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Detection of Antibodies to Non-Vaccinal *Leptospira Serovars* in Dogs in Jos North and Jos South Local Government Area

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ABSTRACT

The study was carried out to determine the prevalence of non-vaccinal Leptospira serovars in dogs in Jos metropolis. A total of 200 blood samples of different dogs were collected from Jos North and Jos South local government areas of Plateau State to detect antibodies to non-vaccinal Leptospira serovars using microscopic agglutination test (MAT) and parameters of age, breed, location and sex were determined. Data obtained were presented using descriptive statistics and Chi-squared Test was used to test for association between the variables. In a total of 129 dogs were positive, with prevalence of 20 (35.71%) in puppies (1-4 month), 72 (83.72%) in growers (4-7 month) and 37 (63.79%) in adult (> 7months). The association between puppies, growers and adults was not statistically significant ($p>0.05$). Although there was statistical significance ($p<0.05$) between females and males examined, 75 (70.09%) of the 93 females examined were positive while 54(58.06%) of the 107 males examined were positive. 67 (54.03%) of 112 exotic breed were positive and 62 (81.58%) of 78 indigenous breed were positive. However there was statistical significance ($p<0.05$) between the exotic and indigenous breeds and also there was statistical significance ($p<0.05$) between Jos North and Jos South local governments with 82 (67.21%) were positive out of 122 from Jos North and 47 (60.26%) out of 78 from Jos South were positive. It is therefore concluded that there is a high prevalence of antibodies to non-vaccinal leptospira serovar in the growers, indigenous and in Jos north local government and therefore it is encouraged that adequate vaccination and proper management to reduce transmission of the leptospira.

***Keywords:** Non-vaccinal Leptospira Serovars, antibodies, Jos, Prevalence.*

INTRODUCTION

Leptospirosis is a zoonotic disease of worldwide veterinary significance in many animal species [1].It is caused by antigenically distinct Serovars of the spirochetes belonging to the genus Leptospira and family Leptospriracea. It is classified serologically into two species, the pathogenic species leptospira interrogans and saprophytic species Leptospira biflexa. The genus have been classified into new species on the basis of genetic relatedness [2].

Leptospira icterohaemorrhagiae and *Leptospira canicola* were identified as the most prevalent *serovar* causing leptospirosis in canine species worldwide [2].Infections with these *serovars* typically cause a hepato-nephric syndrome[2], characterized by acute haemorrhagic diathesis, subacute icterus or subacute uremia in dogs [3].Worldwide use of different brands of commercial bivalent vaccines against two *serovars* has led to decreased incidence of *leptospirosis* in dogs[4].In the last ten years, however, veterinarians have become aware that a number of newly identified *serovars* can cause clinical disease in dogs [4, 5,6].

Clinical manifestation in this cases were more of renal than hepatic involvement before death. Yet, the available bivalent commercial vaccines are always *serovar*-specific in their protection but also protects against clinical diseases only [4].

Leptospirosis is a bacteria disease caused by pathogenic species of the genus *leptospira* [7] characterized by acute multi-organ system febrile disease affecting man and animals. It is considered to be a significant, re-emerging zoonotic disease throughout the world [8, 9, 7, 10, 2].

Leptospires are thin, coiled or spiral shaped organisms with a characteristic terminal hook [11, 9]. Both pathogenic and saprophytic free living species exist [7], which are morphologically indistinguishable [12]. The outer membrane of the organism includes lipopolysaccharide (LPS) and antigenic lipoproteins (LipL21, LipL32, LipL36, LipL41), variation in these outer membrane components allows grouping of *leptospires* into antigenically distinct *serovars* and serogroup [2].

The organism has a worldwide distribution [11, 7, 13], prevalent in temperate and tropical regions of developing and industrialized countries both in rural and urban areas [8].The widespread distribution is a reflection of urinary shedding of *leptospires* into the environment by domestic and wildlife maintenance hosts and the ability of *leptospires* to persist in the environment outside of a host [14]. The risk of host exposure to *leptospires* is dependent on many factors including seasonal variations in climate, density of maintenance hosts and contact between reservoir and accidental hosts [11, 8].

