Concurrent *Leptospira borgpetersenii* Serogroup Ballum and *Ehrlichia canis* Infection in a Dog in Israel: A Case Report

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Abstract

An 11-month-old, intact female Great Dane dog was presented with complaints of vomiting, diarrhea, thrombocytopenia and severe azotemia. The dog has resided in a rural environment. Due to acute kidney injury the dog was hospitalized and treated with intravenous fluids, antibiotics (cefazolin and doxycyline), anti-emetics and diuretics. Serum antibody titer and PCR for *E. canis* were positive. Serum microscopic agglutination test antibodies titers for *Leptospira interrogans sensu lato* serogroups canicola and ballum were 1:50 and 1:100, respectively at day one. A second sample at day 7 yielded a titer of 1:800 for *Leptospira interrogans sensu lato* serogroup ballum with no cross-reactivity with other serogroups. The dog's clinical signs, the thrombocytopenia and serum chemistry abnormalities all improved over seven days of hospitalization, and it was then discharged. This is the first record in the veterinary literature of *L. borgpetersenii* serogroup ballum infection in a dog in Israel and the first record of concurrent canine monocytic ehrlichiosis and leptospirosis in a dog. This dog was likely infected with leptospirosis through drinking stagnant water in a garden pool although it had been fully vaccinated with a commercially available bivalent vaccine, containing bacterins of *L. interrogans* serovars icterohaemorrhagiae and canicola. Leptospirosis should be included in the differential diagnosis of acute azotemia, and its diagnosis can be complicated by concurrent infections, such as in this case. Due the zoonotic potential of leptospirosis, dogs should be kept under hygienic conditions, because the commercially available vaccines do not provide cross protection against most leptospiral serogroups.

Key words: Canine, Leptospirosis, Azotemia, Canine Monocytic ehrlichiosis, Microscopic agglutination test, Thrombocytopenia.

INTRODUCTION

Leptospirosis is an infectious, zoonotic disease of a major worldwide importance of animals and humans (1, 2). It is caused by antigenically distinct serovars of the spirochetal bacterium *Leptospira interrogans sensu lato*. Over 200 serovars of *L. interrogans sensu lato* have been described and have been classified in different species (e.g., *L. interrogans*, *L. kirschneri*, *L. borgpetersenii*) (1-5). Antigenically-related serovars have been traditionally also divided into serogroups, based on serological methods. While serogroups have no taxonomic standing, this classification has proven useful for epidemiological purposes (1-3, 5). From a practical point of view, the protection induced by the currently available bacterins is restricted to the particular serogroup used for their production. The classification of leptospires might be somewhat confusing because there is an overlap of serogroups and newer classifications based on molecular methodologies.

Historically, leptospirosis was a common disease of dogs, and had been most commonly caused by *L. interrogans* se-

rovars icterohaemorrhagiae and canicola. Introduction of bacterin-based bivalent vaccines against these serovars has led to a decrease in the incidence of "classic" leptospirosis in dogs. However, more recently, other leptospiral serovars are more commonly recorded in dogs (1-4, 6, 7).

Leptospires are spread by maintenance, carrier hosts, which usually do not develop clinical signs (1-4, 8). Dogs are maintenance hosts of serovar canicola (9). Bacterial shedding by infected animals occurs mostly via the urine, leading to direct or indirect infection of other animals and humans. Indirect transmission, occurring more often, is through exposure to a contaminated environment, especially water, notably habitats with stagnant or slow moving relatively warm water, which favor leptospiral survival (1-4, 9). Once in a susceptible host, leptospires can potentially invade various organs, but mainly colonize the kidneys, and might cause acute, fatal nephritis, especially in unvaccinated animals (1-4).

Because the clinical signs of leptospirosis can be vague and are sometimes nonspecific, it often poses a diagnostic challenge. Large breed, outdoor, adult dogs are the most commonly affected, although young animals have been reported to manifest the disease more severely (1, 2, 4). Signs of renal and hepatic dysfunction, as well as coagulation defects, usually predominate. Lethargy, vomiting, anorexia and polydipsia have been reported as the most common signs, whereas icterus and fever at presentation are relatively uncommon (8).

