Nigerian J. Anim. Sci. 2022, 24 (1): 67-74

Seroprevalence, isolation and spatial distribution of *Leptospira* serovars in dogs in Sokoto State, Nigeria

[1]Pilau, N.N. and [1]Zaid, Shehu

[1]Department of Veterinary Medicine, Usmanu Danfodiyo University Sokoto

***Corresponding Author:** nicholaspilau@gmail.com **Phone Number:** +2347032409535*

***Target Audience**: Veterinarians, para-veterinarians, animal scientists, laboratory technologists, researchers, biomedical students and animal science enthusiasts*

Abstract

This study undertook a prospective serological and bacteriological survey for Leptospira infection inferred to be endemic by previous sketchy research in most regions of Nigeria. An enzyme conjugate system and culture isolation globally used to screen for Leptospira was used to screen blood and urine samples collected in canine population in studied region. Major objective is to determine potential spatial occurrence of the infection so that quantitative and qualitative risks will be demonstrated for informed public health safety. An overall serological and bacteriological prevalence of 16.4% and 11.75 respectively was recorded. Out of the nine districts studied, only 3 (33.3%) were negative for Leptospira in the State, 6 (66.7%) of the nine districts were positive for isolates constituting a widespread distribution of subsets of canine population closely in contact with humans serving as potential maintenance and dispersal source of leptospires. Two districts presented a statistically significant ($P<0.05$) association in isolation of Leptospira and the region of study essentially due to demographically and socio-economically related variables determining outcomes. This study demonstrates empirical data of circulating leptospires in dogs with widespread distribution. There is therefore a qualitative and quantitative risk for zoonotic spread given an ever-existing canine human bond, this also establishes risks for livestock population given evidence of cross species infection with canine adapted leptospiral serovars

***Keywords**: Leptospira, Spatial, Zoonosis, Infection, Epidemiology, Reservoir*

Description of Problem

Zoonotic diseases are an important threat to public health worldwide as they represent about 70% of the number of emerging infectious diseases in recent time (1). Leptospirosis remains one of the most common and most dreaded zoonotic infection worldwide (2), traditionally associated with certain socio-economic and climatic settings typical in developing regions of the world that favors endemicity in animal vectors and human exposure (2). Leptospires are spirochetes of the genus *Leptospira* (3) that are categorized into three groups comprising saprophytic, intermediate, and pathogenic species (4). Pathogenic leptospires are the causative agent of leptospirosis, which is a worldwide zoonotic disease with a significant impact on both public and veterinary health. The pathogen is maintained in the renal tubules and genital tract of many domestic and wild mammalian species (5).

Many animals may have sub-clinical infections and may be persistent shedders of the organism in their urine (6). In human, more than 500,000 cases of severe leptospirosis are reported each year, with mortality rates exceeding 10% (7)

Most cases of leptospirosis are diagnosed by serology in resource-limited settings (8). The reference standard assay is

the Microscopic Agglutination Test (MAT) in which live antigens representing different serogroups of leptospires are reacted with serum samples and then examined for characteristic *Leptospira* colonial morphology using darkfield microscopy (9).

In Nigeria, four background sero-surveys in healthy people yielded leptospiral prevalence ranging 13.5% in eastern State (10), 18.0% in the Central State of Jos (11) and a typical confirmed case report of a patient presenting non specific febrile illness in Jos about which culture, polymerase chain reaction and phylogenetic analysis through sequencing turned out positive for a *Leptospira* serovars (8). Enzyme conjugated assays are also used to diagnose *Leptospira* based on sequestered group-specific antibodies produced, particularly the IgM which become detectable in acute infections of about 7-days onset. For this purpose, the IgM or IgG *biflexa*-coated enzyme linked immunosorbent assay (ELISA) kit has been used extensively in many studies on Africa for *Leptospira* screening in human and canine populations (3).

