Evaluation of clinical, laboratory, imaging findings and outcome in 99 dogs with leptospirosis

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OBJECTIVE: To report clinical, laboratory and diagnostic imaging features and prognostic factors in dogs with leptospirosis from North-East Germany.

MATERIALS AND METHODS: Medical records of dogs diagnosed with leptospirosis from 2006 to 2013 were evaluated retrospectively.

RESULTS: The study included 99 dogs. At initial presentation, the most common clinical signs were lethargy (96%), anorexia (88%), vomiting (85%), painful abdomen (39%), diarrhoea (38%), oliguria (27%) and tachypnoea (26%). Abnormal laboratory findings included anaemia (63%), thrombocytopenia (63%), leucocytosis (57%), increase of plasma urea (84%) and creatinine concentrations (81%), increased liver enzyme activities (80%), hyperbilirubinaemia (69%), hyperphosphataemia (67%), hyponatraemia (64%), hypoalbuminaemia (55%) and hypokalaemia (29%). Radiological pulmonary changes were detected in 57% of the dogs initially or during the course of disease. Severe dyspnoea, oliguria, azotaemia, hyperbilirubinaemia and severe radiological pulmonary changes were more often found in dogs that did not survive. There was renal, hepatic and pulmonary involvement in 95, 92 and 58% of the dogs, respectively, and multi-organ lesions in 98 dogs (98%); 32 dogs died or were euthanased.

CONCLUSION: Several clinical and laboratory abnormalities were associated with a negative outcome; severe lung involvement was specifically associated with high mortality.

INTRODUCTION

Leptospirosis is a worldwide zoonotic bacterial infectious disease (Smythe 1999, Bharti et al. 2003). This multi-organ disease has been designated as re-emerging zoonosis because of an increasing prevalence in both the human and canine populations over the past 15 years (Jansen et al. 2005, Greene et al. 2012). Acute renal, hepatic, pulmonary (LPHS, leptospiral pulmonary haemorrhage syndrome) disease and haemorrhage are the most frequently reported clinical manifestations in dogs with leptospirosis (Birnbaum et al. 1998, Steger-Lieb et al. 1999, Adin & Cowgill 2000, Goldstein et al. 2006, Geisen et al. 2007, Gerlach & Stephan 2007, Mastrorilli et al. 2007, Kohn et al. 2010, Tangeman & Littman 2013, Major et al. 2014). The prognosis depends on the severity of signs and secondary complications. Renal failure and compromised hepatic and pulmonary function give rise to severe complications and high mortality rates in both humans and dogs infected with leptospirosis (Gilad & Borer 2000, Dolhnikoff et al. 2007, Kohn et al. 2010, Cowgill & Langston 2011,
In dogs with leptospirosis, lung involvement represents a severe complication causing increased case fatality depending on the severity of respiratory distress (Kohn et al. 2010). Furthermore, dogs presenting with azotaemia, increased serum troponin-I concentrations, increased C-reactive protein/haptoglobin-ratio and elevated urine to protein/creatinine-ratio, and reduced serum albumin concentrations have a higher risk of death than dogs without these abnormalities (Rentko et al. 1992, Birnbaum et al. 1998, Adin & Cowgill 2000, Mastrorilli et al. 2007). Until now, there have been only a few studies evaluating clinical, laboratory and radiological signs as prognostic parameters in dogs suffering from leptospirosis (Rentko et al. 1992, Birnbaum et al. 1998, Adin & Cowgill 2000, Mastrorilli et al. 2007). The objectives of this study were: (1) to assess the prevalence and characteristics of clinical, laboratory and imaging features in dogs with leptospirosis and (2) to compare these findings between surviving and non-surviving dogs.

MATERIALS AND METHODS

The medical records of dogs diagnosed with leptospirosis presented between April 2006 and April 2013 were retrospectively reviewed. The clinic treats primary care and referral patients mainly from the Berlin/Brandenburg area. Dogs with acute renal failure, hepatopathy or pulmonary abnormalities on radiographs were suspicious for leptospirosis if other causes were ruled out or seemed unlikely. Dogs were included in the study if the microscopic agglutination test (MAT) (WHO 2011), or the urine or ethylenediamine tetra-acetate anti-coagulated blood polymerase chain reaction (PCR) (OIE 2012) were positive or if Leptospira were detected in renal tissue (Levaditi-staining was used to detect Leptospira organisms) (Kohn et al. 2010). The clinic population presented between 2006 and 2013 was used as control group.