Microscopic agglutination test (MAT) is the standard serologic diagnostic test for leptospirosis (1-4). Organisms grown in liquid media are exposed to serial dilutions of the patients' sera. The end point is the highest serum dilution that agglutinates 50% of the leptospires. Because titers can be negative over the first 10 days of an acute illness, a second, and sometimes even a third serum sample should be obtained at 1- to 2-week intervals. Previous infection or vaccination is usually associated with MAT titers below 1:400, and titers above 1:800 generally are associated with active disease or a subclinical renal carrier state. Leptospires are difficult to culture and may take weeks to months to grow (1-4).

Antimicrobial therapy is essential to terminate bacteremia, and higher success rates for reversal of tissue injury are achieved if antimicrobial agents are administered early in the disease course. Penicillin and its derivatives are the antibiotics of choice for terminating leptospiremia, but do not eliminate the carrier state. The latter can be achieved with other antimicrobials such as tetracyclines, fluoroquinolones or macrolides (10). Other therapeutic measures are mostly supportive, depending on the severity of clinical signs as well as presence and degree of renal and hepatic dysfunction (1-4). Vaccination is considered the frontline defense against leptospirosis and prevents leptospiremia, reduces the severity of clinical signs and prevents urinary leptospiral shedding (1-3). Hygienic habitat conditions are also important in disease prevention and public health.

Israel is considered as an endemic country for leptospirosis, and serologically confirmed cases of leptospirosis in humans, as well as in domestic, farm and wild animals, have been reported (11). *Leptospira borgpetersenii* serogroup ballum infection in Israel, in both humans and rodents, but not in dogs, was previously described in the literature (12, 13). However, only a single case has been recorded in a dog by the Department of Bacteriology and Mycology, Kimron Veterinary Institute, Bet Dagan, Israel (14).

Canine monocytotropic ehrlichiosis (CME) is an important widely distributed canine tick-borne disease, caused by the obligate intracellular rickettsia *Ehrlichia canis* (15, 16). Infection occurs mostly during the warm season, when the vector tick, *Rhipicephalus sanguineus* is highly active. CME is a multi-systemic disease and three clinical stages have been described in experimental infections (15- 17). The acute phase is characterized by fever, depression, lethargy, anorexia, lymphadenomegaly, splenomegaly and hemorrhagic tendencies. Untreated and inappropriately treated (i.e., insufficient antibiotic dose and too short a treatment course) dogs may enter the subclinical phase, in which no clinical signs are evident (15). For incompletely understood reasons, some dogs will progress to the chronic phase, in which symptoms similar to those seen in the acute phase might occur, but are of greater severity and is characterized by bone marrow hypoplasia and pancytopenia. However, in naturally-occurring infection, accurate staging of the disease is difficult (15). Platelets counts have been suggested as a screening test for CME in endemic areas, as thrombocytopenia occurs in most dogs in all phases of the disease (15, 16). The indirect immunofluoresence antibody (IFA) test for anti *E. canis* IgG antibodies is considered the serological 'gold standard' test, indicating exposure to the rickettsia (15, 16, 18). Several enzyme-linked immunosorbent assays (ELISA) were validated and were demonstrated to high correlation with IFA, and are therefore also useful for the diagnosis of CME (16, 19).

However, a definitive diagnosis of CME requires molecular assays of blood and preferably splenic samples (20). Most dogs recover from the acute and subclinical phases and have been proven by molecular assays to clear the infection when adequately treated with tetracyclines (i.e., doxycycline at 10 mg/kg/d PO for 16 days) (15- 17, 20), however, the prognosis of dogs in the chronic phase of CME is grave (21). CME is a well known, widespread disease in Israel and the prevalence of clinically healthy dogs, seroreactive to *E. canis* is high (22). This report describes a case of a concurrent infection *L. borgpetersenii* serogroup ballum and *Ehrlichia canis* in a dog in Israel and focuses mostly on leptospirosis.