Infection requires direct contact with infected tissue or urine, or with contaminated soil or water [12, 15]. Pathogenic *leptospires* can invade damaged skin and intact mucous membranes such as the conjunctiva, spreading rapidly via the bloodstream within minutes [11, 9, 15]. These extracellular pathogens can be highly virulent, adhering to and invading host cells [11].Vascular endothelial damage is the primary disease mechanism, leading to organ damage including but not limited to hepato cellular ischemia, renal tubular necrosis, pulmonary haemorrhage, mycositis, uveitis and meningitis [11, 9]. The degree of injury and severity of clinical signs may vary between serovars and with differing host susceptibility [9]. Localizing within the proximal convoluted of the kidney affords some protection from humoral immunity, thereby allowing persistent infection and urinary shedding of the organism [16, 9, 10]. Post-infection modification of *leptospiral*-like protein epitopes has been demonstrated, suggesting an additional mechanism of immune system evasion that enables persistent infection [17].The persistent and intensity of shedding vary between individuals, host species and infecting serovar [11].

METHODOLOGY

Sample Collection

A total of 200 (two hundred) blood samples of different dogs were collected from Jos North and Jos South local government areas of Plateau State. The dogs' samples were grouped as follows:-from 1-4 months (Puppy), 4-7 months (Grower), and 7 months and above (Adult).The blood was collected through the cephalic vein using sterile needle and syringe. The blood was allowed to stand and the serum was collected into cryo-vials and was taken to the central Diagnostic Laboratory of the National Veterinary Research Institute Vom. Those that were not properly separated were centrifuged for 30 minutes at 2000rpm. The sera were preserved in the freezer at -20^{0}C. The samples were diagnosed using the microscopic agglutination test (MAT) where the specific antigens are reacted with the serum collected. A dilution series of patient's (dogs) serum is mixed with a suspension of live *leptospires* in micro titer plates. After incubating for about 2 hours at 30^{0}C, result is read under the dark field microscope [18].

The microscopic agglutination test (MAT) is the most widely used test for identification of infection in patients with appropriate clinical signs [19, 20]. It is widely available, relatively inexpensive and there is a large body of information available about its use [13]. It has reportedly high sensitivity and specificity, especially when used in the convalescent phase rather than in the acute phase of disease [21] and it is considered a gold standard serological test [22].

A serial dilution of the patient's serum was mixed with cultured *leptospira* organisms from a panel of serovars representing different serogroups [20, 13]. Dark field microscopy was used to assess agglutination of the organisms by antibody (largely IgG and to a lesser extent, IgM) [23, 2]. The titer reported for each serogroup is the greatest dilution of the sera that caused50% agglutination of the organisms representing that serogroup (reported as a reciprocal of the dilution). Positive titers are regarded as exposure (or vaccination) within 12 months of the test [13]. Results were regarded as

serogroup specific, rather than serovar specific as there was significant cross-reactivity between serovars within the same serogroup[2, 13].

There is considerable debate what titers are indicated; a single high MAT titer (≥800) to a non-vaccinal serovar and concurrent negative or low (<400) titers against vaccinal serovars, accompanied by clinical signs of *leptospirosis*, is highly suggestive of current infection according to some researchers [3, 24, 2]. Others suggested a minimum titer 200 [25] or even 1600 [26, 27] is required to confirm infection, especially in vaccinated dogs [25].

A more reliable indicator of acute infection is a fourfold increase in MAT titers. One study reported 45% of *leptospiral* infections in dogs would have been missed if convalescent serology had not been performed [25]. MAT test results are often negative in the first week after infection, especially in younger dogs, therefore a second serum sample should be obtained and MAT tested within two weeks [3, 2, 13]. Negative initial antibody tests can be explained by the delay before IgM production rises and therefore before MAT agglutination can be detected. MAT titre become positive after about one week, peak around 4 weeks, and remain positive for months after both natural infection and vaccination [23, 2]. This peak in MAT titers may be blunted following antibiotic treatment [15, 13] and a convalescent titer of 100 following initial sero negativity in treated animals is also considered indicative of infection by some authors [8].

A titer of ≥100 can be used as evidence of past exposure for epidemiological sero-surveys [13], and for discriminating positive from negative animals, however conclusions about infecting serovars require isolation confirmation as the correlation between the highest MAT and the infecting serovar can be as low as 50% [12, 20, 11, 14]. In addition, vaccinal titer cannot be differentiated from natural exposure using the MAT; some authors will ascribe different cut-off values for vaccinal and non-vaccinal serovars in order to minimize the confounding effect of vaccination [28].

