ORIGINAL STUDY

Clinical evaluation of hyperimmune plasma for treatment of dogs with naturally occurring parvoviral enteritis

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Funding information
This study was funded by Plasvacc USA, Inc.

Results of this study were presented in abstract
form at the 2018 American College of Vet-
erinary Internal Medicine Forum in Seattle,
WA.

Abstract

Objective: To evaluate the clinical efficacy of a single infusion of hyperimmune plasma (HIP) in dogs with canine parvovirus (CPV).

Design: Prospective, randomized, placebo-controlled clinical trial.

Setting: University teaching hospital.

Animals: Client-owned dogs with naturally occurring CPV.

Interventions: Dogs presenting for CPV treatment (n = 31) underwent cardiovascular resuscitation and were randomized to receive a single dose of either HIP (10 mL/kg IV) or placebo (0.9% sodium chloride [10 mL/kg IV]) during the first 6 hours of hospitalization. All dogs were treated with a standardized treatment protocol (IV fluid therapy [120 mL/kg/d isotonic crystalloids], cefoxitin [30 mg/kg IV q 8 h], maropitant [1 mg/kg IV q 24 h], and buprenorphine [0.01–0.02 mg/kg IV q 8 h]) until hospital discharge.

Measurements and main results: Dogs treated with HIP (n = 16) demonstrated a lower shock index at 24 hours (median = 0.77, range: 0.5–1.5) than those treated with placebo (n = 15, median = 1.34, range: 0.5–1.7; $P = 0.02$). Plasma lactate concentration was lower at 24 hours in HIP-treated dogs (median = 1.3 mmol/L, range: 0.9–3.4 mmol/L) than in placebo-treated dogs (median = 2.1 mmol/L, range: 1.1–3.4 mmol/L; $P = 0.01$). There was no difference in duration of hospitalization when comparing HIP-treated dogs (median = 3.2 days, range: 0.83–10 days) to placebo-treated dogs (median = 2.83 days, range: 1–8.38 days; $P = 0.35$). Survival was 16 of 16 (100%) for the HIP group and 14 of 15 (93.3%) for the placebo group ($P = 0.32$).

Conclusions: HIP at 10 mL/kg IV administered to dogs with CPV within the first 6 hours of hospitalization improves markers of shock during the initial 24 hours of hospitalization. No effects were observed on duration of hospitalization or mortality; however, this study was underpowered to evaluate these effects. HIP was well tolerated in this population of critically ill dogs.

KEYWORDS
canine, immunotherapy, shock, shock index

1 | INTRODUCTION

Canine parvovirus (CPV) remains a significant worldwide enteropathogen with high morbidity and mortality among susceptible dogs.[1] No definitive treatment has been identified and current recommendations include hospitalization with aggressive supportive care.[1,2] Although case fatality rate is reduced with supportive care, hospitalization can quickly become cost prohibitive. Numerous targeted therapies have been studied in an effort to reduce disease severity and length of hospitalization including human recombinant granulocyte colony stimulating factor,[3-5] equine antiendotoxin,[6,7] recombinant bactericidal/permeability-increasing protein,[8] oseltamivir,[9] and interferon.[10,11] Therapeutic investigations of these products have produced variable results or required additional investigation, leaving early enteral nutrition as the most commonly cited therapy shown to reduce recovery time and decrease disease morbidity.[12]

Despite adjunctive therapies resulting in limited success, there remains a need for a novel, effective, and easily administered treatment for CPV.[6,11,13] Hyperimmune plasma (HIP) is one such potential therapy that has undergone investigation with mixed results. Administration of CPV-immune plasma has been reported to improve survival and reduce vomiting in dogs with experimentally induced CPV,[14,15] though a more recent study reported that a single dose of immune plasma was not effective at ameliorating clinical signs or hastening hematologic recovery in dogs with naturally occurring CPV.[13] Limitations of the more recent study include lack of a standardized HIP dose based on body weight, and a time lag in HIP administration relative to hospital admission.

A commercial HIP product[*] has recently been introduced in the veterinary market for treatment of CPV. This HIP is produced via plasmapheresis from healthy dogs hyperimmunized against CPV and *Escherichia coli* bacterin. The result is a standardized product that includes specific antibodies against CPV (titers of 1:20 to ≥1:80) as well as anti-endotoxin antibodies (1:10,000 to 1:60,000). The benefit of a commercially available product provided at a standardized dose at the onset of CPV treatment has yet to be evaluated in a clinical setting.

The current study hypothesized that administration of this standardized HIP product at a dose of 10 mL/kg within the first 6 hours of hospitalization would provide short-term cardiovascular benefits and improve the overall outcome of dogs being supportively managed for naturally occurring CPV infection. Study objectives were to determine the effects of HIP treatment on markers of shock within the first 24 hours of hospitalization and to investigate the efficacy of HIP in reducing the duration and severity of clinical signs, length of hospitalization, and morbidity associated with CPV.

2 | MATERIALS AND METHODS

This prospective study identified dogs presenting to a university teaching hospital between June and September 2017 with the chief complaint of CPV infection. This study was performed under a larger umbrella of studies investigating CPV and was approved by the hospital Clinical Review Board prior to study initiation.

