Effects of canine parvovirus strain variations on diagnostic test results and clinical management of enteritis in dogs

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Objective—To estimate the prevalence of canine parvovirus (CPV) strains among dogs with enteritis admitted to a referral hospital in the southwestern United States during an 11-month period and to compare diagnostic test results, disease severity, and patient outcome among CPV strains.

Design—Prospective observational study.

Animals—72 dogs with histories and clinical signs of parvoviral enteritis.

Procedures—For each dog, a fecal sample or rectal swab specimen was evaluated for CPV antigen via an ELISA. Subsequently, fecal samples (n = 42 dogs) and pharyngeal swab specimens (16) were obtained and tested for CPV antigen via an ELISA and CPV DNA via a PCR assay. For specimens with CPV-positive results via PCR assay, genetic sequencing was performed to identify the CPV strain.

Results—56 dogs tested positive for CPV via ELISA or PCR assay. For 42 fecal samples tested via both ELISA and PCR assay, 27 had positive results via both assays, whereas 6 had positive PCR assay results only. Ten pharyngeal swab specimens yielded positive PCR assay results. Genetic sequencing was performed on 34 fecal or pharyngeal swab specimens that had CPV-positive PCR assay results; 26 (76.5%) were identified as containing CPV type-2c, and 9 (26.5%) were identified as containing CPV type-2b. No association was found between CPV strain and disease severity or clinical outcome.

Conclusions and Clinical Relevance—CPV type-2b and CPV type-2c posed similar health risks for dogs; therefore, genetic sequencing of CPV does not appear necessary for clinical management of infected patients. The diagnostic tests used could detect CPV type-2c.


Canine parvovirus was first identified in dogs in the late 1970s, and was named CPV type-2 (CPV-2) to distinguish it from the minute virus of canines, another parvovirus (CPV type-1) that infects dogs. Canine parvovirus type-2 was quickly recognized worldwide but was replaced globally in the early 1980s by an antigenically and genetically distinct strain, CPV type-2a (CPV-2a). Since then, novel antigenic and genetic strains have continued to evolve, often with mutations in the overlapping capsid genes, VP1 and VP2. Two such strains are distinguished by amino acid changes at residue 426 in the VP2 sequence and have been named CPV-2b and CPV-2c. Canine parvovirus type-2 and CPV-2a as well as feline panleukopenia virus have an asparagine at residue 426, whereas CPV-2b has an Asp at residue 426, and CPV-2c has a Glu at residue 426. These amino acid changes alter viral capsid binding by monoclonal antibodies but have only minor effects on polyclonal antibody binding. Additional amino acid changes in the capsid gene have been identified, which have resulted in multiple CPV strains similar to CPV-2a, CPV-2b, and CPV-2c. Most of these strains remain unnamed, and their functional consequences, if any, are not well characterized.

The importance of CPV genetic and antigenic variation on clinical disease and management of disease is unknown. Alteration of viral capsid epitopes could re-

Abbreviations

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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>Asp</td>
<td>Aspartic acid</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>CPV</td>
<td>Canine parvovirus</td>
</tr>
<tr>
<td>Glu</td>
<td>Glutamic acid</td>
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<tr>
<td>VP</td>
<td>Viral capsid protein</td>
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duce the effectiveness of CPV vaccines or affect the performance of rapid in-clinic diagnostic tests for CPV that rely on antibody binding. Even though CPV-2b (Asp at VP2 residue 426) has not been associated with CPV vaccine failures or problems with diagnostic test performance since its recognition in 1984, results of some studies indicate that CPV-2c (Glu at VP2 residue 426) causes more severe disease, infects properly vaccinated dogs, may not be detected by in-clinic diagnostic tests, and frequently results in poor patient outcomes. However, results of other studies indicate that dogs vaccinated with commercially available CPV-2 vaccines are protected when challenge inoculated with CPV-2c, and results of another study indicate that puppies naturally infected with CPV-2c develop only minor signs of disease.

