Leptospirosis is an acute bacterial infection caused by spirochetes of the pathogenic species *Leptospira interrogans* and *Leptospira kirschneri*. It is considered one of the most important reemerging zoonoses worldwide, with increasing seroprevalence and prevalence of clinical illness in humans and dogs over the past several years. The wide spectrum of clinical manifestations in infected dogs varies from subclinical infection to peracute fatal multiorgan involvement dominated by renal, hepatic, hematologic, and pulmonary failure. Given that early and accurate diagnosis of affected dogs is essential to alter the course of the disease with appropriate antimicrobial treatment, the availability of validated diagnostic tools is necessary to confirm a clinical suspicion.

The agglutinating antibodies induced in infected animals by leptospiral outer membrane proteins with variable O-side chain sugars are responsible for the phenotypic classification of leptospires in serogroups and serovars by use of the MAT. Given that early and accurate diagnosis of affected dogs is essential to alter the course of the disease with appropriate antimicrobial treatment, the availability of validated diagnostic tools is necessary to confirm a clinical suspicion.

**Objective**—To determine the diagnostic value of a serologic microagglutination test (MAT) and a PCR assay on urine and blood for the diagnosis of leptospirosis in dogs with acute kidney injury (AKI).

**Design**—Cross-sectional study.

**Animals**—76 dogs with AKI in a referral hospital (2008 to 2009).

**Procedures**—Dogs’ leptospirosis status was defined with a paired serologic MAT against a panel of 11 *Leptospira* serovars as leptospirosis-associated (n = 30) or nonleptospirosis-associated AKI (12). In 34 dogs, convalescent serologic testing was not possible, and leptospirosis status was classified as undetermined. The diagnostic value of the MAT single acute or convalescent blood sample was determined in dogs in which leptospirosis status could be classified. The diagnostic value of a commercially available genus-specific PCR assay was evaluated by use of 36 blood samples and 20 urine samples.

**Results**—Serologic acute testing of an acute blood sample had a specificity of 100% (95% CI, 76% to 100%), a sensitivity of 50% (33% to 67%), and an accuracy of 64% (49% to 77%). Serologic testing of a convalescent blood sample had a specificity of 92% (65% to 99%), a sensitivity of 100% (87% to 100%), and an accuracy of 98% (88% to 100%). Results of the *Leptospira* PCR assay were negative for all samples from dogs for which leptospirosis status could be classified.

**Conclusions and Clinical Relevance**—Serologic MAT results were highly accurate for diagnosis of leptospirosis in dogs, despite a low sensitivity for early diagnosis. In this referral setting of dogs pretreated with antimicrobials, testing of blood and urine samples with a commercially available genus-specific PCR assay did not improve early diagnosis.
false-positive results after vaccination or exposure in endemic areas.8

Paired serologic testing may be impossible in some animals, such as those that die quickly from the severe acute form of the disease, and lack of inclusion of such cases may substantially bias clinical studies toward a conclusion that leptospirosis is a more benign disease than it truly is. In the absence of an agreement on an ideal single-sample cutoff titer as the best compromise between the high sensitivity of a low titer and the high specificity of a high titer, widely different cutoffs have been used in clinical practice and in research studies, ranging from 1:100 to 1:12,800.12,14–17 The World Health Organization and the International Leptospirosis Society recommend that the ideal cutoff for a single specimen be determined individually in a particular area on the basis of the seroprevalence of persistent antibodies in relation to the occurrence of other diseases that induce production of cross-reacting antibodies.22 However, the validity of these recommendations is limited when it comes to diagnosis of the disease in dogs because of the problems relating to vaccine titers. The sensitivity and specificity of a single initial MAT titer of ≥ 1:800 in dogs have been reported in 1 study6 to be 22% to 67% and 69% to 100%, respectively, and marked interlaboratory variations have been found.13 These limitations of serologic testing and its poor ability to predict the infecting serogroup, its cost, and the tedious laboratory methodology have favored the development of other reliable diagnostic methods, especially for early diagnosis.9

