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Leptospiral Antibodies in Vaccinated and Non-Vaccinated Dogs

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Abstract

A serological study on canine leptospirosis was conducted in healthy and suspected cases of (vaccinated and non-vaccinated) house dogs from urban areas of Tiruchirappalli district, Tamil Nadu. Totally 70 dogs blood samples were collected and tested for anti-*Leptospira* antibodies using MAT, 24 (34.3%) were positive. *Leptospira* spp. Serovar Grippotyphosa (11.4%), Canicola (10%) and Icterohaemoragiae (8.6%) has found to be higher predominant in urban areas, which includes the post-vaccinal titres <800 and titres of 800 or greater were classified as suggestive of acute/current infection. Amongst 17 suspected cases of clinical leptospirosis, 12 (70.6%) were seropositive which is 83.3% of the animals were vaccinated and in healthy house dogs 12 (22.8%) of 53 were seropositive and 50% of healthy dogs only vaccinated. Only 7.1% *Leptospira* serovar canicola and Icterohaemoragiae was identified as vaccinal serovars against vaccinated dogs with titre value 1:400 or below.

Keywords: Leptospiral antibodies, Dogs, MAT, Grippotyphosa, Canicola

1. Introduction

Dogs are considered as maintenance hosts for *Leptospira* serovar Canicola, incidental hosts for other serovars, and are a potential source of infection for pet owners [1]. Historically, canine leptospirosis has been associated with serovars Canicola and Icterohaemorrhagiae, but canine serum may contain antibodies specific for a wide range of serovars including Autumnalis, Bratislava, Grippotyphosa, Hardjo, Pomona, and Zanoni [2]. Widespread use of bivalent vaccines produced in the 1980s that are serovar-specific for only Canicola and Icterohaemorrhagiae has resulted in a decreased prevalence of disease associated with those serovars. Renal failure (and less commonly hepatic failure) associated with infection with serovars not present in this bivalent vaccine has become the predominant clinical syndrome in both vaccinated and unvaccinated animals. In animals presenting with renal failure secondary to leptospirosis, lethargy, depression, anorexia, dehydration, and vomiting are common historical complaints with polyuria, polydipsia, and abdominal pain noted less commonly. Other significant physical examination findings may include fever, renomegaly, muscle pain, and icterus [3].

2. Materials and Methods

2.1 Sample Collection and Processing

The blood samples were collected from 70 house dogs in Veterinary Polyclinic, Tiruchirappalli, Tamil Nadu, India, each of apparently healthy (53) and suspected cases (17) of clinical leptospirosis, the animals were classified as vaccinated (59), non-vaccinated (9), irregularly vaccinated (2), and Male (52)

and Female (18). All blood samples were spun down in a centrifuge, the sera harvested aseptically and frozen at -80°C until tested.

2.2 Detection of Leptospiral antibodies in dogs sera

The microscopic agglutination test (MAT) was applied according to the standard method to evaluate the presence of leptospiral antibodies in animal serum [4]. A panel of 12 leptospiral reference serovars, *L. interrogans* serovars Australis (strain Ballico), Autumnalis (strain Akiyami A), Bataviae (strain Swart), Canicola (strain Hond Utrecht IV), Hebdomadis (strain Hebdomadis), Icterohaemorrhagiae (strain RGA), Pomona (strain Pomona), Pyrogenes (strain Salinem) *L. kirschneri* serovar Grippotyphosa (strain Moskva V), *L. borgpetersenii* serovars Ballum (strain Mus 127), Javanica (strain Poi), and Sejroe (strain Hardjoprajitno) were used as antigens. A five to seven days old live *Leptospira* culture was used as antigen at a concentration of 1–2 × 10^8 organisms/mL in MAT. Each serum samples were serially diluted with phosphate buffer saline (1X PBS) in a 96 well microtitre plate and tested in doubling dilutions starting from 1:20, incubated for 2–3 hours at 30°C and the agglutination was observed under dark field microscopy (Nikon, Made in Japan).

3. Results and Discussion

Positive leptospirosis MAT results in dogs may indicate natural infection due to direct or indirect contact with wildlife maintenance hosts or recent vaccination. From MAT analysis, the predominant serovars of *Leptospira* spp. were Grippotyphosa (11.4%), Canicola (10%), Icterohaemorrhagiae (8.6%), Autumnalis and Pomona (5.7%), Javanica (4.3), Australis and Hebdomadis (2.9%). The serovers Ballum, Bataviae, Pyrogenes, Sejroe are reported as negative for leptospira. Postvaccinal titers are usually low (1:100 to 1:400), although they can occasionally rise as high as 1:3,200. The antibodies produced by vaccination (typically serovars canicola and icterohaemorrhagiae) can cross-react with other serovars, but at a low titer (<1:100) and usually for less than 3 months. In this case, the serovar with the highest titer is interpreted as the infecting one, with the lower titers likely representing antibody cross-reaction between serovars (Table 1). Such results reinforce the impact of epidemiologic studies for a better understanding of leptospirosis in dogs.