Dogs were eligible for study inclusion if they had never received a CPV vaccination, weighed >1.5 kg, were demonstrating clinical signs consistent with CPV (eg, lethargy, vomiting, and diarrhea), tested positive for CPV via enzyme-linked immunosorbent assay (ELISA),[†] and had received no more than minimal treatment at another veterinary facility immediately prior to referral. "Minimal treatment" was defined as a single dose of SC maropitant[‡] and less than 15 mL/kg of SC fluids, and "immediately prior to referral" was defined as within 24 hours of study admission. All previous ELISA tests and treatments had to be documented and provided at study admission. Dogs were excluded from the study if they had identifiable comorbidities upon hospital presentation that could influence outcome (eg, concurrent infection), displayed a temperament that could affect study participation, or if owners declined inclusion. Informed owner consent was obtained prior to study enrollment and all costs associated with hospitalization were paid for by the study.

Baseline categorical and historical data obtained from each dog at hospital admission included age, sex, breed, duration of clinical signs prior to presentation, pertinent medical history, vital parameters (rectal temperature, pulse rate, respiratory rate, and systemic arterial blood pressure), body weight, physical examination findings, calculated shock index (SI), and baseline clinical severity score using a previously developed scoring system (Appendix 1).[12] Whole blood was collected for a baseline PCV and total plasma protein concentration, venous blood gas (VBG) and electrolyte panel[§] (including blood glucose and plasma lactate concentrations), CBC,[**] and serum biochemical profile.[††] Admission vital parameters and differential WBC counts were evaluated to identify dogs that met systemic inflammatory response syndrome (SIRS) criteria at the time of hospital admission. Dogs were defined as having SIRS as previously described.[16] A fecal sample was also collected for double centrifugal fecal flotation using Sheather's sugar solution.[‡‡]

A central or peripheral IV catheter was placed in all dogs at hospital admission, followed by IV volume resuscitation using isotonic crystalloid fluids[§§] (15–45 mL/kg IV). The volume of resuscitation fluid provided to each dog was determined using clinical examination, evaluation of patient perfusion parameters, and the estimated intravascular volume deficit.[17,18] Additional IV fluid resuscitation, using crystalloids or colloids[***] (2–5 mL/kg IV), could be administered at the discretion of the lead clinician. The type and volume of fluids administered during fluid resuscitation were recorded for each dog. Vital parameters (rectal temperature, pulse rate, respiratory rate, and systemic arterial blood pressure) and calculated SI were recorded at pre- and postresuscitation time points. If hypoglycemia was identified on the initial VBG and electrolyte panel, a bolus of 50% dextrose[†††] (1–2 mL/kg IV) was supplemented based upon the degree of hypoglycemia. The 50% dextrose was diluted 1:4 using isotonic crystalloid fluids if given through a peripheral catheter, and given over 5 minutes. External warming was also initiated during cardiovascular resuscitation to maintain a rectal temperature >37.2°C (99°F). Once a dog exhibited adequate improvement in perfusion parameters (heart rate, pulse

quality, mentation, mucous membrane color, capillary refill time, and plasma lactate concentration) to indicate appropriate stabilization, the dog was then transitioned into its designated treatment protocol.

Dogs were randomized using a computer program[‡‡‡] and assigned to either the placebo or HIP treatment group. Following initial stabilization, dogs assigned to the placebo group received a single dose of 0.9% sodium chloride (NaCl)[§§§] (10 mL/kg IV) as a constant rate infusion (CRI) over 2 hours, whereas dogs assigned to the HIP group received a single dose of the commercial product (10 mL/kg IV) as a CRI over 2 hours. Both groups were monitored for signs consistent with a transfusion reaction (eg, change in vital parameters, restlessness, vomiting, tachypnea, facial swelling, and collapse) during the drug infusion. Inpatient treatment was the same for both groups following this single treatment infusion. The study was blinded, in that the clinicians administering the study drug were not the same as those providing treatment and recording clinical signs. The clinicians providing treatment and recording data were not aware of the dogs' treatment groups.

All dogs remained in the hospital for the duration of the treatment protocol. Both groups received IV isotonic crystalloid fluids at a base rate of 120 mL/kg/d.[17] Estimated ongoing losses (dictated by the volume and frequency of vomiting/diarrhea) were added to this base rate throughout hospitalization; correction of dehydration using a standard calculation was also performed over the first 24 hours.[18] A standardized dose of 20 mmol/L (mEq/L) KCl[****] was added to maintenance fluids to bring the total to 24 mmol/L (mEq/L) of potassium. The total amount of KCl was subsequently adjusted with additional supplementation added as needed using the dog's daily electrolyte panel and a corresponding chart.[17] Dextrose supplementation was provided to dogs whose blood glucose concentration was <4.44 mmol/L (80 mg/dL) on serial VBG and electrolyte panel measurements. Hypoglycemic dogs received a 25% dextrose bolus (1 mL/kg) IV followed by an additional 2.5–7.5% dextrose CRI as needed to maintain blood glucose above 4.44 mmol/L (80 mg/dL) within the isotonic crystalloid fluid bag. Additional treatments administered to both groups included cefoxitin[††††] (30 mg/kg IV q 8 h), maropitant[c] (1 mg/kg IV q 24 h), and buprenorphine[‡‡‡‡] (0.01 mg/kg IV q 8 h). Dogs that exhibited >3 episodes of vomiting in a 24-hour period or that exhibited persistent ptyalism were provided a 1-time dose of ondansetron[§§§§] (0.5 mg/kg IV). Additional treatments could be administered at the discretion of the primary clinician and were recorded in the medical record. Dogs were first offered a commercial canine convalescence diet[*****] within 24 hours of study admission, and then syringe fed 1 mL/kg PO every 6 hours if they demonstrated no voluntary appetite. If dogs refused to ingest this dose, or if feeding induced vomiting or regurgitation, that feeding attempt was aborted and was attempted at the next treatment time.