Dynamics of leptospires in blood, urine and other body fluids or tissues after natural infection are well studied in the literature (3), but surprisingly is scarcely described for experimental infection, notably on dogs. Urine has remained therefore, a veritable clinical sample for determining chronic infection and elucidating subsets of apparently healthy dogs that may be shedding serovars o*f Leptospira* in the environment

Materials and Methods

Sample Size and Sample Collection

The study was carried out in Sokoto State, northwestern Nigeria (12-13.9667, 4.1333-6.9) as part of a wider study on survey for *Leptospira* serovars in dogs. Sample frame was generated to constitute all Local Government Areas (LGAs) that constitute Sokoto State. Random sampling by pocking an LGA for each zone was used to select locations for sampling. Total Sample Size N was calculated using the formulae $N=Z^2P\ (1-P)/d^2$ (12), taking assumed prevalence (P) to be 33.3% based on a previous study carried out on dogs in 2011 by (13). Total number of samples "N" calculated was 342, it was divided amongst these Local Government Areas (LGAs) to obtain relative sample "n" for the various locations to sample within the sample frame generated. A total of 5ml of urine was collected by cystocentesis using a 25ml eppendorp tubes (genewise®), each collected urine sample was tested on site with a litmus paper, where acidic, another 10ml of bicarbonate buffer was added and then the sample batch was labeled and transported to the laboratory.

Serological Screening

The DAI IgG *Leptospira* Microwell Elisa test kit sourced from Difco Laboratories, Detroit Michigan was used for serological screening of serum samples according to the manufacturer's protocol. Negative controls were measured at 0.0-0.3 OD and positive samples determined as ≥0.5 OD at an absorbance of 450nm.

Culture Isolation and Microscopy

Each urine sample was cultured according to the method described by (4) at the Central Diagnostic Laboratory, National Veterinary Research Laboratory, (NVRI), Vom-Plateau State, Nigeria. The Ellinghausen-McCullough-Johnson Harris (EMJH) (Difco) basal media was prepared by dissolving 2.3g of powdered media per liter, 10% glycerol, PH 7.4. Fetal calf serum was added to commercial leptospiral enrichment (Difco) prepared according to methods described by (4, 14). Final liquid EMJH was prepared by dissolving 100ml enrichment and 10ml fetal calf serum per

liter of EMJH basal media. Media was sterilized through 0.22um filtration system. 2mL of urine samples were inoculated into 18mL EMJH liquid media and incubated at room temperature. Cultures were sub cultured every 10 days and viewed weekly by dark field microscopy using the Olympic® BX 51 Olympic at magnifications 40, 60, 100.

Results

The serological and bacteriological prevalence of *Leptospira* in dogs in Sokoto State with odds ratio describing the degrees of risks to infection for each selected referred location are as shown in Table 1. Relative prevalence of the various LGAs included in the sampling frame is also presented. Canine population sampled recorded an overall serological and bacteriological prevalence of 16.4% and 11.7% respectively. Highest isolation rates seen at Sokoto North, Wamakko and Sokoto South with bacteriological prevalence of 16%, 12% and 17.6% respectively, these Local Government Areas constitute the metropolis of the State with elites resident and abundant canine population. Wamakko and Gwadabawa LGAs presented a statistically significant association ($P<0.05$) with occurrence of detectable antibodies against *Leptospira* in canine population sampled.

Figure 1 shows spatial distribution of infection to *Leptospira* generated by plotting detectable antibodies to *Leptospira* with different Local Government Areas Sampled, A map of the state used with algorithm to describe spatial distribution and relative risks presented by each location based on the results recorded. The result showed Sokoto North, Sokoto South, Dange Shuni and Kware LGAs present elevated comparative risks for infection of leptospires similar to LGAs specified for culture isolation data.

