

# A STUDY ON LEPTOSPIROSIS IN DOGS IN AND AROUND BANGALORE

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Leptospirosis, a zoonotic disease, occurs worldwide in numerous animal hosts, including dogs. The disease is caused by more than 226 known serovars each of which can independently infect any susceptible host species.

Carr *et al.*, (2003) had reported canine leptospirosis as an important cause of morbidity in dogs and man in many parts of the world. In India leptospirosis has been recorded in domestic animals (Arora, 1977; Uppal and Singh 1982). Rodents, wild life, pigs, horse and dogs have been incriminated as reservoirs of leptospires (Upadhyay *et al.*, 1979) and contribute to the spread of the disease. Hence the present study was undertaken to screen the dogs for leptospirosis using different tests.

A total of 70 serum/blood samples collected from dog population in and around Bangalore were initially subjected to dark field microscopy (DFM) for detecting leptospires as described by Chandrasekaran and Pankajalakshmi (1997) with minor modifications.

Then the blood samples were subjected to the polymerase chain reaction (PCR) as per the method of Grave Kamp *et al.* (1993) using primers derived from genomic DNA libraries specific for leptospira i.e. G1 (5' CTG AAT CGC TGT ATA AAA GT 3') and G2 (5' GGA AAA CAA ATG CTC GGA AG 3'). The reaction was set up in 50 µl reaction volumes, containing 5 µl of 10X buffer, 1 µl of primers each, 0.5 µl of dNTPs, 0.5 µl of Taq DNA polymerase, 5 µl of template and final volume was made up using double distilled water.

The PCR was performed in a MJ research thermal cycler, for 32 cycles, each consisting of denaturation at 94°C for 90 sec, annealing at 55°C for 60 sec and polymerization at 72°C for 2 min. Both positive and negative controls are kept for each test to cross check false positive and negative. PCR products were finally electrophoresed on 1.5% agarose gels after staining with ethidium bromide and then visualized with ultraviolet light using Gel documentation system (Biorad). If the template is amplified then it will yield a product of 285bp amplicon.

The serum samples diluted to 1:10 dilution using phosphate buffer saline (PBS) were tested for the presence of antibodies against leptospira by Microscopic agglutination test (MAT) as per Merien, *et al.* (1995) with slight modifications. Prior to the test proper the serum samples were inactivated at 56°C. In the present study eight serovars of *Leptospira interrogans* (*australis*, *autumnalis*, *canicola*, *icterohaemorrhagiae*, *pyrogenes*, *grippotyphosa*, *javanica* and *pomona*) maintained in the EMJH medium (Difco) by the Diagnostic Bacteriology and Mycology, IAH & VB, Hebbal, Bangalore were used. A MAT titre of 1:100 or above is taken as significant as per OIE (2005) manual for leptospirosis. Apart from this the blood samples were filtered using 0.22µ filters and inoculated into EMJH (Difco) semisolid and liquid medium for isolation as per the procedure of Venkatesha (1997). The tubes were incubated at the room temperature for 4-6 weeks and were examined at weekly interval for the growth of leptospires.

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In all the 70 samples leptospira like organisms were not detected by DFM and none of the samples produced an amplicon by PCR indicating that leptospires were not detected in the samples screened. The isolation studies revealed no growth of leptospires even after six weeks. Thus, indicating that all the above tests used for the screening of leptospirosis were correlating to each other. Of the 70 serum samples screened by MAT six (8.57%) showed antibody titres above 1:100 (ranging from 1:3200-1:6400) against five serovars of *Leptospira interrogans* (*icterohaemorrhagiae*, *australis*, *pyrogenes*, *canicola* and *autumnalis*). The result was in agreement with the report of leptospires in dogs by Senthilkumar *et al.*, (2006). Theirman, (1980) reported acute systemic infection in canines with leptospira serovars i.e. *canicola* and *icterohaemorrhagiae* and Brown *et al.* (1996) also reported serovars *canicola*, *icterohaemorrhagiae* and *grippotyphosa* are most commonly associated with leptospires in dogs. As all other test did not reveal the presence of any leptospires but only MAT showed significant antibodies titres in few sera samples against serovars *australis*, *pyrogenes* and *autumnalis* which may be due to past exposure to the infection, however antibodies against *canicola* and *icterohaemorrhagiae* may be due to vaccination or infection as vaccination history was not available.

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