The following data were collected 12, 24, and 48 hours after the treatment infusion: vital parameters (rectal temperature, pulse rate, respiratory rate, and systemic arterial blood pressure), calculated SI, clinical severity score, VBG and electrolyte panel (including blood glucose and plasma lactate concentrations), PCV/TP, and CBC results. A second biochemical profile was performed at 48 hours. For each subsequent day of treatment, dogs received a VBG and electrolyte panel, PCV/TP, and a CBC. Other daily monitoring included physical examination, body weight, systemic arterial blood pressure, clinical severity score, presence/absence of voluntary appetite, and use of rescue antiemetics or other medications. Throughout hospitalization, rectal temperature, pulse rate, respiratory rate, and pain score were recorded every 6 hours.

Hospital discharge occurred when the following criteria were met: (a) no vomiting for ≥24 hours, (b) demonstration of voluntary appetite at least twice within a 24-hour period, (c) clinical severity score of ≤2, (d) a blood neutrophil count of ≥2,500 cells/μL, and (e) maintenance of normoglycemia without dextrose supplementation. Data recorded at hospital discharge for each dog included survival to hospital discharge (yes/no), duration of hospitalization (days), days until clinical severity score was ≤2, change in clinical severity score during hospitalization, and days until return of voluntary appetite. Whole blood was collected at time of discharge for CBC and biochemical profile. Dogs that did not survive until discharge underwent full necropsy.

2.1 | Statistical methods

Commercially available software was used to perform all statistical analyses.[†††††] Datasets were assessed for normality using the Shapiro–Wilk test. If data did not meet normality or if the data were "scores," a nonparametric approach (Wilcoxon test) was used to compare the treatment groups. Two-sided t-tests, including Mann–Whitney when applicable for non-Gaussian distribution, were used to compare the groups in regard to age, body weight at admission, duration of clinical signs prior to hospitalization, and total duration of hospitalization. Categorical variables were described using percentages, and the Fisher's exact test was used to test for differences between the treatment and control groups. Continuous variables not normally distributed are described as median (minimum and maximum) and normally distributed continuous variables are described as mean (standard deviation).

Baseline information regarding segmented neutrophil count, blood lactate concentrations, serum total cholesterol concentration, clinical severity score, pre-resuscitation SI, and postresuscitation SI was compared using 2-sided t-tests. Fisher's exact test for equality was incorporated when looking at the overall success of a treatment protocol, defined as completion of the assigned protocol, including survival and hospital discharge. For the continuous outcomes compared between treatments as well as time points, an "interaction term" of treatment time was included in the linear regression analysis and evaluated for significance. The linear regression was performed on the "ranks" of the outcome due to nonnormal distribution as well as small sample size in each time point. P-values of <0.05 were used to evaluate statistical significance.

3 | RESULTS

Thirty-one dogs were enrolled in the study over a 13-week period and randomized to receive either HIP (n = 16) or placebo (n = 15). Two

TABLE 1 Baseline measured variables (mean with standard deviation for normally distributed data; median with corresponding ranges for nonnormally distributed data) of dogs with canine parvoviral enteritis treated with hyperimmune plasma or placebo, measured at hospital admission

Measured variable	HIP (n = 16)	Placebo (n = 15)	*P*-value
Age (months)	4.5 (2–6)	3 (2–11)	0.05
Body weight (kg)	10.89 ± 7.27	6.11 ± 3.03	**0.03**
Duration of clinical signs prior to treatment (d)	2 (0.5–3)	1.0 (0.25–4.5)	0.25
Clinical severity score	6 (4–9)	6 (1–10)	0.78
Plasma lactate concentration (mmol/L) (mg/dL)	1.95 (0.9–4.1) 17.6 (8.1–36.9)	1.9 (1.2–4.7) 17.1 (10.8–42.3)	0.41
Preresuscitation shock index	1.16 (0.9–1.89)	1.43 (0.5- 2.68)	0.20
Postresuscitation shock index	0.86 (0.63–1.71)	0.99 (0.67–1.83)	0.16
Serum total cholesterol concentration (mg/dL)	257 (180–331)	226 (177–301)	0.08
Segmented neutrophil ($\times 10^9$/L) ($\times 10^3$/μL)	3.45 (0.1–14.1) 3.45 (0.1–14.1)	7.7 (0.2–17.9) 7.7 (0.2–17.9)	**0.02**
Presence of SIRS at admission	11/16	13/15	**0.23**