Generally, diagnostic laboratory–based studies conducted to evaluate CPV strains are performed with specimens from a large geographical area, and there are limited clinical data available for the patients from which the specimens were obtained. Conversely, hospital-based studies conducted to evaluate CPV strains often have extensive clinical data for each patient but rarely include CPV sequence information. The primary objectives of the study reported here were to determine the prevalence of CPV strains obtained from dogs with enteritis that were examined at an emergency referral hospital in the southwestern United States during an 11-month period and to compare the severity of clinical signs and disease outcomes among dogs infected with different CPV strains. Also assessed were the sensitivity of a commercial ELISA for detection of CPV-2c (Glu at VP2 residue 426) causes more severe disease, infects properly vaccinated dogs, may not be detected by in-clinic diagnostic tests, and frequently results in poor patient outcomes. However, results of other studies indicate that dogs vaccinated with commercially availableCPV-2 vaccines are protected when challenge inoculated with CPV-2c, and results of another study indicate that puppies naturally infected with CPV-2c develop only minor signs of disease.

Materials and Methods

Animals—Dogs with a history (signalment, improper or unknown CPV vaccination status, and known or high-risk of CPV exposure) and clinical signs (anorexia, vomiting, and diarrhea) consistent with known or high-risk of CPV exposure), and clinical signs (anorexia, vomiting, and diarrhea) consistent with CPV infection, and whether CPV anti- body, b, and then a conventional PCR assay was performed. Fecal samples and pharyngeal swab specimens were obtained from infected dogs.

Materials and Methods

Diagnostic testing and sample collection—For each dog, a standardized history was obtained from the owner and recorded in the patient’s medical record, and a complete physical examination was performed. In accordance with hospital protocol, a fecal sample or rectal swab specimen was obtained during the initial physical examination and tested for CPV antigen with a commercially available ELISA. Treatment options were provided to each dog’s owner on the basis of results of the physical examination and ELISA, and treatment was administered in accordance with the attending clinician’s recommendations and the owner’s preference and financial constraints. When possible, a second fecal sample was obtained within 24 hours after the initial examination and stored for subsequent evaluation for CPV via ELISA and PCR assay. Later in the study, a pharyngeal swab specimen was also obtained from a subset of dogs when case management allowed, and this swab specimen was stored in 1 to 2 mL of sterile saline (0.9% NaCl) solution for evaluation for CPV via ELISA and PCR assay. All fecal samples and pharyngeal swab specimens were stored at 4°C until analysis.

Various diagnostic tests (determinations of blood glucose and albumin concentrations, CBC, and fecal parasite evaluation) and treatments (IV administration of fluids and fresh-frozen plasma) were performed or provided for study dogs dependent on the recommendations of the managing clinician and the treatment option chosen by the owner. Patient outcomes were recorded when available; however, some dogs that received treatment at home were lost to follow-up.

Clinical severity score—Two investigators (JEM and KMS) independently assigned each dog a clinical severity score retrospectively on the basis of the history and initial physical examination findings recorded in the dog’s medical record by the attending clinician. A 5-point scale (Appendix 1), which was based on each dog’s duration of illness, attitude, ambulatory ability, hydration status, and rectal temperature at the time of initial examination, was used for the clinical severity score.

PCR assay and genetic sequencing—Fecal samples were diluted approximately 1:500 in sterile water. The diluted fecal sample was added to a PCR master mix containing high-fidelity, hot-start DNA polymerase and buffer, a, and then a conventional PCR assay was performed. For pharyngeal swab specimens, a conventional PCR assay was performed directly on the saline solution diluent in which the swab specimens were stored. Forward primer 1F and reverse primer 1R (Appendix 2) were used to detect CPV DNA in all specimens. Each PCR assay underwent 30 amplification cycles (10 seconds at 98°C, 30 seconds at 54°C, and 2 minutes and 15 seconds at 72°C) with a final 10-minute extension at 72°C. The PCR products were then analyzed via gel electrophoresis to identify CPV-positive specimens. For specimens that tested negative for CPV on the initial PCR assay, a range of specimen dilutions (1:10 to 1:1600) were reevaluated via PCR assay. For a specimen that tested positive for CPV by PCR assay, additional PCR reactions were performed using the forward primer 1F and reverse primer 2R to amplify the 3′ proximal region of the VP2 gene (the region that includes residue 426). These PCR products were purified with a PCR purification kit, and primers 1F, 1R, and 2R, along with forward primers 2F and 3F, were used to obtain 2X sequence coverage performed at
a laboratory on an automated sequence analyzer by use of dye-terminator chemistry and DNA polymerase. Sequence contigs were aligned with prototype CPV-2 and CPV-2b genome sequences to generate a consensus sequence for each virus isolate. Each isolate was identified as CPV-2b or CPV-2c on the basis of the sequence at VP2 residue 426.