CASE REPORT

An 11-month-old, 43kg, intact female Great Dane dog was referred to the Hebrew University Veterinary Teaching Hospital (HUVTH) with chief complaints of vomiting, diarrhea and severe azotemia (creatinine, 10.6 mg/dL, reference interval [RI] 0.5-1.5; urea, 309.0 mg/dL, RI 12.6-58.3). The dog has been residing in a rural environment, with a free access to the outside and was occasionally seen to drink water from an ornamental pool in the garden. The dog had been currently vaccinated against canine distemper virus, adenovirus type-2, leptospirosis (*L. interrogans* serovars icterohaemorragiae and canicola), parainfluenza, and parvovirus (Fort Dodge Animal Health, Iowa, USA, given 7 months prior to presentation), as well as for rabies (brand unknown) and was dewormed twice with broad-spectrum preparations (brand unknown). Three months prior to presentation, the dog had an episode of illness, manifested by lethargy, anorexia, fever and lymphadenomegaly, and thrombocytopenia in a complete blood count (CBC). Based on these findings, the dog was tentatively diagnosed with acute ehrlichiosis, and treated with doxycycline (Doxylin, Dexon, Or-Akiva, Israel, 10 mg/kg PO q24h, for 21 days), prednisone (Rekah, Holon, Israel, 1 mg/kg PO q24h, for 10 days) and imidocarb-dipropionate (Imizol, Schering-Plough Animal Health, Middlesex, UK, 6.6 mg/kg SC, q2w, twice) and the dog had recovered. A week prior to presentation to the HUVTH, the dog was presented to the referring veterinarian with complaints of lethargy, pyrexia (40.3˚C), and watery diarrhea. CBC was unremarkable. The dog was treated with sulfamethoxazole/trimethoprim (Resprim forte, Teva, Petah-Tikva, Israel, 30 mg/kg PO q24h, for 7 days) but did not improve. On the following days anorexia and vomiting of billous contents ensued. A CBC showed absolute lymphocytosis and neutropenia, mild normocytic-normochromic anemia, and marked thrombocytopenia (Table 1). Blood smear evaluation showed mature neutrophils with no evidence of cytoplasmic toxicity, bland monocytes and atypical lymphocytes. Serum biochemistry showed severe azotemia, hyperphosphatemia, hypoalbuminemia, as well as increased activity of amylase and muscle enzymes (Table 2). Serum IgG antibody titer for *E. canis* was positive (≥1:80, Immunocomb, Biogal, Galed, Israel). At this point the dog was referred to the HUVTH.

Table 1: Hematological findings of a dog with concurrent *Leptospira borgpetersenii* serogroup ballum and *Ehrlichia canis* infection

Parameter	RDVM[1]	Reference interval
White blood cells (×10³/μL)	8.8	5.2 - 13.9
Red blood cells (×10⁶/μL)	4.76	5.7 - 8.8
Hemoglobin (g/dL)	10.5	12.9 - 18.4
Hematocrit (%)	30.9	37.1 - 57.0
Mean corpuscular volume (fL)	64.8	58.8 - 71.2
MCHC[2] (g/dL)	34.1	31.0 - 36.2
Red blood cell distribution width (%)	13.9	11.9 - 14.5
Platelets (×10³/μL)	44	143 - 400
Mean platelet volume (fL)	24.0	7.0 – 11.0
Neutrophils (×10³/μL)	2.99	3.90 - 8.00
Lymphocytes (×10³/μL)	4.58	1.30 - 4.10
Monocytes (×10³/μl)	0.79	0.20 - 1.10
Eosinophils (×10³/μL)	0.35	0.00 - 0.60
Basophils (×10³/μL)	0.09	0.00 - 0.10

1, obtained by the referring veterinarian 4 days prior to presentation to the hospital; 2, mean corpuscular hemoglobin concentration

At presentation, the dog was quiet, alert and responsive, with body condition score of 3/9 and normal vital signs, hydration status and capillary refill time. Abnormal physical findings included mildly pale mucous membranes, mild lymphadenomegely, and brown pasty stool upon rectal exam. Selected serum biochemistry analytes were measured, with similar results as previously (Table 2). Urine, obtained by cystocenthesis, showed specific gravity (USG) 1.016, pH of 5, hematuria (1-2 red blood cells/HPF), proteinuria (+4) and bacteruria (i.e., cocci were observed in urine sediment). The urine protein to creatinine ratio (UPC) was increased (1.88, RI 0.2-0.5). Urine was not cultured at this point because the dog had received prior antibiotic therapy. The prothrombin