A diagnosis of leptospirosis based on pathogen detection has the potential advantage of early confirmation, independent of the time necessary for a serologic response to occur. The mere presence of leptospiral DNA would still need to be interpreted in the clinical context, considering the possibility of subclinical carriers shedding organisms in their urine. Polymerase chain reaction assays have been recommended for their high intrinsic detection sensitivity. One assay was sensitive but not always specific for the diagnosis of leptospirosis in a small group of affected dogs.23 Various PCR assays have been increasingly offered by commercial veterinary diagnostic laboratories worldwide, with variable analytic validation but typically with minimal or absent clinical validation. Preanalytic variables including patient and disease characteristics, sampling conditions and timing, and nature and preparation of the samples have, to the authors’ knowledge, not been evaluated in a clinical setting of dogs with severe AKI, where an accurate and timely diagnosis is critical. On the basis of data from experimental infections, blood is the recommended substrate in the first 10 days of infection and urine thereafter.10 However, ideal preanalytic conditions are rarely encountered in referral institutions, where most animals are treated with antimicrobials prior to referral and many dogs are anuric.9

The goals of the study reported here were thus to determine the diagnostic value of early and late serologic microagglutination testing, compared with reference paired serologic testing, and to evaluate the diagnostic value of a commercially available Leptospira LipL32 nested PCR assay on urine and blood obtained from dogs with AKI under the conditions of a referral hospital.

Materials and Methods

Case selection and clinical characterization—Guidelines for complete and accurate reporting recommended by the Standards for Reporting of Diagnostic Accuracy Initiative were followed.22 All dogs with AKI evaluated at the University of Bern Vetsuisse Faculty Small Animal Clinic from 2008 to 2009 were enrolled prospectively in the study. The diagnosis of AKI was made on the basis of a complete diagnostic evaluation, including at least medical history, physical examination, CBC, serum biochemical panel, urinalysis (except if anuric), and abdominal ultrasonography. All diagnostic procedures were performed as part of the routine examination, with no additional samples obtained for the study. Acute kidney injury was defined as the combination of sudden onset of uremic signs and consistent laboratory and diagnostic imaging findings, with at least 2 of the following criteria: serum creatinine concentration ≥ 250 µmol/L (2.8 mg/dL), persisting at least 24 hours after correction of prerenal factors; serum creatinine concentration ≥ 100 µmol/L (1.1 mg/dL) or an increase ≥ 100% from documented baseline in the absence of prerenal causes; persistent pathological oligoanuria (< 1 mL/kg/h) after volume repletion; and evidence of renal tubular injury on urinalysis (renal glucosuria and granular casts). Dogs were excluded if the data indicated evidence of underlying chronic kidney disease, including a > 1-month history of polyuria, polydipsia, or weight loss; physical examination indicating small irregular kidneys; or imaging evidence of chronic kidney damage, such as small, irregular, or scarred kidneys.24 Relevant liver involvement was defined arbitrarily as hyperbilirubinemia (> 10 µmol/L (> 0.58 mg/dL)) and at least a 2-fold increase of serum activity of 1 liver enzyme; liver involvement was not a criterion for inclusion in the study. The day of onset of clinical signs was obtained from the standardized history form completed prospectively at initial evaluation.

Serologic MAT and PCR assay—Diagnosis of leptospirosis was made on the basis of serologic testing performed according to the Guidelines of the World Health Organization International Leptospirosis Society12 by the National Reference Laboratory for Leptospirosis, Institute of Veterinary Bacteriology, University of Bern Vetsuisse Faculty. The MAT was conducted with a panel of antigens with 11 ubiquitous and locally prevalent serovars, including L. interrogans serovars Australis, Pomona, Tarassovi, Canicola, Icterohemorrhagiae, Hardjo, Bataviae, Bratislava, Autumnalis, and Sejroe and L. kirschneri serovars Grippotyphosa. Sera were screened initially at a dilution of 1:100, and samples with positive reactions were titrated in serial 2-fold dilutions to a maximum of 1:3,200. A first serum sample (acute phase) was submitted for serologic testing at initial evaluation, and a second sample (convalescent phase) was submitted 1 to 3 weeks later. For seroprevalence data, seropositivity was defined as a titer of ≥ 1:800. This cutoff was chosen as the single serologic testing threshold most commonly used in previous clinical studies.22,23 When a positive test result was obtained for ≥ 1 serovar, the case was attributed to each serovar for which a positive result was found, independent of actual titers.
Seroconversion was defined as a 4-fold increase in MAT titers in 2 to 3 weeks for any of the 11 serovars tested. Further titer increase and seroconversion could not be detected in dogs with positive sample 1 titers reaching the maximal dilution of 1:3,200, and these results were considered unavailable for the interpretation of seroconversion. The rate of cross-reactivity among serovars was defined as the percentage of dogs testing positive for > 1 serovar, independent of their respective titers.

