Leptospirosis with multiple organ dysfunction in a mongoose-scat-detection dog infected with *Leptospira interrogans* serogroup Hebdomadis, Okinawa, Japan

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**ABSTRACT.** A 2-year-old male mongoose-scat-detection dog was diagnosed with leptospirosis by urine PCR. The patient developed acute renal failure, hepatic dysfunction, and disseminated intravascular coagulation. Treatment with antibiotics was administered, including ampicillin and doxycycline, and supportive care management was provided. Seroconversion against serogroup Hebdomadis was observed on day 8. The leptospiral gene *flaB* was detected only in urine collected on day 1, from which *Leptospira interrogans* ST329 was identified by multilocus sequence typing using seven housekeeping genes. *L. interrogans* serogroup Hebdomadis ST329 has been isolated from mongooses and humans in Okinawa, Japan. This patient received early treatment with antibiotics, which may have contributed to the early recovery of renal function and removal of *L. interrogans* from kidney tissue.

**KEYWORDS:** canine leptospirosis, disseminated intravascular coagulation, *Leptospira interrogans* serogroup Hebdomadis, mongoose-scat-detection dog, multilocus sequence typing

Leptospirosis is a zoonotic disease caused by infection with pathogenic spirochetes from the genus *Leptospira*, which affects a wide range of wild and domestic animals and humans [8]. The pathogenic *Leptospira* spp. can colonize the proximal renal tubules of reservoir animals and are excreted in urine [8]. Dogs are infected percutaneously or permucosally with *Leptospira* spp. through contact with the urine of infected animals or with soil or water contaminated with infected urine [8]. Clinical manifestations of canine leptospirosis can vary from subclinical and minimal clinical disease to severe disease involving kidney, liver, and pulmonary tissues, depending on the infecting *Leptospira* strain and host immune status triggered by previous vaccination or previous infection [2, 9, 12]. Since a high index of suspicion is required, canine leptospirosis remains a significant diagnostic challenge for veterinarians [2]. The genus *Leptospira* consists of 64 species divided into 24 serogroups and more than 300 serovars [8, 13]. Dogs are considered as maintenance hosts for *L. interrogans* serovar Canicola worldwide, and accidental infections with serovars/serogroups Australis, Autumnalis, Ballum, Canicola, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Pomona, and Sejroe have been reported [6, 7, 12]. Prevalent *Leptospira* serovars among dog populations vary geographically depending on exposure to wild or domestic reservoir animals [12]. Although *L. interrogans* serogroup Hebdomadis has been isolated from dogs with acute illnesses [6, 7], detailed characteristics of clinical pictures of dogs infected with the Hebdomadis strains have not been reported. In this report, we describe the clinical symptoms, laboratory findings, and serological and molecular findings in a dog infected with *L. interrogans* serogroup Hebdomadis.

A 2-year-old male German Shepherd was presented to the Yanbaru Animal Clinic in August 2021 with clinical signs of fever (39.3°C), diarrhea with hematochezia, vomiting, and dehydration. The owner reported that the patient had exhibited signs of malaise 1 day before the consultation. The patient was a detection dog for scats of small Indian mongoose and was engaged in mongoose exploration in the forest almost every day. The patient was vaccinated with an inactivated bivalent leptospirosis vaccine containing serovars Canicola and Icterohaemorrhagiae in May 2021. The patient returned home after subcutaneous administration of ampicillin...
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(25 mg/kg), enrofloxacin (5 mg/kg), famotidine (1 mg/kg), metoclopramide (0.25 mg/kg), diphenhydramine (1 mg/kg), prednisolone (1 mg/kg), and a combination drug of glycyrhizin, glycine, and cysteine (0.5 mL/kg).

The patient was brought to the clinic again on the next day (day 1) due to food refusal, abnormal urine color (orange-yellow), and vomiting. Body temperature measurement indicated that the patient was afebrile (38.4°C). The blood examination revealed leukocytosis (18,200/μL), neutrophilia (14,860/μL), monocytosis (2,130/μL), thrombocytopenia (35/μL), and decreased hematocrit (35.4%) (Table 1). In addition, serum biochemical examination suggested acute renal failure and hepatic dysfunction based on increased levels of serum creatinine (2.6 mg/dL), blood urea nitrogen (BUN, 53 mg/dL), serum symmetric dimethylarginine (SDMA, 21 μg/dL), serum total bilirubin (6.9 mg/dL), serum C-reactive protein (7 mg/dL), serum alanine aminotransaminase (237 IU/L), and serum alkaline phosphatase (1,225 IU/L). The blood coagulation test suggested disseminated intravascular coagulation (DIC) based on increases in activated partial thromboplastin time (36.7 sec), prothrombin time (8.4 sec), fibrinogen degradation product (27.3 μg/mL), D-Dimer (9.07 μg/mL), and thrombin–antithrombin complex (1 ng/mL). Leptospiral DNA was detected from urine by PCR performed by an outsourcing company (Neopark Okinawa Veterinary Laboratory, Okinawa, Japan) [11]. Based on clinical and laboratory findings and epidemiological information, the patient was diagnosed with leptospirosis. The patient was hospitalized and received intravenous administration of ampicillin. The supportive care management provided included subcutaneous administration of tranexamic acid, phytonadione, and folic acid; intravenous administration of diprophylline, maropitant, tiopronin, ulinastatin, and gluthathione; and oral administration of ursoedocholic acid, benazepril, and camostat mesylate.

