J Vet Sci. 2024 Jan;25(1):e11
https://doi.org/10.4142/jvs.23139
pISSN 1229-845X·eISSN 1976-555X

Journal of Veterinary Science

Original Article
Infectious Disease

Check for updates

Clinical and inflammatory response to antiviral treatments in dogs with parvoviral enteritis

Nergis Ulas [1,*], **Yunusemre Ozkanlar** [2], **Seckin Ozkanlar** [3], **Mehmet Ozkan Timurkan** [4], **Hakan Aydin** [4]

[1]Department of Internal Medicine, Faculty of Veterinary, Ataturk University, Erzurum 25240, Turkey
[2]Department of Internal Medicine, Faculty of Veterinary, Ondokuz Mayis University, Samsun 55139, Turkey
[3]Department of Biochemistry, Faculty of Veterinary, Ataturk University, Erzurum 25240, Turkey
[4]Department of Virology, Faculty of Veterinary, Ataturk University, Erzurum 25240, Turkey

OPEN ACCESS

Received: May 23, 2023
Revised: Sep 11, 2023
Accepted: Sep 24, 2023
Published online: Oct 27, 2023

***Corresponding author:**
Nergis Ulas
Department of Internal Medicine, Faculty of Veterinary, Ataturk University, Erzurum 25240, Turkey.
Email: nergisulas@atauni.edu.tr
https://orcid.org/0000-0003-2340-6882

ABSTRACT

Background: Canine parvoviral enteritis (CPE) is a fatal disease worldwide. The treatment of CPE is based mainly on supportive and symptomatic treatment. Antiviral addition to the treatment may result in a higher survival.
Objectives: This study evaluated the effects of antiviral treatments with a standardized treatment (ST) on the clinical and inflammatory response of dogs with naturally occurring CPE.
Methods: Twenty-eight dogs with CPE caused by canine parvovirus type 2 were divided randomly into treatment groups. The ST group received fluid, antibiotic, antiemetic, and deworming treatments. The antiviral treatment groups received the same ST with an additional antiviral drug, recombinant feline interferon omega (rFeIFN-ω), oseltamivir (OSEL) or famciclovir (FAM).
Results: Compared to the healthy control, the tumor necrosis factor-α, interleukin-1β, interferon (IFN)-α, IFN-γ, haptoglobin, and C-reactive protein values were high ($p < 0.05$) on day zero. At presentation, mild lymphopenia, neutropenia, and a high neutrophil to lymphocyte (LYM) ratio (NLR) were also observed. Adding rFeIFN-ω to the ST produced the best improvement in the clinical score with a decreased NLR, while leucocytes remained low and inflammatory markers stayed high on day three. The survival rates of the groups were 85.7% in ST+IFN, 71.4% in ST+OSEL, 71.4% in ST+FAM, and 57.1% in ST groups on day seven.
Conclusions: Antiviral drugs may be valuable in treating CPE to improve the clinical signs and survival. In addition, the decrease in NLR in favor of LYM may be an indicator of the early prognosis before the improvement of leukocytes, cytokines, and acute phase proteins in CPE.

Keywords: Acute-phase proteins; canine parvovirus; cytokines; leukocyte

INTRODUCTION

Canine parvoviral enteritis (CPE) is a common cause of morbidity and mortality in dogs worldwide, particularly in young animals. The canine parvovirus (CPV) belongs to the genus Protoparvovirus of the Parvoviridae family and is one of the smallest known viruses. The CPV has a non-enveloped icosahedral structure that contains a linear, non-segmented, negative sense, single-stranded DNA genome with 5,323 bases [1,2]. This genome encodes three structural proteins (VP1, VP2, and VP3) and two non-structural (NS) proteins (NS1 and NS2).

ORCID iDs

Nergis Ulas
https://orcid.org/0000-0003-2340-6882
Yunusemre Ozkanlar
https://orcid.org/0000-0002-1281-5413
Seckin Ozkanlar
https://orcid.org/0000-0001-7717-797X
Mehmet Ozkan Timurkan
https://orcid.org/0000-0002-0458-7887
Hakan Aydin
https://orcid.org/0000-0003-2200-1744

Author Contributions

Conceptualization: Ulas N, Ozkanlar Y; Data curation: Ulas N; Funding acquisition: Ozkanlar Y, Ulas N, Ozkanlar S, Timurkan MO, Aydin H; Methodology: Ulas N, Ozkanlar Y, Ozkanlar S, Timurkan MO, Aydin H. Supervision: Ozkanlar Y. Data analysis: Ulas N, Ozkanlar Y. Writing - original draft: Ulas N, Ozkanlar Y. Writing - review & editing: Ulas N, Ozkanlar Y, Ozkanlar S, Timurkan MO, Aydin H.

Conflict of Interest

The authors declare no conflicts of interest.

Funding

This work was supported by the Scientific and Technological Research Council of Turkey - TUBITAK - under the project TOVAG 114O037.

VP2 is the primary structural protein in the capsid and determines its antigenic properties [2]. The CPV exists in two forms (CPV 1 and 2), and CPV type 2 has three genetic variants (CPV-2a, CPV-2b, and CPV-2c) [3]. Despite widespread vaccination, CPV-2 is a significant threat to young dogs [4-6]. The clinical signs of CPE are lethargy, inappetence, vomiting, and diarrhea ranging from soft to mucoid and hemorrhagic. The gastrointestinal tract, marrow, lymphoid tissue, and myocardium are more prone to the CPV, but few studies have reported the skin and nervous system findings [7,8]. The treatment of CPE is largely supportive until the clinical signs resolve to correct dehydration, vomiting, diarrhea, secondary bacterial infection, and systemic inflammation. The survival rate of dogs with CPE can be more than 80% with treatment but as low as 9.1% without [3,9]. Therefore, several studies have investigated treatment protocols to improve inflammatory response and survival rate [10-13].