Seventeen reference strains comprising 14 serogroups and 17 serovars were used for the MAT: Canicola, Pomona, Grippotyphosa, Australis, Bratislava, Ballum, Copenhageni, Autumnalis, Tarassovi, Pyrogenes, Javanica, Sejroe, Icterohaemorrhagiae, Hardjo, Hebdomadis, Bataviae, and Saxkoebing. A greater or equal than fourfold rise of titres within two to three weeks regardless of the vaccination status was considered diagnostic (Birnbaum et al. 1998, Geisen et al. 2007, Gerlach & Stephan 2007, Mastrorilli et al. 2007). MAT titres beyond dilutions of ≥1:800 against all Leptospira serovars in non-vaccinated dogs or against non-vaccine serovars in vaccinated dogs with negative (<1:100) or low vaccination titres (<1:800) were considered highly suggestive for leptospirosis infection. Due to their cross-reactivity, antibody titres against canicola,icterohaemorrhagiae and copenhageni were considered as vaccination titres since none of the dogs had been vaccinated with the novel trivalent or tetravalent leptospirosis vaccines (Levert 2001a, Kohn et al. 2010).

In vaccinated dogs, a titre of ≥1:3-200 against vaccine serovars was considered as highly suggestive for infection. Regular vaccination status was regarded as having a vaccination within the past 12 months.

The medical records were reviewed for signalment, history, physical examination findings, results of the complete blood count (CBC; Cell-Dyn; Abbott Diagnostika), biochemistry (Kone Lab 30i; Thermo Electron GmbH), coagulation profiles including activated partial thromboplastin time (aPTT; Pathromtin SL, Dade Behring Marburg GmbH) and prothrombin time (PT; Hepato Quick; Diagnostica Stago) urinalyses, findings of thoracic and abdominal radiographs, measurement of urine production, central venous pressure, therapy and outcome (cause of death if applicable). Based on respiratory signs, the dogs were grouped as severity grade 1 (mild to moderate dyspnea) or grade 2 (severe dyspnea). Mild to moderate dyspnoea corresponded to laboured breathing and a respiratory rate of >35/minute at rest. Severe dyspnoea corresponded to severely laboured breathing with a respiratory rate of >40/minute, open-mouth breathing, cyanotic mucous membranes with or without haemoptysis (Kohn et al. 2010). Oliguria was determined as urine production <1 mL/kg/h (Cowgill & Langston 2011).

Changes in laboratory parameters were defined along the following scales: anaemia, leucocytosis, thrombocytopenia, increased creatinine, urea and bilirubin concentrations as well as elevated ALT, AST and ALP activities (Kohn et al. 2010, Cowgill & Langston 2011).

Radiographic abnormalities were graded based upon pulmonary patterns and location as grades 1 (caudal interstitial pattern), 2 (generalised mild to moderate reticulonodular interstitial pattern), or 3 (generalised severe reticulonodular interstitial pattern with patchy alveolar consolidations) (Kohn et al. 2010). The radiograph of each dog which displayed the most severe abnormalities was included in the evaluation.

Renal azotaemia was defined by urine-specific gravity (isosmuthria) and increased urea or creatinine values before infusion therapy. Elevated liver enzyme values (ALT; AST; ALP, GLDH) and/or hyperbilirubinaemia were defined as hepatic manifestation.

Multi-organ involvement was defined as renal, hepatic and pulmonary manifestation or combinations of two organ systems. Dogs were divided into two groups (surviving and non-surviving dogs).

STATISTICAL ANALYSIS

Data were evaluated by the statistical software Microsoft Excel 2013 and SPSS 21 (SPSS 14.0 for Windows, Microsoft). Laboratory values were expressed as minimum, maximum and median. Chi-square test has been used to evaluate possible breed over-representation.

