Clinical evaluation of a single dose of immune plasma for treatment of canine parvovirus infection

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Objective—To evaluate the efficacy of administration of a single 12-mL dose of canine parvovirus (CPV)-immune plasma for treatment of CPV enteritis.

Design—Prospective, randomized, double-blinded, placebo-controlled clinical trial.

Animals—14 dogs with naturally occurring CPV enteritis.

Procedures—Dogs were assigned to treatment groups on the basis of randomization tables and were administered a single IV dose of CPV-immune plasma (treatment group) or an equivalent volume of saline (0.9% NaCl) solution (placebo group) within 18 hours after admission to the hospital. Treatment and outcome variables evaluated included neutrophil, monocyte, and CPV counts; number of days of hospitalization; changes in body weight; and cost of treatment.

Results—When dogs treated with CPV-immune plasma were compared with dogs treated with saline solution, there were no significant differences detected among neutrophil or monocyte counts, magnitude of viremia, weight change, number of days of hospitalization, or cost of treatment.

Conclusions and Clinical Relevance—Administration of a single 12-mL dose of immune plasma soon after the onset of CPV enteritis in dogs was not effective in ameliorating clinical signs, reducing viremia, or hastening hematologic recovery. (J Am Vet Med Assoc 2012;240:700–704)

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anine parvovirus is a highly contagious viral agent that spreads between dogs following oronasal exposure or local ingestion. Despite the availability and widespread use of effective vaccines, CPV remains a major cause of morbidity and death in dogs, and it is estimated that up to 1 million dogs are infected with CPV each year in the United States. When left untreated, CPV infection rapidly progresses and causes severe dehydration, disseminated intravascular coagulation, bacterial translocation, and sepsis, with a mortality rate that exceeds 90%. However, with aggressive supportive care, the mortality rate can be reduced to between 0% and 30%. The high cost associated with aggressive and prolonged treatment required for dogs infected with CPV has led to investigation of alternative treatments that can hasten gastrointestinal recovery and the return of hematologic values.

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Abbreviations

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<tr>
<th>Abbreviation</th>
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<tr>
<td>CPV</td>
<td>Canine parvovirus</td>
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<td>qPCR</td>
<td>Quantitative PCR</td>
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The high cost associated with aggressive and prolonged treatment required for dogs infected with CPV has led to investigation of alternative treatments that can hasten gastrointestinal recovery and the return of hematologic values.
and diarrhea in dogs with experimentally induced CPV infection when administered immediately after viral inoculation. Results of 1 study indicated that administration of concentrated lyophilized canine IgG significantly decreased duration of hospitalization in dogs with CPV enteritis. At our veterinary teaching hospital, a single, fixed dose of CPV-immune plasma has been administered as an adjunctive treatment for many years with anecdotal success. However, to our knowledge, a randomized clinical trial conducted to investigate the use of CPV-immune plasma in dogs with naturally occurring CPV infection has not been performed.

Therefore, the purpose of the study reported here was to investigate the clinical effectiveness of immune plasma as adjunctive treatment for dogs with CPV enteritis. We hypothesized that administration of plasma with high anti-CPP antibody titers to dogs with naturally occurring CPV enteritis would significantly decrease circulating viral load and time to hematologic recovery and reduce the duration of hospitalization.

Materials and Methods

Animals—Client-owned dogs with CPV enteritis treated at the Colorado State University Veterinary Teaching Hospital were enrolled in the study between March 2008 and September 2009. Inclusion criteria for entry into the study included positive results on a CPV fecal antigen test, clinical signs of gastrointestinal disease (vomiting, diarrhea, and anorexia), and age < 1 year. Exclusion criteria included vaccination with a commercial vaccine containing CPV or treatment with corticosteroids or oseltamivir within 1 week prior to hospital admission. Client consent was obtained from each dog’s owner prior to enrollment in the study. The study protocol was approved by the Institutional Animal Care and Use Committee at Colorado State University.