The gold standard for treating CPE involves administering IV fluids to restore intravascular fluid volume, replenish fluid losses, and maintain hydration, along with ancillary therapies, such as antiemetics, antibiotics, and nutrition [3]. Previous studies have suggested that antiviral drugs may be included in the treatment of CPE to reduce clinical signs and mortality [3,14,15]. Interferons (IFNs) have antiviral, antiproliferative, and immunomodulatory effects. Human IFN has been implemented in veterinary medicine [16,17]. Evidence suggests that recombinant feline IFN omega (rFeIFN-ω) has beneficial effects in treating CPE [18] because of the rapid improvement of clinical signs, lowering mortality [19]. Oseltamivir (OSEL) has been shown to prevent weight loss and improve white blood cells (WBCs) in CPE with no major adverse effects [15]. Therefore, OSEL and rFeIFN-ω may be an antiviral therapy for CPE [3,19]. Famciclovir (FAM) treats feline rhinotracheitis caused by the herpes virus in cats [20]. The drug can be used in dogs because of the safety of the metabolic and pharmacokinetic activities, but there have been few studies [21].

CPV causes leukopenia with regard to lymphopenia, and neutropenia in severe cases. An assessment of blood leucocyte changes provides valuable prognostic information because CPV causes lymphoid atrophy and marked bone marrow hypocellularity. The systemic inflammatory response of the body to infection can be varied in each individual with different innate and adaptive immunity, while treatment is essential for improving the survival rate. Therefore, prognostic indicators [11] and specific biomarkers [12] may help determine the clinical severity and progression of the disease associated with systemic inflammatory response [22] in CPE. This study detected CPV positivity in the laboratory (by polymerase chain reaction [PCR]) and the clinical findings. Therefore, this randomized clinical trial evaluated the systemic inflammatory responses and clinical efficacies of possible antiviral treatments as an adjunct to the standardized treatment (ST) of dogs with CPE. A prospective evaluation of the clinical severity scores, blood leucocytes, cytokines, and acute phase protein response was also conducted.

MATERIALS AND METHODS

Animals

Client-owned dogs were eligible for study inclusion, demonstrating the naturally acquired clinical signs of CPE (i.e., lethargy, vomiting, and diarrhea). The dogs were excluded from the study if they had a CPV vaccination history. The dogs with a CPE infection participating in this trial had no comorbidity except for ascarid infestation. All CPE dogs received the same antiparasitic therapy for deworming. A specific diagnosis of CPV-2 was made by a PCR assay

of samples at the virology laboratory of the veterinary faculty following the positive result of a rapid enzyme-linked immunosorbent assay (ELISA) test. This study was approved by the Animal Experiments Local Ethics Committee of the University (HADYEK, decision No: 2013/162).

Study design and treatments

The present study was a prospective and randomized clinical trial in dogs with naturally occurring parvoviral enteritis. The dogs with CPE were employed randomly in groups to evaluate the clinical efficacies of treatment protocols compared to the ST. **Table 1** lists the study design and drug administrations in the groups. Twenty-eight one-to-six-month-old puppies of different breeds and genders with natural CPE caused by a CPV-2 infection were divided randomly into four treatment groups. In addition, seven healthy two-to-six-month-old puppies were used as controls for the hematological and biochemical comparisons. **Table 2** lists the breed, age, and sex of the animals included in the study. The dogs with CPE were hospitalized for seven days in individual cages, and an IV catheter was inserted to administer fluids and medications to maintain the same inpatient protocol in all dogs. Clinical data were recorded, and blood sampling was done on days zero, three, and seven. All dogs were discharged from

Table 1. Study design and drug administrations in the groups

Groups	No.	Administrations and treatments
Healthy control	7	N/A
CPE		
ST	7	Fluid (lactated Ringer's solution plus dextrose) Antibacterial (trimethoprim plus sulfadoxine) Antiemetic (metoclopramide) Deworming (fenbendazole, pyrantel, praziquantel)
ST+IFN	7	ST plus recombinant feline interferon omega
ST+OSEL	7	ST plus oseltamivir
ST+FAM	7	ST plus famciclovir

CPE, canine parvoviral enteritis; ST, standardized treatment; IFN, interferon; OSEL, oseltamivir; FAM, famciclovir; N/A, not available.

Table 2. The breed, age and sex of the animals in the groups

Dog No.	Healthy control	CPE			
		ST	ST+IFN	ST+OSEL	ST+FAM
1	Mixed breed 1-month-old Male	Kangal 6-month-old Male	Rottweiler 2-month-old Female	Kangal 2-month-old Male	Mixed breed 1.5-month-old Female
2	Mixed breed 1.5-month-old Male	Boxer 2.5-months-old Male	Rottweiler 2-month-old Male	Kangal 2-month-old Male	Mixed breed 1.5-month-old Female
3	Mixed breed 2-month-old Female	Mixed breed 3-month-old Female	Kangal cross 2-month-old Male	Terrier 2-month-old Female	Mixed breed 2-month-old Male
4	Mixed breed 1-month-old Male	Mixed breed 3-month-old Female	Kangal cross 2-month-old Female	Kangal cross 1.5-month-old Female	Kangal 3-month-old Female
5	Rottweiler 1.5-month-old Female	Kangal 1.5-month-old Male	Kangal 1-month-old Male	Pointer cross 3-month-old Male	Mixed breed 3-month-old Female
6	Kangal 1.5-month-old Female	German Shepherd 3-month-old Male	Golden retriever 2-month-old Male	Kangal cross 1-month-old Female	Kangal 1-month-old Male
7	Kangal 4-month-old Male	Rottweiler 2-month-old Female	German Shepherd 2-month-old Male	Doberman 2.5-month-old Male	Kangal 1-month-old Female

CPE, canine parvoviral enteritis; ST, standardized treatment; IFN, interferon; OSEL, oseltamivir; FAM, famciclovir.

https://doi.org/10.4142/jvs.23139

the hospital on day seven because all surviving dogs ceased vomiting and diarrhea after one week of hospitalization. The dogs were followed up for 14 days after the discharge to confirm the survival of the dogs.