RESULTS

Leptospira diagnosis (Table 1) – 99 dogs with leptospirosis were included in the study. In 98 dogs the MAT was performed; in total 72 of 98 dogs (71%) had diagnostic titres at the day of admission or when the second MAT was performed after 10 to
were no differences between survivors and non-surviving dogs. There were no significant differences between surviving and non-surviving dogs suffering from leptospirosis with respect to age (median 6-9 versus 6-0 years), body weight (median 23.5 vs. 19 kg) or sex (female 39%/13% versus male 29%/19%). 80/99 (80%) of the dogs were regularly vaccinated with bivalent leptospirosis vaccines during the previous 12 months.

History
The 99 dogs were presented because of lethargy (96%), inappetence (88%), vomiting (77%), diarrhea (32%), reluctance to move (20%), laboured breathing (18%), or polydipsia and polyuria (11%). Less commonly, adipsia (8%), abdominal pain (8%), weight loss (6%) and brown-red urine (4%) were noted by the owners.

Clinical signs
Clinical signs (Table 2) on the day of admission included lethargy (96%), anorexia (88%), vomiting (85%), abdominal pain (39%), diarrhea (38%), oliguria (27%) and dyspnoea/tachypnoea (26%). 13 from 26 dogs suffered from mild to moderate (grade 1; 50%) and 13 dogs from severe dyspnoea (grade 2; 50%). Less common were a delayed capillary refill time (18%), pale (17%) or icteric mucous membranes (10%), fever (15%) or hypothermia (15%), peripheral lymphadenopathy (10%) or a stiff gait (8%). Comparison of the dogs revealed that non-survivors were significantly more often presented with oliguria (P=0.00001), icterus (P=0.0001), or delayed capillary refill time (P=0.0004). 57 dogs developed respiratory signs during the course of disease. Respiratory distress was mild to moderate in 20 (group 1/survivors: 28%; group 2/non-survivors: 3%), and severe in 37 dogs (group 1: 18%; group 2: 78%).

Signalman
67% of the dogs were pure-bred and 33% were mixed-breed. Jack Russell terrier (8%), golden retriever (6%) and German shepherd dog (6%) were the most common breeds. Less common breeds included wire-haired dachshund (4%), Rottweiler (4%) and beagle (3%). Jack Russell terriers were over-represented when compared to the hospital population (8 versus 3%). The age of the dogs ranged from 2-5 months to 15 years (median 6-0 years). The body weight ranged from 1-1 to 53 kg (median 19-8 kg). 51 dogs were female (52%; 14% spayed) and 48 dogs were male (48%; 15% castrated). There were no significant differences between surviving and non-surviving dogs suffering from leptospirosis with respect to age (median 6-9 versus 6-0 years), body weight (median 23.5 vs. 19 kg) or sex (female 39%/13% versus male 29%/19%). 80/99 (80%) of the dogs were regularly vaccinated with bivalent leptospirosis vaccines during the previous 12 months.

14 days. All dogs diagnosed via MAT except one had a convalescent titre with an at least fourfold rise. In 55 dogs the diagnosis was based solely on the MAT titres. 22 of 73 (30%) dogs had a positive PCR result in either the urine (12/22; 5%) or blood (9/22; 40%). In seven dogs the diagnosis was based solely on a positive urine or blood PCR. 15 dogs displayed a combination of a diagnostic MAT titre and a positive urine/blood PCR.

In 11 dogs renal tissue was examined histopathologically for leptospiral organisms, in seven of these dogs (64%), leptospiral organisms were detected by Levaditi staining. In one dog, the diagnosis based solely on positive Levaditi staining.

