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Prevalence of canine leptospirosis in Tiruchirappalli, Tamil Nadu

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Leptospirosis is a worldwide zoonosis caused by pathogenic *Leptospira* species. Worldwide around 500 000 persons are affected by leptospirosis annually (Gansen *et al*. 2007). Historically, leptospirosis was recognized as a disease of dogs before it was known in any other animal species, including humans (Faine 1994). *Leptospira interrogans* serovars Canicola and icterohaemorrhagiae are considered as the most significant and predominant serovars causing 'Stuttgart' and 'Weil's disease' respectively. The acute disease may result in the death of the dogs and if they survive may harbor the leptospires in kidneys becoming persistent shedders of the bacteria in urine and thus an important source for human leptospirosis (Levett 2001). Due to the coexistence and close contact between dogs and humans, the transmission of the etiological agent may occur among veterinarians, dog breeders and dog owners (Greene and Miller 1998). Infections of humans with either canicola or Icterohaemorrhagiae have been connected with contact with the urine of infected dogs. For this reason, a study of the prevalent serovars among dogs is important to update the epidemiology of this disease in any area. It is also important to emphasize on a rapid method for the diagnosis of canine leptospirosis. Therefore, the present study was carried out to determine the incidence and prevalence of canine leptospirosis in Tiruchirappalli, Tamil Nadu, India by a rapid 16S rRNA nested PCR based method.

Study area and samples: The study was conducted in Tiruchirappalli city situated in central part of Tamil Nadu. The samples were collected from Government Veterinary Hospital, Palakkarai, Tiruchirappalli where the dogs are brought regularly by their owners because of illness. The study was approved by the Department of Animal Husbandry, Government of Tamil Nadu (Approval number: 77721/JJ/09 dated 2.12.2009). A total of 135 samples were collected from clinically suspected domestic and stray dogs for leptospirosis. Blood was collected on sterile containers by cephalic vein puncture or sephaneous vein puncture and transported to the laboratory on ice. The blood was allowed to clot and centrifuged at 3000 rpm for 5 min. The serum thus resulting was stored at –20°C until processing. Age, sex and breed of the canine populations were also recorded. Inclusion criteria were followed as per Birnnaum *et al.* (1998). Vaccinated dogs were excluded from the study.

Serology: To establish a titre rise or seroconversion the MAT was applied according to standard method (Faine *et al.* 1999). A battery of 12 leptospiral strains used including Australis (serovar Australis, strain Jez-Bratislava), Autumnalis (serovar Autumnalis, strain Akiyami A), Ballum (serovar Ballum, strain Mus 127), Bataviae (serovar Bataviae, strain Swart), Canicola (serovar Canicola, strain Hond Utrecht IV), Icterohaemorrhagiae (serovar Icterohaemorrhagiae, strain RGA), Grippotyphosa (serovar Grippotyphosa, strain Moskva V), Hebdomadis (serovar Hebdomadis, strain Hebdomadis), Javanica (serovar Poi, strain Poi), Pomona (serovar Pomona, strain Pomona), Sejroe (serovar Hardjo, strain Hardjoprajitno) Pyrogenes (serovar Pyrogenes, strain Salinem) were used as antigens. ELISA was performed as described previously (Terpstra *et al*. 1985). The sonicated whole cell lysate of Patoc I was used as antigen in ELISA

DNA extraction: The leptospiral genomic DNA was extracted from serum samples using the methods described previously (Boom *et al.*1990). The extracted DNA was visualized on a 0.8% agarose gel stained with ethidium bromide. The quantity and quality of the DNA was analyzed biophotometerically.

Nested-PCR for 16S rRNA gene amplification: Primer selection and PCR assay were performed as previously described (Djadid *et al.* 2009). The extracted DNA was amplified using primers 5′GGC GGC GCG TCT TAA ACA TG 3′ and 5′GTC CGC CTA CGC ACC CTT TAC G 3′ for

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the first amplification and the second amplification was performed with primers 5′CAA GTC AAG CGG AGT AGC AA 3′ and 5′CTT AAC CTG CTG CCT CCC GTA 3′. PCR was performed in a total volume of 25 µl. Each experiment was carried out in duplicate and a negative control was maintained for each experiment. The amplified fragments were separated on 2% agarose gel stained with ethidium bromide and visualized in a gel documentation system.