For some dogs, the initial ELISA was performed on a fecal sample or rectal swab specimen that was different from the specimen evaluated via the PCR assay. Therefore, all specimens evaluated by PCR assay were subsequently evaluated via ELISA to ensure sensitivity and specificity calculations were performed with test results (PCR assay and ELISA) obtained from the same sample or specimen.

Statistical analysis—All statistical analyses were performed with a statistical software program, and values of P < 0.05 were considered significant. Comparisons were made between dogs infected with CPV-2b and those infected with CPV-2c. For categorical variables (eg, hydration status, prognosis, and clinical signs), comparisons were performed with a Fisher exact test (for small sample size) or a χ² test of independence (when the expected value in all cells was ≥ 5). For continuous variables (eg, clinical severity score and duration of hospitalization), comparisons were performed using the Wilcoxon rank sum test. The Spearman rank coefficient (ρ) was used to determine the correlation of the clinical severity scores assigned to each dog by the 2 investigators.

Results

Animals—A convenience sample of 72 dogs were enrolled in the present study. A fecal sample from each dog was tested for CPV antigen via ELISA as part of the initial physical examination, of which 53 tested positive for CPV antigen (Table 1). Subsequently, fecal samples were obtained from 42 dogs and pharyngeal swab specimens were obtained from 16 dogs and tested for CPV via ELISA and PCR assay. A total of 56 of 72 (77.8%) study dogs had positive results for CPV as determined by ELISA or PCR assay performed on fecal samples. The 56 dogs that tested positive for CPV belonged to 52 owners; 2 owners each had 2 CPV-positive dogs, and 1 owner had 3 CPV-positive dogs.

Of the 56 dogs that tested positive for CPV, ages ranged from 1 to 36 months; 25 (44.6%) were ≤ 4 months old, 23 (41.1%) were 5 to 11 months old, and 8 (14.3%) were ≥ 12 months old. Twenty-eight (50%) dogs were female, of which 3 were spayed; and 28 (50%) were males, of which 2 were neutered. The most common breeds represented were Chihuahua (n = 11 [19.6%]) and pit bull–type dogs (8 [14.3%]), with the remaining dogs (37 [66.1%]) representing various purebred and mixed breeds. Thirty-six (64.3%) dogs were reportedly vaccinated for CPV at least once; however, information regarding the number, date, and manufacturer of the CPV vaccines received by each dog was unavailable. Information regarding previous veterinary examination was available for 53 dogs, of which 15 (28.3%) had been seen by a veterinarian for clinical signs consistent with enteritis prior to being examined at the referral hospital. Information regarding travel was provided for 51 dogs, of which only 1 (2.0%) had traveled outside of Arizona in the 2 months prior to examination at the referral hospital.

