INTRODUCTION

Leptospirosis is a zoonotic disease with worldwide distribution, caused by infection with pathogenic spirochaetes of the genus *Leptospira* (Nevett et al., 2006). In the current literature, canine leptospirosis is well documented in the United States, Canada, Australia, Germany and Italy (Geisen et al., 2007; Goldstein, 2005; Greenlee et al., 2004; Langston and Heuter, 2003; Mastrorilli et al., 2007; Miller et al., 2007; Prescott et al., 2002, Ward, 2002; Ward et al., 2004). Previously, the typical clinical signs of leptospirosis were considered to be icterus and hemorrhagic diathesis. However, the most common clinical signs seen today are those related to acute renal failure (Langston and Heuter, 2003).

Historically, most clinical cases were caused by infection with the *Leptospira* serovars Icterohaemorragiae and Canicola (Goldstein, 2005). These serovars are included in commercially available bivalent vaccines, which reduce clinical disease and the urinary shedding (Schreiber et al., 2005). In the last decade, several reports documented an increased incidence of the disease, though with few cases of the serogroups Icterohaemorrhagiae and Canicola. The most common serovars causing clinical disease in the United States today belong to serogroups Grippotyphosa, Pomona and Australis (Goldstein, 2005; Prescott et al., 2002). An increased incidence of serogroup Autumnalis has been reported as well (Langston and Heuter, 2003).

ABSTRACT

Recent information regarding clinical leptospirosis in Europe is sparse in comparison with information about this disease in the United States and certain other countries. A retrospective study describing seven clinical cases of dogs diagnosed with leptospirosis on the basis of a MAT (Microscopic Agglutination Test), serology or autopsy at the Department of Medicine and Clinical Biology of Small Animals, Ghent University, between January 2003 and October 2005 is presented. All the dogs had non-specific clinical signs and presented with acute renal failure, with or without signs of liver insufficiency and/or bleeding tendencies. In the three dogs in which a MAT was performed, the highest antibody titers were found against *Leptospira* serogroup Pomona (n=2) and Javanica (n=1). It is recommended to treat every patient with clinical signs and laboratory abnormalities of acute renal failure without a known cause with penicillin derivatives until specific testing confirms or rules out leptospirosis.

SAMENVATTING

Recente informatie aangaande klinische leptospirose in Europa is schaars in vergelijking met de informatie over deze ziekte in de Verenigde Staten en enkele andere landen. Een retrospectieve studie van zeven klinische gevallen van honden gediagnosticeerd met leptospirose op basis van een MAT (Microscopic Agglutination Test), serologie of een autopsie bij de vakgroep Medische en Klinische Biologie van de Kleine Huisdieren, Universiteit Gent, tussen januari 2003 en oktober 2005 wordt beschreven. Alle honden vertoonden aspecifieke klinische tekenen ten gevolge van een acute nierinsufficiëntie, met of zonder tekenen van leverinsufficiëntie en/of bloedingsneiging. Van de drie honden waarbij een MAT werd uitgevoerd, was de hoogste antistoffiterversus *Leptospira* serogroep Pomona (n=2) en Javanica (n=1). Elke patiënt met klinische tekenen en laboratoriumafwijkingen die wijzen op acute nierinsufficiëntie zonder gekende oorzaak dient behandeld te worden met penicillinederivaten totdat specifieke testresultaten leptospirose bevestigen of uitsluiten.
al., 2007), while the serogroup Australis appears to be common in Italy (Mastrorolli et al., 2007). Infection with serogroup Javanica has been described in dogs and humans in Europe (Cacciapuoti et al., 1994; Vandenbroek et al., 1991). However, no reports have been found correlating infection with this serovar to clinical disease in dogs.

The prevalence of serogroup Canicola seems to be declining in European countries as well, possibly because of widespread vaccination. Although both vaccinal serogroups (Icterohaemorrhagiae and Canicola) are expected to induce agglutinating antibodies, the seroprevalence of Icterohaemorrhagiae antibodies is higher than those of Canicola in canine serum samples in France (André-Fontaine, 2006). This can be explained by the higher infection pressure of the former strains, compared to the latter strains in the environment. Dogs are the maintenance hosts for the serogroup Canicola, while rodents play that role for Icterohaemorrhagiae (Langston and Heuter, 2003; van de Maele et al., accepted for publication).