On the basis of the number of serologic tests performed and their results, dogs were allocated into 3 groups: dogs with paired serologic testing and seroconversion for any serovar were categorized as having confirmed leptospirosis, dogs with paired serologic testing without seroconversion for any serovar were categorized as having confirmed nonleptospirosis AKI, and dogs with only a single serologic test were categorized as undetermined. Dogs with an initial titer of 1:3,200 were only included in the leptospirosis group if they had seroconversion for at least 1 other serovar.

Urine samples collected by cystocentesis, catheterization, or free-catch and blood samples collected at initial referral were sent to a commercial laboratory, following the laboratory’s conditioning and shipping instructions. Whole EDTA-anticoagulated blood (2 mL) and unprocessed urine (5 mL) were mailed on ice after overnight storage at 4°C. The DNA extraction and PCR amplification products. The lower detection limit was 10 leptospires/mL of urine, as determined with a positive control and for the external and internal PCR were run with each assay.

The PCR products were detected by UV light transillumination of electrophoresis gels. Specificity of positive test results was not verified further by sequencing PCR amplification products. The lower detection limit was 10 leptospires/mL of urine, as determined with a dilution series of the positive control serovar Bratislava in urine.

Urine and blood PCR testing was discontinued during the second half of the study because of the low yield of positive results in dogs with leptospirosis. The serologic testing and the PCR laboratories were unaware of the results of each other. When possible, kidney and liver tissues were obtained at necropsy from the diseased dogs. Specimens were collected with sterile disposable blades, immediately frozen, and sent overnight to the same laboratory for DNA isolation and PCR testing with the same assay as described.

Data and statistical analysis—Because multiple sets of data were not normally distributed, clinical and laboratory characteristics of the dogs with AKI were reported as median (IQR) for the whole study population and compared between the leptospirosis and the nonleptospirosis AKI groups via a Wilcoxon rank sum test for numeric data and a Fisher exact test for categorical data.

Seroconversion and prevalence of seroconversion for the various *Leptospira* serovars were analyzed in the group of dogs with a confirmed diagnosis of leptospirosis. Assessment of the diagnostic value of acute and convalescent single serologic testing was performed in the 2 groups of dogs with confirmed diagnoses (leptospirosis and nonleptospirosis AKI) and included computation of sensitivity, specificity, and positive and negative predictive values, diagnostic OR, and diagnostic accuracy. McNemar’s test of diagnostic accuracy were expressed with their respective 95% CIs.23 Diagnostic accuracy of urine and blood PCR assay was assessed on the same criteria in the 2 groups with confirmed diagnoses (leptospirosis and nonleptospirosis AKI). Because of the low number of positive results, specificity and positive predictive value were not determined. A 2-graph ROC curve analysis and a standard ROC curve analysis were used for the determination of the optimal diagnostic cutoff and the area under the curve, respectively.24 Statistical analyses were performed with commercial software, and values of *P* < 0.05 were considered significant.

### Results

**Dogs and clinical data**—Seventy-six dogs had AKI and met the inclusion criteria. There were 63 purebred dogs from 46 breeds and 13 mixed-breed dogs. Sex distribution included 46 males (22 castrated) and 30 females (18 spayed) with a median body weight of 19.9 kg (43.8 lb; IQR, 9.8 to 30.0 kg [21.6 to 66.0 lb]) and median age of 6.5 years (IQR, 3.4 to 9.6 years) at initial evaluation. Dogs with leptospirosis (median age, 7.2 years [IQR, 4.2 to 9.7 years]) were significantly (*P* = 0.04) older than dogs with AKI from other causes (median age, 4.4 years [IQR, 1.1 to 6.3 years]). All dogs were brought for initial care to their local veterinarian a median of 2 days (IQR, 1 to 3 days) after the first clinical signs were noticed by the owner. They were referred to the small animal teaching hospital a median of 4 days (IQR, 2 to 5 days) after the first signs. All dogs received antimicrobials as part of their initial treatment prior to referral; however, records of drugs, dosages, and duration were lacking for too many of them for appropriate statistical analysis.

