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DETECTION OF ANTIBODIES TO A NON-VACCINAL *LEPTOSPIRA* SEROVAR IN DOGS IN SOKOTO STATE, NIGERIA.

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ABSTRACT
A prospective serological investigation for leptospiral antibodies and risk factors associated with the infection were carried out in dogs in Sokoto metropolis, Sokoto State-Nigeria. The Leptospira IgG Microwell ELISA was used for the serological confirmation of infections in sera of 196 dogs sampled. The influence of Sex, Breed, and Season as multiple predictors or risk factors of Leptospira infection was determined using multiple logistic regression analysis at P≤ 0.05. A total of 66 (33.7%) sera tested positive for Leptospira antibodies in sampled dogs. Sera with high antibody titre were further evaluated for antibodies to the prevailing serovars of leptospires by the Microscopic Agglutination Test (MAT) at a cut off titre of 1:100. Antibodies to Leptospira Canicola, L. Icterohemorrhagiae, L. Pomona and L. Bratislava were detected. Dog breed and season were significantly (p<0.05) and positively correlated with the prevalence of Leptospira antibodies in the study area. The detection of Leptospira antibodies in the dogs suggests that non vaccinal Leptospira serovars are likely present in dogs within the study area and these may serve as potential sources of zoonosis to man.

***Keywords*:** Dogs, *Leptospira,* Antibodies, Prevalence, Serum, Sokoto

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INTRODUCTION

Leptospirosis is a highly contagious, infectious and zoonotic disease of worldwide significance in human and virtually all species of animals in which it causes abortion, still birth, infertility, decreased milk production and death in domestic and wild animals [1, 4]. The disease has varying prevalence in different regions of the globe [2, 3]. Leptospirosis is caused by infections with highly pathogenic, antigenically distinct serovars of *Leptospira interogans* [5].

The organism is divided into two species, *Leptospira biflexa* and *L. interogans*, the former being saprophytic. All pathogenic strains of *Leptospira* belong to a single species *Leptospira interogans* [6]. Primary reservoirs of *Leptospira* organisms are the rodents while infected animals like dogs, pigs and cattle can act as reservoirs after recovery or those having subclinical infections [7]. Studies have shown that *Leptospira* organisms are maintained in nature by any of the following rodents: rats, mouse, raccoons, opossums, hedgehogs, vole, foxes, skunks, bandicoots, and weasels [8]

For centuries, serovars Canicola and Icterohemorrhagiae were regarded as the sole agents of canine leptospirosis. Canine leptospirosis associated with these serovars have been reported in the United States of America and Canada for more than a century [9]. By the 1990s however, an increased

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incidence of *Leptospira interogans* serovar Grippotyphosa and serovar Pomona was observed [10]), thus suggesting a changing trend in the epidemiology of the disease. It is speculated that these changes in serovar prevalence are related to two primary factors that may strongly influence the epizootiology of leptospiral serovars [10]). These factors include vaccination which has virtually eradicated clinical infection as a result of serovars Icterohemorrhagiae and Canicola in dogs. Secondly, increasing urbanization associated with greater contact between pets and wildlife may result in unknown serovars like *L.* Grippotyphosa and *L.* Pomona becoming important in the epidemiology of leptospirosis. Veterinarians and researchers have become aware of the emergence of t a number of previously unknown ones. For instance, recent serological studies on pets and wildlife reservoirs in the United States suggest that *L. Grippotyphosa* and *L. Pomona* have replaced *L. Icterohemorrhagiae* and *L. Canicola* [11].

Leptospires are transmitted between animals by direct contact with body fluids (urine, blood, saliva and milk), venereal and placental transfer, and bite wounds. Ingestion of infected meat, contaminated water and vegetables as well as contaminated beddings are also important sources of infections [12, 13]. This research was conceived therefore, to determine the overall prevalence of leptospiral antibodies and serovars in serum samples of dogs from various parts of Sokoto metropolis.

MATERIALS AND METHODS

Study Area

This study was conducted in Sokoto metropolis, Sokoto State, located in Northwestern Nigeria between longitudes 4^0 8´E and 6^0 54´E, and latitudes 12^0 N and 13^0 58´N [14].