The frequency of agglutinins to the 12 serovars of *Leptospira* tested with various categories of dogs is shown in Table 2. Overall, 70 serum samples tested, 24 were seropositive for leptospirosis, had mixed infections by *Leptospira* serovars and had current/acute infections respectively. *Leptospira* infection was highest in suspected cases 70.6% (vaccinated 83.3% and non-vaccinated 16.7%) and lowest in healthy dogs 22.6% (vaccinated 50%, non-vaccinated 33.3% and irregular vaccination 16.7%). The female dogs (38.9%) were at significantly higher risk of leptospirosis than were male dogs (32.7%) has significant p value <0.05. In addition, dogs in age groups of above 10 years (50%) of age were at significantly greater risk than were dogs younger than 10 years (21%) of age. Vaccinated dogs were 27.1% (16/59) seropositive compared with a seroprevalence of 66.7% (6/9) for non-vaccinated dogs, a difference which is statistically significant with p value <0.05 for vaccinated dogs. These results may represent both vaccination titre and infected state.

In this study, the *Leptospira* serovars canicola, icterohaemorrhagiae and grippotyphosa were serologically predominant for healthy and suspected cases of leptospirosis. Serovars canicola and icterohaemorrhagiae have been reported to be the predominant serovars responsible for canine leptospirosis leading most commercially available vaccines to include both serovars [5,6]. However, other serovars may be responsible for *Leptospira* infections depending on the geographical location,

thereby resulting in ineffective vaccination [7]. It is therefore, the animals were positive for serovar icterohaemorrhagiae, and serovar canicola which is one of the vaccine serovars used by all veterinary clinics in the country. On the other hand, serovar grippotyphosa was most frequently detected in both healthy dogs and in suspected cases of leptospirosis. Vaccination for animal has the only adopted strategy in control of canine leptospirosis but this has not always been successful because protection is short lived and it does not provide cross-protection against other serovars [8]. Although all vaccines used for prevention of canine leptospirosis in the country contain serovars canicola and icterohaemorrhagiae, serovar grippotyphosa has mostly been associated with infection and disease in our study area. From this study, we concluded that the presence of only 7.1% *Leptospira* spp in vaccinated dogs with lower titre value and the titre may raise during infection state with the distributions of serovar variations also.

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5. References

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Table 1 – Leptospiral antibodies identified by MAT in vaccinated and non-vaccinated dog sera samples from Tiruchirappalli district, TN

Serovars	MAT Titres					Total (%)
	1:200	1:400	1:800	1:1600	1:3200	
Australis	01	01	-	-	-	02 (2.9)
Autumnalis	-	02	02	-	-	04 (5.7)
Ballum	-	-	-	-	-	-
Bataviae	-	-	-	-	-	-
Canicola	02	02	03	-	-	07 (10)
Grippotyphosa	02	-	02	03	01	08 (11.4)
Hebdomadis	01	01	-	-	-	02 (2.9)
Icterohamorragiae	01	01	03	01	-	06 (8.6)
Javanica	01	01	01	-	-	03 (4.3)
Pomona	01	01	02	-	-	04 (5.7)
Pyrogenes	-	-	-	-	-	-
Sejroe	-	-	-	-	-	-

Table 2 – Association of *Leptospira* seropositive and explanatory variables ($p \leq 0.05$) among different dog groups

Parameters	Category	No. Tested	No. Positive	% Positive	*P*-value*
Age	< 1 yr	08	03	37.5	0.2048
	1 - 5 yrs	31	11	35.5	0.0577
	5- 10 yrs	19	04	21.0	0.1560
	> 10 yrs	12	06	50.0	0.1051
Gender	**Male**	**52**	**17**	**32.7**	**0.0374***
	Female	18	07	38.9	0.0903
Vaccination program	**Vaccinated**	**59**	**16**	**27.1**	**0.0397***
	Non-vaccinated	09	06	66.7	0.1051
	Irregularly Vaccinated	02	02	100	0.2951
Clinical status	healthy	53	12	22.6	0.0529
	infected	17	12	70.6	0.0529