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Scientific Report

Isolation of *Leptospira canicola* from a dog in Iran: first report

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Summary

A 5-year-old male cross-bred guard dog with signs of anorexia, vomiting and jaundice was referred to Small Animal Hospital, Faculty of Veterinary Medicine, University of Tehran. Initial diagnostic laboratory tests revealed leukocytosis, thrombocytopenia, haemoconcentration, azotaemia, high liver enzyme activities, proteinuria and bilirubinuria. Aggressive therapeutic procedures failed to improve the animal deteriorated condition and finally the animal died. After necropsy, specimens were collected for microscopic examination as part of completing diagnostic procedures. Results of urine bacterial culture confirmed leptospirosis as a causative agent. Furthermore, numerous large spiral bacteria were also observed under dark-field microscope. This article summarizes and characterizes the historical and physical findings, laboratory data, bacterial culture and pathological diagnostic features of leptospirosis in the cross-bred dog. Based on published data, this is the first case of confirmed *Leptospira* isolation from a dog in Iran.

Key words: *Leptospira canicola*, Dog, Iran

Introduction

Leptospirosis is an important infectious disease affecting almost all mammals (Palaniappan *et al*., 2005). Although leptospires are ubiquitous and potentially lethal, they are often not diagnosed (Wagenaar *et al*., 2004). The disease is a common zoonosis acquired by exposure to body fluids, tissues of infected animals, or contaminated soil or fresh water (Edwards and Levett, 2004). Multiple organ involvement may be encountered in leptospirosis, and early renal involvement is very common, characterized by tubulo-interstitial nephritis and tubular dysfunction (Yang *et al*., 2001). Treatment with penicillin may significantly improve organ failure and is considered the drug of choice in managing of leptospiral infections (Markum, 2004).

Case history

Clinical findings

A 5-year-old male cross-bred guard dog was referred to Small Animal Hospital, Faculty of Veterinary Medicine, University of Tehran with a 5-day history of anorexia, lethargy, general weakness and vomiting. In the initial examination, the animal was recumbent and semi-comatose, without any response to the external stimulus. Icterus was highly evident in mucous membranes of eyes, buccal cavity, anal region and in the external surface of penis. The dog was hypothermic (37°C) and tachycardia (213 beats/min) was the main finding in cardiovascular examination. The dog had been vaccinated just against rabies without

any record of other routine vaccination programs. The animal was dehydrated of about 7-8% based on skin elasticity and sunken eyes appearance. The dog was kept in out door area and the owner had not any information about possible urinary disorders.

Fluids replacement (infusion of Ringers solution), combined with antibiotic therapy (procaine penicillin G 40000 U/kg BID) was performed. Despite treatment, the dog's condition continued to deteriorate over the next hours. One dose of rapid-acting corticosteroid (100 mg hydrocortisone succinate) was administered but the dog did not respond to therapy and finally died.

Laboratory findings

Laboratory abnormalities included leukocytosis (23000 cells/µl), thrombocytopenia (100000/µl), haemoconcentration (PCV = 60%), azotaemia (BUN = 77 mg/dl) and high liver enzyme activities (ALT = 250 U/L, ALP = 300 U/L). Urinalysis showed proteinuria (3^+), and high bilirubin concentration (0.8 mg/dl) along with the presence of large numbers of leukocytes, erythrocytes and granular casts in high power microscopic field.

With respect to presence of icterus in clinical examination and possibility of leptospirosis as a causative factor, urine sample was collected by cystocentesis and inoculated into Fletcher culture media and then incubated at 30°C for 2 weeks. Two weeks after initial inoculation, a drop of the culture was examined by dark-field microscopy which revealed large numbers of moving leptospires (Fig. 1).

Histopathological findings

The animal was submitted for necropsy inspection. On gross examination, poor body condition and general muscle atrophy was observed. Following skin removal from ventral mid-line, marked icterus was also obvious on subcutaneous tissues. Intra-abdominal findings included splenomegaly, hepatomegaly and slightly enlarged pale kidneys. Excised specimen were fixed in formalin and submitted for histopathological examination. After paraffin embedding, sections were cut at 5 µm and stained with haematoxylin and eosin for microscopic examination. Histologically, the most striking lesion in the liver was shrinkage of hepatic cells and their dissociation, break up the normal columns of liver cells. The affected liver cells had granular and eosinophilic cytoplasm and fatty changes of varying degrees were obvious in their cytoplasm. Furthermore, the plug of bile retention that is mentioned as cause of icterus in leptospirosis was found.

In kidneys, there were not any significant changes in glomerules, but histologically tubules had some alterations in two different patterns. Some tubules were swollen, coarsely granular and deeply eosinophilic and some were vacuolated due to hydropic changes. There was also focal interstitial nephritis in the renal cortex. The interstitial spaces of the affected tubules were infiltrated by lymphocytes, plasma cells, and macrophages.