Abbreviations: HIP, hyperimmune plasma; SIRS, systemic inflammatory response syndrome. P-values of <.05 were used to evaluate statistical significance

additional dogs were evaluated for study enrollment but were deemed ineligible and rejected due to concurrent severe hookworm infection and external parasite infestation. A variety of breeds were represented in the study, with the majority of dogs being mixed breed. Dog breeds were categorized into overall groups based upon their predominant breed conformational characteristics. Breeds represented within the HIP group included terrier-type dog (4/16, 25%), pit bull-type dog (4/16, 25%), Pug (2/16, 12.5%), and 1 each of Rottweiler, Beagle, Deerhound, Labrador Retriever, Border Collie, and German Shepherd dog (1/16, 6.25%). Breeds represented in the placebo group included Mastiff (4/15, 26.7%), terrier-type dog (3/15, 20%), Pug (2/15, 13.3%), German Shepherd dog (2/15, 13.3%), and 1 each of Dachshund, Dalmatian, Doberman Pinscher, and pit bull-type dog (1/15, 6.7%).

The HIP group included 8 intact males and 8 intact females, whereas the placebo group included 13 intact males and 2 intact females. There was no difference in gender between the 2 groups (*P* = 0.05). Median age for the HIP group was 4.5 months (range, 2–6 months) and 3 months (range, 2–11 months) for the placebo group (*P* = 0.52). The mean body weight at admission was significantly higher for the HIP group (10.9 ± 7.3 kg) compared to the placebo group (6.1 ± 3.0 kg; *P* = 0.03). The median duration of clinical signs prior to presentation was 2 days (range: 0.5–3 days) for the HIP group and was and 1.0 day (range: 0.25–4.5 days) for the placebo group (*P* = 0.25). One dog each in the control and HIP groups had received a single dose of maropitant[‡] prior to study admission.

Baseline information was compared between the 2 groups to estimate disease equivalence upon study admission (Table 1). There was no significant difference in baseline clinical severity score, plasma lactate concentration, serum total cholesterol concentration, preresuscitation SI, or postresuscitation SI when comparing HIP and placebo dogs. There was no difference in the proportion of dogs that met the diagnostic criteria for SIRS at study admission between the placebo group (13/15, 86.7%) and the HIP group (11/16, 68.8%; *P* = 0.23). Seven of 16 dogs (43.75%) in the HIP group and 3 of 15 (20%) in the
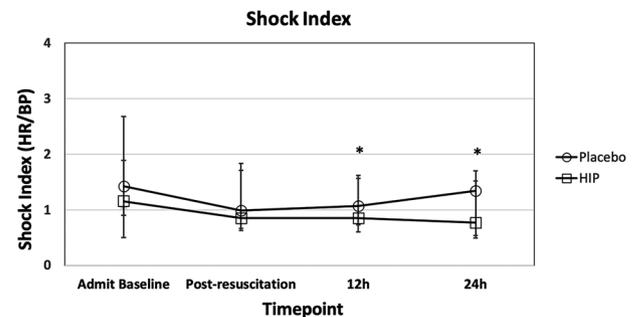

FIGURE 1 Median and range of shock index in dogs with canine parvoviral enteritis treated with hyperimmune plasma or placebo at admission, postresuscitation, and 12- and 24-hour postadmission. Hyperimmune plasma, n = 16; Placebo, n = 15
BP, systolic systemic arterial blood pressure; HIP, hyperimmune plasma; HR, heart rate

placebo group were neutropenic on presentation and study admission (*P* = 0.25). Baseline neutrophil count was significantly lower in the HIP group (median = 3.45×10^9/L [3.45×10^3/μL], range: 0.1–14.1×10^9/L [0.1 -14.1×10^3/μL]) compared to the placebo group (median = 7.7×10^9/L [7.7×10^3/μL], range: 0.2–17.9×10^9/L [0.2–17.9×10^3/μL]; *P* = 0.02). Fecal results were available for 18 dogs and 2 of 18 (11.1%) dogs were positive for intestinal parasites. One dog from the HIP group had confirmed *Toxocara canis*, and 1 dog from the placebo group had *Cystoisospora ohioensis*-like oocysts (coccidia).

Markers of shock (calculated SI and plasma lactate concentration) were compared between groups at baseline, postresuscitation, and at 12 and 24 hours (Figures 1 and 2). Plasma lactate concentration was also compared at the 48-hour mark and at hospital discharge. HIP-treated dogs demonstrated a lower SI at the 12-hour mark (median = 0.85, range: 0.6–1.6) compared to placebo-treated dogs (median = 1.08, range: 0.7–1.6; *P* = 0.04). Dogs treated with HIP continued to have a lower SI at 24 hours (median = 0.77, range: 0.5–1.5) compared to
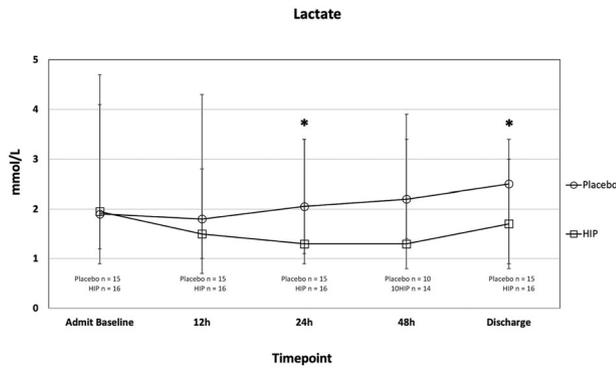
FIGURE 2 Median and range of plasma lactate concentration in dogs with canine parvoviral enteritis treated with hyperimmune plasma or placebo at admission, 12-, 24-, and 48-hour postadmission, and at hospital discharge
HIP, hyperimmune plasma