In this retrospective study, seven clinical cases of dogs diagnosed with leptospirosis at the Department of Medicine and Clinical Biology of Small Animals, Ghent University, between January 2003 and October 2005 are presented.

**MATERIALS AND METHODS**

Retrospective screening of the medical records database of all dogs examined at the Ghent University Department of Medicine and Clinical Biology of Small Animals from January 2003 until October 2005 identified 7 cases that had been given a diagnosis of leptospirosis. The information extracted from each record included breed, gender, age at diagnosis, geographic area, month of diagnosis, clinical signs, physical examination findings, results of leptospiiral serology, complete blood count (CBC), serum biochemical analyses, urinalysis, abdominal ultrasound, treatment, time to resolution of clinical signs and outcome.

Suspicion of leptospirosis was raised based on history, clinical signs, CBC and serum biochemical analysis. Diagnosis relied on the demonstration of antibodies in serum samples with a human agglutination test using a heat stable antigen (Bio-Rad, Leptospira serology 79623). By physical processes, disappearance of the serogroup specificity is obtained, making it a useful screening test for leptospirosis. Sera were also tested in a MAT in 3 cases for the detection of antibodies against the serogroups Gryppotyphosa, Canicola, Pomona, Icterohaemorrhagiae, Javanica, Bratislava, Bataviae, Pyrogenes, Tarassovi, Sejroe, Celledoni, Cynopteri, Panama, Shermani, Djasiman, Hebdomadis, Louisiana, Mini, Manhao and Ranarum. The etiologic serogroup was defined as the serogroup against which the highest antibody titer was detected. In one case this was diagnosed at autopsy, when an immunofluorescence test was performed to confirm the diagnosis.

**Results**

**Signalment**

Seven dogs were diagnosed with leptospirosis during the study period. The following breeds were represented: Cavalier King Charles Spaniel, giant poodle, Groenendael, Maltese, Samoyed, Shih tzu and Viszla. Five were intact males, two were intact bitches. They ranged in age from four months to nine years, five dogs were less than two years old, and the remaining two dogs were six and nine years, respectively. Little was known about the environment the dogs lived in. One dog had visited a rural area three weeks before clinical symptoms occurred. Two of the dogs were diagnosed in January, one in June, three in September and one in October.

**Clinical signs and vaccination status**

Clinical signs included anorexia (n=5), vomiting (n=5), lethargy (n=5), melena (n=1), and lameness (n=1). Physical examination revealed icterus (n=3), abdominal pain (n=2), bleeding tendencies (n=2), bradycardia (n=2), tachycardia (n=1), systolic heart murmur (n=1), pulmonary crackles (n=1), weak pulses (n=1) and edema (n=1). One out of the seven dogs was hypothermic (< 37°C) and one was hyperthermic (> 39°C). Oliguria (urine output < 0.5 ml/kg/h) was seen in one dog.

The vaccination status was known for six of the seven dogs. Two dogs (dog 1 and 3) had been vaccinated less than one year previously with an inactivated vaccine containing the serogroups Icterohaemorrhagiae and Canicola (Vanguard® lepto, Pfizer A.H. or Nobivac® lepto, Intervet). The four-month-old puppy (dog 7) had only received a primo vaccination with an inactivated vaccine containing both serogroups (Vanguard® DA2Pi-CPV-lepto, Pfizer A.H.) at eight weeks of age. Three dogs (dog 2, 4 and 5) had had their last Leptospira vaccination more than one year before presentation.

**Diagnosis**

The abnormalities seen in the hematology and biochemistry profiles are shown in Table 1. CBC results were retrieved from five cases. One dog was mildly anemic (Hct = 31.8%) and one dog showed severe anemia (Hct = 18%). Leucocytosis was present in five out of five dogs. Two were thrombocytopenic (range 34 x 10³/µl and 120 x 10³/µl). Unfortunately, coagulation profiles were not determined.