A total of 135 dogs satisfied the inclusion criteria. The collected samples came from 89 males and 46 females of age ranging 1 month to 8 years with a mean age of 17 months. The common clinical manifestations observed were fever (92%), sudden illness (52%), anorexia (49%), vomiting (46%), depression (39%), subnormal rectal temperature (<37 ±1.2) (32%), shock (27%), dehydration (22%), oliguria (22%), anuria (16%), renomegaly (12%), and uremia (11%).

The performance of MAT provided a strong evidence of current leptospiral infection. Seroepidemiological analysis indicated out of 135 serum samples 51 (37.8%) were positive for leptospirosis. Leptospirosis cases were mostly males (27%) but there was no significant difference between the infection of leptospirosis in male and female cases.

Highest titre was detected against Autumnalis (25.5%), Australis (7.8%), Icterohaemorrhagiae (19.6%), Bataviae (5.9%), Canicola (21.6%), Hebdomadis (3.9%) and Javanica (15.7%). The antibody titre were in the range of 1:80 and above. The highest titre was observed against the serovar Autumnalis with a median titre of 1 in 640 (Table 1) followed by Icterohaemorrhagiae and Canicola.

The frequency of leptospiral infection was higher for the breed Boxer (100%) followed by Daschund (80%) and Doberman (75%). The decreased frequency of infection was observed among German Shepard (28.6%). Statistical analysis showed significant differences between the leptospiral infections among different breeds. The evaluation of breed wise prevalence of leptospiral serovar showed the frequency of Autumnalis infection to be higher in Doberman (33%), Australis in Pomeranian, Icterohaemorrhagiae in Boxer (50%), Bataviae in Non-discript (12.5%), Canicola in Doberman (33%), Hebdomadis

Table 1. Distribution of Leptospiral serovars among dogs with median antibody titre by microscopic agglutination test (MAT)

Serovars	Seropre valence (n=51)	Percentage	Median titre
Autumnalis	13	25.5	640
Australis	4	7.8	160
Icterohaemorrhagiae	10	19.6	320
Bataviae	3	5.9	80
Canicola	11	21.6	320
Hebdomadis	2	3.9	80
Javanica	8	15.7	160

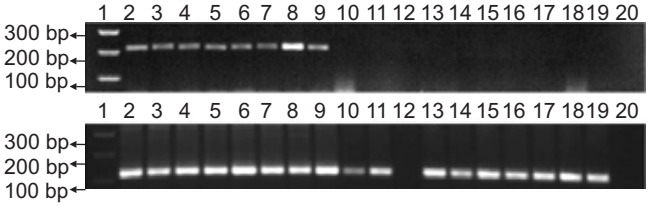

Fig. 1: Nested PCR based detection of canine leptospirosis
A: 16SrRNA primary amplification; B: 16SrRNA nested amplification; Lane 1, 1 Kb DNA marker; Lane 2, *L. interrogans* serovar Autumnalis (positive control); Lane 3–19, Amplification from dog serum samples; Lane 20, Negative control.

in Daschund (25%) and Javanica in Boxer (50%). The IgM ELISA performed also provided evidence of current leptospiral infection.

Further confirmation of leptospiral infections were achieved by subsequent nested PCR analysis of the serum samples. The amplified product at 289 bp was detected for the positive cases. Out of the 135 cases 69 were PCR positive (Fig. 1).

The sensitivity, specificity, negative predictive value, positive predictive value for IgM ELISA, and PCR Vs the gold standard test MAT showed point estimates of sensitivity and specificity ranged from 92% and 73–93% respectively. Test specificity was notably higher for MAT vs PCR with point estimate as 92.8%.