MAT titres and Leptospira serovars
In 72 dogs with diagnostic MAT titres against at least one Leptospira serogroup, the highest titres were against the following serogroups: Australis (44/72; 61%; 1:800-1:25,600), Grippotyphosa (47/72; 65%; 1:400-1:12,800) and Pomona (43/72; 60%; 1:800-1:6400). Icterohaemorrhagiae (20/72; 28%; 1:200-1:6400) and Autumnalis (13/72; 18%; 1:800-1:1600) were less common. Rarely, high MAT titres were found against serogroups Javanica (3/72; 4%; 1:400-1:16000), Bataviae (2/72; 3%; 1:800), Javanica (2/72; 3%; 1:800), Pyrogens (1/72; 1%; 1:800), Bal- lum (1/72; 1%; 1:800), and Sejroe (1/72; 1%; 1:400). There were no differences between survivors and non-surviving dogs regarding Leptospira serogroups detected by MAT.

Table 2. Clinical signs and thoracic radiographs in surviving (group 1) and non-surviving (group 2) dogs with leptospirosis on admission day

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Survivors group 1 (n=67)</th>
<th>Non-survivors group 2 (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lethargy</td>
<td>64 (96)</td>
<td>31 (97)</td>
</tr>
<tr>
<td>Anorexia</td>
<td>59 (88)</td>
<td>28 (88)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>56 (84)</td>
<td>28 (88)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>26 (39)</td>
<td>13 (41)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>22 (33)</td>
<td>16 (50)</td>
</tr>
<tr>
<td>Oliguria</td>
<td>11 (16)</td>
<td>16 (50)</td>
</tr>
<tr>
<td>Dyspnoea grade 1*</td>
<td>7 (10)</td>
<td>6 (19)</td>
</tr>
<tr>
<td>Dyspnoea grade 2*</td>
<td>12 (18)</td>
<td>25 (78)</td>
</tr>
<tr>
<td>Delayed capillary refill time</td>
<td>7 (10)</td>
<td>11 (34)</td>
</tr>
<tr>
<td>Pallor</td>
<td>12 (18)</td>
<td>5 (16)</td>
</tr>
<tr>
<td>Fever</td>
<td>10 (15)</td>
<td>5 (16)</td>
</tr>
<tr>
<td>Hypothermia</td>
<td>11 (16)</td>
<td>4 (13)</td>
</tr>
<tr>
<td>Icterus</td>
<td>2 (3)</td>
<td>8 (25)</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>6 (9)</td>
<td>4 (13)</td>
</tr>
<tr>
<td>Stiff gait</td>
<td>6 (9)</td>
<td>2 (7)</td>
</tr>
<tr>
<td>Severe thoracic radiographic abnormalities*</td>
<td>8 (12)</td>
<td>16 (50)</td>
</tr>
</tbody>
</table>

* Respiratory rate of >35/minute
† Respiratory rate of >40/minute, open mouth breathing, cyanotic mucous membranes, with or without haemoptysis
‡ Generalized severe reticulonodular interstitial pattern with patchy alveolar consolidations
Imaging results
Thoracic radiographs were available in all 99 dogs on the day of admission and pulmonary abnormalities were detected in 49 dogs (49%). In the course of disease 57 dogs (57%) developed pulmonary abnormalities in total. Radiological pulmonary changes grades 1, 2, and 3 were present in 12, 21 and 24 dogs, respectively. Thoracic radiographs were available in 48 dogs on the day when dyspnoea was most severe and in nine dogs radiographs were taken the day before. Eight dogs had dyspnoea but no abnormal thoracic radiological findings. Non-surviving dogs more often had severe radiological pulmonary changes than surviving dogs (group 1: 8/67, 12% versus group 2: 16/32, 50%; P<0.0001).

Radiographs of the abdomen were performed for all dogs on the day of presentation. 15 dogs had splenomegaly (15%), 7 hepatosplenomegaly (7%), 5 hepatomegaly (5%) and 14 lack of detail (14%). Ultrasonography of the abdomen was performed in 80 of 99 (80%) dogs. Abnormalities of the liver and/or gallbladder (53%; 42/80), spleen (31%; 25/80) and kidney (14%; 11/80) were most common. In 24 dogs with abnormal findings of the gallbladder, sludge (24/80), thickening of the gallbladder wall (8/80) or calculi (32%; 27/85), crystals (22%; 19/85) and granulated epithelial cells (71%; 60/85), leucocytes (58%; 49/85) and prostatic hypertrophy (1%; 1/80) were rare abnormalities.

