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Canine leptospirosis: Clinical and molecular diagnosis associated therapeutic management

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Abstract
Canine leptospirosis is an important zoonotic disease caused by the species of the spirochete genus Leptospira. There are more than 250 serovars of Leptospira were identified throughout the world and atleast ten of them including Australis, Autumnalis, Canicola, Grippothyphosa, Hardjo, Icterohaemorrhagiae, Pomona, Saxkoebing and Sejroe are being reported in association of canine leptospirosis throughout the globe. Four dogs of different age group and breeds with the history of pyrexia, anorexia, vomiting and enteritis were reported to the infectious disease unit of Veterinary Clinical Complex, Veterinary College and Research Institute, Namakkal for therapeutic intervention. Pale pink mucous membrane, hematochezia, pyrexia, tachypnea, shivering, dehydration, oliguria with mild cough were the predominant clinical examination findings in all animals and one had icterus. Anemia (mean - 8.2 $\pm$ 2.1 g/dl), leucocytosis (25.1 $\pm$ 4.2 x 10^3/ µl) mild thrombocytopaenia (mean - 1.2 $\pm$ 0.2 x 10^3/ µl) were notifiable haemato-biochemical changes noticed in all animals. Extracted template DNA from sera of all dogs were subjected to polymerase chain reaction targeting Lip L 32 gene specific for pathogenic Leptospires detected positive for infection. Doxycycline @ of 10 mg/kg body weight once daily for 21 days along with supportive therapy ended in uneventful recovery of all four dogs.

Keywords: canine leptospirosis, PCR, therapy

Introduction
Leptospirosis, an under reported zoonotic disease caused by spirochaetal bacteria of the genus Leptospira known to infect human and lower vertebrates (Cilia *et al*., 2020) [6] and reported in almost every continent (Marami *et al*., 2021) [11]. The genus Leptospira classically divided into two species namely *L. interrogans* sensu lato containing all pathogenic strains and the another species containing all saprophytic stains, *L. biflexa*. Within these two species, more than 250 serovars were identified by their distinct antibody reactivity to different carbohydrate moieties of the lipopolysaccharides in the outer membrane and further classified into antigenically related serogroups (Greene and Decaro, 2012) [8] and among this 10 serogroups are important for dogs and cats. Most common serovars of Leptospira reported in dogs are Australis, Autumnalis, Canicola, Grippothyphosa, Hardjo, Icterohaemorrhagiae, Pomona, Saxkoebing and Sejroe (Sykes *et al*., 2011) [15]. The disease is frequently reported in tropical and sub - tropical regions of the world (Lau *et al*., 2017) [10] and endemic in southern states of India including Kerala (Ambily *et al*., 2013) [3] and Tamil Nadu (Senthil *et al*., 2013) [14]. Canine leptospirosis can be either clinical or subclinical and usually exhibited as lethargy, anorexia, vomiting and the clinical expression is influenced by different infecting serovars and their pathogenicity, geographical area and host immunity (Alder, 2010) [2]. Due absence of specific clinical presentation of canine leptospirosis, confirmatory diagnosis is need of the hour for successful therapeutic regimen initiation and prevention of carrier status of affected dogs thus curtailing transmission cycle. This paper describes the molecular diagnosis based successful therapeutic management of canine leptospirosis.

Materials and Methods
Four dogs of different breeds and different age groups were presented to Infectious disease unit of Veterinary Clinical Complex, Veterinary College and Research Institute, Namakkal, Tamil Nadu for treatment with history of fever, inappetance, vomiting and enteritis. Pale pink mucous membrane, hematochezia, pyrexia, tachypnea, shivering, dehydration, oliguria with mild cough were the predominant clinical examination findings in all animals and one had mild icteric mucous membrane. Blood sample was collected aseptically from the jugular vein in EDTA vials and clot activator vials (2ml) for haematobiochemical analysis in automated

biochemical analyser (Vetscan2, Abaxis, United Kingdom and Biosystems Diagnostics Pvt. Ltd., India) and for polymerase chain reaction (PCR). Template DNA was extracted from the serum samples by hot and cold method as described by Dashtil *et al.* (2009) with minor modifications. Extracted template DNA was subjected to PCR by using following lipL32 primer (Major outer surface lipoprotein of pathogenic leptospires) as per the method recommended by Patricia *et al*. (2014) [13].

LipL32 Forward primer - 5'-CGC TGA AAT GGG AGT TCG TAT GAT T-3'

LipL32 Reverse primer - 5'-CCA ACA GAT GCA ACG AAA GAT CCT TT-3'

Detection of 423 bp PCR product on agarose gel electrophoresis of amplified template nucleic acid confirmed the positivity of all four dogs for Leptospirosis. All dogs were treated with doxycycline (10 mg/kg body weight, Per os) principally for 21 days with anti emetics (ondansetron @ 0.2 mg/kg), anti ulcer drugs (pantaprazole @1 mg/kg), fluid therapy with crystalloids based on clinical evaluation of dehydration and anaemic status and all were uneventfully recovered after 21 days of treatment.