(PT) and activated partial thromboplastin (aPTT) times were within RI (7.35 sec, RI 6.0-8.4 and 12.1 sec, RI 11.0-17.4, respectively), but hyperfibrinogenemia (516 mg/dL, RI 200-400) was noted. Basal cortisol was within RI (2.96 µg/dL, RI 1.3-7.2). Blood samples were sent for PCR of *E. canis* DNA and serum antibodies titers for several *L. interrogans sensu lato* serogroups. Abdominal ultrasound showed moderate splenomegaly, increased echogenicity of both renal cortices, although the size of both kidneys was normal (9-10 cm), and no evidence of dilation of their pelvices was present. No other abnormalities were observed. Indirect arterial blood pressure (ABP, Cardell, 9401BP, Sharn Veterinary Inc., Tampa, FL, USA) was markedly increased (systolic 216 mmHg, RI 90-140; diastolic 136, RI 50-80; mean 164, RI 60-100).

The dog was hospitalized and initially treated with IV fluids (Hartmann's solution, Teva-Medical, Ashdod, Israel, 4.5 ml/kg/hr), cefazolin (Cefamezin, Teva, Petach-Tikva, Israel, 11 mg/kg IV q12h), doxycycline (Doxylin, Dexon, Or-Akiva, Israel, 10 mg/Kg PO q24h), metoclopramide (Pramin, Rafa Labs., Jerusalem, Israel, 1 mg/kg/d IV at constant infusion rate [CRI]), maropitant (Cerenia, Pfizer, Sandwich, UK, 1 mg/kg, SC q24h), famotidine (Baxter, Deerfield, IL, 0.25 mg/kg IV q24h) and amlodipine (Teva, Petach-Tikva, Israel, 0.4 mg/kg PO q12h). The dosages of drugs that are mainly excreted via the urine were reduced based on serum creatinine, in light of decreased renal function. Venous blood gas, obtained 12 hours post initiation of IV fluid therapy, showed borderline metabolic acidosis and hypocarbemia (pH 7.35, RI 7.34-7.43 and HCO_3 - 17.2 mmol/L, RI 20-24) with respiratory compensation (pCO_2 31.8 mmHg, RI 35-45), although no increase in respiratory rate or effort were noted.

Over the following week the dog remained hospitalized, was alert, had normal vital signs, ate well, and no episodes of vomiting or diarrhea were observed. Arterial BP remained mildly increased despite amlodipine treatment (systolic 120-208 mm Hg; diastolic 82-154 mm Hg). Although urine production rate was deemed normal, 24 hours post initiation of IV fluid therapy, the dog's body weight had increased above the target body weight, presumably due to overhydration. Thus, diuretics were added, including furosemide (Alfasan, Woerden, Holland, 2 mg/Kg IV, once) and mannitol (Osmitrol 20%, Baxter, Deerfield, IL, USA, 0.5 gr/kg IV bolus, followed by 1 mg/kg/min IV at CRI for 5 days). On day 2 of hospitalization, platelet count increased ($147x10^3/\mu L$). Serum creatinine concentration improved gradually, and was 2.34 mg/dL at day 7. The PCR tested positive for *E. canis*. Serum MAT antibody titers for *L. interrogans sensu lato* serogroups canicola and ballum (*L. canicola* and *L. ballum*, respectively) were 1:50 and 1:100, respectively (Department of Bacteriology and Mycology, Kimron Veterinary Institute, Bet Dagan, Israel). A second blood sample for leptospiral MAT titer, at day 7, was positive for *L. ballum* (1:800), with no cross-reactivity with other serogroups, including *L. canicola*.

The dog was discharged from the HUVTH after seven days of hospitalization. Treatment at home included amoxicillin-clavulonate (Augmentin, SmithKline, Brentford, UK, 15

Table 2: Serum biochemistry and coagulation results of a dog with concurrent *Leptospira borgpetersenii* serogroup ballum and *Ehrlichia canis* infection