Study design and sample size calculation—The study was a prospective, randomized, double-blinded, placebo-controlled clinical trial. It was designed to achieve a power of at least 80% on the basis of an expected 25% difference in ≥ 1 of 5 predetermined variables (neutrophil and monocyte counts, magnitude of viremia, percentage change in weight, number of days of hospitalization, and cost of treatment) compared between CPV-immune plasma–treated and placebo–treated dogs, with an expected sample SD of 30% and an α of 0.05. Calculations made by use of power calculation software indicated that 7 dogs/treatment group should be enrolled.

Preparation of immune plasma—Pooled, antibody-rich anti-CPP plasma was obtained from healthy blood donor dogs at our veterinary teaching hospital that had recovered from CPV infection. The donor dogs were also screened and had negative results for common infectious diseases of dogs prior to collection of blood samples for plasma harvest. Plasma included in the pooled immune plasma bank was obtained from dogs with anti-CPP titers (mean, 1:7,000) as determined by a commercial laboratory. Pooled immune plasma was stored in 12-mL aliquots and frozen at –80°C. Plasma was thawed in a warm water bath until it reached room temperature (22.2°C [72°F]) and then immediately administered.

Treatment protocol—An investigator who was not involved in daily patient care or treatment decisions was involved in assigning dogs to receive CPV-immune plasma or a placebo (an equivalent volume of saline [0.9% NaCl] solution). Dogs were randomly assigned to each treatment group by use of a predetermined block randomization design. This investigator also administered the CPV-immune plasma or saline solution to the enrolled patients. Dogs in the treatment group received a single IV infusion of 12 mL of CPV-immune plasma administered over a period of 20 minutes, whereas dogs in the placebo group received an IV infusion of 12 mL of saline solution administered over a period of 20 minutes. Dogs were monitored during and after the infusions for signs of adverse reactions.

The decision to use a single 12-mL dose of CPV-immune plasma regardless of each dog’s body weight was reached on the basis of several considerations. First, this dose of plasma would deliver approximately 2.5 mL of plasma/kg (1.14 mL of plasma/lb) for a dog with the mean body weight (4.3 kg [9.5 lb]) of the dogs in the study, which is consistent with recommendations for passive plasma administration in other infectious diseases. Second, there was no available method for determining the actual amount of CPV-immune plasma to administer to achieve a potential therapeutic endpoint and we were unaware of any study that could have provided guidance for an effective starting point. Third, suggested starting doses for passive immunization with immune plasma include an extremely large range from 0.2 to 150 mL/kg (0.09 to 68.2 mL/lb). Finally, we had a considerable amount of clinical experience with the use of a fixed dose of CPV-immune plasma for treatment of young dogs with CPV enteritis.

All dogs in the study received supportive care that consisted of IV administration of crystalloid fluid supplemented with potassium chloride. Additionally, all dogs in the study were treated with antimicrobials. Specific treatment decisions were made at the discretion of the attending critical care clinician. Dogs in the study also received antiemetics, antacids, anthelmintics, glucose, colloids, blood, and analgesic medications as deemed necessary by the attending clinician. Clinicians involved in direct patient care were unaware of the treatment group to which the dogs were assigned. Patient care was overseen and approved by a board-certified critical care specialist who also was unaware of the study group to which dogs were assigned, and this clinician made the final decision with regard to when each dog was discharged from the hospital.

Patient monitoring—At enrollment into the study, a blood sample was collected for a CBC. Additional collection of blood samples for CBCs was performed daily throughout the treatment period. In small dogs, smaller volumes of blood were collected with microweather blood collection tubes, and all blood sample collections were approved by the supervising clinician after reviewing each dog’s daily PCV and total solids concentration. If a dog became anemic, blood collection was suspend-
ed until the supervising clinician approved resumption of sample collection. Neutrophil and monocyte counts were obtained from the daily CBC results. Serum was obtained from blood samples and frozen at −80°C for quantitation of CPV viremia. Dogs were weighed every 12 hours, and body temperature and pulse and respiratory rates were determined. Duration of fever was defined as the time that body temperature exceeded 39.2°C (102.5°F). Episodes of vomiting, regurgitation, and diarrhea were recorded. Number of days of hospitalization was also recorded. Cost of treatment was determined at the time of discharge and included the cost of medications dispensed at discharge. Percentage change in body weight was determined as follows: (body weight at hospital admission – body weight at discharge)/body weight at hospital admission.