On day 4, acute pancreatitis was suspected due to elevated lipase levels (>1,000 IU/L), for which treatment using fusazapladib sodium hydrate (0.4 mg/kg), ceftoxime (50 mg/kg), and bunopenrine (0.015 mg/kg) was started. Appetite normalized on day 7. Ampicillin was changed to minocycline (5 mg/kg) and cefoxetaxime to meropenem (12 mg/kg) for the treatment of catheter site infection and prevention of secondary infections due to acute pancreatitis, respectively, on day 11. Bowel evacuation normalized on day 18, and the patient was discharged on day 32 because of improvements in appetite and blood examination findings and resolution of vomiting.

Table 1. The result of complete blood count, serum biochemical profile, blood coagulation test

| Laboratory examination | Unit | Nominal range |
|------------------------|------|---------------|
| White blood cell count | K/μL | 5.05–16.76 |
| Lymphocytes | K/μL | 1.01–5.1 |
| Monocytes | K/μL | 0.16–1.12 |
| Neutrophils | K/μL | 2.95–11.64 |
| Eosinophils | K/μL | 0.06–1.23 |
| Basophils | K/μL | 0–0.1 |
| Hematocrit | % | 37.3–61.7 |
| Red blood cell count | M/μl | 5.65–8.87 |
| Hemoglobin | g/dL | 13.1–20.5 |
| Platelets | K/μL | 148–484 |
| Serum biochemical profile | | |
| Glucose | mg/dL | 75–128 |
| Blood urea nitrogen | mg/dL | 7–27 |
| Creatinine | mg/dL | 0.1–1.8 |
| Sodium dimethylarginine | μg/dL | 0–14 |
| Total bilirubin | mg/dL | 0.1–0.5 |
| Total protein | g/dL | 5–7.2 |
| Albumin | g/dL | 2.6–4 |
| Alanine aminotransaminase | IU/L | 17–78 |
| Alkaline phosphatase | IU/L | 0–89 |
| Creatine phosphokinase | IU/L | 49–166 |
| Calcium | mg/dL | 9.3–12.1 |
| Inorganic phosphorus | mg/dL | 1.9–5 |
| Lipase | IU/L | 10–160 |
| C-reactive protein | mg/dL | <0.7 |
| The blood coagulation test | | |
| Activated partial thromboplastin time | Sec | 10–16 |
| Prothrombin time | Sec | 6–8 |
| Fibrinogen | mg/dL | 86–375 |
| Fibrinogen degradation product | μg/mL | 0–5 |
| Antithrombin activity | % | 102–156 |
| D-Dimer | μg/mL | 0–2 |
| Thrombin-antithrombin complex | ng/mL | 0–0.2 |

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Upon discharge, faropenem (8 mg/kg) was administered perorally in place of meropenem until day 70 to prevent the recurrence of catheter site infection and pancreatitis. Doxycycline (5 mg/kg) was administered perorally in place of minocycline from day 49 to 70 and again from day 98 to 105 because of mild leukocytosis observed on day 98. Body weight decreased from 25.2 kg on day 0 to 18.8 kg on day 21, but gradually increased to reach 26.6 kg on day 98.

Detailed laboratory tests for leptospirosis were performed at the Okinawa Prefectural Institute of Health and Environment: blood and urine culture using liquid Ellinghausen–McCullough–Johnson–Harris (EMJH) medium, liquid EMJH medium supplemented with sulfamethoxazole, trimethoprim, amphotericin, fosfomycin and 5-fluorouracil (STAFF) [1], and liquid Korthof’s medium; DNA detection for pathogenic Leptospira spp. via nested-PCR targeting the leptospiral gene flaB; and anti-Leptospira antibody detection by microscopic agglutination test (MAT) were conducted as previously described [4, 5]. Blood culture on day 1 and urine culture on Days 1, 9, 11, 14, 18, 23, 26, 29, 32, 44, 62, 77, 91, 119, 146, 175, and 203 were negative. The flaB gene was detected from urine, but not blood, collected on day 1. DNA detection from urine collected on the other days was negative. The nucleotide sequence of the flaB amplicon was determined using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), and the sequence obtained (DDBJ accession number LC713068) was identical to that of L. interrogans (CP044513). Multilocus sequence typing (MLST) of the flaB-positive urine DNA was performed using seven housekeeping genes (glmU, pntA, sucA, tpiA, pfkB, mreA, and caiB) [4, 6, 7], and the sequence type was identified as ST329 (DDBJ accession numbers LC713061-LC713067). This ST has been detected from L. interrogans serogroup Hebdomadis isolated from mongooses and humans in Okinawa, Japan (https://pubmlst.org/organisms/leptospira-spp) [10]. Antibody detection was performed on Days 1, 8, 11, 13, 15, 18, 20, 23, 26, 29, 32, 49, 70, 84, 98, 105, 113, 144, 175, and 203. Anti-Hebdomadis antibodies were seroconverted to reciprocal MAT titer 1,280 on day 8; peaked at 2,560 on Days 11, 13, and 15; and decreased to <40 on day 203 (Fig. 1). Results for culture, DNA detection using blood and urine samples and antibody detection performed at 4 days after the onset of the patient were negative for five dogs living together with the patient and for two dogs who were close contacts at work.