RESULT

The tables below show the relationship and distribution of detection of antibodies to non vaccinal *leptospira* serovars in relation to age, sex, breed, and location

Table1: Age distribution of *leptospira* in dogs in Jos north and south local government areas of plateau state.

Age in month	Positive	Negetive	Total
1- 4 (puppies)	20 (35.71)	36 (64.23)	56 (28%)
4- 7 (growers)	72 (83.72)	14 (16.28)	86 (43%)
> 7 (adult)	37 (63.79)	21(36.20)	58(29%)
Total	129	71	200

X^2=0.38 P=0.08746 d/f=2

Table2: Sex distribution of *leptospira* in dogs in Jos north and south local government areas of plateau state.

SEX	POSITIVE	NEGETIVE	NUMBER OF SAMPLE
MALE	54 (58.06%)	39 (41.94%)	93 (46.50%)
FEMALE	75 (70.09%)	32 (29.96%)	107 (53.50%)
TOTAL	129 (64.5)	71 (35.5%)	200 (100%)

X^2=0.8519 P=0.02092 d/f=2

Table3: Distribution of *leptospira* base on breed in dogs in Jos north and south local government areas of plateau state.

BREED	POSITIVE	NEGETIVE	NUMBER OF SAMPLES
EXOTIC BREED	67 (54.03%)	57 (45.97%)	122 (62%)
INDIGENOUS BREED	62 (81.58%)	14 (18.42)	78 (38%)
TOTAL	129 (64.5%)	71 (35.5%)	200 (100%)

X^2= 0.3997 P= 0.1926 d/f=2

Table 4: Distribution of *Leptospira* in Dogs Base on Location.

LOCATION	POSITIVE	NEGETIVE	NUMBER OF SAMPLES
JOS NORTH	82 (67.21%)	40 (82.79%)	122 (61%)
JOS SOUTH	47 (60.26%)	31 (39.74%)	78 (39%)
TOTAL	129 (64.5%)	71 (35.5%)	200 (100)

X^2= 0.3311 /X^2/= 0.05806 d/f=2

DISCUSSION

*Leptospirosis*is a re-emerging zoonotic disease is an important cause of vasculitis, renal and hepatic disease and should be a potential diagnosis for dogs presenting with hemorrhagic uremic or icteric symptoms, particularly in endemic areas. It is a serious disease in dogs with a reported case fatality rate of 10-20% [2].

Based on the finding in dogs that were presented at the clinics in National Veterinary Research Institute, bukuru and Jos and environs, blood samples were collected and their sera were separated to detect antibodies to non *leptospira* serovars apart from those in the vaccine (*canicola* and *icterohaemorrhagiae*). In the table, age variation of 1-4 months (puppies), 4-7 months (Growers) and 7 above (adult), the growers were found to have more number of positive than the number of negative. According to Greene *et al*., [13] and Sykes *et al.,* [2] younger dogs less than six (6) months seem to develop more signs of hepatic dysfunction in an outbreak of leptospirosis, however, acute renal failure in young dogs is often associated with *L. gripptotyphosa* and more than one form of leptospirosis may occur in given animals and the clinical manifestation can vary among outbreak and geographical areas with a given serovar. Also related to age there was no significant difference using the one way ANOVA as statistical analysis which shows the p value is greater than 0.05.

This study showed that prevalence of *Leptospirosis* in dogs based on sex is significant ($p<0.05$). It is found out that the sero positive is relatively higher in females than males due to the geographical areas although the result reveals contrary to Rentko *et al*., [4] and Ward *et al*., [29] whose study shows a greater susceptibility of female dogs than in males because male dogs spend more time outside compared to females.

The result of this detection is convincing enough that *leptospirosis* is still endemic in the north than the southern part of Plateau state according to the survey done. But considering the study location which is cold and windy, *leptospirosis* maybe transmitted from one location to another. And the result was subjected to a method of statistical analysis (one way ANOVA) which shows that there is no significant base on location ($P>0.05$).

While the results of this survey in no way suggested that serovars *icterohaemorrhagiae* and *canicola* are no longer prevalent in the two local government areas. It has presented a host range of prevailing serovars against which vaccinal protection should be directed.

Epidemiologically, current *leptospiral* vaccination with the commercial bivalent vaccines (*Canicola* and *Icterohaemorrhagiae*) is bedeviled with two problems: it has a shorter duration of immunity about 6 months [2] than the viral antigens in combination with it and also has a high risk of post-vaccinal hyper sensitivity. Yet, it is only commercially available in this combination with the distemper antigen in particular that has recently been recommended for fewer vaccination protocols [30]. Should this be widely adopted, a growing subpopulation of unprotected dogs and wildlife to all serovars would emerge in both urban and suburban cities.