**CPV quantitation**—A qPCR assay was used for CPV viral quantitation as described in another study. Briefly, the qPCR assay amplified a conserved region of the CPV genome, and a plasmid vector containing the cloned CPV gene served as an internal control sample for the qPCR amplification.

**Statistical analysis**—Neutrophil, monocyte, and plasma CPV counts were compared between treatment groups with a repeated-measures ANOVA. Comparisons between cost of treatment, number of days in the hospital, and percentage change in body weight were determined at the time of discharge and included the cost of medications dispensed at discharge. Percentage change in body weight was determined as follows: (body weight at hospital admission – body weight at discharge)/body weight at hospital admission.

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### Results

Fourteen dogs were enrolled in the study, with 7 dogs in each of the CPV-immune plasma–treated and placebo-treated groups. Most of the dogs enrolled in the study were mixed-breed dogs (n = 6); other breeds included Labrador Retriever (2), Chihuahua (2), American Pit Bull Terrier (1), Yorkshire Terrier (1), Australian Shepherd Dog (1), and Miniature Bull Terrier (1). The age of affected dogs ranged from 2 to 11 months. All dogs in the placebo-treated group were sexually intact (4 females and 3 males). The CPV-immune plasma–treated group consisted of 6 sexually intact dogs (4 females and 2 males) and 1 neutered male dog. At study enrollment, the body weight for the placebo-treated group ranged from 1.2 to 17 kg (2.6 to 37.4 lb; median, 9.5 kg [20.9 lb]) and that for the CPV-immune plasma–treated group ranged from 0.86 to 26 kg (1.9 to 57.2 lb; median, 4.3 kg). Three dogs in the placebo-treated group and 4 dogs in the CPV-immune plasma–treated group had not been vaccinated against CPV prior to hospital admission. The duration of clinical signs prior to hospital admission ranged from 12 to 48 hours in the placebo-treated group and from 12 to 72 hours in the CPV-immune plasma–treated group. Significant differences in variables between the 2 groups were not detected (Table 1).

One dog died during the study. That dog was assigned to the placebo-treated group and went into cardiopulmonary arrest 4 days after admission into the hospital. Cardiopulmonary and cerebral resuscitation were successful, but the owner chose to euthanize the dog. Histopathologic findings included almost complete loss of lymphoid tissue and intestinal villi, hepatic congestion, and evidence of eosinophilic pulmonary edema.

The effect of treatment on neutrophil count over time in CPV-immune plasma–treated and placebo-treated dogs during hospitalization was assessed (Figure 1). In both groups, neutrophil counts remained relatively constant during the first 4 days following initiation of treatment. There was no significant ($P = 0.69$) difference in the neutrophil counts between the 2 groups.

Changes in monocyte counts in CPV-immune plasma–treated and placebo-treated dogs over time were also assessed (Figure 1). Monocyte counts decreased over the first 4 days of treatment. Similar to neutrophil counts, there was not a significant ($P = 0.43$) difference in mean monocyte counts between the 2 groups.

The magnitude of CPV viremia remained relatively constant for the placebo-treated group but increased over the first 3 days of treatment for the CPV-immune plasma–treated group (Figure 2). However, the magnitude of CPV viremia was not significantly ($P = 0.13$) different between the 2 treatment groups.

The number of days of hospitalization for the placebo-treated dogs ranged from 1 to 8 days, with a median of 4 days (Table 1), whereas that for the immune plasma–treated dogs ranged from 1 to 8 days, with a median of 4 days. There was no significant ($P = 0.94$) difference in median number of days of hospitalization between the 2 treatment groups.