Study Design

This was based on a prospective serological survey for the antibodies against *Leptospira* in serum samples of dogs in Sokoto metropolis, Sokoto State, Nigeria. Blood samples were taken from four locations within the metropolis: the Veterinary Teaching Hospital attached to the Usmanu Danfodiyo University, Sokoto (VTHUDUS), the Sokoto State Zonal Veterinary Clinic, Sokoto, the Mammy Market in the Army Barrack, Sokoto, and from the residence of clients in different parts of the metropolis. A simple random sampling was done of every available dog with bias for unvaccinated dogs of all ages and all breeds encountered. Samples were taken over a 7 month duration (April, 2010-October, 2010), in two seasons (Dry and Wet). The breed, sex, age, season, and address of each dog sampled were documented. The sample size for the study was calculated using the formula:

$$\mathbf{N= (z\alpha)^2 P (1-p)/d^2 \quad =196 \text{ Samples.}}$$

Where N is the minimum sample size; $(z\alpha)^2$ =1.96 at 95% confidence interval; P is the estimated prevalence 50% (0.5) and d^2 is the allowable precision of 5% (0.05) [15].

Sampling Technique

Stratified random sampling was employed by dividing the study area into four strata or locations on the basis of availability of dogs to sample in these locations. The four locations chosen were: The Sokoto State Veterinary Clinic located in Sokoto metropolis, the Veterinary Teaching Hospital, Usmanu Danfodiyo University Sokoto (VTHUDUS), the Mammy Market in the Army Barrack and clusters of clients widely distributed in Sokoto metropolis. The ages of the sampled dogs were obtained from either their clinic records or by direct dentition as described by de Lahunta and Habel [16].

Sample Collection and Preservation

About 5 ml of blood samples were aseptically collected from the cephalic vein into appropriately labeled sample tubes without EDTA (Becton Incorporated, USA) [8]. The samples were left in a slanting position at 45º for 30 minutes to harvest the serum which were immediately refrigerated at -20ºC in the Large Animal Unit of The Veterinary Teaching Hospital, Usmanu Danfodiyo University Sokoto until use [6].

Serum samples were screened using a commercially available *Leptospira* IgG Microwell ELISA Kit (Diagnostic Automation, Inc, Calabasas-USA). The serology was carried out using the protocol provided by the manufacturer while the assay was done in the Bacteriology Laboratory of the National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria.

Statistical Analysis
Statistical analysis and comparison between control and tests wells as well as investigating disease associations with sex, breed, and season was done using univariate and multivariate logistic regression models contained in Stata C 10® by StataCorp, Lakeway Drive, College Station, Texas, USA. The Likelihood ratio test (LRT) was used to evaluate the difference between nested models, using a stepwise backward exclusion of variables starting with the least significant (i.e. highest *P* value). [17].

Serotyping the *Leptospira*
To determine antibodies for prevailing leptospiral serovars in the sera, a purposive non probability sampling technique was used with bias for strongly positive samples as determined by the ELISA test. These included samples having optical absorbance (Optical Density: OD readings) close to or higher than the positive control. Only 12 (2.5%) such samples satisfied the inclusion criteria and were used for this study.

The 12 samples were serologically analyzed using the Microscopic Agglutination Test (MAT), to determine the serovars present in these samples with six reference serovars (Icterohemorrhagiae, Canicola, Grippotyphosa, Pomona, Bataviae, and Bratislava) obtained from a month old culture of leptospires in Ellinghausen-McCullogh-Johnson-Harris (EMJH) medium incubated at 25^0C [18]. The strain or serovar that caused 50% agglutination at the cut off titer used was recorded as the serovar that elicited antibody production in the test serum and in the dog sampled. [5].

RESULTS AND DISCUSSION
A total of 66 (33.3%) samples tested positive to *Leptospira* antibodies out of the 196 samples used for the study (Table I). This is lower than the seroprevalence of 46.2% reported by Okewole and Ayoola [19] in Ibadan, Southwestern Nigeria. In another survey in dogs in the same study area, Agunloye *et al*. [20] reported a seroprevalence of 31.1%; thus suggesting an increasing prevalence pattern in that region.

The low prevalence recorded in our study in Sokoto, a semi-arid region in comparison with the high prevalence recorded in Southwestern Nigeria (a humid region) may be due to the climatic variations in the two regions. Previous studies show that damp and coastal areas with heavy rainfall favour the survival of leptospires in the environment and hence a high likelihood of infections among pets [19].