ST protocol

The ST protocol was fluid resuscitation, antibacterial drugs, antiemetic therapy, and deworming. Fluid therapy was lactated Ringer's solution plus 5% dextrose with potassium chloride (Polifarma, Turkey). The amount of fluid was estimated when the dog submitted to the clinics based on the degree of dehydration (ranging from 5%–12%). The initial volume (L) of fluid required has been calculated as the percentage of dehydration times body weight (kg) to achieve clinical rehydration, and fluid infusion was continued daily (120 mL/kg/d IV). The antibacterial drug was trimethoprim 4 mg/kg IM q24h plus sulfadoxine 20 mg/kg IM q24h for five days (Animar, Ceva, Turkey). Antiemetic therapy was metoclopramide 2 mg/kg IV q24h for five days (Metpamid, Sifar, Turkey). Antiparasitic therapy for deworming was fenbendazole, pyrantel plus praziquantel (Caniverm, Bioveta, Turkey) on the first day. All dogs were fed the same food (gastrointestinal formula) throughout the study to maintain consistency in nutrition. The dogs in the ST group (n = 7) had the ST protocol as described above.

Antiviral treatments

The dogs in the antiviral treatment groups had ST protocol with an additional antiviral medication. The antiviral drugs in the groups were rFeIFN-ω 2.5 MU/kg IV q24h for three days (rFeIFN-ω, Virbagen omega, Virbac, France) in the ST+IFN group (n = 7), OSEL 2 mg/kg PO q12h for five days (Enfluvir, Atabay, Turkey) in the ST+OSEL group (n = 7), and FAM 40 mg/kg PO q12h for five days (Famvir, Novartis, Turkey) in the ST+FAM group (n = 7). All orally used drugs were given IV antiemetic therapy to ensure drug swallowing, which was confirmed by a visual inspection of the oral cavity after administration.

Clinical scoring

After obtaining anamnesis from the owners, the clinical findings were recorded daily during the study. A score was assigned from 0 to 3 or 4 based on the degree of each finding as follows: rectal temperature (0, 1, 2, and 3 for 37.5–39.4, ≥ 39.5, 37.1–37.4, and ≤ 37 °C, respectively), general condition (1, 3, and 4 for lethargy, depression, and coma, respectively), dehydration degree (1, 3, and 4 for 5, 5–10, > 10% dehydration, respectively), physical consistency of feces (2 and 4 for liquid-mucoid and hemorrhagic, respectively), abdominal pain (1 and 2 for mild and severe, respectively), and vomiting (3). The maximum score was 20 for death, as described previously [23]. A daily clinical score is the sum of the normalized score of two observations per day, and the total clinical score is the sum of daily scores obtained on days zero, three, and seven. Clinical scoring findings are presented according to the days of blood sampling to evaluate the findings.

Rectal swab and blood collection

A pair of rectal swab samples were obtained directly from the rectum of each dog before treatment (day 0) for the rapid ELISA and PCR assays. The blood samples were collected from the dogs on days zero, three, and seven of treatment. Blood was drawn from the antebrachial cephalic vein into a pair of spray-coated vacutainers with anti-coagulant K2EDTA (Becton Dickinson, USA) for hematological and virological analyses. Blood was also drawn into spray-coated silica vacutainers containing a polymer gel without an anti-coagulant (Becton Dickinson) for biochemical analysis.

Virological analysis

Fecal samples of the dogs showing the clinical signs of CPE were first evaluated for the positivity of the viral antigens of CPV with a rapid ELISA test in the clinic. A PCR assay was performed in the samples to confirm the diagnosis made by the in-clinic assay and determine the type of CPV antigen [24].

Rapid ELISA test of fecal samples

A solid phase chromatographic immunoassay was used to detect the CPV and canine coronavirus (CCV) antigens in feces using a rapid ELISA test kit (Anigen Rapid CPV/CCV Ag, RG11-05, Bionote, Korea). Briefly, fecal swab samples from dogs before treatment were dipped into the diluent and mixed for 10 sec. Subsequently, four drops of the sample solution were dropped onto the wells of the cassette to determine the immunochromatographic positivity within 5–10 min for the viral antigens of the CPV and CCV, as described by the manufacturer's instruction. The positive dogs for solely CPV were eligible for a further PCR assay.

Preparation of fecal and blood samples for PCR assay

The fecal samples were vortexed after treatment with the phosphate-buffered saline (PBS) buffer (pH 7.5) and centrifuged at 3,000 rpm for 10 min at 4°C (Hettich 38R, Hettich Zentrifugen, Germany). The upper phase was allocated into sterile tubes for analysis. Blood samples with EDTA were added to an equal volume of ficoll solution (F5415-50 mL, Sigma–Aldrich, USA) and centrifuged at 2,000 rpm for 10 min at 4°C. Peripheral blood mononuclear cells were separated from the gradient layer between the plasma and ficoll solution, which was then washed twice with PBS to obtain the leucocytes for analysis.

PCR assay

DNA extraction was performed according to the silica-based extraction model using 'High Pure Viral Nucleic Acid' kit procedures (Cat. No:11858874001, Roche Life Science, Germany). The reaction was performed in a total volume of 30 μL consisting of 3 μL of extracted DNA template, 1 μL of each primer (10 μM), 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.5 U Taq DNA polymerase, and nuclease-free water (Thermo Scientific, USA). Primers for CPV DNA were used to target the VP2 capsid protein-encoding gene. A previously sequenced CPV sample was used as a positive control. The thermal condition of the PCR assay was performed, as described previously [25]. The PCR products were applied to agarose gel electrophoresis to determine the product size at 555 bp for the first PCR and at 629 bp for the second PCR analysis. A second PCR analysis was performed to check the positivity of CPV. **Table 3** lists the PCR primers and product sizes in agarose gel. The dogs that tested positive for CPV-2 in the fecal swabs and blood samples by PCR assay were included in the study.