Laboratory results
CBC and biochemistry analyses were performed for all dogs every one to two days during hospitalisation (Table 3). Abnormal findings of the CBC at admission included thrombocytopenia (63%; 62/99; ≤165 G/L), anaemia (63%, 62/99; 50-421 L/L) and leucocytosis (57%; 56/99; ≥14.0 G/L). The aPTT was prolonged in 53% (40/75) and the PT in 22% (17/76) of the dogs. Biochemistry revealed an increase of plasma urea (84%; 82/98; ≥107 μmol/L) and creatinine (81%; 80/99; ≥107 μmol/L) concentration, hyperbilirubinaemia (>5.2 μmol/L) was present in 69% (68/99) of the dogs. ALP (298 U/L), AST (≥241 U/L) and ALT (≥771 U/L) activities were increased in 68% (65/95), 63% (59/94) and 54% (52/97) of the dogs, respectively. Hyperalbuminaemia (55%; 52/94; <28 g/L), hypoponataemia (64%; 63/99; <140 mmol/L), hyperphosphataemia (67%; 64/96; >1.6 mmol/L) and hypokalaemia (29%; 29/99; <3.6 mmol/L) were further common findings on admission day.

In the course of disease leucocytosis, anaemia, and thrombocytopenia were the most common CBC abnormalities. Differential cell count revealed neutrophilia (88%; 76/86), a left shift (34%; 29/86), monocytosis (72%; 62/86) and lymphopenia (50%; 43/86). Lymphocytosis (10%; 9/86) and eosinophilia (5%; 4/86) were occasionally detected. Biochemistry showed an increased creatinine and urea as well as elevated ALP, AST and ALT concentrations. Hyperbilirubinaemia, hypoalbuminaemia, hypoponataemia, hyperphosphataemia, hypokalaemia and hyperproteinaemia were further common findings during the course of disease. Urinalysis revealed microscopic haematuria (85%; 80/94), glucosuria (83%; 78/94) and elevated urine–protein/creatinine-ratio (78%; 50/64). Erythrocytes (92%; 78/85), epithelial cells (71%; 60/85), leucocytes (58%; 49/85), hyaline cylinders (32%; 27/85), crystals (22%; 19/85) and granulated cylinders (16%; 14/85) were detected in the sediment analysis.

In non-surviving dogs higher peak creatinine (635 versus 300 mmol/L; P=0.0001) and urea (61.5 versus 35.8 mmol/L; P=0.0015) concentrations, AST (180 versus 721 U/L; P=0.003) levels, bilirubin (28 versus 11.4 μmol/L; P=0.0001), and phosphate (5.0 versus 2.6 mmol/L; P=0.003) levels were detected during course of disease. The serum potassium concentration (3.8 versus 2.9 mmol/L) was lower in dogs that survived.

Therapy
All dogs were treated with an antibiotic (solely amoxicillin/clavulanic acid in 49 dogs, amoxicillin/clavulanic acid followed by doxycycline in 50 dogs) and intravenous crystalloid fluids. Symptomatic treatment included H₂-receptor antagonists or proton pump inhibitors (92%; 92/99; ranitidine, omeprazole); antiemetics (65%; 62/96; metoclopramide, maropitant, ondansetron); and diuretics (total 51/99; 51 furosemide, 6 mannitol in addition). Oxygen was administered via a nasal tube (36/99) in cases of respiratory distress. 36 of 37 dogs with severe dyspnoea received oxygen via nasal tubes. In none of the dogs was a ventilator used. Glucocorticoids (33/99) and theophylline (28/99) were administered in dogs with respiratory distress and LPHS. Two dogs were treated with haemodialysis due to severe renal failure.