Table 1: Serological and bacteriological prevalence of *Leptospira* in dogs based on the different locations in the study area

	ELISA		CULTURE			
Variables	**Positive**	**Negative**	**Positive**	**Negative**	**P_E**	**P_C**
Location(s)						
Shagari (n=11)	0(0.0%)	11(100.0%)	0(0.0%)	11(100.0%)	0.23	0.33
Gwad. (n=19)	0(0.0%)		1(5.3%)	18(94.7%)	0.03*	0.20
Bodinga(n=12)	19(100.0%)		0(0.0%)	12(100.0%)	0.57	
Wurno (n=16)	1(8.3%)	11(91.7%)	0.29			
Kware (n=9)	1(6.2%)	15(93.8%)	0(0.00)	16(100.0%)	0.34	
D/Shuni (n=85)	2(22.2%)	7(77.8%)	0.19			
S/South (n=83)	19(22.4%)	66(77.6%)	0(0.0%)	8(88.9 %)	0.78	0.91
Wamakko(n-32)	17(20.5%)	66(79.5%)	1(11.1%)	70(82.4%)	0.87	0.95
S/North[a] (n=75)	1(3.2%)	31(96.8%)	15(17.6%)	76(91.6%)	0.90	0.22
Total	15(20.0%)	60(80.0%)	4(12.9%)	7(87.1%)	0.03*	0.92
	56(16.4%)		12(16.0%)	64(84.0%)	1.00	1.00
	286(83.6%)		40(11.7%)	302(88.3%)		

OR (Shagari 95% Cl) 0.00 (0.00, 1.406), **OR (Gwadabawa 95%CI)** 0.00 (0.00, 0.774), **OR (Bodinga 95%CI)** 0.367 (0.016, 2.421), **OR (Wurno 95% CI)** 0.269 (0.012, 1.711), **OR (Kware 95% CI)** 1.141 (0.149, 5.759), **OR (D/Shuni 95% CI)** 1.151 (0.534, 2.505), **OR (S/South 95% CI)** 1.030 (0.469, 2.274), **OR (Wamakko 95% CI)** 0.135 (0.006, 0.814), **OR (S/North 95% CI)** 1.00[a]Reference category, *Significant, OR=Odd Ratio, CI=Confidence Interval, P_E=P-value(Elisa), P_C=P-value (Culture)

