



# CLINICO-PATHOLOGICAL STUDIES ON LEPTOSPIROSIS IN DOGS IN THRISSUR\*

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Received : 23.03.2017

Accepted : 01.06.2017

## Abstract

The present study was carried out in eleven animals that was diagnosed with leptospirosis. Observation of jaundice, myalgia, bloody diarrhea, haematuria and signs of renal failure formed the criteria for inclusion in the study. Clinico-pathological and ultrasonographic evaluation was carried out to study the changes in blood and internal organs. Confirmation of leptospirosis was done by dark field microscopy (DFM) and microscopic agglutination test (MAT). The findings were compared with control animals.

**Keywords:** Jaundice, Leptospirosis, Dark Field Microscopy, Microscopic Agglutination Test.

Leptospirosis is an anthroponozoonotic infection occurring worldwide caused by a pathogenic spirochetal organism belonging to the genus *Leptospira* (Greene *et al.*, 2012). The southern states of India, mainly Kerala and Tamil Nadu has been found to be endemic

for leptospirosis. (Balakrishnan *et al.*, 2008; Abhinay *et al.*, 2012). Kerala gets heavy rains and intermittent floods from April to November. Intermittant flooding of low lying areas led to repeated flushing of the rodent burrows into water sources like ponds, streams, rivers and canals where leptospires could survive for months and cause the disease (Kuriakose *et al.*, 1997). Canine leptospirosis offers a great risk to humans because of the close association between the pet and its owner.

## Materials and Methods

The study was conducted among 11 dogs brought to Teaching Veterinary Clinical Complex, Mannuthy and University Veterinary Hospital, Kozhikode. Clinically observable yellowish discolouration of sclera and visible mucous membranes, fever, myalgia, bloody diarrhea, haematuria and signs of renal failure were the criteria for inclusion in the present study.

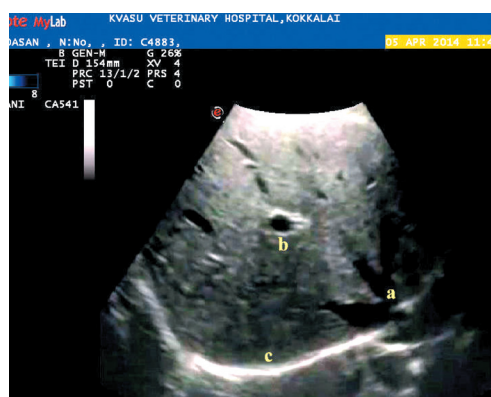
\*Part of MVSc thesis submitted by first author to KVASU

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Detailed clinical examinations of the selected cases were conducted. The haematological variables included haemoglobin, PCV, total erythrocyte count (TEC), total leukocyte count (TLC), differential leukocyte count, platelet count and erythrocyte morphology. The serum biochemical variables included alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), total, direct and indirect bilirubin, cholesterol, glucose, blood urea nitrogen, serum creatinine, serum total protein, albumin and globulin. Confirmation of leptospirosis was done by dark field microscopy (DFM) and microscopic agglutination test (MAT). The live *Leptospira* organisms were identified by their typical morphology and motility in DFM. Twenty microlitre of the sample was placed on a clean grease free slide and examined under dark field microscope without coverslip using low power and high power objectives for typical cork screw motility. The MAT was carried out as described by Faine *et al.* (1999). All the leptospiral serovars prevalent in the region were included in the battery of cultures used for MAT. Hepatic and renal ultrasonography was done to evaluate parenchymal changes in liver and kidney according to the method described by Nyland *et al.* (2002).

## Results and Discussion

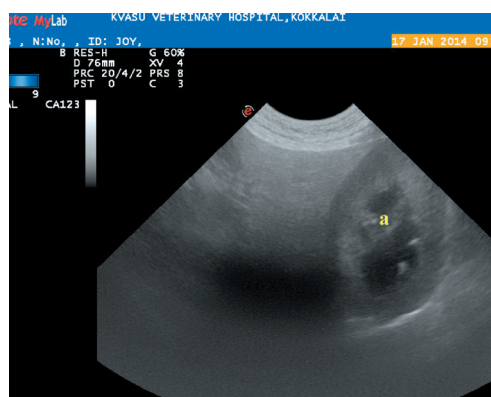
The age of the dogs diagnosed with leptospirosis ranged from 3 months to 9 years with a mean age of 4.5 years. The breeds diagnosed with leptospirosis were Labradors (n= 3), German Shephard (n = 3) one each in Rottweiler, Spitz, Basset hound, Bull mastiff and Dachshund. Seven cases were found to be males and four were females. The clinical signs were fever (mean rectal temperature  $103.65 \pm 0.25^{\circ}\text{F}$ ), icterus, anorexia, lethargy, vomiting, myalgia (which was reported as leg pain and refusing to stand up), bloody diarrhoea, haematuria and shivering. The clinical signs observed were in accordance with Greene *et al.* (2012). Sagittal and transverse ultrasound scan of the liver showed diffusely hypoechoic parenchyma (Fig. 1). Kurtz *et al.* (1980) observed an overall reduction in echogenicity of the liver parenchyma and attenuated



**Fig. 1:** Ultrasonographic appearance of liver. The decreased hepatic echogenicity (hypoechoic parenchyma) indicates hepatitis and congestion. Image shows a) hepatic veins b) portal veins c) diaphragm.