The most effective control measure, therefore, would be to eliminate the "carrier state". Unfortunately, wild animal reservoirs and the sub clinically infected animals, including vaccinated dogs, continue to harbor and shed organisms. Also, control of rodents, rats and mice in kennels, as well as the maintenance of environmental conditions that exclude the survival of *leptospiral* organisms, are all part of the effective control methods.

Furthermore, prompt isolation of infected dogs, good kennel hygiene that stresses clean food and water, and prompt doxycycline treatment of the isolated cases further enhance control. In view of the emerging new serovars, a polyvalent commercial envelope vaccine like Duramune (Fort-Dodge, USA) or the pentavalent-outer envelope vaccine [31] that includes the new serovars might be more protective than the old bivalent types.

CONCLUSION AND RECOMMENDATION

In view of the effect of *leptospirosis* which is a zoonotic disease, measures should be taken to curtail the transmission of the organism (*leptospires*) through contact with contaminated urine and blood, especially in animal husbandry, those who engage in water sportpeople whose occupation expose them to wildlife and domestic animal host. Further investigation of

leptospirosis, diagnosis can be done using the following methods.

- Polymerase chain reaction (PCR) in identification of *leptospiral* DNA (do, FA staining of urine dark field microscopy.
- Direct detection of bacterium which may be done by culture of urine or blood culture.

REFERENCES

[1] Daher, EF, Soares DS, de Menezes Fernandes ATB, Girão MMV, Sidrim PR, Pereira EDB. Risk factors for intensive care unit admission in patients with severe leptospirosis: a comparative study according to patients' severity BMC Infect Dis, 2016: 16: 40 View Record in Scopus

[2] Greene CE, Sykes JE, Moore GE, Goldstein RE, Shultz RD. Leptospirosis. In: Greene CE, ed. *Infectious Diseases of the Dog and Cat*. 4th ed. St. Louis, Mo: Saunders Elsevier; 2012: 431–447.

[3] Adin CA, Cowgill LD. Treatment and outcome of dogs with Leptospiorosis: 36 cases(1999-1998). *Journal of the American Veterinary Medicine Association*. 2000: 216(3): 371-375.

[4] Rentko VT, Clark N, Ross LA, Schelling SH. Canine leptospirosis. A retrospective study of 17 cases. *Journal of veterinary Internal Medicine*.1992: 6(4), 235-244

[5] Brown CA, Roberts WA, Miller-Liebi MA. Leptospirain terrogans serovars grippotyphosa infection in dogs. *Journal of America Veterinary Medicine Association*.1996: 209: 1265-1267.

[6] Harkin KR, Roshta YM, Sullivan JT. Clinical application of a polymerase chain reaction assay for disease of leptospirosis in dogs. *Journal of the American Veterinary Medical Association*.2003:22(9), 1224-1229.

[7] WHO. Human Leptospirosis: guidance for diagnosis, suivellance and control. Geneva: *World Health Organisation*. 2003.

[8] Bharti AR, Nally JE, Ricaldi, JN, Matthias M.A, Diaz, MM, Lovett MA. Leptospirosis: a zoonotic disease of global importance. *Lancet Infectious Diseases*.2003: 3(12), 757-771.

[9] Langston CE, Heuter KJ (2003).Leptospirosis: A re-emerging zoonotic disease. Veterinary Clinics of North America: *Small Animal practice*.2003: 33(4), 791-807.

[10] Stokes RA and Forrester SD. New and unusual causes of acute renal failure in dogs and cats. *Veterinary Clinics of North America. Small Animal Practice*. 2004: 34(4), 909-922.

[11] Levette PN. Leptospirosis. *Clinical Microbiology Reviews*.2001:14(2), 296.

[12] Faine S. Guidelines for the control of leptospirosis. Geneva: *World Health Organisation*. 1982.

[13] Sykes JE, Hartmann K, Lunn KF, Moore GE, Stoddard RA, Goldstein RE. 2010 ACVIM small animal consensus statement on leptospirosis: diagnosis, epidemiology, treatment, and prevention. *Journal of Veterinary Internal Medicine*. 2011: 25: 1–13.

[14] Harkin DA, Levette PN. Leptospirosis in Humans *Leptospira* and leptospirosis. 2015: 65-97.