All seven dogs were initially azotemic. An elevated serum AST was present in four out of five dogs, ALT in three out of five dogs and ALP in four out of six dogs. Hypoprotenemia was present in two out of five dogs, and hyperalbuminemia in two out of six dogs. Four out of four dogs displayed hypochloremia, while four out of five had hypokalemia at first presentation and one out of five initially had hyponatremia.

In five cases, the results of urinalysis were re-
Three dogs had isosthenuria (USG 1.007-1.015). Proteinuria was detected in three dogs (urine protein/creatinine ratio of 1.77, 3.14 and 7.66, reference range < 1) without an active sediment. Two dogs had glucosuria with normoglycemia. Hemoglobinuria and hematuria were found in one dog each.

Abdominal ultrasound testing was performed in six dogs. Hyperechoic kidneys were seen in four cases, one of which also displayed a dilatation of the renal pelvis. The renal architecture and echogenicity were considered normal in two dogs.

In all cases, the diagnosis of leptospirosis was made either upon a positive screening agglutination test result (n=6) or by immunohistochemistry of hepatic and renal tissue after autopsy (n=1). Single serum samples were obtained from all dogs. Serum antibody titers with a MAT were highest in the serogroup Pomona in two dogs (dogs 1 and 4) and in the serogroup Javanica in one dog (dog 7). Unfortunately, in three dogs a positive screening test result was not followed by a MAT for identification of the etiologic serovar.

Six dogs received treatment, and one dog died at initial presentation before treatment could be instituted. All dogs received intravenous crystalloid solutions (four NaCl 0.9% and two Hartmann). One dog was supplemented with potassium chloride to treat hypokalemia. Amoxicillin (Clamoxyl® 20 mg/kg i.v. q 8 h or Duphamox® 10 mg/kg p.o. q 12 h) or a combination of amoxicillin with clavulanic acid (Synulox®) 8.5 mg/kg s.c. q 12 h was administered to all dogs for 2 weeks. Doxycyclin (Ronaxan®) 10 mg/kg p.o. q 12 h, was administered to five dogs for two weeks, following amoxicillin treatment. One dog also received enrofloxacin (Baytril 2.5%) 5 mg/kg s.c. q 24 h and metronidazole (Flagyl®) 30 mg/kg i.v. q 24 h because of hyperthermia and suspicion of sepsis at presentation. A histamine (H2)-receptor-blocker, ranitidine (Zantac®) (initially 1-2 mg/kg i.v., after which the dose was reduced to avoid accumulation due to lower renal clearance), combined with metoclopramide (Primperan®) (0.1-0.3 mg/kg q 6-8h or at a constant infusion rate of 0.1-0.2 mg/kg/h), was used in all dogs. A gastric feeding tube was placed in one dog because of the high catabolic state and persistent anorexia. One dog was euthanized after five days of hospitalization because of its deteriorating state. The five remaining dogs recovered completely. During follow-up consultations, none of them had developed either clinical or clinicopathological evidence of chronic renal failure.

### DISCUSSION

In contrast to a plethora of articles in the literature describing leptospirosis in the USA and Canada, only two recent articles describe its prevalence, clinical presentation, treatment and prevention in Europe. In the present article, seven dogs diagnosed with leptospirosis in Belgium are described in detail.

In agreement with a previous study by Stokes and Forrester (2004), the affected dogs in this study were predominantly young (< 2 years) middle to large breed dogs. Other recent studies do not comment on size and breed of the affected dogs and did not find a predominantly young population (Miller et al., 2007; Geisen et al. 2007). A sex predisposition has not been a consistent finding in previous retrospective studies (Harkin et al., 1996). Most cases were diagnosed during the second half of the year. A retrospective study performed in the United States and Canada (Ward, 2002) found a significant correlation between the occurrence of leptospirosis and periods of high rainfall prior to diagnosis, and this is probably the reason why most cases are seen during late summer to fall. Leptospires have a higher rate of survival in watery areas and moist soil (Ward, 2002).