The key feature in the transmission of leptospirosis between animals, as well as between animals and humans, is most probably through the shedding of infectious leptospires in the urine of the carrier animal. Thus the shed leptospires serves as a means of direct transmission of the infection via contamination of mucous membranes of other animals or in direct transmission via contamination of the environment. Rats are considered to be the most significant reservoirs of leptospires worldwide. Since leptospirosis is one of the complicated zoonotic diseases that infects most of the warm blooded animals and dogs with no exception. Canine leptospirosis has been reported from different geographical areas of the world (Green 1996, Zeinali 2000). The dogs were considered as the maintenance host for serovar Canicola and is considered as an incidental host for a variety of other serovars. Canine leptospirosis is usually caused by *L. interrogans* serovar Canicola and *L. interrogans* serovar Icterohaemorrhagiae.

The overall seroprevalence was found to be 37.7% (51/135). In South India, serological evidence of leptospirosis among peripheral dogs showed a seropositivity of 62.5% in Chennai. The predominant serovars encountered in the present study were Autumnalis, Icterohaemorrhagiae, Canicola and Javanica followed by Australis, Bataviae and Hebdomadis. Most clinical cases of leptospirosis in dogs are caused by serovars Canicola, Icterohaemorrhagiae, Grippotyphosa and Pomona (Rentko *et al.*1992). In addition

other serovars namely Australis, Javanica, Ballum, Hardjo, Autumnalis and Pyrogenes were recorded by Senthilkumar *et al.* (2006).

In recent days ELISA proved to be a test with good sensitivity and specificity. It can sufficiently be sensitive to be used as test sieve or screening for the detection of leptospiral antibodies in dogs. However, positive tests should always be confirmed later on with MAT again which becomes tedious. Serological surveys often report more than 20% of examined canine sera to contain antibodies specific for pathogenic *Leptospira* serovars. However it is difficult to correlate serological findings with prevalence of acute infection which has been reported low or negative antibody titre (Faine *et al.*1999, Ellis *et al.* 1981) and the presence of circulating serovars. Thus there is a need for a definitive early diagnosis for the prompt treatment of the disease. In this study a 16S rRNA specific nested PCR for the amplification of the leptospiral DNA from the serum samples was designed. The technique exponentially showed a good sensitivity on comparison with MAT. Twenty two MAT negative cases were positive by nested PCR and the possible explanation for this among the clinically suspected leptospirosis cases may be due to the presentation of the cases during the acute phase of the disease.

Usually during the acute phase the detectable level of the antibody were found to be low and the presence of leptospires may circulate in the blood. Considering this fact the nested PCR may be an alternative incredible choice for the early and accurate diagnosis of leptospirosis cases in animals. 16S rRNA nested PCR has been well evaluated among the human cases earlier (Djadid *et al.* 2009) and the utility of the PCR has been evaluated among the canine cases through this investigation. Thus a combination of clinical signs and PCR can provide a definitive diagnosis of leptopsirosis during the acute phase of the disease which facilitates early treatment.

Canine leptospirosis surveillance serve as an important tool to detect the circulating serovars and it is very essential for the preparation of new vaccine candidates and also to assess the human risk of exposure and may provide insights into which serovars are currently of clinical importance. Considering this fact the nested PCR may be an alternative incredible choice for the early and accurate diagnosis of leptospirosis in animals.

SUMMARY

Canine leptospirosis is prevalent worldwide and generally associated with infection by *Leptospira interrogans* serovar Canicola and Icterohaemorrhagiae. Present study was aimed to determine the incidence and prevalence of canine leptospirosis and also to determine the utility of the 16S rRNA sequence based diagnosis of canine leptospirosis in comparison with the standard microscopic agglutination test (MAT). In total 135 blood samples were collected consisting of both domestic and stray dogs attending the veterinary polyclinic for the treatment of febrile illness. The leptospiral infection was determined by MAT, ELISA and nested PCR analysis. The present investigation showed an overall seroprevalence of 37.7% (51/135) with the predominant serovar to be Autumnalis 9.6% (13/135), followed by Icterohaemorrhagiae 8.14% (11/135), Canicola 8.14% (11/135) and Javanica 6.66% (9/135). Thus the surveillance of canine leptospirosis is an important tool for detecting the risk of exposure in human and it provide an insight about the important prevailing circulating serovars. In the present study 16S rRNA nested PCR for the diagnosis of canine leptospirosis showed a sensitivity 92%, specificity 73%, PPV 68%, NPV 93%, respectively on comparison with MAT which envisaged the detection of canine leptospirosis at the acute phase of the disease.

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