Hematological analysis

The complete blood count (CBC) was performed in blood samples with an anti-coagulant (K2EDTA) by an automated blood count device (Abacus Junior Vet5, DiatronTM, Hungary). All CBC parameters were recorded, and the WBC (total leukocyte), lymphocyte (LYM),

Table 3. Polymerase chain reaction primers and product sizes

Primers	Primer directory (5′ → 3′)	Size (bp)
Hfor	CAGGTGATGAATTTGCTACA	
Hrev	CATTTGGATAAACTGGTGGT	629
555for	CAGGAAGATATCCAGAAGGA	
555rev	GGTGCTAGTTGATATGTAATAAACA	555

bp, base pair.

https://doi.org/10.4142/jvs.23139

neutrophil (NEU), and platelet (PLT) counts were presented for blood count changes of CPE in the study. The PLT-to-LYM ratio (PLR, absolute number of PLTs divided by absolute number of LYMs) and NEU-to-LYM ratio (NLR, absolute number of NEUs divided by absolute number of LYMs) were also calculated.

Cytokine and acute phase protein analysis

After clotting at room temperature, blood samples without anti-coagulant were centrifuged for separation at 4,000 rpm for 10 min at 4°C (Beckman Coulter centrifuge, Allegra X-30R, USA). The serum phase was allocated into sterile tubes and stored at −80°C until analysis. The solid phase sandwich ELISA method [26,27] was used to determine the cytokine and acute phase proteins in sera samples with the following kits: tumor necrosis factor-α (TNF-α; Canine TNF-alpha, ELC-TNFa, RayBio, USA), IL-1β (Canine IL-1 Beta, ELC-IL1b, RayBio), IFN-α (Canine IFN-alpha, CA1581, Biotang, USA), IFN-γ (Canine IFN-gamma, ELC-IFNg, RayBio), haptoglobin (Canine Haptoglobin, ECH2003-1, Assaypro, USA), and C-reactive protein (CRP; Canine CRP, E-EL-C0020, Elabscience, USA) according to the manufacturer's instructions. The absorbances were measured in an ELISA reader (BioTek, μQuant, USA).

Statistical analysis

The data revealed a normal distribution with a coefficient variation of less than 20%. The data within and between the groups were compared using one-way ANOVA with Tukey's post-hoc test (SPSS for Windows, Ver. 22.00, IBM, Chicago, IL, USA) for the blood counts, acute phase proteins, and cytokines. Pearson's Chi-square analysis was used to compare the clinical scores. Kaplan–Meier analysis examined the survival over time in the treatment groups. A p value <0.05 was assigned as significant. The data are presented as the mean ± standard error of the mean (SEM) in the graphs to make simple conclusions by a visual inspection because SEM is closely related to the confidence interval and p value [28].

RESULTS

Conventional PCR and rapid ELISA assays confirmed CPE in the dogs

The CPV-2 DNA was determined using a PCR assay of fecal or blood samples after a positive immunochromatographic result for the CPV antigen using a rapid ELISA test of fecal samples of the dogs with CPE. **Fig. 1** presents a positive result in rapid ELISA and PCR product of CPV-2 nucleic acid in agarose gel.

Clinical findings and scoring

The prominent clinical findings were apathy, lethargy, diarrhea, vomiting, and dehydration on day 0 in all dogs with CPE. For illustration, **Fig. 2** presents the clinical appearances of CPE. Diarrhea varied from soft to mucoid to liquid and hemorrhagic. The frequency of vomiting decreased after the treatments. The appetite and tendency to drink water improved after the cessation of nausea and vomiting. Some dogs from each group showed fecal excretion of adult roundworms (i.e., ascarids) approximately one day after antiparasitic drug administration. The degrees of the findings differed by treatments that reflected clinical severity scores in the groups during the study.

The clinical scores were determined according to the clinical severity, such as the rectal temperature, general condition, dehydration degree, stool consistency, abdominal pain and vomiting, and survival. **Fig. 3** shows the daily clinical scores on days zero, three, and seven

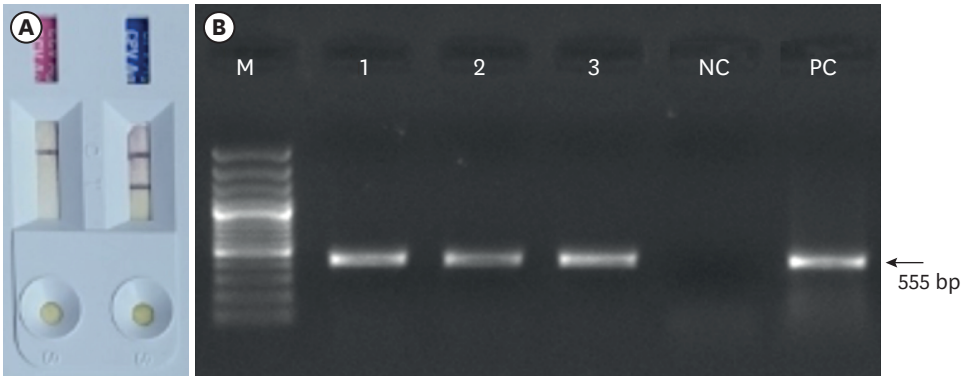

Fig. 1. Rapid ELISA test and gel electrophoresis of the PCR products. (A) Rapid ELISA test shows positive results for the CPV antigen (double bands in blue line) and negative results for canine coronavirus antigen (single band in red line) in fecal samples. (B) PCR products of CPV-2 in agarose gel were observed at 555 pb bands (1, 2, and 3). Note that all dogs with CPE used in this study were positive for CPV-2 according to the PCR assay.
M, marker; NC, negative control; PC, positive control; bp, base pair; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; CPV, canine parvovirus.