Leptospirosis status could be determined by paired serologic testing in 42 dogs, which were grouped according as leptospirosis (*n* = 30) and nonleptospirosis AKI (12). Convalescent serologic testing could not be obtained from the remaining dogs, and their status remained undetermined (*n* = 34). The clinical and laboratory characteristics of the main study groups (leptospirosis and nonleptospirosis AKI) were summarized (Table 1) and indicated a similar degree of renal disease in both groups. Thirty-one of 42 (74%) dogs with determined leptospirosis status survived the AKI, with a similar outcome in dogs with leptospirosis and in dogs with AKI from other causes (*P* = 0.24). Dogs with undetermined leptospirosis status had a significantly (*P* < 0.001) worse outcome, with only 7 of 34 (21%) surviv-
Vaccination history indicated that 75 of 76 (99%) study dogs had been vaccinated with a divalent leptospirosis vaccine, including the serovars Canicola and Icterohemorrhagiae. Five of 76 (7%) dogs had received the last vaccination within 1 month, 33 of 76 (43%) within 6 months, and 73 of 76 dogs (96%) within 12 months. Vaccination rates were similar for dogs with a determined leptospirosis status (leptospirosis and non-

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reference range</th>
<th>Leptospirosis (n = 30)</th>
<th>Nonleptospirosis AKI (n = 12)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>—</td>
<td>7.2 (4.2–9.7)</td>
<td>4.4 (1.1–6.3)</td>
<td>0.04*</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>—</td>
<td>20.7 (9.5–30.6)</td>
<td>21.6 (11.1–32.0)</td>
<td>0.8</td>
</tr>
<tr>
<td>Oligoanuria (No. [%])</td>
<td>—</td>
<td>16 (53)</td>
<td>8 (67)</td>
<td>0.51</td>
</tr>
<tr>
<td>Liver involvement (No. [%])</td>
<td>—</td>
<td>8 (27)</td>
<td>1 (8)</td>
<td>0.25</td>
</tr>
<tr>
<td>Hemodialysis (No. [%])</td>
<td>—</td>
<td>18 (60)</td>
<td>8 (67)</td>
<td>0.74</td>
</tr>
<tr>
<td>Survival (No. [%])</td>
<td>—</td>
<td>24 (80)</td>
<td>7 (58)</td>
<td>0.24</td>
</tr>
<tr>
<td>PCV (L/L)</td>
<td>0.39–0.57</td>
<td>0.31 (0.28–0.38)</td>
<td>0.35 (0.31–0.38)</td>
<td>0.42</td>
</tr>
<tr>
<td>Platelets (X 10^9/L)</td>
<td>150–400</td>
<td>139 (108–214)</td>
<td>212 (178–264)</td>
<td>0.08</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>53–120</td>
<td>585 (383–938)</td>
<td>774 (588–1,165)</td>
<td>0.15</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>0.6–1.4</td>
<td>6.6 (4.3–10.6)</td>
<td>8.7 (6.6–13.2)</td>
<td></td>
</tr>
<tr>
<td>Phosphorus (mmol/L)</td>
<td>3.5–11.1</td>
<td>49.9 (38.5–65.5)</td>
<td>44.3 (28.2–66.8)</td>
<td>0.73</td>
</tr>
<tr>
<td>Potassium (mg/dL)</td>
<td>2.8–5.9</td>
<td>10.8 (7.1–16.4)</td>
<td>11.5 (9.0–16.1)</td>
<td></td>
</tr>
<tr>
<td>Bilirubin (µmol/L)</td>
<td>0.6–4.3</td>
<td>5.1 (3.7–9.6)</td>
<td>6.0 (3.6–11.0)</td>
<td>0.88</td>
</tr>
<tr>
<td>(µg/dL)</td>
<td>0.03–0.25</td>
<td>0.30 (0.21–0.56)</td>
<td>0.35 (0.21–0.64)</td>
<td></td>
</tr>
</tbody>
</table>

Data are reported as proportions or median (IQR). *Values are significantly (P < 0.05) different. — = Not applicable.