The 56 dogs that tested positive for CPV were initially examined at the referral and emergency hospital because of diarrhea (n = 43 [76.8%]), vomiting (51 [91.1%]), lethargy (53 [94.6%]), and inappetence (48 [85.7%]); 34 dogs each had 4 of these clinical signs, 15 dogs each had 3 clinical signs, 7 dogs each had 2 clinical signs, and no dogs had only 1 clinical sign. Abnormal physical examination findings included pyrexia (n = 22 [39.3%]), signs of abdominal pain (31 [55.4%]), and dehydration (43 [78.2%]; hydration status of 1 dog unknown). A blood sample was obtained during initial physical examination from each of 24 of the CPV-positive dogs. Blood glucose concentration was determined via glucometer (3 dogs; glucose concentration, 18, 85, and 116 mg/dL) or an in-clinic analyzer (21 dogs; median glucose concentration, 112 mg/dL; range, 41 to 184 mg/dL), and 3 (12.5%) dogs were hypoglycemic (reference range, 74 to 143 mg/dL). For 21 CPV-positive dogs, serum albumin concentration (median concentration, 2.3 g/dL; range, 1.5 to 3.9 g/dL) was determined via an in-clinic analyzer; 9 (42.9%) dogs were hypoalbuminemic (reference range, 2.3 to 4 g/dL).

A CBC was performed for 19 CPV-positive dogs; med-

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. tested</th>
<th>ELISA</th>
<th>PCR assay</th>
<th>No. positive on both ELISA and PCR assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Initial fecal sample</td>
<td>72</td>
<td>53</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Subsequent fecal sample</td>
<td>42</td>
<td>27</td>
<td>15</td>
<td>33</td>
</tr>
<tr>
<td>Pharyngeal swab specimen</td>
<td>16</td>
<td>0</td>
<td>16</td>
<td>10</td>
</tr>
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</table>

In accordance with hospital policy, fecal samples (initial fecal sample) were obtained from all 72 dogs during initial physical examination for CPV antigen detection via a commercially available ELISA. From a subset of 42 dogs, another fecal sample (subsequent fecal sample) was obtained within 24 hours after initial physical examination and tested for CPV via ELISA and PCR assay. From a subset of 16 dogs that tested positive for CPV via an ELISA performed on 1 or both fecal samples, pharyngeal swab specimens were obtained and tested for CPV via ELISA and PCR assay. For each fecal sample and 1 pharyngeal swab specimen that was obtained from a dog from which no subsequent fecal sample was available that had a CPV-positive result via PCR assay, genetic sequencing of PCR products was used to identify the virus strain.

— Not applicable.
dian leukocyte count was $5.84 \times 10^3$ WBCs/µL (range, $0.72 \times 10^3$ WBCs/µL to $14.41 \times 10^3$ WBCs/µL), and 10 (52.6%) dogs were leukopenic (reference range, $5.5 \times 10^3$ WBCs/µL to $16.9 \times 10^3$ WBCs/µL). Evaluation of fecal samples for gastrointestinal parasites was performed for 12 dogs; hookworms and Giardia were detected in fecal samples from 2 dogs.

Of the 56 CPV-positive dogs, 23 (41.1%) were hospitalized, and median duration of hospitalization was 4 days (range, 2 to 9 days). Six of these hospitalized dogs received fresh-frozen plasma. Twenty-nine (51.8%) CPV-positive dogs were treated at home, and 4 dogs were dead on arrival at the referral and emergency hospital or euthanized immediately after initial physical examination.

Outcome was available for 42 of the 56 (75.0%) CPV-positive dogs; 30 (71.4%) dogs recovered, 8 (19.0%) dogs were euthanized, and 4 (9.5%) dogs were reported by their owners to have died at home. Four dogs were euthanized because of deteriorating clinical condition despite maximum supportive treatment, and 4 were euthanized because of owners’ financial constraints. The remaining 14 (25.0%) dogs were lost to follow-up.

CPV strains detected—Of 33 fecal samples that tested positive for CPV via PCR assay, 9 (27.3%) were identified as CPV-2b (Asp at VP2 residue 426), and 24 (72.7%) were identified as CPV-2c (Glu at VP2 residue 426); CPV-2a (asparagine at VP2 residue 426) was not detected in any sample. For 1 dog, a pharyngeal swab specimen, but no fecal sample, was available for PCR testing and genetic sequencing; this specimen was identified as CPV-2c, which resulted in 25 dogs identified as infected with CPV-2c and 34 samples that tested positive for CPV via PCR assay. Similar to reports in other studies, 5,6 additional single nucleotide polymorphisms were identified in the capsid genes of these samples; these variants remain unnamed, and their functional consequences are unknown.