This report describes clinical features and laboratory findings of a mongoose-scat-detection dog infected with L. interrogans. Although culture was not successful, MLST and serological reaction results strongly indicated that the infecting strain was L. interrogans serogroup Hebdomadis. Canine leptospirosis due to infection with L. interrogans serogroup Hebdomadis often occurs in Japan, but its clinical pictures have not been described in detail [6]. Initial manifestations of this case included fever, diarrhea with hematochezia, vomiting, dehydration, and malaise, which are common clinical signs of canine leptospirosis [2]. The patient developed acute renal failure, hepatic dysfunction, and DIC. BUN and creatinine levels are elevated in more than half of leptospirosis cases [9], and successful treatment is associated with a gradual return of the parameters to normal reference ranges within 10–14 days [12]. In the present case, increased serum creatinine and BUN were observed only on day 1 and during the period from day 1 to day 23, respectively, indicating successful treatment. On the other hand, the concentration of SDMA which is a more sensitive biomarker for renal dysfunction than creatinine continued higher than the normal range during the period except day 70, suggesting the renal function of the patient may be impaired even after the recovery. In addition, no sequelae requiring dialysis remained in this case, whereas a previous study showed that approximately 50% of the dogs surviving acute leptospirosis had impaired renal function more than 1 year after hospital discharge [9]. Early antimicrobial therapy is essential to improving the survival rate and duration of hospitalization [2].
Moreover, *Leptospira* spp. might be shed for months in urine if appropriate antimicrobial treatment is not initiated [12]. The patient in the present case received ampicillin on day 0, which may have contributed to the early recovery of renal function and the removal of *L. interrogans* from kidney tissue.

In Okinawa Prefecture, small Indian mongooses are invasive species that cause severe damage to native wild animal populations and have thus become the target of capture and extermination. Mongoose-scat-detection dogs play a role in understanding the distribution of mongooses and increasing the efficiency of mongoose capture, with the work performed mainly in forest areas. *L. interrogans* serogroup Hebdomadis has been isolated from mongooses and mice, and antibodies against serogroup Hebdomadis have been detected in wild boars in the northern part of the main Okinawa Island [3]. In the present case, MLST showed that the patient was infected with *L. interrogans* ST329, which has been previously isolated from mongooses and humans, suggesting that the patient contracted the disease through the work environment, possibly through fresh water and soil contaminated with mongoose urine.

The patient had been vaccinated with the bivalent vaccine against serovars Canicola and Icterohaemorrhagiae but became infected with the Hebdomadis strain, enforcing the serovar-specific effectiveness of killed whole cell vaccines. Some canine leptospirosis vaccines protect against serogroups Canicola, Icterohaemorrhagiae, Grippotyphosa, and/or Pomona; however, no vaccines are available against serogroups Australis, Autumnalis, and Hebdomadis, which are prevalent in Japan [2, 6, 9, 12]. Among the above serogroups, *L. interrogans* serogroup Hebdomadis is the most prevalent. A study isolated *L. interrogans* serogroup Hebdomadis from 21/45 infected dogs (46.7%) and detected antibodies in 34/59 infected dogs (57.6%) [6]. However, canine leptospirosis caused by serogroup Hebdomadis is not listed as a notifiable disease by the Act on Domestic Animal Infectious Diseases Control in Japan. It is necessary to accurately monitor the serological distribution of canine leptospirosis and develop a vaccine suitable for each region.

In conclusion, this report demonstrated that *L. interrogans* serogroup Hebdomadis infection caused multiple organ dysfunction in a dog. Early diagnosis and early treatment require the identification of specific biomarker(s) for severe canine leptospirosis. In addition, a vaccine tailored according to geographic region or a universal vaccine effective against all serovars is needed for the effective prevention of canine leptospirosis.

CONFLICT OF INTEREST. The authors declare that they have no conflicts of interest.

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