Results and Discussion

Leptospirosis is a most common and widely reported zoonotic disease in tropical countries of the world caused by systemic infection of dogs with pathogenic spirochaetes of the genus Leptospira which are flexible, motile, spiral shaped bacteria with hook ends and wide range of mammalian animals are being infected by this spirochaete and excrete the organism in urine (Greene and Decaro, 2012) [8]. Etiological agents of canine leptospirosis are the pathogenic species of *Leptospira Interrogans* and *Leptospira kirschneri* and more than 250 serovars are defined based on the antigenic variation of lipopolysaccharide (LPS) antigen (Sykes *et al*., 2011) [15]. Canicola, Icterohaemorrhagiae are the most common serovars affecting canines around the past three decades and after usage of bivalent vaccines there are increased numbers of the Leptospiral serovars affecting dogs (minimum of 10 serovars) including Canicola, Icterohaemorrhagiae, Pomona, Bratislava, Grippotyphosa and Australis (Campbell, 2007) [5] and numerous reports of various serovars isolation from canines indicates wide contact between dogs with other domestic farm and wild animal reservoirs. Dogs may get infected by the spirochaete by direct contact with other reservoir hosts and their urine, either drinking or swimming in the environmental water sources and in sewage (Meeyam *et al*., 2006) [12].

Infection of canines with leptospires does not always end up in overt clinical signs and severity of signs depends upon age, immune response of the host, environmental factors like endemicity, virulence of the serovar that infects (Langston and Heuter, 2003) [9]. Peracute, acute and subacute/chronic manifestations of leptospirosis was described by various researchers and per acute form is characterised by sudden death. Acute form is characterised by pyrexia, shivering, muscle weakness, vomiting, dehydration and shock, and tachypnoea where as in sub acute form, a most common form consists of the clinical signs mentioned earlier along with lethargy, abdominal pain, myalgia and enteritis. Vasculitis associated ecchymoses or petechiae and icterus are the clinical presentations also reported in sub acute form (Van de Maele *et al*., 2008) [16]. All dogs in the present study had the similar symptoms described by the above authors and icterus was noticed one dog. Birnbaum *et al*. (1998) [4] reported that endothelial damage and disordered coagulation associated with thrombocytopaenia are the pathophysiological mechnaisms associated with leptospriosis which usually exhibited clinically in the form of haematochezia /melena, reported in the dogs in the present study. Anemia (mean - 8.2 $\pm$ 2.1 g/dl), leucocytosis (25.1 $\pm$ 4.2 x 10^3/µl) mild thrombocytopaenia (mean -1.2 $\pm$ 0.2 x 10^3/µl) were the predominant blood biochemical findings of the dogs which was in concurrent with the reports of Abdullathief *et al*. (2018) [1], who reported that leucocytosis and mild anaemia were reported in dogs affected with leptospirosis.

Detection of leptospiral antigen in the serum and urine by dark field microscopy, culture and PCR using primers specific for pathogenic leptospires are the recommended by various authors for diagnosis of leptospirosis. Among these PCR considered to be more sensitive and specific when compared to the other tests like dark field microscopical examination and culture as it takes considerable time, maintenance of live culture of leptospiral serovars and low sensitivity. Hence, PCR was carried out using serum samples by targeting lipL 32 primer specific for pathogenic leptospires and all animals were positive for leptospirosis by PCR by detection of 423 bp amplified PCR product on electrophoresis (Figure – 1). Molecular diagnostic assays like PCR have the advantages of diagnosing early phase of infection compared with serological tests with poor sensitivity and specificity (Sykes *et al*., 2011) [15] which can be used as a tool for taking appropriate decisions for initiation of early therapeutic management of affected dogs.

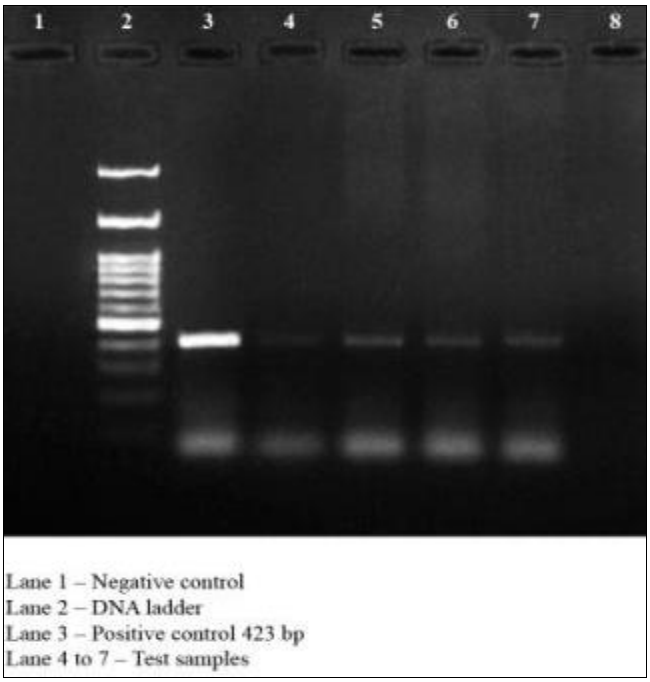

Fig 1: Leptopspirosis PCR

All the dogs were treated with doxycycline @ dose of 10 mg/kg body weight (Per os) for 21 days as per the recommendation of Greene and Decaro (2012) [8] along with supportive therapy including antiemetics (Ondansetron @ 0.2 mg/kg for five days) and anti ulcer drugs (Pantaprazole @ 1 mg/ kg for 21 days) with clinical evaluation of rehydration with anaemic status and appropriate crystalloid fluid therapy for five days and haematinics. All dogs were recovered uneventfully after 21 days. As the leptospirosis is a zoonotic disease, accurate early diagnosis and treatment of affected

canines with suitable therapeutic regimen coupled with periodical vaccination of healthy dogs with inactivated leptospiral vaccines are highly recommended for efficient control and prevention of canine leptospirosis at the field level.

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