Parameter	RDVM[1]	Day 0	Day 11	Reference interval
Glucose (mg/dL)	83			65-118
Cholesterol (mg/dL)	254			135-280
Urea (mg/dL)	309.0	266.9	99.55	12.6-58.3
Albumin (g/dL)	2.5	2.75	2.62	2.60-4.00
Alkaline phosphatase (U/L)	118			0-150
Alanine aminotransferase (U/L)	23			0-60
Aspartate aminotransferase (U/L)	43			0-50
Amylase (U/L)	1558			200-1480
Calcium (mg/dL)	11.3			9.0-11.7
Creatine kinase (U/L)	348			50-200
Creatinine (mg/dL)	10.6	9.28	2.03	0.5-1.5
Phosphorus (mg/dL)	12.0	12.63	5.94	2.50-6.20
Triglycerides (mg/dL)	70			50-100
Protein (g/dL)	5.5			5.4-7.5
Globulin (g/dL)	3.0			2.0-3.8
Total bilirubin (mg/dL)	0.2			0.1-0.5
γ-glutamyltransferase (U/L)	0			0-6
Lactate dehydrogenase (U/L)	563			50-320
Chloride (mmol/L)	106	110.3		102-117
Potassium (mmol/L)	4.3	4.14	4.9	3.8-5.6
Sodium (mmol/L)	146	147.4		142-159
Prothrombin Time (sec)		7.35		6.0-8.4
Activated Partial thromboplastin time (sec)		12.1		11.0-17.4
Fibrinogen (mg/dL)		516		200-400

1, obtained by the referring veterinarian 4 days prior to presentation to the hospital.

mg/kg PO q12h), famotidine (Gastro 20, Teva, Petach-Tikva, Israel, 0.85 mg/kg PO q24h), doxycycline, amlodipine and subcutaneous fluids (Hartmann's solution, 10 ml/kg q12h). On a follow-up, four days post discharge, the dog was bright and alert. The platelet count had increased ($170x10^3$/μL) and serum creatinine was 2.03 mg/dL. Improvement in other serum biochemistry analytes was also noted (Table 2). Blood pressure was 153/96 mmHg. On a telephone interview 150 days later, the referring veterinarian had reported that the dog was healthy and doing well.

DISCUSSION

This is the first report of *L. ballum* infection in a dog in Israel, although previously reported in humans and rodents in the country (12, 13). *L. ballum* is a well-recognized worldwide pathogen, mostly transmitted by rodents (5, 23). It has been infrequently diagnosed in dogs, mostly in the Caribbean Islands and South America (5, 16-31) and has been estimated to be of low significance (30). Several large-scale surveys, conducted in Australia, USA, Canada and Japan were all negative for this serovar, supporting its uncommon infection in dogs (32-35). The pathogenicity and characteristic clinical signs of *L. ballum* infections have not been extensively investigated and reported. A recent report has characterized four new isolates of *L. ballum* from *Mus musculus* (domestic mouse) with varying degrees of virulence in a hamster model. One strain was highly virulent and led to pulmonary hemorrhage (36).

Leptospirosis in dogs is an uncommon disease in Israel. Forty-eight leptospirosis cases were diagnosed in dogs between 1997 and 2009 (four cases or less annually, on average). The particular serovars involved in these infections were mostly not reported (37), although a single case of *L. ballum* infection was diagnosed in 2007 in a dog (14). Nevertheless, the epidemiology of leptospirosis in dogs in Israel has never been published in the veterinary literature. In a previous epidemiological study of leptospirosis in human patients in Israel, *L. ballum* was more prevalent in rural environment (7/12 cases), and infections were associated with the patients' occupation (i.e., more common in farmers and sewage workers) (11). The present dog comes from a rural environment, and could thus have been exposed to various foci of leptospiral contamination, although the suspected source of infection was considered to be the stagnant water in the ornamental garden pool.

The present dog was currently vaccinated with the commercially-available bi-valent vaccine, containing bacterins of serovars icterohaemorrhagiae and canicola. Other vaccines, containing two additional bacterins, of serovars grippotyphosa and pomona, are now available in the United States, but not in Israel (1-3). Vaccines made by the killed whole cell method are serogroup-specific; hence, they do not stimulate strong immunity against *L. ballum*. Thus, the available vaccines could not have been expected to protect the present dog against this particular leptospiral serovar. In Cuba, where *L. ballum* is prevalent and is the most common cause of human leptospirosis, killed whole-cell vaccines were formulated based on two clinical isolates of this serogroup. Immunization of hamsters with these vaccines was protective against infection (38). New efforts are now being made in analysis of the *L. interrogans sensu lato* whole-genome, in order to identify potential antigens, which can produce heterologous protection, and might thus provide cross-protection in future vaccines (39-41).

