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Incidence of *Leptospira Interrogans* serovar autumnalis in a non-descript pet dog- a case report

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Abstract

Leptospirosis is one of the major diseases in pet animals caused by a spirochete belonging to genus Leptospira. The animals once infected evince leptospiremia in the initial stages which later leads to vascular damage, multiple organ dysfunction and finally may cause death. Animals may become chronic shedders even after successful treatment due to renal colonization of the organisms. An eight year old unvaccinated dog with the history of vomiting, oliguria and constipation was later diagnosed to be as a leptospira infection by microscopic agglutination test (MAT). The major clinical signs included pyrexia, myalgia and jaundice with laboratory findings such as leukocytosis, anemia and thrombocytopenia. Damage to the kidney and liver were evident on ultrasonography and enzyme function tests (ALT, ALP, BUN and Creatinine). The dog was successfully treated with Benzyl Penicillin and supportive medications over a period of one month. Periodic vaccination can reduce the incidence of leptospirosis in dogs to a greater extent. As the identified serovar was *Leptospira Interrogans* serovar autumnalis which is absent in the commercial vaccines being used in India, thrust is given to include other serovars in the vaccine in endemic areas. Absence of cross-protection between the serovars may further aid in the incidence of new serovars and cross reactivity in the MAT should also be considered during diagnosis. As there is high chance of zoonotic transfer of the disease, it is important to vaccinate the dog regularly to avoid the disease in handlers and pet parents.

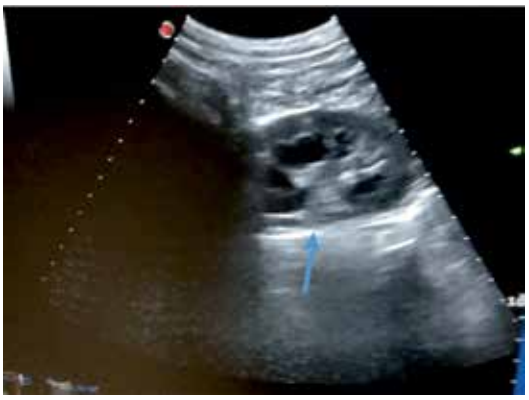
Keywords: *Leptospira Interrogans* serovar autumnalis, Microscopic Agglutination Test, Benzyl Penicillin

Leptospirosis is one of the zoonotic diseases which spreads among dogs mainly through contaminated urine and could be easily transferred to the handlers and pet parents through direct contact with urine and blood. The disease is caused by a spirochete of the species leptospira among which the serovars of *Leptospira interrogans* sensu lato comprised the major pathogens, majority of which are found to be endemic in Kerala. The organisms can survive in the environment in soil and water logged areas and can spread easily to the people working in those areas by coming in contact with contaminated water. The disease can also be transmitted through bite wounds and by ingestion of infected tissues whereas transveneral transmission and placental transfer has also been reported. The disease is most commonly found during winter or rainy season and in areas where there is poor drainage of water. The organism can penetrate intact mucous membranes and abraded skin areas, enters kidney through circulation and is excreted in the urine. Clinical manifestations include coagulopathy, jaundice, renal failure, vascular damage, thrombocytopenia and the animal may become chronic shedders of leptospires.

Clinical History, Observations and Treatment

An eight year old female non-descriptive dog was presented to the University Veterinary Hospital, Kokkalai with history of anorexia, vomiting, decreased urine output and was passing tarry feces. The dog was recumbent when brought to the hospital and was experiencing myalgia. On detailed physical examination, pyrexia with 103.5°F was observed with dehydration, icteric mucous membranes and no lymphadenopathy. The dog had no prior history of illness but was treated for the same condition with a dose of amoxirum forte and supportive medication by a local veterinarian. Vaccination and deworming was not followed regularly.

Laboratory examination revealed leukocytosis ($21.9x10^3/\mu l$), thrombocytopenia ($105x10^3/\mu l$) and anemia (RBC-$4.67x10^6/\mu l$, haemoglobin- 9.8g/dl). Serum analysis revealed increase in the levels of BUN (58.64mg/dl), creatinine (2.26mg/dl), ALT (140.8IU/L), ALP (2055.5IU/L), bilirubin (5.35mg/dl) and total protein levels in normal range (6.54g/dl). Serum sample was sent for detecting Leptospiral antibodies.