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**Table 3. Laboratory parameters in surviving (group 1) and non-surviving (group 2) dogs with leptospirosis (at the time of maximal deviation from the reference range)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Survivors group 1 (n=67)</th>
<th>Non-survivors group 2 (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>median (range)</td>
<td>median (range)</td>
</tr>
<tr>
<td>Platelets (G/L)</td>
<td>98 (7 to 478)</td>
<td>50 (5 to 639)</td>
</tr>
<tr>
<td>Haematocrit (L/L)</td>
<td>0-31 (0.16 to 0.65)</td>
<td>0-33 (0.20 to 0.5)</td>
</tr>
<tr>
<td>Leucocytes (G/L)</td>
<td>24.7 (9.1 to 87.9)</td>
<td>23.1 (6.4 to 104)</td>
</tr>
<tr>
<td>aPTT (s) (n=75)</td>
<td>18.1 (9.2 to 31.4)</td>
<td>21 (12.1 to 43)</td>
</tr>
<tr>
<td>PT (s) (n=76)</td>
<td>18.7 (14.3 to 50)</td>
<td>18.6 (12.3 to 31.2)</td>
</tr>
<tr>
<td>Creatinine (μmol/L) (n=99)</td>
<td>300 (70 to 1608)</td>
<td>635 (137 to 1273)</td>
</tr>
<tr>
<td>Urea (mmol/L) (n=98)</td>
<td>35.8 (4-7 to 111)</td>
<td>61.6 (7.6 to 300)</td>
</tr>
<tr>
<td>ALT (U/L) (n=97)</td>
<td>142 (36 to 16.176)</td>
<td>173 (20 to 757)</td>
</tr>
<tr>
<td>AP (U/L) (n=97)</td>
<td>238 (23 to 4590)</td>
<td>343 (33 to 3298)</td>
</tr>
<tr>
<td>AST (U/L) (n=93)</td>
<td>72 (20 to 16.733)</td>
<td>180 (16 to 1499)</td>
</tr>
<tr>
<td>Bilirubin (μmol/L) (n=97)</td>
<td>11.4 (1-6 to 604)</td>
<td>28 (3 to 765)</td>
</tr>
<tr>
<td>Albumin (g/L) (n=94)</td>
<td>27 (19 to 35)</td>
<td>25 (21 to 47)</td>
</tr>
<tr>
<td>Total protein (g/L) (n=97)</td>
<td>74 (40 to 98)</td>
<td>68 (41 to 90)</td>
</tr>
<tr>
<td>Sodium (mmol/L) (n=99)</td>
<td>136 (114 to 182)</td>
<td>134 (118 to 149)</td>
</tr>
<tr>
<td>Chloride (mmol/L) (n=64)</td>
<td>99 (81 to 124)</td>
<td>97 (87 to 108)</td>
</tr>
<tr>
<td>Phosphate (mmol/L) (n=97)</td>
<td>2.6 (0.73 to 9.3)</td>
<td>5.0 (1.3 to 10.1)</td>
</tr>
<tr>
<td>Potassium (mmol/L) (n=99)</td>
<td>2.9 (1.7 to 6.0)</td>
<td>3.8 (2.3 to 6.9)</td>
</tr>
<tr>
<td>Calcium (mmol/L) (n=96)</td>
<td>2.5 (1.0 to 3.9)</td>
<td>2.6 (1.6 to 3.8)</td>
</tr>
<tr>
<td>Urine protein/creatinine ratio (n=64)</td>
<td>2.1 (0.24 to 18.4)</td>
<td>2.5 (0.45 to 11.3)</td>
</tr>
</tbody>
</table>

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*Case study of leptospirosis in 99 dogs*
Organ manifestation and outcome

There was renal, hepatic and pulmonary involvement in 95, 92 and 58%. 98 dogs (98%) had multi-organ involvement: kidney/liver/lung in 49 dogs (50%; 28/49 survived), kidney/liver in 41 dogs (41%; 34/41 survived), kidney/lung in 5 dogs (5%; 1/5 survived), liver/lung in 3 dogs (3%; 3/3 survived). One dog that survived, showed evidence of liver disease only. In total, 67 of 99 dogs (68%) survived whereas 15 dogs (15%) died and 17 dogs (17%) were euthanased due to progression of disease.

Based on clinical signs, laboratory, imaging and histopathology findings, pulmonary involvement (24/32; 75%) was the most common cause for death. 7 of 32 dogs (22%) died (5/32) or were euthanised (2/32) due to renal failure. One dog (3%) presenting with lethargy, anorexia, haematemesis, abdominal pain and severe azotaemia died peracute on the admission day, presumably due to sepsis.