The dog's previous illness was presumptively as a result of monocytic ehrlichiosis. Because both leptospirosis and ehrlichiosis commonly manifest as fever, lethargy, anorexia and thrombocytopenia, and since a molecular assay to detect ehrlichial DNA was not performed at that time, it cannot be ruled out that the preceding episode of illness was rather due to leptospiral infection and not necessarily due to ehrlichiosis, or that both bacteria had concurrently infected the dog at that time. Clinical improvement would have been expected in either disease with doxycycline therapy, as both leptospires and rickettsiae are highly susceptible to doxycycline (10, 15). The presence of a positive anti-ehrlichial IgG titer, as in this dog, could only serve as an indication of a previous exposure to the rickettsia, and does not necessarily indicate presence of an active infection, especially in an endemic area such as Israel (15, 22, 19). It should be pointed out that a three-week doxycycline treatment, as presently prescribed prior to presentation to the HUVTH, is supposed to be sufficient to eliminate the carrier state in dogs (1, 2). However, prednisone that was prescribed might have immunosuppressed the dog, thus preventing clearance of infection, or had made the dog susceptible to leptospirosis. The initial sulfamthoxazole/trimethoprim therapy during the present episode probably failed to improve the dog's condition because leptospires are resistant to sulfonamides and their combination with trimethoprim (2, 10, 42).

The dog was proven to be co-infected with *E. canis*, diagnosed by PCR. If the prior illness of this dog was indeed also caused by the rickettsia, a 21-day course of doxycycline should possibly have led to its clearance (15, 20). Thus, ehrlichial reinfection may have probably occurred which would account for the present episode of ehrlichiosis. A persistent, sub-clinical disease, due to inappropriate therapy (i.e., poor owner compliance and a short treatment course) (15) or the immunosuppressive effects of steroids cannot be ruled out, although there is no evidence to support this scenario in the present case. The concurrent presence of both infections in a single dog could be unrelated; however, immunosuppression by one infection might have led to increased susceptibility to the other.

In the present case, the higher MAT titer observed for *L. ballum* compared to *L. canicola* at presentation increased the clinical suspicion of leptospirosis. This is because the available antileptospiral vaccines do not cross-protect against the former, while they do protect against *L. canicola*. Therefore, titers against *L. canicola* would have been expected to be higher compared to those against *L. ballum* in response to vaccination or in presence of a subclinical exposure to the organism (1, 2). However, the eightfold increase in MAT titer against *L. ballum* strongly supports the diagnosis of an infection by this leptospire. This is in agreement with previous reports stating that the causative serovar is more likely to be specifically identifiable in animals' convalescent sera (2). Cross-reactivity of leptospiral serological reactions tends to occur mostly during the acute disease phase (2). Additionally, it has been reported previously in serological studies that *L. ballum* and *L. canicola* tended to cross-react (24, 30). These findings can account for the low MAT titer against *L. canicola* (1:50) detected in the first serum sample in this dog, which was later negative. Because post-vaccination MAT titers do not usually persist for more than 3 months (1-3), it is less likely that the titer for *L. canicola* detected in the first serum sample was due to prior vaccination, because this dog has received its last vaccination seven months prior to presentation to the HUVTH. Nevertheless, this cannot be ruled out, because MAT titers as high as 1:800 have been observed to persist for as long as nine months post vaccination (1, 2).

In conclusion, this is, to the best of knowledge, the first record of *L. borgpetersenii* serogroup ballum infection in dogs in Israel to be published in the veterinary literature. In addition, it is the first report of concurrent infection of *L. ballum* and *Ehrlichia canis* in a dog. It exemplifies that leptospirosis, although uncommonly diagnosed in Israeli dogs, does occur, and should be included in the differential diagnosis list for acute azotemia. Its diagnosis and therapy can be complicated by concurrent infections. Maintenance of proper hygiene at the immediate environment is important in prevention of leptospirosis, because the currently available vaccines do not provide protection against most leptospiral serovars. Due to the zoonotic potential of leptospirosis, dogs, especially from rural households, presenting nonspecific acute signs, such as fever, malaise, anorexia and depression, in addition to azotemia, should be serologically screened for leptospirosis, and in cases with borderline serology, a second, convalescent sample should be tested.

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