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**Table 1. Mean values, standard deviations and reference ranges of the hematologic and biochemical blood results of the patients with Leptospirosis in the study.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean values</th>
<th>Standard deviation</th>
<th>Reference ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hct (%)</td>
<td>37.4</td>
<td>10.3</td>
<td>35.0 -50.0</td>
</tr>
<tr>
<td>WBC (/µl)</td>
<td>18.10</td>
<td>1.7</td>
<td>6.20 – 8.70</td>
</tr>
<tr>
<td>PLT (10⁹/µl)</td>
<td>306</td>
<td>231</td>
<td>150 – 500</td>
</tr>
<tr>
<td>Alb (g/l)</td>
<td>22.7</td>
<td>5</td>
<td>20 – 40</td>
</tr>
<tr>
<td>T-Pro (g/l)</td>
<td>61</td>
<td>15</td>
<td>60 – 80</td>
</tr>
<tr>
<td>BUN (mmol/l)</td>
<td>37.28</td>
<td>23.9</td>
<td>3.33 - 8.32</td>
</tr>
<tr>
<td>Cre (µmol/l)</td>
<td>529</td>
<td>292</td>
<td>60µmol +BW</td>
</tr>
<tr>
<td>Na (mmol/l)</td>
<td>146</td>
<td>9</td>
<td>&gt; 140.0</td>
</tr>
<tr>
<td>K (mmol/l)</td>
<td>3.83</td>
<td>0.93</td>
<td>4.0 - 6.0</td>
</tr>
<tr>
<td>Cl (mmol/l)</td>
<td>101</td>
<td>6</td>
<td>&gt; 110</td>
</tr>
<tr>
<td>ALP (IU/l)</td>
<td>3519</td>
<td>5445</td>
<td>&lt; 147</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>221</td>
<td>145</td>
<td>&lt; 94</td>
</tr>
<tr>
<td>AST (IU/l)</td>
<td>473</td>
<td>572</td>
<td>&lt; 44</td>
</tr>
</tbody>
</table>

Icterus and bleeding tendencies were once considered to be typical symptoms of leptospirosis, especially related to infection with serogroup Icterohaemorrhagiae (Langston and Heuter, 2003; Navarro and Kociba, 1982). Previous studies (Navarro and Kociba, 1982; Ward et al., 2004) show a shift away from the clinical features of liver insufficiency and bleeding tendencies and towards the clinical signs of acute renal failure. A recent study (Goldstein et al., 2001) suggested that infection with different serogroups only causes minor differences in clinical symptoms. Leptospira serogroup Pomona was associated with more severe renal disease and a worse outcome compared with disease caused by other serogroups. In this study, the most common clinical signs of leptospirosis were non-specific and included mainly gastro-intestinal signs such as anorexia (n=5), vomiting (n=5), abdominal pain (n=2) and melena (n=1). Physical examination revealed icterus and bleeding tendencies in only three dogs.

The most common laboratory abnormalities encountered were leucocytosis, azotemia, electrolyte disturbances, increased liver enzymes (ALT, ALP or AST), isosthenuria and proteinuria. While all seven dogs suffered from acute renal failure, only three showed an increase in serum AST and ALT concentrations. Furthermore, the high mean and SD value of ALP is primarily caused by one outlier (the dog with a final diagnosis made on autopsy). Without this patient’s values, the mean and SD for ALP would become 252 and 141, respectively. The leukocytosis and electrolyte abnormalities are typical findings in patients with acute renal failure and are likely to reflect this disease.

Although the results of the abdominal ultrasound tests were also non-specific, they revealed abnormalities in the echogenicity or architecture of the kidneys in four out of the six cases. This is in accordance with the findings of Forrest (1998). Therefore, this test is considered to be a useful complementary technique in the diagnostic workup.

The definitive diagnosis was made either by the results of a screening agglutination test or by an immunofluorescence test on histopathology. In three dogs with positive antibody titers, a MAT was performed for further serogroup specification. MAT is considered the gold standard for the diagnosis of leptospirosis in dogs and ideally would have been performed in all the dogs. However, many owners declined an additional MAT. This refusal was based on financial restraints, lack of a sufficient amount of serum in the initial sample or the owner’s feeling that the results would not significantly alter the therapy and/or the prognosis. The time the results would be available. The potential drawbacks of the MAT are twofold. First, it has been reported that antibody titers against leptospirae may only be identified three weeks after infection in some cases (Branger et al., 2005). Therefore, convalescent serum samples, showing a fourfold rise in titer are recommended (Goldstein, 2005). Secondly, vaccination with commercially available inactivated vaccines can induce antibody titers to serogroups Icterohaemorrhagiae and Canicola. However, antibody titers higher than 1:800 are rarely seen after vaccination and rarely persist for longer than three months. Consequently, a titer of higher than 1:800 to vaccinal and non-vaccinal serovars is considered to be consistent with a diagnosis of leptospirosis (Goldstein, 2005).