DISCUSSION

This study represents an overview of 99 cases of clinical canine leptospirosis from Berlin/Brandenburg (North-East Germany) over a seven-year period. The dog is the natural reservoir for serovar Canicola. However, during the period that dogs have been regularly vaccinated the infecting *Leptospira* serogroups have changed. Most commonly reported serogroups nowadays are Australis, Grippotyphosa and, occasionally, Pomona (Birnbaum et al. 1998, Steger-Lieb et al. 1999, Adin & Cowgill 2000, Goldstein et al. 2006, Geisen et al. 2007, Gerlach & Stephan 2007, Kohn et al. 2010, Barmettler et al. 2011, Mayer-Scholl et al. 2013). These findings are consistent with our data. Interestingly, serogroup Pomona infection is more common in dogs in our study than in previous reports (Geisen et al. 2010, Barmettler et al. 2007, Tangeman & Littman 2013). Pulmonary abnormalities in 35 of 50 (70%) patients were described in an earlier publication from North-East Germany (Kohn et al. 2010). There was not always a relationship between clinical respiratory signs and radiological pulmonary changes.

Seven dogs had radiological pulmonary changes without dyspnoea and six dyspnoeic dogs had no radiological lung abnormalities. However, tachypnoea or dyspnoea can also be caused by acidosis or pain (Kohn et al. 2010). Haematological abnormalities are common in dogs with leptospirosis, and the most common finding is thrombocytopenia (Greene et al. 2012). Thrombocytopenia was found in 87% of the non-surviving and in 46% of the surviving dogs. Possible causes for thrombocytopenia in these patients are platelet activation and aggregation as well as sequestration in cases of splenomegaly (Davenport et al. 1989, Nicodemo et al. 1997, Kohn et al. 2010). Immune-mediated platelet destruction might also play a role (Davenport et al. 1989). However, a flow cytometry platelet-bound antibody test was negative in five dogs suffering from leptospirosis (Kohn et al. 2010). Disseminated intravascular coagulation (DIC) has been noted as another possible cause of thrombocytopenia in leptospirosis (Edwards et al. 1986, Yang et al. 2006). DIC was suspected in only 20% of the cases in this study based on elevated coagulation parameters (PT, aPTT) in combination with thrombocytopenia. However, coagulation parameters were not examined in all dogs and PT and aPTT values were usually only measured once during the course of disease. Other factors, such as antithrombin or fibrinogen, were only measured in a few dogs, and were thus not included in data analysis.

Comparison of groups 1 and 2 revealed that a similar percentage of dogs suffered from anaemia. Anaemia was attributed to various causes, including haemolysis due to *Leptospira* infection, blood loss from the gastrointestinal tract due to uraemia, pulmonary haemorrhage and inhibition of haematopoiesis due to an inflammatory response (Berneimer & Bey 1986, Thompson & Manktelow 1986b, Lee et al. 2002, Greene et al. 2012).

In this study, serum creatinine and urea measurements were higher in non-survivors compared to survivors. As mentioned in earlier studies, severe azotaemia was associated with a poor prognosis in dogs with leptospirosis (Rentko et al. 1992, Birnbaum et al. 1998, Adin & Cowgill 2000, Mastrorilli et al. 2007). AST values were higher in non-surviving dogs compared to those of survivors. However, AST is not considered to be a specific indicator of liver function as it is also localised in myocardial and disease and coagulation abnormalities. Recently an increase in pulmonary disease, closely resembling the severe pulmonary form of leptospirosis (LPHS) described in humans, has been noted in dogs (Dolhnikoff et al. 2007, Kohn et al. 2010). Case fatality rates of 13 to 60% have been described in human patients with severe manifestation of the lung form and pulmonary involvement has become the main cause of death in some countries (Tevejo et al. 1998, Dolhnikoff et al. 2007).