Increased echogenicity in renal medulla

Increased cellularity in urinary bladder

Increased granularity in liver, hepatomegaly, rounded appearance of liver, thickened cortex, formation of slurry in gall bladder

MAT- positive agglutination reaction

Abdominal utrasonography revealed hepatomegaly, thickened capsule and rounded appearance of liver with altered granularity. Thickening was observed in the medulla of kidney and cellularity in the urinary bladder.

Blood smear and buffy coat smear was negative for hemoparasites. Microscopic agglutination test (MAT) of the serum sample showed agglutination at 1 in 400 dilution for antigens of *Leptospira interrogans* serovar autumnalis.

As there was no clinical improvement with the use of intravenous amoxicillin, drug of choice, benzyl penicillin was given at the rate of 40,000 IU/Kg body weight/IV twice daily for 7 days. Mannitol (20 %) @ 0.5 g/kg was given intravenously to correct oliguria along with pantoprazole (0.5mg/kg/IV/day). Silymarin and ursodeoxycholic acid (15mg/kg/day) was supplemented orally, as there was change in echogenicity of liver and gallbladder. Cremaffin was given orally to correct constipation.

The animal started taking food after few days of therapy and started passing soft stool. Hydration status was maintained initially with intravenous crystalloids and dog showed improvement in condition. The treatment was shifted to oral doxycycline (10 mg/kg body weight/ day) for two weeks with supportive therapy.

Animal showed improvement at the end of treatment with decrease in the leucocytes ($14x10^3/\mu l$) and decrease in creatinine value (1.8 mg/dl) and slight improvement in the levels of anemia.

Discussion

Leptospirosis is one of the prevalent zoonotic diseases in Kerala. The Integrated Disease Surveillance Project (IDSP) report (2018) revealed 1318 confirmed cases of human leptospira in Kerala with 4 per cent death rate. Although, the incidence rates are misjudged due to lack of awareness and subclinical infection, the Kerala State Health Department reported 570 confirmed cases with death rate of 3.2 per cent after the incidence of

2018 floods in Kerala (James *et al*., 2018). The serovars in human beings depends on the animal reservoirs and maintenance hosts (house rats) whereas pet dogs may also play a major role in the transmission of the disease to human beings (Bharti *et al*., 2003). Increased renal carriage among dogs leads to excretion of virus in the urine which could easily come in contact with handlers and help in the transmission of the disease.

Antibodies against, *Leptospira interrogans* serovar icterhaemorrhagiae, seriovar canicola, serovar pomona, serovar autumnalis, serovar habdomadis, serovar grippotyphosa, serovar javanica has been detected among dogs brought to University Veterinary hospital, Kokkalai and Teaching Veterinary Clinical Complex, Mannuthy, Kerala (Ambily *et al*., 2013).

Since the animal was ailing for the past 2 weeks before being presented to the hospital, vascular damage was evident with the increase in values of liver function tests and kidney function tests. As the animal was dehydrated and recumbent, the increase in the alkaline phosphatase levels in serum was noticed. Increase in the levels of BUN, creatinine and ultrasonography of kidney revealed renal damage which may indicate renal colonization of the organism with shedding of the organisms in the urine. Increased cellularity in the urinary bladder may be due to proteinuria but there was no biochemical variation in the levels of proteins in serum which accounts for hypoprotenemia. Damages to liver, spleen and kidney had been documented through ultrasound scanning in 98 per cent of the dogs with leptospirosis with variable degrees of damage to different organs (Knopfler *et al*., 2017).

Immunization has been considered as one of the effective ways in reducing the occurrence and severity of leptospirosis in dogs. Initial immunization in canines with killed leptospira vaccines can be carried out at the age of 9 weeks with booster dosage after 2-4 weeks (Greene *et al*., 1998). Killed vaccine containing antigens from four main serogroups- canicola, icterohaemorrhagiae, grippotyphosa, and pomona is being used in India but does not provide cross protection against other serovars. Vaccination also decreases renal colonization and shedding of the organism. Cross reaction has also been observed in between the serovars in MAT.

Vaccine strains, serovars grippotyphosa and pomona antigens may disproportionately increase titers for serovars autumnalis and bratislava on MAT, leading to false positive reaction (Greene *et al*., 1998). Since the animal was not vaccinated within the past 2 years of clinical illness, cross reaction of antigens was ruled out with vaccinated strains.

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