placebo-treated dogs (median = 1.34, range: 0.5–1.7; $P = 0.02$). There was no significant difference in plasma lactate concentration at the 12-hour mark between HIP-treated dogs (median = 1.5 mmol/L, range: 0.7–2.8) and placebo-treated dogs (median = 1.8 mmol/L, range: 1.0–4.3; $P = 0.13$). However, plasma lactate concentration was significantly lower at the 24-hour mark in HIP-treated dogs (median = 1.3 mmol/L, range: 0.9–3.4 mmol/L) when compared to the placebo-treated dogs (median = 2.1 mmol/L, range: 1.1–3.4 mmol/L; $P = 0.01$). There was no significant difference in plasma lactate concentration at the 48-hour mark between HIP-treated dogs (median = 1.3 mmol/L, range: 0.8–3.9) and those given placebo (median = 2.2 mmol/L, range: 1.4–3.4; $P = 0.10$). However, plasma lactate concentration was significantly lower at hospital discharge in the HIP group (median = 1.7 mmol/L, range: 0.8–3.0) when compared to the placebo group (median = 2.5, range: 0.9–3.4; $P = 0.04$).

Changes in serum albumin concentration did not appear to be responsible for the observed changes in markers of shock, as no differences were present between groups during hospitalization (Table 2). The total dose of isotonic crystalloids administered during initial fluid resuscitation and during the first 24 hours of hospitalization was not significantly different between the HIP group (81.3 mL/kg ± 46.3 mL/kg) and the placebo group (102.5 mL/kg ± 28.7 mL/kg; $P = .14$). This total dose of isotonic fluids excluded the placebo dose of 0.9% NaCl. No dogs received other

artificial or synthetic colloids during the first 24 hours of hospitalization, and there were no observed clinical signs consistent with acute transfusion reaction in any dogs receiving HIP.

Rescue antiemetics were used in both groups, but their overall use was low. There was no difference ($P = 0.6$) in the number of HIP-treated dogs requiring ondansetron during hospitalization (1/16, 6.25%) compared to the placebo dogs (2/15, 13.3%). Pain was closely monitored in all dogs, and every dog received buprenorphine according to treatment protocol. Additional pain medications could be provided per clinician discretion. One dog in the placebo group also received a ketamine CRI, and 1 dog in the HIP group received a lidocaine CRI. Additional medications used in the HIP group included metronidazole[‡‡‡‡‡] (1/16, 6.25%) and metoclopramide[§§§§§] (1/16, 6.25%). Additional medications and treatments used in the placebo group included metronidazole (2/16, 13.3%), metoclopramide (1/16, 6.25%), enrofloxacin[******] (1/16, 6.25%), ampicillin–sulbactam[††††††] (1/16, 6.25%), and supplemental oxygen therapy (1/16, 6.25%). All dogs received early enteral nutrition support within 24 hours of study admission, and no dogs received parenteral nutrition.

Of those dogs presenting nonneutropenic at study admission, 7 of 9 (77.8%) in the HIP group and 4 of 12 (33.3%) in the placebo group became neutropenic during hospitalization ($P = 0.08$). For those dogs that presented or became neutropenic during hospitalization (n = 14 for HIP group; n = 7 for placebo group), there was no difference in time to resolution of neutropenia (days) between groups ($P = 0.43$).

PCV was compared between groups at baseline, 24 and 48 hours posttreatment, and at hospital discharge. There was no significant difference in baseline PCV between HIP-treated dogs (median = 44%, range: 28–57%) and those that received placebo (median = 38%, range: 28–54%; $P = 022$), and there remained no difference at 24 hours ($P = 0.11$). Dogs in the HIP group had a higher PCV (median = 42%, range: 23–48%) at 48 hours than those in the placebo group (median = 36%, range: 19–40%; $P = 0.01$). PCV in the HIP-treated dogs remained higher (median = 46%, range: 35–52%) than placebo-treated dogs (median = 35%, range: 23–42%; $P = 0.01$) at hospital discharge.

There was no difference in clinical severity scores between groups at any time point during the study (Table 3). There was no difference in duration of hospitalization when comparing dogs in the HIP group (median = 3.2 days, range: 0.8–10 days) to those in the placebo group (median = 2.8 days, range: 1–8.4 days; $P = 0.35$). There was also no difference when comparing time to return of voluntary appetite between

TABLE 2 Median and range of serum albumin concentration in dogs with canine parvoviral enteritis treated with hyperimmune plasma or placebo at baseline, 24- and 48-hour posttreatment, and at hospital discharge

Serum albumin concentration	Baseline	24 hours	48 hours	Hospital discharge
HIP (g/L) (g/dL)	29.5 (20–37) 2.95 (2–3.7)	24.5 (17–30) 2.45 (1.7–3)	25.5 (19–32) 2.55 (1.9–3.2)	23.5 (18–32) 2.35 (1.8–3.2)
Placebo (g/L) (g/dL)	28 (23–37) 2.8 (2.3–3.7)	24 (16–34) 2.4 (1.6–3.4)	24.5 (22–27) 2.45 (2.2–2.7)	25.5 (10–37) 2.55 (1–3.7)
P-value	0.77	0.83	1.0	0.93

Abbreviation: HIP, hyperimmune plasma.