Pulmonary changes were previously described in dogs with leptospirosis (Rentko et al. 1992, Harkin & Garrrell 1996, Birnbaum et al. 1998, Goldstein et al. 2006, Mastrorilli et al. 2007, Tangeman & Littman 2013). Pulmonary abnormalities in 35 of 50 (70%) patients were described in an earlier publication from North-East Germany (Kohn et al. 2010). There was not always a relationship between clinical respiratory signs and radiological pulmonary changes. Seven dogs had radiological pulmonary changes without dyspnoea and six dyspnoeic dogs had no radiological lung abnormalities. However, tachypnoea or dyspnoea can also be caused by acidosis or pain (Kohn et al. 2010). Haematological abnormalities are common in dogs with leptospirosis, and the most common finding is thrombocytopenia (Greene et al. 2012). Thrombocytopenia was found in 87% of the non-surviving and in 46% of the surviving dogs. Possible causes for thrombocytopenia in these patients are platelet activation and aggregation as well as sequestration in cases of splenomegaly (Davenport et al. 1989, Nicodemo et al. 1997, Kohn et al. 2010). Immune-mediated platelet destruction might also play a role (Davenport et al. 1989). However, a flow cytometry platelet-bound antibody test was negative in five dogs suffering from leptospirosis (Kohn et al. 2010). Disseminated intravascular coagulation (DIC) has been noted as another possible cause of thrombocytopenia in leptospirosis (Edwards et al. 1986, Yang et al. 2006). DIC was suspected in only 20% of the cases in this study based on elevated coagulation parameters (PT, aPTT) in combination with thrombocytopenia. However, coagulation parameters were not examined in all dogs and PT and aPTT values were usually only measured once during the course of disease. Other factors, such as antithrombin or fibrinogen, were only measured in a few dogs, and were thus not included in data analysis.
skeletal muscle. Therefore, high AST values can also be attributed to muscle damage. Low potassium in leptospirosis infection can be due to renal or gastrointestinal electrolyte losses (Rentko et al. 1992, Goldstein et al. 2006, Mastrorilli et al. 2007). Hypokalaemia has also been associated with leptosomal glycoliprotein, which inhibits the expression and functions of the tubular sodium–potassium-adenosine triphosphate (Na–K-ATPase) (Younes-Ibrahim et al. 1995, Nakou et al. 2000, Wu et al. 2004).

Serum potassium concentrations decrease later in the course of disease, which explains the lower values in surviving compared to non-surviving dogs during course of disease.

 Reported mortality in dogs with leptospirosis has been reported to range from 11 to 48% (Rentko et al. 1992, Harkin & Gartrell 1996, Birnbaum et al. 1998, Steger-Lieb et al. 1999, Adin & Cowgill 2000, Prescott et al. 2002, Bourtiff et al. 2003, Goldstein et al. 2006, Geisen et al. 2007, Gerlach & Stephan 2007, Mastrorilli et al. 2007). In our study, 32% (32/99) of the dogs died or had to be euthanased due to severe clinical manifestation.

A limitation of this study is its retrospective design. Not all of the dogs had medical records for the preceding year. Therefore, it is possible that they were vaccinated but the vaccine was not reported by the owner. Clinical, laboratory and diagnostic imaging have not been obtained at the same time for all dogs. There was no treatment standardisation among the patients and that could have affected survival. Furthermore, the main cause of death was determined based on clinical, laboratory and radiographic findings; post-mortem histopathology was performed only in a few dogs.

In conclusion, dogs with multi-organ (kidney/liver/lung) or pulmonary involvement had the worst outcome. Therefore, strict monitoring of the respiratory rate as well as thoracic radiographs on admission and during course of disease are important in dogs suffering from leptospirosis. Dogs with mainly renal manifestations had a somewhat better prognosis than dogs with severe pulmonary involvement. Only seven dogs died or had to be euthanased due to acute renal failure. However, as described in earlier studies, dogs with severe azotaemia had a higher risk of death compared to dogs with mild to moderate increases in creatinine concentrations (Goldstein et al. 2006, Mastrorilli et al. 2007). Therefore, renal replacement therapy such as haemodialysis should be considered early in the course of disease.

Conflict of interest
None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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