Comparisons between CPV-2b- and CPV-2c-infected dogs—Median age (5 months; range, 2 to 12 months) of the 9 dogs infected with CPV-2b did not differ ($P = 0.83$) from that (4 months; range, 2 to 36 months) of the 25 dogs infected with CPV-2c. Also, the proportion of dogs > 4 months old that were infected, compared with that of dogs ≤ 4 months old that were infected, did not differ ($P = 0.70$) between dogs infected with CPV-2b and CPV-2c. Similarly, the proportion (6/9 [66.7%]) of dogs infected with CPV-2b that had received at least 1 CPV vaccination did not differ ($P = 0.70$) from that (13/24 [54.2%]; vaccination status of 1 dog unknown) of dogs infected with CPV-2c.

Dogs infected with CPV-2c did not have more severe disease than those infected with CPV-2b. During initial physical examination, the proportion (9/9 [100%]) of dogs infected with CPV-2b that were dehydrated did not differ ($P = 0.07$) from that (16/24 [69.6%]; hydration status of 1 dog unknown) of dogs infected with CPV-2c. The extent of dehydration during initial physical examination was estimated and recorded for 20 dogs. Similarly, the proportion (3/6 [50.0%]) of dogs infected with CPV-2b that were severely (≥ 8%) dehydrated did not differ ($P = 0.30$) from that (3/14 [21.4%]) of dogs infected with CPV-2c. A CBC was performed for 6 dogs infected with CPV-2b and 12 dogs infected with CPV-2c. Median leukocyte count (5.03 $\times 10^3$ WBCs/µL; range, $0.72 \times 10^3$ WBCs/µL to $11.92 \times 10^3$ WBCs/µL) of CPV-2b–infected dogs did not differ ($P = 0.88$) from that (3.22 $\times 10^3$ WBCs/µL; range, $0.82 \times 10^3$ WBCs/µL to $14.41 \times 10^3$ WBCs/µL) of CPV-2c–infected dogs.

Comparison of the clinical severity scores independently assigned to each dog by 2 investigators revealed that the dogs were scored similarly ($P = 0.78$). The median clinical severity score assigned to dogs infected with CPV-2b was 3 (range, 2 to 5) for 1 investigator and 3 (range, 2 to 4) for the other investigator. The median clinical severity score assigned to dogs infected with CPV-2c was 2 (range, 2 to 5) for 1 investigator and 3 (range, 1 to 5) for the other investigator. Within each investigator, clinical severity scores did not differ ($P > 0.41$) between dogs infected with CPV-2b and CPV-2c.

Three dogs infected with CPV-2b and 14 dogs infected with CPV-2c were hospitalized for treatment. Median duration of hospitalization (4 days; range, 4 to 9 days) for CPV-2b–infected dogs did not differ ($P = 0.91$), compared with that (5 days; range, 3 to 8 days) for CPV-2c–infected dogs.

Of the 34 dogs (33 dogs determined CPV-positive by PCR assay on fecal samples and 1 dog determined CPV-positive by PCR assay on a pharyngeal swab specimen) for which the infecting strain of CPV was sequenced, 1 dog infected with CPV-2b and 3 dogs infected with CPV-2c were lost to follow-up. The proportion (4/8 [50.0%]) of dogs infected with CPV-2b that recovered did not differ ($P = 0.20$) from that (17/22 [77.3%]) of dogs infected with CPV-2c that recovered. Similarly, the proportion of dogs that were dehydrated during initial physical examination and subsequently recovered did not differ significantly ($P = 0.67$) between dogs infected with CPV-2b and dogs infected with CPV-2c.