Table 1: Prevalence of Leptospiral antibodies in dogs in Sokoto, Nigeria

Variables		Number examined	No. (%) positive	Relative prevalence %)
All dogs		196	66 (33.7)	100.0
Breed	Local	113	18 (15.9)[a]	27.3
	Caucasian	3	1 (33.3)[a]	1.5
	Rottweiler	15	1 (6.7)[a]	1.5
	Alsatian	65	46 (70.7)[b]	69.7
Sex	Male	98	26 (26.5)[a]	39.4
	Female	98	40 (40.8)[a]	60.1
Season	Wet	120	59 (49.2)[a]	89.4
	Dry	76	7 (9.2)[b]	10.6

[ab] Figures in the same column with different superscripts within breed, sex or season are significantly different ($P < 0.05$)

The German shepherd breed showed a significant association (P<0.05) with prevalence of leptospiral antibodies. A relative prevalence of 69.7% was obtained for this breed (Table 1). Okewole and Ayoola [19] recorded 46.2% in the same breed in 2009. The preponderance of high prevalence of leptospiral antibodies in the German shepherd breed remains speculative whether it is inherent in that breed as maintenance hosts for specific but undetermined serovars. It is tempting from the result of this study to deduce strong correlations between the prevalence of leptospiral antibodies and the German shepherd breed. However, Raju *et al*. [21] found no basis for leptospires to have particularly high affinity for any specific dog breed.

There was a relatively higher prevalence of *Leptospira* antibodies in female than male dogs; a relative prevalence of 60.1% in females against 39.4% in males (Table 1). This is similar to the reports of Harkins and Gatley [22] who found a high prevalence among female than male dogs in South Africa. The authors based their observations on the probability of females being associated with frequent sniffing of damp areas where urine of rodents are found which if contaminated can lead to infection. Although this may probably be the reason for the high relative prevalence recorded in the female group, Miller et al. [23] noted that in Australia young male dogs were relatively more frequently infected than females.

Regarding the two seasons used for the study (Dry and Wet seasons), higher prevalence was recorded in the Wet season particularly about August , the peak of the rains. Significantly ($p < 0.05$) higher prevalence (89.4%) was recorded during the Wet than Dry (10.6%) season (Table I).

Table 2: Distribution of *Leptospira* serovars obtained from detectable antibodies in dogs examined at Sokoto, Nigeria.

Serovars	No. (%)	Location	Breed	Mean MAT titre
Leptospira canicola	6 (50.0)	A, B, C	Rottweiler	1:300
L. icterohaemorrhagiae	3 (25.0)	B, D	Alsatian	1:300
L. pomona	1 (8.3)	A	Alsatian	1:200
L. bratislava	1 (8.3)	C	Caucasian	1:300

A = Mammy market slaughter slab at Sokoto Army Barrack; B = State Veterinary Clinic, Sokoto; C = Sokoto South Metropolis and D = Veterinary Teaching Hospital, Usmanu Danfodiyo University, Sokoto.

In Nigeria, information on human and canine leptospirosis are limited; physicians and veterinarians hardly consider leptospirosis in their differential diagnosis of cases. The potential economic losses through abortions and deaths of animals as well as the role of this disease as an important zoonosis have been ignored for a long time. This unfortunate development is in contrast to the situation in other regions of the world where active and continuous research is carried out on the surveillance and prevention of this zoonotic disease.

This study has shown that serovars Canicola, Icterohemorrhagiae, Pomona and Bratislava are present in Sokoto metropolis (Table 2). The serovars (Canicola, Icterohemorrhagiae and Pomona are already known to be causes of leptospirosis in the study area while the non vaccinal serovar Bratislava is being reported for the first time in the study area. This serovar (Bratislava) has earlier been identified in Ibadan and cities other than the study area [19].
The results of this study have far reaching implications for pets and humans in the state, since close association between pets and humans may predispose the latter to infection with the identified serovars. It is highly probable that, vaccinal serovars may be or are gradually being replaced by other serovars not covered by the bivalent and quadrivalent vaccines, the only commercially available vaccines for protective immunization against canine leptospirosis. This disturbing conclusion of the existence of non vaccinal serovars is shared by researchers in South Africa and other parts of the world, where serovars Bataviae, Copenhageni and Autumnalis have been documented [24].

The same reality and concern is shared globally that, should vaccination be continued with the present bivalent and quadrivalent types amidst new serovars emerging and replacing the popular serovars known as causes of canine leptospirosis, then a growing subpopulation of unprotected dogs and wildlife to unknown non-vaccinal serovars would emerge which would portend danger for public health.

CONCLUSION

The results of this study support reports from other parts of Nigeria that leptospirosis is still prevalent in dogs and that serovars known to be causes of this disease exists in Sokoto State. The study also suggest that breed and season may be important multiple predictors of the prevalence of the disease in dogs in the study area. In the light of this, there is need for a widely and co-ordinated prospective seroepidemiological study to profile and document vaccinal and non-vaccinal emerging serovars in Sokoto State that may compare with other records in other regions on the importance of leptospirosis as a global zoonosis and the necessity of re-appraisal of the present day vaccines.

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