Finally, we also assessed the effects of treatment on weight loss during the treatment period and the cost of

### Table 1—Median ± SEM values of descriptive variables for 14 dogs with CPV enteritis treated with 12 mL of CPV-immune plasma (n = 7) or 12 mL of saline (0.9% NaCl) solution (placebo [7]) within 18 hours after hospital admission in addition to standard supportive care.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CPV-immune plasma–treated group</th>
<th>Placebo-treated group</th>
<th>P value*</th>
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<tbody>
<tr>
<td>Age (mo)</td>
<td>3.0 ± 1.52</td>
<td>3.5 ± 0.89</td>
<td>0.95</td>
</tr>
<tr>
<td>Body weight at admission (kg)</td>
<td>4.3 ± 3.4</td>
<td>9.5 ± 2.1</td>
<td>0.54</td>
</tr>
<tr>
<td>Duration of clinical signs prior to hospital admission (d)</td>
<td>24 ± 7.9</td>
<td>24 ± 4.4</td>
<td>0.68</td>
</tr>
<tr>
<td>Dose (mL/kg)</td>
<td>2.79 ± 1.9</td>
<td>1.26 ± 1.25</td>
<td>0.54</td>
</tr>
<tr>
<td>Weight loss during hospitalization (%)</td>
<td>2 ± 4.1</td>
<td>0 ± 4.2</td>
<td>0.56</td>
</tr>
<tr>
<td>Time in hospital (d)</td>
<td>4 ± 0.53</td>
<td>4 ± 0.79</td>
<td>0.95</td>
</tr>
<tr>
<td>Cost of treatment ($)</td>
<td>1,424 ± 161</td>
<td>1,665 ± 279</td>
<td>0.82</td>
</tr>
</tbody>
</table>

*Represents results of a Mann-Whitney U test; values were considered significant at $P < 0.05$. To convert mL/kg to mL/lb, divide by 2.2.
treatment. The percentage of weight loss during treatment was 2% for the CPV-immune plasma–treated dogs and 0% for the placebo-treated dogs, but this difference was not significant (Table 1). The mean ± SEM cost of hospitalization was $1,424 ± 161 for the CPV-immune plasma–treated dogs, compared with $1,665 ± 279 for the placebo-treated dogs. Again, these values did not differ significantly ($P = 0.62$).

**Discussion**

In the study reported here, we found no evidence that the administration of a single dose of 12 mL of CPV-immune plasma to dogs within 24 hours after onset of clinical signs of CPV enteritis had any effect on the return of hematologic values to the reference range, CPV viremia, duration of hospitalization, or cost of treatment. This was despite the fact that use of CPV-immune plasma for CPV enteritis has been recommended on the basis of anecdotal evidence and the analogy that use of immune plasma is effective for the treatment of other viral diseases.15

A number of reasons may explain why we failed to detect an effect from the administration of CPV-immune plasma to dogs with CPV infection. Because of the relatively small number of dogs evaluated in this study, it is possible there was a type II error, possibly as a result of group assignment bias. A survival prediction index could have been used to determine whether most of the more severely affected dogs were assigned to one of the treatment groups. Calculation of the survival prediction index requires serum albumin and creatinine concentrations, and unfortunately, a serum biochemical analysis was not performed on all dogs at admission. Therefore, we were unable to calculate the survival prediction index and confirm whether there was group assignment bias. However, when we evaluated data that were available, we did not detect any differences in the composition of the CPV-immune plasma–treated versus placebo-treated dogs with respect to age, breed, sex, duration of illness, or number of ancillary treatments administered.