TABLE 3 Median and range of clinical severity scores in dogs with canine parvoviral enteritis treated with hyperimmune plasma or placebo at baseline, 24- and 48-hour posttreatment, and at hospital discharge

Clinical Severity Score	Admission	24 hours	48 hours	Hospital discharge
HIP	6 (4–9)	5 (1–8)	4 (1–9)	2 (0–2)
Placebo	6 (1–10)	3 (0–8)	3 (0–7)	1 (0–2)
P-value	0.78	0.12	0.43	0.91

Abbreviation: HIP, hyperimmune plasma.

the HIP group (median = 2.4 days, range: 0.3–5.5 days) and placebo group (median = 0.9 days, range: 0.33–8.4 days; $P = 0.06$) or when comparing time to resolution of vomiting between the HIP group (median = 1.3 days, range: 0.25–9 days) and placebo group (median = 1.6 days, range: 0–6.7 days; $P = 0.73$).

Overall treatment success, defined as survival to hospital discharge, was 16 of 16 (100%) for the HIP group and 14 of 15 (93.3%) for the placebo group ($P = 0.32$). The dog that died experienced respiratory arrest 4 days after admission. Histopathologic findings included necro-hemorrhagic enteritis, diffuse bone marrow hypoplasia, evidence of severe fibrinous pleuropneumonia, and pleural effusion.

4 | DISCUSSION

To the authors' knowledge, this is the first randomized, placebo-controlled study aimed to determine the clinical efficacy of a commercially available HIP product in dogs with CPV. This study demonstrated that HIP administered to CPV dogs at a dose of 10 mL/kg IV within the first 6 hours of hospitalization appears to be safe and improved markers of shock (SI, plasma lactate concentration) during the initial hospitalization period.

The calculation of a SI, which is defined as the ratio of heart rate to systolic arterial blood pressure, was developed as a simple method to quantify the severity of shock on presentation to the emergency room.[19] A SI of >0.9 or 1.0 is reported to be specific and sensitive for detection of moderate to severe shock in people and dogs.[19-21] In veterinary studies, a SI > 1.0 accurately identified dogs presenting to an emergency room in shock compared to both healthy dogs and those presenting to an emergency room not in shock.[21] When combined with a lower plasma lactate concentration, these parameters suggest that HIP treatment was associated with improved cardiovascular stability and perfusion early in the hospitalization period.

The combination of lower values for both SI and plasma lactate concentration in the HIP versus placebo group suggests that HIP treatment was associated with improved cardiovascular stability and perfusion by 24 hours after hospitalization. The lower SI at the 12-hour and 24-hour marks suggest that HIP was associated with rapid initial improvements in cardiovascular stability, whereas the delay in improvement of lactate until the 24-hour mark may indicate

a lag in improvement in global perfusion. The lower plasma lactate concentration in the HIP group at hospital discharge may be associated with a sustained improvement in tissue perfusion in this group. However, the slightly higher plasma lactate concentration in the placebo group at discharge may have been due to an unrelated mild relative hyperlactatemia from trembling, exercise, or restraint. Blood lactate concentration was not a criterion for hospital discharge. Although volume replacement may have contributed to improved markers of shock in the HIP group, both HIP and placebo groups received similar fluid resuscitation prior to randomization, and there was no significant difference in total fluid volume administered by 24 hours. Therefore, other factors should be considered, such as the anti-inflammatory effects of passive immunity and the role of plasma in preservation of the endothelial glycocalyx layer.

Endothelial hyperpermeability is a hallmark of SIRS and sepsis that directly contributes to high morbidity and mortality in critically ill patients.[22] In a previous study,[23] 36% of dogs with parvoviral enteritis met the diagnostic criteria for SIRS on admission, and the higher mortality in this subgroup highlights the role of SIRS in the pathophysiology of CPV. Sepsis and inflammatory endothelial injury lead to ubiquitous degradation of the glycocalyx, endothelial hyperpermeability, a marked decrease in systemic vascular tone, hypoalbuminemia, and disruption of the microcirculation that leads to distributive shock and multiorgan dysfunction.[22,24] Recent in vitro studies support an endothelial stabilizing role of fresh frozen plasma (FFP) as it reduces vascular endothelial cell permeability, partially restores the damaged endothelial glycocalyx and syndecan-1 expression,[25,26] and decreases expression of endothelial adhesion markers[27] and endothelial leukocyte binding.[25,27,28] Although specific markers of endothelial hyperpermeability were not measured in the current study, dogs severely affected with CPV with SIRS and sepsis likely develop endothelial hyperpermeability and may benefit from the endothelial stabilizing role of plasma.