Figure 1—Relative seroprevalence for 11 serovars of leptospires at initial evaluation (sample 1 [S1]) and convalescent testing (sample 2 [S2]), and overall prevalence of seroconversion in 30 dogs with acute leptospirosis. AUS = Australis. AUT = Autumnalis. BAT = Bataviae. BRA = Bratislava. CAN = Canicola. GRI = Grippotyphosa. HAR = Hardjo. ICT = Icterohemorrhagiae. POM = Pomona. SEJ = Sejroe. TAR = Tarassovi.
leptospirosis AKI; 3/42 within 1 month, 18/42 within 6 months, and 40/42 within 12 months) and for the dogs with undetermined leptospirosis status.

**Serovars and serologic testing**—Initial serologic testing (sample 1) was performed a median of 4.5 days (IQR, 3 to 5 days) after the first recorded clinical signs. A second serologic testing (sample 2) was performed for 42 dogs a median of 10 days (IQR, 7 to 15 days) later or a median of 14 days (IQR, 11 to 20 days) after the first clinical signs. A diagnosis of leptospirosis was confirmed in dogs with seroconversion (leptospirosis AKI group [n = 30]), and AKI from other causes was diagnosed in the dogs with no seroconversion (nonleptospirosis AKI group [12]). Other causes of AKI identified in nonleptospirosis AKI group dogs included grape toxicosis (n = 2), unidentified toxic nephrosis (2), and acute leishmaniasis (1). The etiology could not be identified in the remaining 7 dogs. Convalescent sample 2 could not be obtained from 34 dogs because of early death (n = 27) or discharge from hospital and lack of follow-up (7). Of these dogs with undetermined leptospirosis status, 11 were seropositive (≥ 1:800 for at least 1 serovar), and 23 were seronegative for all tested serovars.

Results of serologic testing for the 30 dogs with a definitive diagnosis of leptospirosis were determined (Figure 1). A high rate of cross-reactivity was observed between the tested serovars, with most dogs testing positive for > 1 serovar (30% on sample 1, 83% on sample 2, and 83% on sample 2 with seroconversion). The median number of serovars with positive results was 0.5 for sample 1 and 2 for sample 2. Seroconversion occurred for a median of 3 serovars. Antibody titers against serovars Australis and Bratislava were the most prevalent, with > 35% dogs seropositive on sample 1 and > 70% on sample 2. In contrast, results were positive for serovar Autumnalis, Grippotyphosa, and Pomona in < 15% of the 30 dogs on sample 1 and in 35% to 45% on sample 2. Only rare dogs were seropositive for the vaccinal serovars Canicola (1/30 for sample 1; 2/30 for sample 2) and Icterohemorrhagiae (70% on sample 2). In contrast, re-

![Figure 2—Two-graph ROC curves representing sensitivities and specificities of serologic testing (MAT) for any of 11 serovars on acute (top) and convalescent (bottom) serum samples from 42 dogs with AKI, with MAT seroconversion as the reference method.](image)

Table 2—Diagnostic value (with 95% CI) of acute and convalescent serologic testing (MAT) and blood and urine PCR assay testing in dogs with AKI.