Sensitivity and specificity of CPV ELISA—Results for CPV obtained via the ELISA were compared with those obtained via the PCR assay to calculate the sensitivity and specificity of the ELISA. Both assays were performed on the same fecal samples obtained from 42 dogs. All of the 27 samples that yielded positive results for CPV via ELISA also yielded positive results via PCR assay (Table 1). Fifteen samples yielded negative results for CPV via ELISA; of those, 6 samples yielded positive results via PCR assay. The overall sensitivity and specificity of the CPV ELISA for fecal samples were 81.8% (95% CI, 36% to 94%) and 100% (95% CI, 35% to 100%), respectively. The sensitivity (100%; 95% CI, 35% to 100%) of the ELISA for the detection of dogs infected with CPV-2b (n = 9) did not differ ($P = 0.16$) from that (75%; 95% CI, 47% to 90%) for detection of dogs infected with CPV-2c (24).

Hospital versus at-home treatment for CPV—Treatment outcome was available for 42 of the 56 CPV-positive dogs. The proportion (18/23 [78.3%]) of dogs that recovered after treatment in the hospital did not differ ($P = 0.32$) from that (12/19 [63.2%]) of dogs that recovered after treatment at home.
Prognostic indicators for clinical outcome of CPV infection—Multiple variables were individually compared with patient outcome to evaluate the usefulness of each variable as a prognostic indicator for outcome of CPV infection. Dogs that were euthanized because of owners’ financial constraints were excluded from these analyses. Variables not significantly associated with patient outcome in the present study included patient age ($P = 0.53$), breed ($P = 0.46$), sex ($P = 0.72$); possible exposure to CPV prior to initial physical examination at the emergency and referral hospital ($P = 0.70$); leukocyte count ($P = 0.43$); serum albumin concentration ($P = 0.53$); blood glucose concentration ($P = 0.36$); signs of abdominal pain ($P = 0.47$), diarrhea ($P = 0.33$), vomiting ($P = 1.00$), lethargy ($P = 0.57$), inappetence ($P = 1.00$), and ptyalism ($P = 1.00$) during initial physical examination; and administration of fresh-frozen plasma ($P = 0.58$). The only variable that was significantly ($P = 0.04$) associated with patient outcome was hydration status, which was known for 55 of the 56 CPV-positive dogs. Of the 41 dogs with known outcomes and hydration status, all 10 that were not dehydrated during initial physical examination recovered, whereas only 20 of 31 (64.5%) dogs with known outcomes that were dehydrated during initial physical examination recovered.

Detection of CPV from pharyngeal swab specimens—Pharyngeal swab specimens were obtained and tested for 16 dogs that were CPV-positive as determined via ELISA performed on a fecal sample. Of the 16 specimens, 0 yielded positive results for CPV via ELISA, whereas 10 yielded CPV-positive results via PCR assay (Table 1).

Discussion
To our knowledge, the study reported here is the first in which investigators evaluated clinical information and CPV sequence data from multiple dogs infected with CPV that were treated at a single referral hospital in the southwestern United States. Results indicated that CPV-2c was more prevalent in the study population than was CPV-2b; however, disease severity and outcome of infection did not differ significantly between the 2 strains.

Recognition of CPV-2c in 2001 prompted concern that the newly identified strain may cause disease of greater severity, be able to infect properly vaccinated dogs, and evade detection via diagnostic tests that relied on identification of antibody against viral antigens. Similar to other CPV strains (CPV-2a, CPV-2b, and an unnamed CPV strain with an alanine substitution for serine at VP2 residue 297), CPV-2c has quickly achieved global dissemination. Rapid dissemination of CPV-2c indicates positive selection pressure for that particular strain, which in turn suggests that the Glu mutation at VP2 residue 426 must provide some advantage for that strain of CPV. However, that advantage does not necessarily equate to an ability to cause more severe disease or infect properly vaccinated dogs. It may be that CPV-2c can replicate faster, yield a greater volume of virus for shedding, be transmitted more efficiently between dogs, or cause less severe disease than the other known CPV strains.

Twenty-five of 56 (44.6%) CPV-infected dogs were ≤ 4 months old at the time of initial physical examination; thus, they were within the window of susceptibility during which puppies can become infected with CPV even if they have been inoculated with a modified-live CPV vaccine. Investigators of 1 study hypothesized that CPV-2c caused disease in adult dogs more frequently than did CPV-2a and CPV-2b. That hypothesis was not supported by results of the present study; however, the sample size in our study was small, and most of the dogs were < 1 year old, so there may not have been enough power to detect such a difference if it existed.