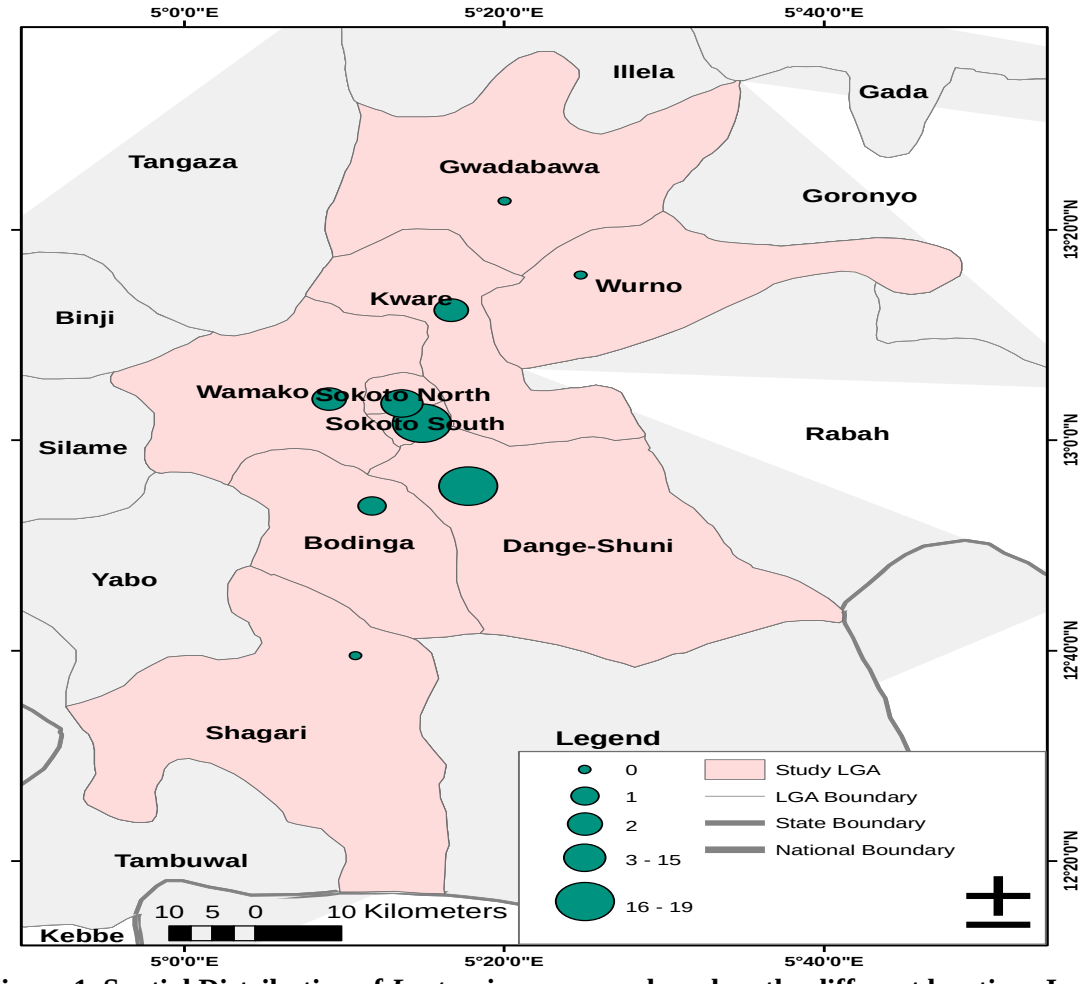

Figure 1. Spatial Distribution of *Leptospiro* serovars based on the different locations In Sokoto State

Discussion

The overall bacteriological prevalence of 11.7% appears lower compared to a previous study by (13) that reported 33.3% using microscopic agglutination test (MAT) to test clinical samples against predetermined serovars. Both studies used different techniques with expectedly, different outcomes. The time difference of a decade may have changed demographic variables that affect disease or infection outcomes as well. The present study is pivotal in developing preventive programs and building capacity to predict relative risks in locations. *Leptospira* in many respects is like *Yersinia*, both being pathogens which should not be found circulating in incidental, maintenance or accidental hosts for likelihood of dispersal of the organism in the population. Continuous screening is therefore imperative in symptomatic or asymptomatic animals using rapid diagnostics or, where available, culture isolation. The data generated in this study therefore underscores the importance of focusing the spotlight to prevent possible

epidemic or deaths from pyrexia of unknown origin (PUO) often associated with leptospiral outbreaks.

Most studies in Nigeria on *Leptospira* focus on isolation of the organism and sequencing targeted genes of *Leptospira* serovars. Studies on the spatial patterns of distribution and associated predictive variables that establishes risks of infection are quite few. The relative prevalence of the various towns and LGAs used in the study raises important research questions, which can be developed in a wider study to understand the variables associated with differences in risks between various locations in a State. The high prevalence obtained in Sokoto South, Sokoto North, Wamakko and Kware relative to other districts may be associated with abundance of canine hosts in those areas located in the center of the metropolis, other considerations not captured in the design of this study may be vaccination and management factors.

Awareness has increased internationally over the past decade that leptospirosis is a globally important public health threat, both in developing countries and industrialized countries (15). In a global concerted effort to control the spread of leptospirosis in its fascinating re-emerging forms, focus is now shifted to Africa where typical socio-economic, cultural, demographic and ecological factors prevail for maintenance and perpetuation of leptospires. This underscores prospective serological screening and where possible, culture isolation studies to generate basic epidemiological data that will be referral and directional to track the origin, spread and evolution of *Leptospira* serovars in regions of developing countries.

In most countries and human settlements in Sub Saharan Africa, the urban and suburban areas are densed and characterized by decayed infrastructure, notably slums, poor waste disposal, minimal drainages with consequential water lodging. These remediable conditions provide ideal ecological basis for rapid disease transmission and may precipitates sporadic epidemics in the event of floods. The data in this research of varying infection rate in the different regions may not be unconnected with the variations in climatic, socio-economic and demographic determinants. Sanitation, population density, socio-economic and educational factors informing such things as vaccination, confinement and visitation to veterinary hospitals differ. One limitation of this study is the lack of associated questionnaire to test educational, socio-economic and cultural determinants of *Leptospira* infection in the populace