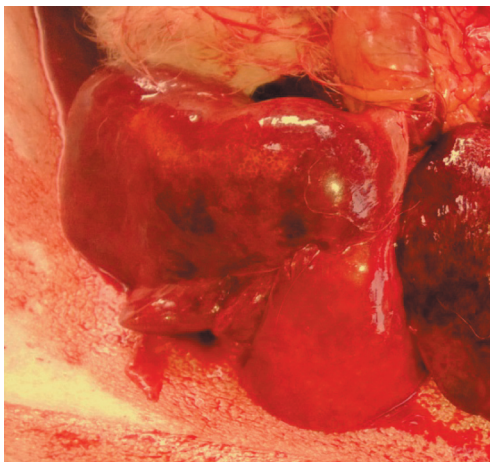
brightness and more extensive demonstration of the portal veins in acute leptospiral hepatitis as observed in the present study. Birnbaum *et al.* (1998) observed hepatomegaly in dogs with leptospirosis. Gall bladder was observed as a pear shaped anechoic structure on the right side of the liver with no apparent abnormalities. The generalized reduction in echogenicity might be associated with congestion. Increase in size of the liver and smooth margins might be associated with hepatitis. On scanning the kidneys, there was an enlargement in the size of the kidneys and loss of corticomedullary distinction indicating acute nephritis (Fig. 2).

The haematological and biochemical values of the affected dogs were compared with six apparently healthy dogs brought for routine vaccination and check up. The total serum



**Fig. 2 :** Ultrasonographic appearance of Kidney showing a loss of corticomedullary distinction (a).

bilirubin was elevated to  $5.18 \pm 0.7$  out of which the conjugated and unconjugated bilirubin were  $3.22 \pm 0.49$  and  $1.96 \pm 1.25$  mg/dl respectively. Kidney and liver were the major parenchymatous organs targeted by the leptospire, therefore jaundice observed in the present study might be the result of hepatocellular and vascular injury along with intrahepatic cholestasis, hepatocytic swelling and inflammation which occluded the small bile ducts and increased the reflux of bilirubin into circulation by increasing the membrane permeability to bilirubin. The pattern of jaundice observed in the present study were in accordance with the findings of Greenlee *et al.* (2004), Geisen *et al.* (2007) and Sykes *et al.* (2010).



**Fig. 3:** Appearance of liver on post mortem examination showing extensive congestion in the liver along with rounding of margins



**Fig. 4:** Appearance of kidney on post mortem examination showing nephritis and adherence of capsule to cortex.

The mean total leucocyte count in diseased animals was  $29.18 \pm 2.48$  ( $\times 10^3$  cells/cmm) with  $80.46 \pm 1.69$  per cent neutrophils,  $16.74 \pm 1.77$  per cent lymphocytes,  $1.14 \pm 0.14$  per cent eosinophils and  $1.57 \pm 0.25$  per cent monocytes. The mean total erythrocyte count (TEC) of diseased animals was  $4.57 \pm 0.45$  ( $\times 10^6$ /cmm). The mean values of haemoglobin and PCV were  $10.92 \pm 1.01$  g/dl and  $33.75 \pm 2.87$  per cent respectively. The mean platelet count of diseased animals was  $1.57 \pm 0.2$  ( $\times 10^5$ /cmm). The mean serum protein of diseased animals was  $5.5 \pm 0.29$  g/dl. Albumin, globulin and albumin to globulin ratio were  $2.18 \pm 0.22$ ,  $3.31 \pm 0.3$  g/dl and  $0.72 \pm 0.09$  respectively. Serum ALT and AST values of diseased animals were  $1295.82 \pm 356.44$  and  $905.73 \pm 183.07$  IU/L respectively. Serum ALP and GGT activities were  $291.36 \pm 51.05$  and  $12.09 \pm 1.69$  IU/L respectively. Mean serum creatinine of diseased animals was  $3.43 \pm 0.51$  mg/dl and blood urea nitrogen was  $144.45 \pm 18.31$  mg/dl. Mean serum glucose in diseased animals were  $72.82 \pm 3.78$  mg/dl and mean serum cholesterol was  $168.09 \pm 17.58$  mg/dl. On urinalysis, proteinuria was observed in 7 out of 11 cases, glucosuria was observed in 5 cases and bile pigments were observed in all cases. Benzidine test for detection blood in urine was positive in three cases. Microscopical examination of sediments revealed presence of erythrocytes in three cases. Screening of faecal sample revealed absence of parasitic ova thus ruling out gastrointestinal nematodiasis. Possibility of parvo viral enteritis was ruled out by screening the faecal samples of the cases with bloody diarrhoea using immune chromatographic assay (ScanVet™, Intas)