Several weaknesses in the present study could be addressed in a subsequent study. For example, a follow-up study might more appropriately include a treatment group that received non–CPV-immune canine plasma, rather than a saline solution placebo as was used in the present study. It is possible that administration of CPV-immune plasma could exert treatment effects unrelated to the anti-CPV antibodies, which would make administration of non–CPV-immune plasma a better control treatment. If positive effects from administration of CPV-immune plasma had been detected, then a follow-up study to compare the effectiveness of CPV-immune and non–CPV-immune plasma would be indicated.

Another limitation of the present study was the potential that the dose of CPV-immune plasma administered was too low or the titer of anti-CPV antibodies was insufficient to adequately neutralize CPV in the circulation or tissues of infected dogs. The mean dose of CPV-immune plasma we used was similar to that recommended for passive immunotherapy for other infectious agents.18 Detailed studies conducted to evaluate various doses of immune plasma would be difficult to perform and were beyond the scope of the present study. In regard to insufficient anti-CPV antibodies, an antibody titer of 1:80 is considered protective.23 The immune plasma administered to the dogs in the present

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**Figure 1**—Mean ± SEM values for neutrophil (A) and monocyte (B) counts for 14 dogs with CPV enteritis treated with 12 mL of CPV-immune plasma (n = 7; triangles) or 12 mL of saline (0.9% NaCl) solution (placebo [7]; circles) within 18 hours after hospital admission in addition to standard supportive care. Neutrophil counts were determined daily for each dog. Day 0 represents the value determined before treatment was administered on the day each dog was admitted to the hospital. Changes in values over time were not significantly different between the 2 groups for neutrophil ($P = 0.69$) or monocyte ($P = 0.43$) counts.

**Figure 2**—Mean ± SEM number of serum CPV particles as determined by use of a qPCR assay for 14 dogs with CPV enteritis treated with 12 mL of CPV-immune plasma (n = 7; triangles) or 12 mL of saline solution (placebo [7]; circles) within 18 hours after hospital admission in addition to standard supportive care. Serum was obtained daily from blood samples that were collected from each dog. The magnitude of viremia over time was not significantly ($P = 0.15$) different between the 2 groups. See Figure 1 for remainder of key.
study had a mean CPV antibody titer of 1:7,000 as determined by use of a hemoagglutination inhibition assay, which is generally considered a very high antibody titer. Therefore, we do not believe that use of plasma with a higher concentration of anti-CPV antibodies would have substantially changed the outcome of the present study. However, we acknowledge that a dosing scheme for CPV-immune plasma determined on the basis of body weight for each dog may be warranted in a subsequent study, particularly if a reliable biological marker of plasma administration can be identified.

It is also possible that more frequent administration of immune plasma could have improved the effectiveness of treatment. Although this may be a theoretical concern, we believe it is unlikely more frequent administration would improve treatment response because infused antibodies typically have a relatively long half-life (weeks). 23 The present study was designed with a single administration of CPV-immune plasma because that is how the treatment would most probably be used in clinical practice.

The timing of administration is also a possible cause of failure to detect a treatment effect from administration of CPV-immune plasma. The CPV disease in the study dogs may have been too advanced at the time CPV-immune plasma was administered to change the clinical course of disease. However, the plasma was administered shortly after the dogs were admitted to the hospital, which is realistically the only setting in which administration of CPV-immune plasma would be expected to have an effect. It is also possible cellular immunity plays a critical role in controlling acute CPV infection, and augmenting humoral immunity cannot overcome defects in cellular immunity created during the early phases of CPV disease in dogs.

For the present study, we concluded that administration of CPV-immune plasma as adjunctive treatment for CPV enteritis in dogs did not result in substantial clinical benefits in terms of the time required for return of hematologic values to the reference range, reduction in serum viremia, or duration of hospital stay. However, additional studies with larger numbers of dogs and various doses of CPV-immune plasma may be necessary to fully resolve the issue.

Unresolved questions that should be addressed in future research studies conducted to investigate the use of CPV-immune plasma for treatment of acute CPV infections include determination of an effective plasma dose and proper timing of administration and identification of appropriate biomarkers for treatment effects.

References