Fig. 2. Clinical appearance of the dogs with canine parvoviral enteritis. (A) apathy and lethargy, (B) soft to mucoid diarrhea (arrow), (C) hemorrhagic mucoid diarrhea (arrow), (D) hemorrhagic diarrhea with ascarid excretion (arrow). Note that all dogs are dehydrated.

https://doi.org/10.4142/jvs.23139

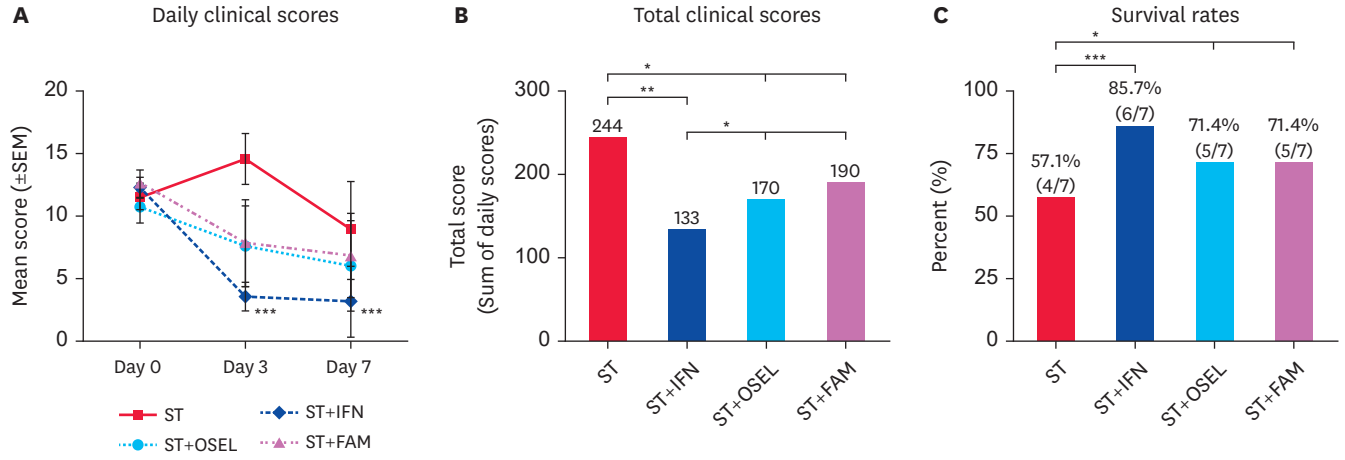

Fig. 3. Clinical scores and survival rates of the dogs with canine parvoviral enteritis in treatment groups. (A) Similarity of clinical scores among the groups on day zero indicates uniformity of clinical randomization of the groups. Note that clinical score is most improved in ST+IFN on day three. (B) IFN led to the best improvement of the total clinical score (sum of zero, three, and seven days) compared to its baseline (from 12.3 ± 0.8 on day zero to 3.6 ± 1.1 and 3.1 ± 2.8 on days three and seven, respectively). (C) Survival rate is higher in the ST+IFN than in other groups. The survival rates were the same in the ST+OSEL and ST+FAM groups, but higher than the ST group while the total clinical score was higher in the ST+FAM group than in the other antiviral groups.
ST, standardized treatment; IFN, interferon; OSEL, oseltamivir; FAM, famciclovir; SEM, standard error of the mean.
*p < 0.05, **p < 0.01, ***p < 0.001.

and the total clinical scores (sum of days zero, three, and seven) in the treatment groups. Daily clinical score was first improved in the ST+IFN group compared to its baseline (from 12.3 ± 0.8 on day zero to 3.6 ± 1.1 on day three, $p < 0.001$) and found the lowest in degree on day seven (from 12.3 ± 0.8 on day zero to 3.1 ± 2.8 on day seven, $p < 0.001$). The clinical score in ST was highest on day three. The total clinical score in the ST group (244) was higher than in the antiviral treatment groups ($p < 0.05$). The total clinical score in the ST+IFN group (133) was lower than in the ST+OSEL group (170) and ST+FAM group (190) ($p < 0.05$). **Fig. 3C** shows the survival rates in the groups. Four of the seven dogs (57%) in the ST group, six of the seven dogs (86%) in the ST+IFN group, five of the seven dogs (71%) in the ST+OSEL group, and five of the seven dogs (71%) in the ST+FAM group survived after the treatment. The survival rates were significantly higher in the ST+IFN group ($p < 0.001$), ST+OSEL group ($p < 0.05$), and ST+FAM group ($p < 0.05$) than in the ST group after treatment.

Hematological findings

Fig. 4 shows the leukocyte and PLT values. The WBC values on day zero in the ST (8.8 ± 0.9), ST+IFN (7.7 ± 2.2), ST+OSEL (8.9 ± 1.1), and ST+FAM (8.2 ± 3.6) groups were relatively low in value ($p > 0.05$) compared to healthy dogs (14.4 ± 1.9) and did not improve on day three ($p > 0.05$) compared with their baselines (on day 0). On day seven, the WBC level increased in the ST+IFN group ($p \leq 0.01$), ST+OSEL group ($p \leq 0.01$), and ST+FAM group ($p < 0.05$) but not in the ST group ($p > 0.05$) compared to their baselines. The WBC level was also higher in the ST+IFN and ST+FAM groups than in the ST group ($p < 0.05$), whereas the ST+OSEL tended to increase ($p > 0.05$).