Contacts with pets and livestock in Northern Nigeria poses an increased risk of *Leptospira* infection (16). It is evident dogs in different parts of Sokoto State in the extreme northwest sharing a border with Niger Republic are serving as maintenance host for *Leptopira* serovars (Table 1). It is undetermined if the *Leptospira* isolates were pathogenic or saprophytic as both group I, group II and group III *Leptospira* strains can grow in the selective medium used. However, previous studies have established saprophytic serovars of *Leptospira* are incapable of prolonged colonization as they have very low infectivity, mostly found in the environment and not in mammalian infection (17). It is also undetermined which specific serovars the isolates were as that will require sequencing studies or a molecular assay with specific designed primers. The data shows quantitative and qualitative risks of animal to human transmission of *Leptospira* across the greater population in Sokoto State Nigeria.

Understanding the epidemiological features of leptospirosis is a critical step in designing interventions for reducing risks of

transmission (18).

Due to the complexity of disease transmission, prevention of leptospirosis is difficult in low and middle-income tropical countries where the ecology provides an ideal environment for spread of leptospires (19). Basic data on prevalence and factors driven infection is the first step in prevention. Monitoring and surveillance will target areas with known risks for transmission and maintenance of the organism. Most expert researches on *Leptospira* advocate delineating reservoirs of the disease and a quantitative as well as a qualitative assessment of human associations with those reservoirs (20). The present study studied dogs because of their historical and continuous associations with humans.

Pets and other livestock are considered an important reservoir that contributes substantially to the transmission of leptospirososis (16). Proximity to animals by virtue of a person's lifestyle or occupation is generally associated with the re-emergence and escalated risks of exposure to zoonotic pathogens, thus, occupations like farmers, nomads, hunters, veterinarians, butchers, animal handlers and abattoir workers presents relative risks for infection with *Leptospira* from animal sources in Nigeria. While the present study did not quantify risks based on occupational exposure, the data of relative risks in different regions will inform policy managers on animal health and public health. A wider study is advocated to determine which serovars were isolated through generated amplicons on targeted genes or whole genome sequencing of isolates. Identifying the serovars will enable tracing origin, course and dispersal of the isolates as well as interrupting transmission.

Conclusion and Applications

This study, based on empirical data established the following:

1. *Leptospira* is circulating in both symptomatic and asymptomatic canine population in different Local Government Areas of Sokoto State.
2. Overall prevalence of *Leptospira* recorded was 11.7%. The spatial distribution of infection is complimentary for both serological and bacteriological data.
3. The areas with highest infection rate and therefore with the greatest risk for human infection were Sokoto North, Sokoto South, Wamakko and Dange Shuni. The data from this study can be applied to prioritize the regions in Sokoto State for advanced studies on the ecological and demographical determinants establishing this increased risk. Isolation and prophylactic treatment of all dogs as well as selective curative treatment can be embarked since *Leptospira* is sensitive to a number of antibiotics with doxycycline been the choice drug. The data will serve as a wake up call to diagnosticians and clinicians to increase suspicion index for *Leptospira* that before now is rarely considered

References

1. Cutler, S.J., Fooks, A.R.., van der Poel, W.H.M.(2010). Public health threat of new reemerging and neglected zoonosis in the industrialized world. *Emerging Infectious Disease* 16: 1-7.
2. Pappas, G., Papadimitriou, P., Siozopoulou, V., Christou, L., Akritidis, N. (2008) The globalization of leptospirosis: worldwide incidence trends. *International Journal of Infectious Diseases.* 12:351-357.
3. Ahmed, A., Grobusch, M.P., Klatser, P.R., Hartskeerl, R.A. (2011).

Molecular approaches in the detection and characterization of *Leptospira*. *Journal of Bacteriology and Parasitology*. 3: 133-137.