<table>
<thead>
<tr>
<th>Test</th>
<th>Specificity (%)</th>
<th>Sensitivity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Diagnostic OR</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute serologic testing (n = 42)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 1:400 any serovar</td>
<td>83 (55–96)</td>
<td>60 (42–75)</td>
<td>90 (70–97)</td>
<td>45 (27–65)</td>
<td>7.5 (1.4–46)</td>
<td>67 (52–79)</td>
</tr>
<tr>
<td>≥ 1:800 any serovar</td>
<td>100 (76–100)</td>
<td>50 (33–67)</td>
<td>100 (80–100)</td>
<td>44 (28–63)</td>
<td>25.0 (1.4–460)</td>
<td>64 (49–77)</td>
</tr>
<tr>
<td>≥ 1:1,600 any serovar</td>
<td>100 (76–100)</td>
<td>37 (22–55)</td>
<td>100 (74–100)</td>
<td>39 (24–56)</td>
<td>14.7 (0.8–273)</td>
<td>55 (40–69)</td>
</tr>
<tr>
<td>≥ 1:800 AUS</td>
<td>100 (76–100)</td>
<td>37 (22–54)</td>
<td>100 (74–100)</td>
<td>39 (24–56)</td>
<td>14.7 (0.8–273)</td>
<td>55 (40–69)</td>
</tr>
<tr>
<td>≥ 1:800 BRA</td>
<td>100 (76–100)</td>
<td>47 (30–64)</td>
<td>100 (78–100)</td>
<td>43 (27–61)</td>
<td>22.0 (1.2–405)</td>
<td>62 (47–75)</td>
</tr>
<tr>
<td>≥ 1:800 AUS or BRA</td>
<td>100 (76–100)</td>
<td>47 (30–64)</td>
<td>100 (78–100)</td>
<td>43 (27–61)</td>
<td>22.0 (1.2–405)</td>
<td>62 (47–75)</td>
</tr>
<tr>
<td>Convalescent serologic testing (n = 42)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 1:400 any serovar</td>
<td>92 (65–99)</td>
<td>100 (89–100)</td>
<td>97 (84–99)</td>
<td>100 (74–100)</td>
<td>488 (18–12,326)</td>
<td>98 (88–100)</td>
</tr>
<tr>
<td>≥ 1:800 any serovar</td>
<td>92 (65–99)</td>
<td>100 (89–100)</td>
<td>97 (84–99)</td>
<td>100 (74–100)</td>
<td>488 (18–12,326)</td>
<td>98 (88–100)</td>
</tr>
<tr>
<td>≥ 1:1,600 any serovar</td>
<td>100 (76–100)</td>
<td>90 (74–97)</td>
<td>100 (88–100)</td>
<td>80 (55–93)</td>
<td>196 (9.4–4,097)</td>
<td>93 (81–98)</td>
</tr>
<tr>
<td>PCR assay</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood (n = 23)</td>
<td>—</td>
<td>0 (0–20)</td>
<td>—</td>
<td>35 (19–55)</td>
<td>0.5 (0–30)</td>
<td>35 (19–55)</td>
</tr>
<tr>
<td>Urine (n = 13)</td>
<td>—</td>
<td>0 (0–20)</td>
<td>—</td>
<td>23 (8–50)</td>
<td>0.3 (0–20)</td>
<td>23 (8–50)</td>
</tr>
</tbody>
</table>

AUS = Serovar Australis. BRA = Serovar Bratislava. NPV = Negative predictive value. PPV = Positive predictive value. See Table 1 for remainder of key.
with the reference paired serologic testing, was assessed in the 42 dogs with a confirmed diagnosis of leptospirosis or nonleptospirosis AKI, both overall for any serovar and for each individual serovar separately. Relevant results were summarized (Table 2; Figure 2). The best overall diagnostic test performance independent of disease prevalence, estimated with the diagnostic OR, was obtained with a cutoff of 1:800 for acute serologic testing (diagnostic OR, 25) and 1:400 or 1:800 with convalescent serologic testing (diagnostic OR, 468). An ROC curve analysis gave an area under the curve of 0.81 (95% CI, 0.64 to 0.90) for sample 1 and 1.00 (95% CI, 0.96 to 1.00) for sample 2 serologic testing, indicating good and excellent discriminative values for the diagnosis of acute leptospirosis, respectively. Given that >70% of dogs seroconverted for serovars Australis and Bratislava in this dog population, single early serologic testing with a cutoff of 1:800 restricted to these 2 serovars would have resulted in a low sensitivity (Australis, 37%; Bratislava, 37%) and diagnostic accuracy (Australis, 53%; Bratislava, 53%) when evaluated separately (Figure 3). The diagnostic performance was close to that of the complete 11-serovar panel, when restricted to these 2 serovars in combination at sample 1 (sensitivity, 47%; diagnostic accuracy, 62%).