The ELISA used in the present study was a commonly used, commercially available, in-clinic diagnostic test for CPV. The ELISA manufacturer reports that it has high specificity and sensitivity for the detection of CPV-2b. In the study reported here, the calculated specificity and sensitivity of the ELISA were 81.8% and 100%, respectively, for the detection of CPV-infected dogs when results of a conventional PCR assay for CPV were used as the gold standard for comparison. The ELISA successfully detected both CPV-2b and CPV-2c strains in fecal samples, and the sensitivities for detection of each strain did not differ significantly. However, the sample size for each strain was small and the CIs were very large, so there may not have been enough power to detect a difference. The sensitivity (75.0%) of the ELISA for detecting CPV-2c in the present study was similar to that (77.0%) calculated by investigators of another study in which the sample size was 100. Regardless of the sensitivity of any diagnostic test used, a dog with a history and clinical signs consistent with parvoviral enteritis should be treated as if it were infected with CPV.

In the present study, dogs infected with CPV that received treatment at home were just as likely to recover as dogs infected with CPV that received treatment in the hospital. Therefore, treatment at home may be a reasonable option for some dogs with parvoviral enteritis. However, these findings may have been biased because most of the dogs that were lost to follow-up were dogs that received treatment at home. Furthermore, there is the possibility that dogs with severe disease were more likely to be hospitalized, whereas dogs with less severe disease were more likely to receive treatment at home.

Results of other studies indicate that various factors such as leukopenia, gastrointestinal parasites, and the lack of early enteral nutrition are associated with negative outcomes for dogs with parvoviral enteritis, and these factors can be used as prognostic indicators for CPV-infected dogs. However, in the present study, the only variable associated with patient outcome was hydration status. Dogs that were dehydrated during initial examination were less likely to recover from CPV infection.

The initial site for CPV replication is the lymphoid tissue of the oropharynx, and CPV has been detected in the tongues and tonsils of infected dogs. Therefore, it may be possible to detect CPV in specimens obtained from the oropharynx before overt clinical signs develop and viral shedding in the feces begins. Identification of CPV-infected dogs before they have signs...
of illness would be beneficial for kennel populations because, once identified, infected dogs could be immediately isolated, which would reduce the risk for CPV transmission to other dogs in the kennel. In the present study, CPV was detected in 10 of 16 pharyngeal swab specimens via a PCR assay, but was not detected via an ELISA performed on the same specimens. Storing the swab specimens in saline solution may have diluted the volume of CPV below the threshold of detection for the ELISA. Whether the use of the ELISA on undiluted pharyngeal swab specimens could detect CPV at an earlier or more consistent time during infection than on fecal swab specimens is unknown and warrants further investigation.

The present study was conducted at an Arizona emergency and referral veterinary hospital, in part, because CPV-2c had been identified in that region of the country.17 The comparison of disease severity and outcome between dogs infected with CPV-2b or CPV-2c from the same geographic region that were treated by the same hospital should have reduced bias from confounding factors such as population demographics and variability in hospital protocol and record keeping. Other potential sources of bias for this present study included enrollment of dogs only from an emergency and referral hospital, enrollment of dogs by different clinicians, financial constraints of owners, and loss of some patients to follow-up. However, because the strain of CPV that infected each dog was unknown during initial examination and treatment, sources of potential biases should have been distributed similarly among CPV-2b- and CPV-2c–infected dogs. The prevalence of CPV-2b and CPV-2c could have been biased in the present study if there was a difference in disease severity between the 2 strains and if CPV-infected dogs that were treated by general veterinary practitioners had less severe disease than those treated by veterinary practitioners at an emergency and referral hospital. Despite small sample sizes that limited the analyses and inferences made, we found no evidence that dogs infected with CPV-2c had more severe disease or worse outcomes than dogs infected with CPV-2b.