The NEU and LYM levels were low with the same manner of WBC in the groups on days zero and three. NEU level on day seven increased in the ST+IFN and ST+FAM groups ($p < 0.05$) but not in the ST and ST+OSEL groups ($p > 0.05$) compared to the baselines. The LYM level on day seven increased in the ST+IFN and ST+OSEL groups ($p < 0.05$) but not in the ST and ST+FAM groups ($p > 0.05$) compared to the baselines. The PLT levels were relatively low in the CPE dogs on days zero and three compared to the healthy dogs ($p > 0.05$). The PLT level increased only in the ST+IFN groups on day seven compared to its baseline ($p < 0.01$).

https://doi.org/10.4142/jvs.23139

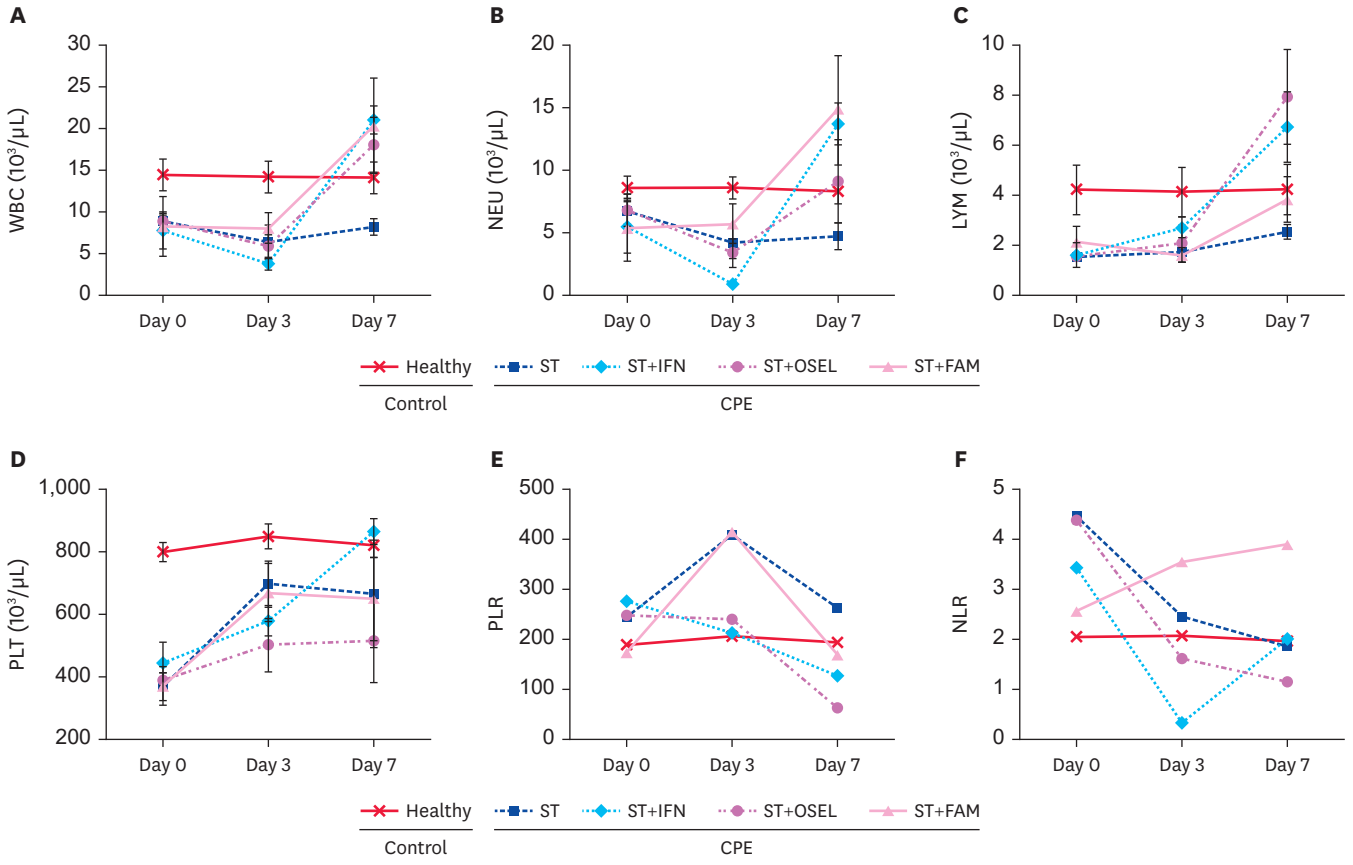

Fig. 4. Mean leucocyte and PLT values of the dogs with CPE in treatment groups and healthy dogs. (A-C) WBC, NEU, and LYM values in CPE dogs were relatively low on days zero and three; WBC were higher in the ST+IFN ($p \leq 0.01$), ST+OSEL ($p \leq 0.01$) and ST+FAM groups ($p < 0.05$) but not in the ST group ($p > 0.05$) in terms of the NEU and LYM levels on day seven. (D) The PLT levels were relatively low in the CPE dogs on days zero and three; PLT was high in the ST+IFN groups on day seven compared to its baseline ($p < 0.01$). (E) PLR values were high in the CPE dogs on day zero and in the ST and ST+FAM groups on day three, while the ratio is the lowest in the ST+OSEL and ST+IFN groups on day seven. (F) NLR values in CPE dogs were higher than in healthy dogs on day zero, with a dramatic decrease in NLR on day three in the ST+IFN ($p < 0.01$) and ST+OSEL ($p < 0.05$) groups than the healthy dogs.
WBC, white blood cell; NEU, neutrophil; LYM, lymphocyte; PLT, platelet; PLR, platelet to lymphocyte ratio; NLR, neutrophil to lymphocyte ratio; CPE, canine parvoviral enteritis; ST, standardized treatment; IFN, interferon; OSEL, oseltamivir; FAM, famciclovir.