4. Freitas, J.C.D., Silva, F.G.D., Oliveira, R.C.D., Delbem, Á.C.B., Müller, E.E., Alves, L.A. (2004). Isolation of *Leptospira* spp from dogs, bovine and swine naturally infected. *Ciência Rural*. 34:853-6.
5. Ellis, W.A. (2010). "Control of Canine Leptospirosis in Europe: Time for a Change?" *Vet Rec* 16: 602-605.
6. Levett, P.N. (2001). Leptospirosis. *Clinical Microbiology Review*. 14:296-326
7. Fragas, T.R., Barbosa, A.S. and Isaac, L. 2011). Aspects of Innate immunity, immunopathogenesis and immune evasion from the complement system. *Scandinavian Journal of immunology* 73: 408-419
8. Isa, S.E., Onyedibe, K.I., Okolo, M.O., Abiba, A.E., Mafuka, J.S., Simji, G.S., Nathan, S.Y., Udoh, U.A., Awang, S.K., Egah, D.Z., Banwat, E.B., Newport, M. and Ahmed, A. (2014). A-21-year old student with fever and profound jaundice. *PLOS Neglected Tropical Diseases* 8: 2534-2540
9. Levett, P.N. (2007). *Leptospira* In: Murray, P.R., Baron E.J., Jorgensen, J.H. Eds, Manual of Clinical Microbiology, 9th edition, American Society for Microbiology Press, Washington, USA. Pp 963-970.
10. Onyemelukwe, N.F. (1993). A serological survey for leptospirosis in Enugu area of eastern Nigeria among people at occupational risk. J*ournal of Tropical Medicine and Hygiene.* 96:301-304.
11. Ezeh, A.O., Kmety, E. Ellis, W.A., Addo, P.B., Adesiyun, A.A. (1991). A new leptospiral serovar in the Pyrogenes serogroup isolated in Nigeria. *Review of Science Technology*. 9:195-6
12. Thursfield M. Veterinary Epidemiology 3rd Edition ed: Wiley-Blackwell, p29
13. Pilau, N.N., Daneji, A.I., Mera, U.M., Adamu, Y.A., Magaji, A.A. and Okoli, C.E. (2011). Detection of Antibodies to a non-vaccinal *Leptospira* serovar in dogs in Sokoto. *Journal of Veterinary and Applied Science*. 1:7-10.
14. Wuthiekanun, V., Amornchai, P., Paris, D.H., Langla, S., Thaipadunpanit, J., Chierakul, W. (2012). Rapid isolation and susceptibility testing of *Leptospira* spp. using a new solid medium, LVW agar. *Antimicrobial Agents and* Chemotherapy, 57: 297-302.
15. Bharti, A.R., Nally, J.E., Ricaldi, J.N., Matthias, M.A., Diaz, M.M., Lovett, M.A. (2003). Leptospirosis: A zoonotic disease of global importance. *Lancet Infectious Disease*. 3: 757-771.
16. Omitola, O.O. and Taylor-Robonson, A.W. (2020). Emerging and re-emerging bacterial zoonoses in Nigeria: current preventive measures and future approaches to intervention. *Heliyon*, 6: 4095-4105.
17. Ganoza, C.A., Matthias, M.A., Saito, M., Cespedes, M., Gotuzzo, E. and Vinetz, J.M. (2010). Asymptomatic renal colonization of humans in the peruvian Amazon by *Leptospira*. *PLoS Neglected Tropical Diseases.* 4: 612-622.
18. Assimina, Z. and Fatoula, B. (2008): Leptospirosis: Epidemiology and preventive measures. *Health Sciences Journal* 2: 75-82.
19. Pereira, M.M., Da-Silva, J.J., Pinto, M.A., Da-Silva, M.F., Machado, M.P., Lenzi, H.L. and Marchevsky, R.S (2005). Experimental leptospirosis in

marmoset monkeys (*Callithrix jacchus*): a new model for studies of severe pulmonary leptospirosis. *American Journal of Tropical Medicine and Hygiene.* 2005: 72:13–20.

20. de Vries, S.G., Visser, B.J., Nagel, I.M., Goris, M.G.A., Hartskeerl, R.A., Grobusch, M.P. (2014). Leptospirosis in Sub-Saharan Africa: A systematic review. *International Journal of Infectious Diseases*. 28:47-64.