PCR assay—Thirty-six blood samples (leptospirosis group, 15; nonleptospirosis AKI group, 8; dogs with undetermined leptospirosis status, 13) and 20 urine samples (leptospirosis group, 10; nonleptospirosis AKI group, 3; dogs with undetermined leptospirosis status, 7) were submitted for PCR assay a median of 5 days (IQR, 3 to 7 days) and a median of 6 days (IQR, 4 to 9 days) after the first clinical signs, respectively. Only 1 blood sample and 1 urine sample yielded positive results. Both came from seropositive dogs from the undetermined leptospirosis status group, with the highest MAT titer for serovar Bratislava (1:3,200). Computed diagnostic value of blood and urine PCR testing for acute canine leptospirosis was summarized (Table 2).

Kidney tissue samples from 11 dogs (leptospirosis group, 1; dogs with undetermined leptospirosis status, 10) were submitted for PCR testing. The dog with confirmed leptospirosis was tissue PCR negative; however, samples were taken after 20 days of antimicrobial treatment. The other samples yielded positive results on 2 seropositive and 2 seronegative dogs with undetermined leptospirosis status. The 2 seropositive dogs had been on antimicrobial treatment for 2 days each. Additional liver tissue samples were taken from these 2 dogs and were PCR positive also.

**Discussion**

Reliable prognostic indicators are especially important for therapeutic decisions in dogs with AKI because invasive and expensive renal replacement treatments are commonly needed. Apart from the grade of functional loss and the presence of oligoanuria, the underlying etiology is one of the major prognostic determinants for potential clinical recovery from AKI. A diagnosis of leptospirosis is usually considered relatively favorable, compared with other infectious, toxic, or polysystemic causes of AKI, and thus it may influence treatment decisions. Furthermore, successful treatment of acute leptospirosis in dogs depends largely on early antimicrobial and supportive treatment and therefore on a reliable and timely diagnosis. The ability to reach an early diagnosis with a high degree of accuracy is also crucial for the safety of the dog owners and the hospital staff, considering that leptospirosis is considered the most widespread zoonosis worldwide and dogs are potential vectors. The Consensus Statement of the American College of Veterinary Internal Medicine published in 2011 has clarified the recommended approach and the use of the available methods for the diagnosis of leptospirosis in dogs. However, few data are currently available on the field evaluation of the diagnostic test performance for the 2 most commonly used assays offered through commercial laboratories: the MAT and PCR assay. Thus, this study was performed to evaluate the value of single serologic testing and PCR assay in urine and blood as early diagnostic tools for leptospirosis in dogs under the field conditions of a referral center in a highly endemic area of central Europe.

The pattern of serologic reactivity for leptospirosis is generally variable between geographic areas, with adaptation of particular strains to reservoir hosts and vaccination protocols. In a study conducted in southern Germany, antibody titers to the serovar Grippotyphosa predominated in 13 of 42 dogs with leptospiro-
sis, with only 3 dogs having the highest titer to serovar Bratislava, whereas a study performed in Italy found 20 dogs with predominant antibody titers to serovar Australis. Despite the poor predictive accuracy of the MAT to indicate the infecting serovar, the serologic pattern from the present study revealed a high prevalence of the 2 nonvaccine serovars Australis and Bratislava in the canine population in Switzerland.

Although serologic testing is not typically considered adequate for early diagnosis because 2 to 4 weeks are necessary for mounting an immune response, results of the present study indicated that use of a 1:800 cutoff with early sampling at initial evaluation yielded a diagnostic accuracy of 64%, with no false-positive diagnoses of leptospirosis. However, 15 (50%) dogs with leptospirosis would have been incorrectly determined to not have leptospirosis. A lower cutoff of 1:400 might even be indicated for clinical use, considering that it would yield a similar accuracy (67%) with improved sensitivity (from 50% to 60%), albeit at the cost of decreased specificity (from 100% to 83%), positive predictive value (from 100% to 90%), and overall diagnostic performance. A cutoff ≥ 1:800 is not indicated under these conditions of dogs with AKI, even for research studies. Despite the highly endemic nature of leptospirosis in the study area and its expected risk of previous exposure and thus of false-positive results, the diagnostic specificity of a titer ≥ 1:800 was still 100%.

