LEPTOSPIROSIS IN DOG: DARK FIELD MICROSCOPY AND ISOLATION

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Abstract

A total of 110 canine blood and urine samples were collected from dogs with clinical manifestations suggestive of leptospirosis, presented at the University Veterinary Hospitals, Mannuthy and Kokkalai, Thrissur. The samples were screened for leptospirosis by Dark Field Microscopy (DFM), of which 56 were found positive. All the DFM positive samples were subjected to isolation trials using modified Ellinghausen-McCullough-Johnson-Harris medium (EMJH). Growth of leptospires could be obtained from 11 samples.

Keywords: *Leptospirosis, DFM, isolation, EMJH*

Leptospirosis is a reemerging zoonotic disease of worldwide distribution. It is considered as one of the most dreadful zoonoses in developing countries like India. Dogs act as reservoir host for *Leptospira interrogans* serovar Canicola and is considered as an incidental host for several other serovars. In dogs, the disease is characterised by fever, vomiting, dehydration, collapse, hepatitis, nephritis and death (Faine, 1982).

Laboratory diagnosis of leptospirosis could be made by DFM, isolation, molecular methods and serological tests (Levett, 2003). Approximately 10^4 leptospires per millilitre are necessary for one cell per field to be visible by dark field microscopy (Ahmad *et al.,* 2005). The most definite method of confirming leptospirosis is isolation of the organisms, but the procedure is time consuming.

Adler (1986) stated that the most important factors for isolation of *Leptospira* are aseptically collected material, quick processing, culture medium suitability and selective antibiotics. The objective of this study was to diagnose leptospirosis by DFM and to isolate leptospires from positive cases.

Materials and Methods

A total of 110 canine blood and urine samples collected from clinical cases suspected for leptospirosis, presented during the period from December 2011 to February 2013 at University Veterinary Hospitals, Mannuthy and Kokkalai, Thrissur were used in the study. Approximately three millilitre of blood or urine sample was collected from suspected cases. Urine sample from canines were collected by catheterization in vials containing equal volumes of sterile phosphate buffered saline (PBS). One to two drops of whole blood or a drop of plasma was inoculated in the medium with aseptic precautions and inoculum was thoroughly mixed. A drop of urine was inoculated into two or more tubes as described for blood.

Leptospira EMJH culture medium base (Difco) with supplement was employed for isolation of leptospires. Ellinghausen-McCullough-Johnson-Harris medium containing

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100 µg of 5 fluorouracil/ mL and supplemented with bovine albumin (SRL) was used. Bovine albumin supplement was enriched with zinc sulphate, calcium chloride, magnesium chloride, ferrous sulphate, copper sulphate, tween 80, vitamin B_{12} and sodium pyruvate.

Leptospira semi-solid medium was prepared by adding 0.2 per cent bacteriological agar to *Leptospira* liquid medium.

All inoculated culture tubes were incubated at 37ºC for 24 h and then at 28 to 30ºC for a period of two to three months. Tubes were examined at weekly intervals for the presence of leptospires by DFM. Tubes showing growth of leptospires were subcultured into fresh medium.

Results and Discussion

Darkfield microscopy of the samples showed that 50.90 per cent of the total collected blood and urine samples were positive for leptospires. Cells with typical morphology and motility resembling leptospires were observed. Dark field microscopy was useful in early diagnosis of leptospirosis, which was in accordance with Chandrashekaran and Pankajalakshmi (1997) who demonstrated leptospires in blood of dog.

Out of total 110 samples tested, 56 were found DFM positive. As shown in Table. 2, from 56 samples *Leptospira* isolation was successful in 11 (19.64 per cent) samples. Out of 52 blood samples, *Leptospira* spp. could be isolated from ten (19.23 per cent) samples. De-Feritas *et al*. (2004) isolated *Leptospira* spp. from naturally infected dogs, cattle and pigs using modified EMJH medium. Four urine samples were inoculated for isolation trials, from which one (25 per cent) had shown the growth of leptospires. Similar results were obtained by Natarajaseenivasan and Ratnam (2000), who isolated 12 *Leptospira* isolates from various sources like ailing human urine, dead albino mice, dead Wistar rats, field and house rats. Inoculated tubes in which growth of leptospires were detected after 8 to 12 weeks, were further subcultured and maintained. Other culture tubes having contamination and which could not show the growth of leptospires, were discarded as negative after 16 weeks. The methodology used in this study was found to be efficient in the isolation of *Leptospira* from clinical cases of canines. The identification of these isolates will allow new epidemiological and prophylactic studies of leptospirosis in Thrissur.

Table 1. Result of DFM examination of clinical samples

Type of sample	Dark field microscopy		
	No. tested	No. positive	% positive
Blood	89	52	58.42
Urine	21	4	19.04
Total	**110**	**56**	**50.90**

Table 2. Results of isolation of *Leptospira* from DFM positive clinical samples

Type of sample	Isolation in EMJH		
	No. tested	No. positive	% positive
Blood	52	10	19.23
Urine	4	1	25
Total	**56**	**11**	19.64

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