Statistical analysis of the haematological parameters revealed a significant elevation ( $p \leq 0.01$ ) in the level of total WBC count along with neutrophils and a significant reduction ( $p \leq 0.01$ ) in the lymphocyte and monocytes. A significant reduction was observed in the levels of TEC ( $p \leq 0.05$ ), haemoglobin ( $p \leq 0.05$ ) and mean platelet count ( $p \leq 0.01$ ) of diseased animals when compared to healthy animals. The serum ALT, AST and ALP in diseased animals were significantly higher ( $p \leq 0.01$ ) compared to healthy animals but no significant difference

were observed in the level of GGT. There was a significant increase ( $p \leq 0.01$ ) in the level of creatinine and BUN while no significant difference were observed in the level of serum glucose and cholesterol.

Significant neutrophilic leukocytosis and thrombocytopenia have been reported by Birnbaum *et al.* (1998) and Greenlee *et al.* (2004). Greene *et al.* (2012) reported an anaemia of mild severity in leptospira infected dogs and attributed it mainly to decreased RBC production resulting from chronic renal failure (CRF) or oliguric acute renal failure (ARF), generalized inflammatory disease, blood loss due to thrombocytopenia, gastric ulceration and haematuria. Thrombocytopenia observed in the present study might be due to vasculitis, increased platelet consumption as a result of excessive intravascular coagulation and a reduced rate of platelet production in the bone marrow (Greenlee *et al.*, 2004). Increased ALT, AST and ALP values were observed in hepatic insult resulting from leptospirosis by Birnbaum *et al.* (1998) and Sykes *et al.* (2010). This might be due to the Leptospiral toxin induced damage to hepatocellular tissue and vascular endothelium leading to hepatocyte swelling and intrahepatic cholestasis as opined by Greene *et al.*, 2012. Elevated creatinine values along with proteinuria and increased blood urea nitrogen consistent with acute renal failure. Non suppurative interstitial nephritis and tubular nephrosis were observed by Tochetto *et al.* (2012).

A confirmatory diagnosis of leptospirosis can be done by molecular methods like polymerase chain reaction (PCR), serological methods like MAT or bacteriological isolation of the organism Sykes *et al.* (2010). Bolin (1996) found that DFM examination required a skilled observer to differentiate between leptospires and artifacts. A detection of leptospires using bacteriological isolation of the organism is time consuming making it impractical for delivering quick diagnosis. Molecular detection methods like PCR required well equipped laboratory facilities and skilled personnels (Levett, 2003). Microscopic Agglutination Test (MAT) is considered as the gold standard serological test for the diagnosis

of leptospirosis (Faine *et al.* 1999 ; Quinn *et al.*, 2002). MAT results in the present study showed more prevalence of *L.interrogans* serovar Australis (n=5) followed by serovar Autumnalis (n=4) and Canicola (n=2). MAT proved to be the most specific test which could identify the serogroup identity of the infecting leptospira (Ahmed *et al.* 2005). Abhinay *et al.* (2012) reported that the most prevalent leptospira serovar affecting dogs in Thrissur district was Australis whereas Vamshikrishna *et al* (2013) observed Autumnalis as the most prevalent serovar in Thrissur. In a study conducted by Soman (2004) in Thrissur, Pomona and Australis were detected in 38.63 and 36.36 per cent sera samples respectively.

Three of the affected animals in the present study were vaccinated with commercially available multi component vaccine within nine months. All the cases were treated with Inj. benzyl penicillin @ 40,000 IU/ Kg BW intravenously twice a day. Supportive therapy was given with pantoprazole @ 1mg/kg, vitamin B complex injections and isotonic fluids which was selected depending on the degree of dehydration and the magnitude of renal compromise. Out of 11 animals, four died during the course of the treatment and the remaining showed improvement after 6 days of treatment. They were treated with Tab doxycycline at the dose rate of 10 mg/ Kg BW orally daily for ten days along with Tab. pantoprazole, Tab silymarin, vitamin E and B-complex.

Post mortem examination of dogs revealed icterus of the subcutaneous fat and omentum. There was extensive congestion in the liver along with rounding of margins (Fig. 3). Liver was enlarged and friable. Kidneys were enlarged and the capsule was adhered to the cortex (Fig. 4). Petechial haemorrhages were present on the renal cortex.

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