In the present study, the prevalence of dogs infected with CPV-2c was approximately 2.8 times the prevalence of dogs infected with CPV-2b. No differences were detected between dogs infected with CPV-2c and those infected with CPV-2b in terms of signalment, initial physical examination findings, disease severity, or disease outcome. Thus, the use of genetic sequencing to identify specific viral strains is unnecessary for the treatment and management of dogs with parvoviral enteritis at this time. However, it remains important for diagnostic laboratories and researchers to continue monitoring and characterizing CPV strains that affect dogs. For the CPV-infected dogs of the present study, complete compliance with recommended CPV vaccine protocols was infrequent, and prevention of CPV through the administration of modified-live CPV vaccines is still recommended.20 Nevertheless, even for puppies that have received the recommended CPV vaccination protocol, there often remains a window of susceptibility to CPV, which varies among individuals. Therefore, it is important for owners of puppies ≤ 16 weeks old to avoid exposure of those pets to high-risk areas where the virus is likely to be present.

For dogs that develop parvoviral enteritis, prompt supportive care is imperative for the patient’s survival, and thorough cleaning and disinfection of the patient’s environment is crucial for the prevention of disease transmission to other dogs.

References


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**Appendix 1**

Five-point clinical severity scale used to retrospectively assign scores to each of the 72 study dogs on the basis of the history and initial physical examination findings recorded in each dog’s medical record by the attending clinician.

<table>
<thead>
<tr>
<th>Clinical severity score</th>
<th>Duration of illness (d)</th>
<th>Attitude</th>
<th>Ambulatory ability</th>
<th>Estimated hydration status</th>
<th>Rectal temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt; 1</td>
<td>Bright, alert, and responsive</td>
<td>Normal</td>
<td>Euhydration</td>
<td>Normothermic</td>
</tr>
<tr>
<td>2</td>
<td>1–3</td>
<td>Quiet, alert, and responsive to signs of depression</td>
<td>Normal</td>
<td>5% dehydration</td>
<td>Normothermic to febrile</td>
</tr>
<tr>
<td>3</td>
<td>2–3</td>
<td>Signs of depression</td>
<td>Possibly weak</td>
<td>6%–7% dehydration</td>
<td>Normothermic to febrile</td>
</tr>
<tr>
<td>4</td>
<td>&gt; 3</td>
<td>Signs of depression to obtunded</td>
<td>Weak and reluctant to walk</td>
<td>7%–8% dehydration</td>
<td>Normothermic to febrile</td>
</tr>
<tr>
<td>5</td>
<td>&gt; 3</td>
<td>Stuporous</td>
<td>Recumbent</td>
<td>8%–10% dehydration</td>
<td>Hypothermic</td>
</tr>
</tbody>
</table>

Dogs represented a convenience sample of dogs with histories and clinical signs consistent with paroviral enteritis that were examined at an emergency and referral hospital in the southwestern United States from August 2008 through June 2009.

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**Appendix 2**

Canine parovirus-specific primers used in PCR assays or genetic sequencing (or both) that was performed on fecal samples and pharyngeal swab specimens obtained from dogs with histories and clinical signs consistent with paroviral enteritis that were examined at an emergency and referral hospital in the southwestern United States from August 2008 through June 2009.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primer 1F</td>
<td>5′-GAAAACGGATGCTGGAGATCACAGC-3′</td>
</tr>
<tr>
<td>Primer 1R</td>
<td>5′-TATTTGGAATATCTTATTGGC-3′</td>
</tr>
<tr>
<td>Primer 2F</td>
<td>5′-CAAGGCAAAGAAATGGCCCTTGA-3′</td>
</tr>
<tr>
<td>Primer 2R</td>
<td>5′-CTAGGGCGAAACCAACCAACCAACCA-3′</td>
</tr>
<tr>
<td>Primer 2F</td>
<td>5′-AGATGTAATAATACCATAGCATTGTTGAC-3′</td>
</tr>
<tr>
<td>Primer 3F</td>
<td>5′-ACAGGAGAAGAACACCTTGGAGATTTA-3′</td>
</tr>
</tbody>
</table>