Definitive identification of the causative serovar can be achieved by bacteriological culture. However, leptospiral organisms are difficult to culture and may require weeks of incubation to obtain a result (Wild et al., 2001). PCR techniques based on the 23S rDNA gene sequence or on the gene hapl have been used for serovar identification in dogs (Branger et al., 2005; Harkin et al., 2003). Recent advances have made it an easy, rapid and more sensitive technique (Branger et al., 2005). However, PCR for leptospirosis is not yet readily available to practitioners. Therefore, the combination of clinical signs and high antibody titers to a pathogenic serovar continues to be routinely used in practice to confirm the diagnosis of leptospirosis.

Treatment consists of specific and supportive therapy (Langston and Heuter, 2003). Specific therapy, consisting of antibiotics, minimizes organ damage and quickly clears the leptospiremic phase (Green et al., 2006). High doses of penicillin, ampicillin or amoxicillin and tetracyclines are believed to be very effective in treating this phase. Oral administration of tetracyclines can be difficult in a vomiting, debilitated patient. Therefore, intravenous penicillin derivatives are mostly used in the leptospiremic phase. Against the leptospiruric phase, doxycycline, aminoglycosides and macrolides seem to be efficient (Alt et al., 2001). Doxycycline is most commonly used in dogs, given together with or two weeks after a penicillin derivative (amoxicillin) (Goldstein, 2005). Aminoglycosides are contraindicated in patients with renal impairment. All dogs in the study initially received amoxicillin for two weeks, followed by doxycycline for two weeks. Sometimes, a combination of amoxicillin combined with clavulanic acid or amoxicillin with enrofloxacin and metronidazole was used in debilitated patients, pending a definitive diagnosis, in order to broaden the spectrum. Hemodialysis has been shown to improve the prognosis of dogs with leptospirosis and severe azotemia (Adin et al., 2000). However, hemodialysis is not available for dogs in Belgium.

The prognosis of leptospirosis in dogs is fair to poor, depending on the clinical state of the patient at initial presentation and on the causative leptospiral serovar. Survival rates of 80% for dogs with acute renal failure due to leptospirosis have been reported (Adin et al., 2000). Five of the seven dogs in this study made complete recovery with regard to renal function. This compares favorably to the average prognosis of patients presenting with acute renal failure. It is recommended to treat every patient with clinical signs and laboratory test results indicating acute renal failure of unknown cause with penicillin derivatives until specific tests rule out or confirm leptospirosis infection. Clinical suspicion for the disease should remain high and appropriate treatment should be instigated whenever indicated.
An important drawback of this study is its retrospective nature, leading to unrecovered data. This might also have caused bias in the population of dogs tested for leptospirosis. Furthermore, only a very limited number of patients were included. Not all dogs were tested by the MAT, which is considered the gold standard. It is strongly advised that in the event leptospirosis is suspected, a definitive diagnosis can be obtained by performing the MAT, and ideally a convalescent titer is checked three weeks later.

A shift from infection with vaccinal serogroups towards other serogroups such as Grippotyphosa and Pomona may lead not only to changes in clinical signs, but also to calls for the development of a multivalent vaccine offering immunity to these serovars as well. Although bivalent vaccines against serogroups Icterohaemorrhagiae and Canicola continue to be useful, no vaccine currently available in Europe can offer protection against all possible serovars, due to the lack of cross-reactivity between serovars. A new vaccine now into a vaccinal preparation. Nevertheless, it is strongly advised that in the event leptospirosis is suspected, a definitive diagnosis can be obtained by performing the MAT, and ideally a convalescent titer is checked three weeks later.

References


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