The PLR values tended to be high in the CPE dogs on day zero. The ratio increased in the ST and ST+FAM groups on day three and returned to their baselines. A decreasing trend was observed for PLR in the ST+IFN group. PLR was the lowest in the ST+OSEL and ST+IFN groups on day seven. The NLR values were higher in the CPE dogs than in the healthy dogs on day zero. A dramatic decrease in NLR on day three was observed in the ST+IFN ($p < 0.01$) and ST+OSEL ($p < 0.05$) groups, with a tendency to decrease in the ST group compared to the healthy dogs. The NLR value in the ST+IFN group on day seven was similar to the healthy dogs, while ST+FAM remained high during the study.

Cytokines and acute-phase proteins

Fig. 5 shows the mean values of cytokine and acute phase protein response. Compared to the healthy control, the TNF-α values in dogs with CPE were high in ST, ST+IFN, ST+OSEL, and ST+FAM groups on days zero, three, and seven ($p < 0.05$). In addition, the TNF-α values were similar among the treatment groups on days zero, three, and seven ($p > 0.05$) except for the ST+FAM group, which was higher than the ST group on day seven ($p < 0.05$). Compared to the healthy control, the IL-1β values in dogs with CPE were high in the ST, ST+IFN, ST+OSEL, and ST+FAM groups on days zero, three, and seven ($p < 0.05$) except for the ST+IFN group on

https://doi.org/10.4142/jvs.23139

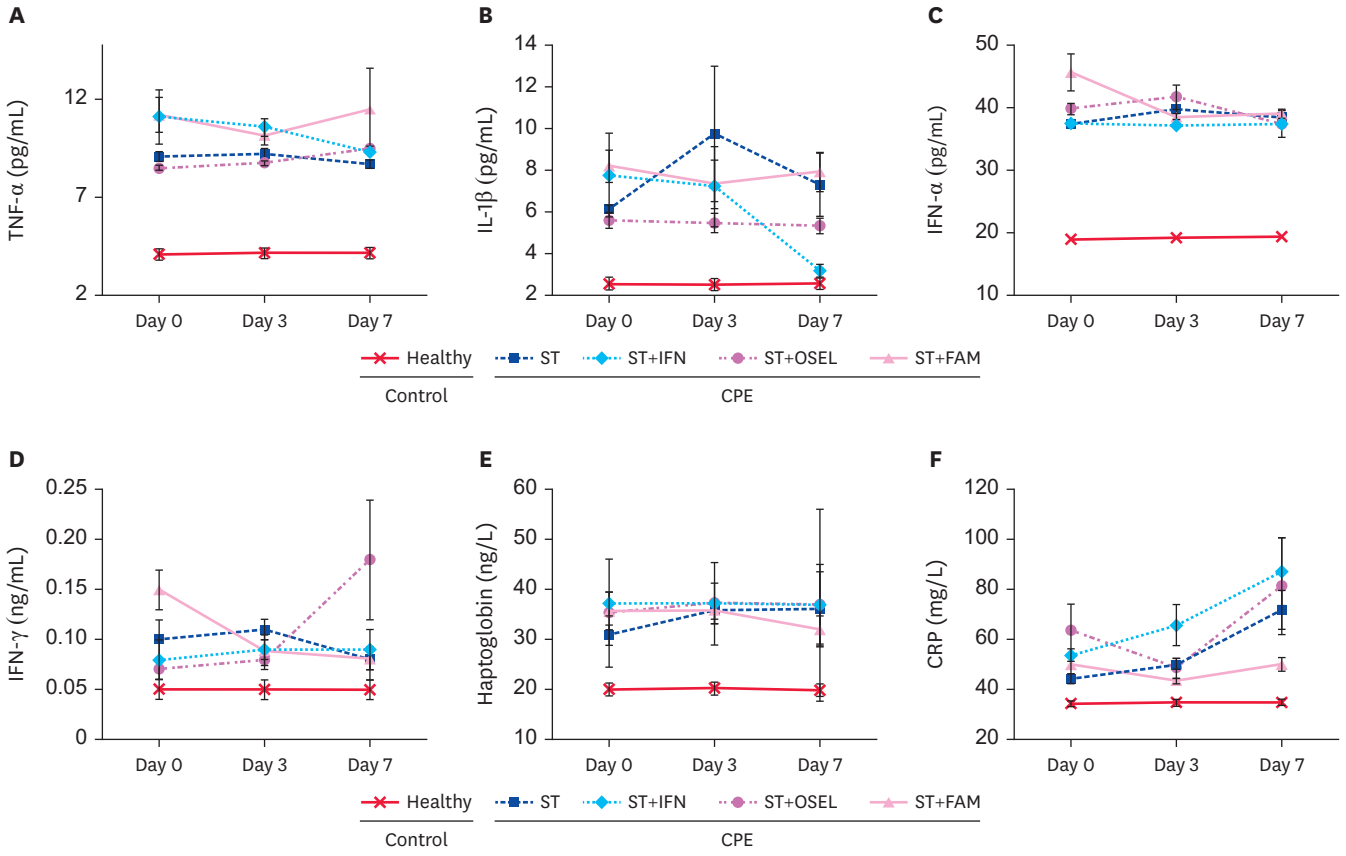

Fig. 5. Mean cytokine and acute phase protein values of the dogs with CPE in treatment groups and healthy dogs. TNF-α (A), IL-1β (B), IFN-α (C), IFN-γ (D), haptoglobin (E), and CRP (F) in treatment groups with CPE were higher or tended to be higher than healthy dogs on days zero. Compared with healthy control, TNF-α, IFN-α, and haptoglobin in treatment groups were high on days zero, three, and seven ($p < 0.05$). On day seven, IL-1β decreased while CRP increased in the ST+IFN group compared to the baselines on day 0 ($p < 0.05$).
TNF-α, tumor necrosis factor-α; IL, interleukin; IFN, interferon; CRP, C-reactive protein; CPE, canine parvoviral enteritis; ST, standardized treatment; OSEL, oseltamivir; FAM, famciclovir.