Endotoxin, a unique lipopolysaccharide (LPS) present in the outer cellular membranes of normal gram-negative bacterial gut flora, is a potent bacterial toxin that plays an integral role in the pathophysiology of gram-negative sepsis and CPV infection.[29,30] Previous studies evaluating neutralization of LPS endotoxin with equine-derived antiendotoxin antibody have produced conflicting results,[2,6,31] and equine-origin proteins may be associated with anaphylaxis.[32] Therefore, a canine-derived product with standardized anti-LPS activity may have clinical utility in treating dogs with CPV.

Plasma transfusion therapy has additional potential benefits as an adjunct therapy in CPV patients. Plasma can serve as a source of albumin and associated oncotic support, immunoglobulins, and serum protease inhibitors.[2,13,33,34] Anecdotal reports of using convalescent serum from previously CPV-infected dogs as a source of passive immunization have been described.[33] In an older study, CPV-infected dogs were passively immunized with IV convalescent canine serum 24 hours after oral CPV inoculation.[15] The passively immunized dogs did not develop CPV-associated clinical signs, lymphopenia, or fecal virus excretion, and had no evidence of intestinal CPV infection at necropsy.[15] Infused antibodies in CPV-immune plasma could

theoretically neutralize free virus in plasma and suppress viral spread by inhibiting entry into new target cells.[8,13] In a more recent study, however, a single 12-mL dose of CPV-immune plasma as adjunctive treatment of symptomatic CPV dogs failed to improve time to hematologic recovery, magnitude of viremia, weight change, or duration of hospitalization.[13] The current study also did not demonstrate improvements in duration of hospitalization, survival to hospital discharge, or return of voluntary appetite in dogs administered HIP. To the authors' knowledge, however, this is the first study documenting improved markers of shock in CPV-infected dogs administered HIP.

There were no significant differences in serum albumin concentration between groups at any time point, and there was no difference in the total dose of crystalloid fluids administered within the first 24 hours of hospitalization. However, plasma has a higher colloid osmotic pressure than the saline placebo, and it is possible that the albumin and globulins within the HIP product resulted in an increase in intravascular colloid osmotic pressure that contributed to improvement in SI and plasma lactate concentration reported in the HIP group.[17] Differences in fluid colloid osmotic pressure and their associated effects on IV volume could have been responsible for some of the cardiovascular benefits observed in the treatment group during the first 48 hours of treatment. However, given the lack of difference in serum albumin between groups, it seems unlikely that colloid osmotic pressure alone is responsible for the observed benefits of HIP administration.

Baseline and 24-hour PCVs were similar between HIP and placebo groups. However, HIP-treated dogs had higher PCV at 48 hours and at hospital discharge when compared to those treated with placebo. Possibilities for this difference may include a combination of age, blood sampling techniques, fluid therapy during hospitalization, gastrointestinal bleeding, and other mechanisms that have been described with anemia of inflammation.[35] Placebo-treated dogs had a median PCV of 35% at hospital discharge; however, lower PCV is unlikely responsible for the higher plasma lactate concentration reported at hospital discharge in the placebo dogs. In experimental studies of euvolemic hemodilutional anemia, PCV < 15% was necessary to increase plasma lactate concentration.[36]

There was no significant difference in duration of hospitalization or mortality between dogs treated with HIP and placebo in this study; however, the study was not powered to fully evaluate these effects. It is possible that type II error contributed to the lack of statistical significance as post hoc power analysis suggests that 260 dogs would be needed to detect a difference in mortality between the HIP and placebo groups. Because survival is already favorable in dogs with CPV that receive appropriate supportive care, other outcome markers or analysis of HIP in more severely affected CPV populations might provide better insights regarding the benefits of HIP. Future studies could evaluate the role of HIP in preventing morbidity in CPV-exposed, asymptomatic dogs, or the role of HIP in symptomatic dogs that have not yet become neutropenic.

Disease equivalence was assessed using a number of baseline parameters, including body weight and neutrophil count. There was a significant difference between these 2 parameters when comparing

HIP and placebo groups at baseline; dogs in the HIP group had higher mean body weight and lower neutrophil count at hospital admission. Additionally, 77% of dogs treated with HIP went on to develop neutropenia compared to 33% of placebo-treated dogs, though this difference was not statistically significant. Previous studies have identified changes in leukocyte count as a predictor of outcome in dogs with CPV,[37] suggesting that HIP-treated dogs may have had more significant CPV infection. Because the timing of HIP administration was not standardized according to timing of CPV exposure, this observation cannot be linked directly to lack of product efficacy, but may suggest a more compromised population. However, low baseline serum total cholesterol concentration has also been proposed to be an index of disease severity and negative prognostic indicator in parvoviral enteritis,[38] and there was no significant difference in baseline serum total cholesterol concentrations between placebo and HIP groups in this study.