Presence of leptospires in histopathological samples was also

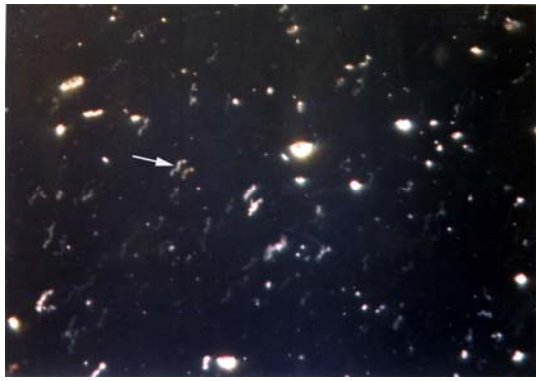

Fig. 1: Leptospiral organisms in dark-field microscope (×400). The organisms can be visualized in black ground contrast (arrow)

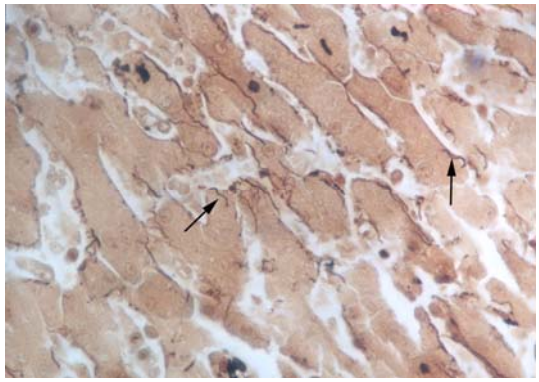

Fig. 2: Levaditi's stain demons-trates spirochetes inside of hepatocytes and in sinusoids (×400)

confirmed by silver impregnation technique. In this staining technique, the organisms were seen in sinusoids and within liver cells as well as in the pulps of spleen as black spiral organisms in orange-brown coloured fields (Fig. 2).

Serology: serological test was performed by microscopic agglutination test (MAT) in Razi Vaccine and Serum Research Institute, Karaj, Iran. The serum sample was tested using a battery of 16 *Leptospira* antigens and the rate of agglutination was assessed under dark-field microscope. It was positive at a dilution of 1/400 against *L. canicola*.

Discussion

Leptospirosis is a major public health problem throughout the world (Sipos *et al*., 2003). The disease may vary in severity from an asymptomatic infection to severe and fatal illness. The presence of acute renal failure with jaundice should aware clinician of leptospirosis (Cetin *et al*., 2004). Renal involvement (acute renal failure), interstitial nephritis with oedema and mononuclear cellular infiltration are the usual findings in affected animals (Daher *et al*., 2004; Wu *et al*., 2004).

Azotaemia, proteinuria, and presence of large numbers of leukocytes, erythrocytes and granular casts in urinary sediment in this case are closely related to interstitial nephritis which is characteristic pathologic finding in acute renal failure associated with leptospirosis.

All of the reports in Iran by now were restricted to isolation of leptospires in cattle, goat, sheep and mouse and this is the first one in the dog. In the last bacteriologic and serologic studies of canine leptospirosis in Tehran and its suburban areas, 300 blood samples were tested by MAT and IFA and in 31%, the serum samples were positive against one, two or three leptospiral serogroups, but in none of the urine samples leptospiral organism was isolated (Rad *et al*., 2004). Isolation of leptospires has some difficulties that lead to incomparable results with serologic studies. Because of fastidious growth requirements and susceptibility to adverse environmental conditions, proper timing and technique are essential for recovery of leptospires. Samples should also be taken before initiation of antibiotic therapy. Dogs are usually leptospiraemic during the first week of infection, but the numbers of circulating organisms subsequently decrease as serum antibody titres increase. Urine is the ideal fluid for culture, however, multiple sampling is required because of intermittent shedding of organisms. Catheterized or voided urine sample is frequently contaminated by normal flora that interferes with the growth of leptospires, therefore, cystocentesis is preferred. The other probable factor may be related to acidic conditions of urine transport, because leptospires cannot survive in such condition for more than a few hours.

In spite of isolation of leptospires in this study, the serological test was positive at the dilution of 1/400 against *L. canicola*. For serologic confirmation of an acute disease such as leptospirosis, demonstration of a four-fold rise in MA titre is classically required. However, in a single specimen, like the animal of the present study, the disease would be diagnosed due to the presence of serological titre along with compatible clinical signs and knowing the fact that there has not been any vaccination against leptospirosis. On the other hand, it should be considered that the magnitude of rise in titre does not always parallel the severity of clinical illness. It is suggested that dogs infected with *serovar canicola*, to which they are well adapted, may be actively infected and excreting organisms with titres less than 100. It should also be considered that positive sera in MAT may have cross-reaction to a variety of serovars. So the numerous antigens must be used to identify the serovar that cause disease. Further two-fold dilutions are done against positive-reacting concentration. The highest titre is interpreted as the infecting one (*canicola* serovar in this animal) and lower titres represent antibody cross-reactivity between serovars. At present time, MA test is considered as the standard serologic test in diagnosis of leptospirosis. ELISA appears to be more sensitive in detecting antibody and more serovar specific for determining very early infection in dogs than the MA test but it is not widely available for clinical application. Use of techniques such as polymerase chain reaction (PCR) and

genetic probes also would be helpful (Green, 1998).

Isolation of leptospires in a dog for the first time in Iran with respect to zoonotic importance of the disease and close contact of dogs with their owners, highlights the need for vaccination of dogs against leptospirosis in a regular manner. Based on a report in the United States, widespread use of a bivalent vaccine against *L. canicola* and *L. icterohaemorrhagiae* is led to near extinction of leptospirosis in the canine population. Now, they have problem with cases infected with other serovars such as: *L. pomona*, *L. bratislava* and *L. grippotyphosa* that available bivalent vaccine does not provide immunity to these emerging serovars, leaving the canine population unprotected and at risk for infection (Brinbaum *et al*., 1998; Harkin and Gastrell, 1996). Considering the widespread dispersion of leptospirosis, it should be included routinely in the differential diagnosis of all dogs with acute renal and hepatic failure.

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