On a single late serologic specimen collected 11 to 20 days after the onset of clinical signs, use of a lower cutoff of 1:400 had almost the same diagnostic value as paired serologic testing with seroconversion. Although this result is usually received too late to influence clinically relevant therapeutic decisions, it is of value for retrospective confirmation of a clinical suspicion and for epidemiological decisions concerning exposure of other animals and humans previously in contact with the diseased dog.

Molecular diagnosis of leptospiral infection with PCR assay has been documented and recommended with blood samples for acute leptospirosis and urine samples for chronic infections with urinary shedding. These recommendations are mostly based on experimental infections with known timing of inoculation and absence of antimicrobial treatment. The clinical setting as encountered in the present study differs from the simplified experimental setting in many regards, including variable unknown size of the inoculum, possibility of previous and repeated exposure, variable shedding patterns, possible presence of comorbidities, variable clinical course and manifestations, variable immune response, and widespread antimicrobial pretreatment (100% in this study). One goal of the present study was to evaluate the diagnostic value of PCR testing on blood and urine in dogs with AKI and a suspicion of acute leptospirosis in the clinical setting of a referral hospital in a highly endemic area, following the protocols for sampling and shipping recommended by a major commercial diagnostic laboratory. The results of PCR testing in the present study were in contrast with previous reports. With only 1 positive urine PCR assay result in 20 dogs with acute leptospirosis, the present study differs markedly from the findings from Harkin et al, who found a sensitivity of 100% and a specificity of 88.3% for PCR assay performed on urine samples of 132 dogs with clinical signs or laboratory changes possibly associated with leptospirosis. However, only 16 of those dogs were in acute renal failure, and only 1 of them had seroconverted, hampering a direct comparison with the results of the present study.

Although the causative serovars were not characterized in the present study, the positive results obtained with tissue samples via the same technique by the same laboratory seem to rule out inappropriate choice of primers and missing the relevant serovars and to favor inadequate preanalytic conditions, such as inappropriate time of sampling, loss or degradation of DNA during shipping and preparation, or interference of sample components with the assay. Although sampling would be performed ideally at initial evaluation before any therapeutic intervention, few data are available in the literature concerning the influence of antimicrobial treatment on the ability to detect DNA from infectious organisms, especially for dogs with leptospirosis.

Samples submitted for PCR assay were not specially prepared or extracted for shipment to the diagnostic laboratory. Whole EDTA-anticoagulated blood and unprocessed urine were mailed on ice after overnight storage at 4°C, according to laboratory instructions. Although this method seems appropriate for other samples, it may not be optimal for animals with AKI or leptospirosis because of the presence of degrading enzymes or other substances that interfere with the assay. Sample preparation protocols such as centrifugation of the urine samples or use of nucleic acid stabilizing solutions should therefore be evaluated with appropriate clinical material from a representative setting before this test can be recommended further. This exemplifies the need for a detailed validation procedure of an assay, not restricted to its sole analytic performances, but extended to the characterization of valid and realistic preanalytic conditions similar to those encountered in clinical practice and confirmed by a field trial to evaluate the diagnostic performances of the test.

Limitations of the present study were mostly related to the specific clinical situation and the heterogeneous nature of the clinical cases from a referral hospital. The difficulties encountered are common in a clinical setting, requiring a validation of these commonly used diagnostic tests under field conditions. The systematic use of antimicrobials early in prerereferal treatment may be good therapeutic practice in dogs with AKI and a suspicion of leptospirosis, but it may create diagnostic difficulties. Collection and storage of appropriate samples prior to initiating treatment could easily circumvent these difficulties and facilitate the diagnostic approach. Isolation and identification of the infecting serovars may have shed light on the adequacy of the PCR primers used in this study. However, cultures of leptospires are typically of low yield, with reported sensitivities of 5% to 50%, and they are even more sensitive to preanalytic variables such as antimicrobial pretreatment.

Although results of the present study cannot necessarily be extrapolated to different clinical, epidemiolog-
References