[15] Van de Maele I, Claus A, Haesebrouck F, Daminet S. Leptospirosis in dogs: a review with emphasis on clinical aspect. *The Veterinary Record*. 2008: 163(14), 409-413.

[16] Marshall RB, Manktelow BW.Fifty years of leptospirosis Research in New Zealand: a perspective. *New Zealand Veterinary Journal*. 2002: 50, 61-63.

[17] Witchell TD, Eshghi A, Nally JE, Hof R, Boulager M J, Wunder Jr E A, Cameron C E.Post-translational modification of LipL32 during Leptospirain terrogans infection. PLoS neglected Tropical Diseases. 2014: 8(10), 3280.

[18] Goris, M.G.A., Leeflang, M.M.G., Loden, M., Wagenaar, J.F.P. Prospective evaluation of three rapid diagnostic tests for diagnosis of human leptospirosis. PLoS Neglected Tropical Diseases. 2013: 7 (7): e2290. Gussenhoven, G.C.,

[19] O'Keefe J, Jenner J, Sandifer N, Antony A, Williamason N .A serosurvey for antibodies to leptospira in dogs in the lower North Island of New Zealand. *New Zealand Veterinary Journal*. 2002: 50(1), 23-25.

[20] O'Keefe JS. A brief review on the laboratory diagnosis of leptospirosis. *New Zealand Veterinary Journal*. 2002: 50(1), 9-13

[21] Bajani MD, Ashford DA, Bragg SL, Woods CW, Aye T, Spiegel RA, Wegant RS. Evaluation of four commercially available rapid serologic tests for diagnosis of Leptospirosis. *Journal of clinical Microbiology*.2003: 4(2), 803-809.

[22] Nally JE, Chantranuwat C, Wu XY, Fishbein MC, Pereira MM, Da Silva JJ, Blanco DR, Lovett MA. Alveolar septal deposition of immunoglobulin and complement parallels pulmonary hemorrhage in a guinea pig model of severe pulmonary leptospirosis. Am J Pathol. 2004;164:1115–1127.

[23] Hartman EG, Van Houten M, Frik JF, Van der Donk JA. Humoral immune response of dogs after vaccinated against Leptospirosis measured by an IgM and IgG-specific ELISA. *Veterinary Immunology and Immunopathology*. 1984: 7(3-4), 245-254.

[24] Harkin KR, Roshto YM, Sullivan JT, Purvis TJ, Chengappa MM. Comparison of polymerase chain reaction assay, bacteriologic culture, and

serologic testing in assessment of prevalence of urinary shedding of leptospires in dogs. *Journal of America Veterinary Medical Association*; 2003: 222(9):1230–1233.

[25] Tangeman, LE, Littman MP. Clinicopathologic and a typical features of naturally occurring Leptospirosis in dogs: 51 cases (2000-2010). *Journal of the American Veterinary Medical Association.*2013: 243(9), 1316-1322.

[26] Gautam R, Wu C-C, Guptill LF, Potter A, Moore GE. Detection of antibodies against *Leptospira* serovars via microscopic agglutination tests in dogs in the United States, 2000–2007. *Journal of America Veterinary Medical Association*. 2010: 237(3):293–298.

[27] Miller MD, Annis KM, Lappin MR, Lunn KF. Variability in results of the Microscopic Agglutination Test in dogs with clinical leptospirosis and dogs vaccinated against leptospirosis. *Journal of veterinary Internal Medicine*. 2011: 426-432.

[28] Stokes JE, Kaneene JB, Schall WD. Prevalence of serum antibodies against six *Leptospira* serovars in healthy dogs. *Journal of America Veterinary Medical Association*; 2007: 230(11):1657–1664.

[29] Ward MP, Guptill LF, Prahl A, Wu CC. Serovar-specific prevalence and risk factors for Leptospirosis among dogs: 90 cases (1997-2002). *Journal of the American Veterinary Medical Association.*2004: 22(1), 53-58.

[30] Olson, P., H. Finnsdottir, B. Klingeborn, A. Hedhammar. Duration of antibodies elicited by canine distemper virus vaccinations in dogs. Vet. Rec. 1997: 141, 654-655.

[31] Bey RF, Johnson RC. Humoral immune response of dogs vaccinated with Leptospiral pentavalent outer envelop and whole culture vaccines. *America Journal of Veterinary Research*1978: 39, 831-836.

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