This study has limitations. For example, the placebo group received 0.9% NaCl instead of nonhyperimmune FFP. Isotonic crystalloids were chosen as the placebo because a practitioner would likely be choosing between a colloid versus crystalloid for continued resuscitation of a given CPV case, and 0.9% NaCl has a lower cost and greater availability than HIP. Theoretical benefits of HIP over FFP include high concentrations of antibodies against CPV and E. coli endotoxin and rigorous quality control procedures to ensure product potency. Future studies should consider comparing HIP to FFP to better assess these theoretical benefits. The small study sample size and the potential limitations this has on detection of differences between groups for some parameters have already been noted. Canine Acute Patient Physiologic and Laboratory Evaluation scores could have been determined for each patient at admission to improve objective evaluation of baseline illness severity differences between treatment groups.[39] The Clinical Severity Score used in this study is a previously developed, disease-specific score that accounts for attitude, gastrointestinal signs, and appetite.[12] However, this clinical severity score has not been externally validated.

Additionally, neither the timing nor the dose of HIP was standardized to the development of clinical signs or other potential cytokine or LPS measures but rather to the time of hospital presentation or the dog's body weight, respectively. The HIP manufacturer recommends that the product be administered at a dose of 10 mL/kg over 1 hour. Local hospital policy recommends administration of blood product transfusions over 2–4 hours in order to ensure patient safety and facilitate careful observation for transfusion reactions. Therefore, the HIP product was administered at a dose of 10 mL/kg as a continuous IV infusion over 2 hours to conform to hospital guidelines. Further investigation into the ideal timing and dosing of HIP administration may lead to improved efficacy.

Additionally, CPV or LPS antibody titers within the HIP product were not quantified during the current study. The manufacturer states that CPV antibody titers range from 1:20 to ≥1:80, and antiendotoxin antibodies titers range from 1:10,000 to 1:60,000. It is unknown what antibody titers would be ideal for the management of CPV and related sepsis, and it is possible that the antibody titers were insufficient to adequately neutralize CPV in the circulation of infected dogs. It is also

possible that repeated administration of HIP could have improved its efficacy. The present study was designed with a single administration of CPV-immune plasma, which would be a reasonable treatment strategy for either inpatient or outpatient CPV treatment. Measurement of specific pro- or anticoagulant factors was not measured in this population of dogs. It is possible that various components of HIP, including pro- and anticoagulant factors, could have played a role in the clinical benefits observed in the treatment group. Finally, although both treatment groups received early enteral nutritional support, the exact nutritional intake was not quantified for each dog.

In conclusion, dogs with canine parvoviral enteritis that received HIP within the first 6 hours of hospitalization demonstrated improved markers of shock during the first 24 hours of hospitalization. Larger studies are needed to confirm these findings and to determine the effects of HIP on duration of hospitalization and mortality. Future studies evaluating HIP dose and timing of HIP administration are needed to better define the possible clinical benefits of this product. HIP was safely provided to dogs with CPV with no noted adverse effects in this population of critically ill dogs.

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ENDNOTES

* Caniplas, Plasvacc Australia (Plasvacc), Kalbar, Queensland, Australia.
† SNAP Parvo Test, Idexx, Westbrook, ME.
‡ Maropitant citrate (Cerenia), Pfizer Animal Health, New York, NY.
§ ABL 800 Flex Blood Gas Analyzer, Radiometer, Bronshoj, Denmark.
** CBC Advia 120 Hematology System, Siemens Healthcare Diagnostics, Inc, Newark, DE. Manual differentials using Wright–Geimsa stain were performed by a DACVP.
†† Cobas C501 analyzer, Diamond Diagnostics, Holliston, MA.
‡‡ Sheather's sugar solution, Jorgensen Labs, Loveland, CO.
§§ Lactated Ringer's Injection, Baxter, Deerfield, IN.
*** Hetastarch, Abbott Laboratories, North Chicago, IL.
††† Dextrose, Hospira, Inc, Lake Forest, IL.
‡‡‡ Microsoft Excel, Redmond, WA.
§§§ 0.9% Sodium chloride injection, Baxter, Deerfield, IN.
**** Potassium chloride, APP Pharmaceuticals, Schaumburg, IL.
†††† Cefoxitin, Apotex Corporation, Weston, FL.
‡‡‡‡ Buprenorphine, Reckitt Benckiser Pharmaceuticals, Inc, Richmond, VA.
§§§§ Ondansetron, West-Ward, Eatontown, NJ.
***** Hill's a/d, Hill's Pet Nutrition, Topeka, KS.
††††† SAS v9.4, SAS Institute Inc., Cary, NC.
‡‡‡‡‡ Metronidazole injection, Baxter, Deerfield, IN.
§§§§§ Metoclopramide injection, Pfizer, Gladstone, NJ.
****** Enrofloxacin (Baytril) injectable, Bayer, Whippany, NJ.
†††††† Ampicillin-Sulbactam (Unasyn) injectable, West-Ward, Eatontown, NJ.

ACKNOWLEDGMENT

CONFLICT OF INTEREST

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APPENDIX 1

CLINICAL SEVERITY SCORE FOR DOGS WITH CANINE PARVOVIRAL ENTERITIS[12]

Scoring categories	0	1	2	3
Attitude	Normal	Mild-moderate depression	Severe depression	Collapsed or moribund
Appetite	Normal	Voluntarily eats small amounts	No interest	N/A (Not applicable)
Vomiting episodes	Absent	Mild (1 in 12 h)	Moderate (2–5 in 12 h)	Severe (≥6 in 12 h)
Feces	Formed	Soft/pasty	Watery diarrhea, nonbloody	Watery diarrhea, bloody