day seven ($p > 0.05$). The IL-1β values were similar among the treatment groups compared to the healthy control on days zero, three, and seven ($p > 0.05$), except that IL-1β in the ST+IFN group was lower than the ST and ST+FAM groups on day seven ($p < 0.05$). Compared to the healthy control, the IFN-α values were high in the treatment groups with CPE on days zero, three, and seven ($p < 0.01$). The IFN-α value on day seven decreased in the ST+OSEL group compared to its time point on day three ($p < 0.05$). The IFN-α value on days three and seven was lower in the ST+FAM group than its baseline on day zero ($p < 0.05$). The IFN-γ values on day zero in the treatment groups with CPE were not higher than the healthy control ($p > 0.05$) except for the ST+FAM group ($p \leq 0.01$). IFN-γ value on day seven was higher in the ST+OSEL group than in its baseline, control, ST, and ST+FAM groups ($p < 0.05$). A decrease in IFN-γ value was detected in the ST+FAM group on day seven compared to its baseline ($p < 0.05$).

The CRP levels were high in the treatment groups with CPE on days zero, three, and seven. Compared to the healthy control, the CRP value was high in the ST ($p < 0.05$), ST+IFN ($p<0.05$), and ST+OSEL ($p<0.05$) groups on day seven. In addition, the CRP value increased in the ST+IFN group on day seven compared to its baseline ($p < 0.05$). The CRP value in the ST+FAM group was lower than in the ST+IFN and ST+OSEL groups ($p < 0.05$). Compared to the healthy control, the haptoglobin values were high in the ST, ST+IFN, ST+OSEL, and ST+FAM groups on days zero, three, and seven ($p < 0.05$). The haptoglobin values in the

https://doi.org/10.4142/jvs.23139

ST group on days three and seven were higher than their baseline on day 0 ($p < 0.05$). The haptoglobin values in the ST+IFN, ST+OSEL, and ST+FAM groups did not change compared to their baselines during treatment. On day seven, the haptoglobin level in the ST+FAM group was lower than in the ST, ST+IFN, and ST+OSEL groups ($p < 0.05$).

DISCUSSION

CPE is characterized by lethargy, vomiting, diarrhea, and dehydration, with high morbidity and mortality in dogs. The main therapeutic approach is based on symptomatic treatment (i.e., ST) that includes fluid therapy, which is essential to replenish fluid loss, accompanied by ancillary treatments with antibiotic, antiemetic, and antiparasitic drugs addressing the acute gastrointestinal signs [3,10,19,29]. On the other hand, failure of innate and adaptive immunity combined with bacteremia and viremia may worsen the systemic inflammatory response and septic shock in young dogs [22,30]. This study examined the inflammatory response of unvaccinated puppies with naturally occurring CPE during ST and possible antiviral therapy.

Fecal shedding of the CPV usually precedes the onset of diarrhea and continues for several days throughout the clinical phase of CPE. A definitive diagnosis depends on detecting viral particles by ELISA, PCR, electron microscopy, hemagglutination, and virus isolation [3,31], even though the tentative diagnosis of CPE might be possible according to the clinical signs, e.g., hemorrhagic diarrhea and vomiting. A DNA-based PCR assay is considered one of the most sensitive and specific methods for virus detection [24,25]. CPV-2b and CPV-2c are detectable in diagnostic tests [32,33]. Therefore, CPV strain variations and genetic sequencing may not be necessary for the clinical treatment of infected dogs because CPV-2b and CPV-2c pose similar health risks for dogs [33]. In this study, CPV-2 infection was determined on the first day of sampling by PCR assay using primers targeting the VP2 capsid protein in specimens after a cage-side immunochromatographic ELISA Ag test. Accordingly, the dogs with a CPV-2 infection confirmed by a PCR assay were enrolled in the CPE treatment groups.

rFeIFN-ω has been shown to reduce vomiting, diarrhea, and mortality in dogs with a CPV-2 infection [34,35]. Using rFeIFN-ω is further recommended to improve the clinical signs of CPE [18,19]. rFeIFN-ω administration rapidly alleviates the clinical severity scores, resulting in a higher survival rate in the ST+IFN group than in ST alone. OSEL, an antiretroviral drug, is a neuraminidase inhibitor against viruses containing neuraminidase. Unlike the influenza virus, the proliferation of CPV is not based on neuraminidase. OSEL is suggested to reduce the bacterial permeability of the mucin layer in respiratory and intestinal epithelial cells and prevent bacterial passage that leads to endotoxemia, sepsis, systemic inflammatory response, and organ failure [15,36]. Therefore, OSEL appears to act independently from its direct antiviral effect in CPE. Healing of the clinical score and lowering the mortality rate in the ST+OSEL group compared to the ST alone group may indicate the efficacy of OSEL in CPE.

FAM is used to treat herpesvirus in cats [20] and is safe with regard to metabolic and pharmacokinetic activities in dogs [21]. To the authors' knowledge, this is the first report of FAM administration to dogs with CPV infection and symptomatic treatment. Interestingly, FAM deteriorated the clinical scores on day three, while it has improved the survival rate compared to ST. There is a limitation to concluding the efficacy of FAM without previous reports because of the worsened clinical signs on day three in this randomized clinical trial,

even though mortality was decreased. rFeIFN-ω and OSEL decrease the severity of the clinical scores and increase the survival rates compared to ST. Therefore, the efficacies of rFeIFN-ω and OSEL are related to the healing of the clinical scores and improving the survival rates, which are consistent with the previous reports mentioned above. These findings suggest that antiviral addition (e.g., rFeIFN-ω and OSEL) to ST has potentially beneficial effects, and further antiviral candidates need to be evaluated in a CPV infection [37].

Mild leukopenia was observed in dogs with CPE upon admission. In most infections, one could expect a leukocytosis finding as a reaction against pathogens, even though leucopenia caused by neutropenia and lymphopenia in CPE were observed. A CPV infection causes mild to severe leukopenia due to destruction of the thymus, lymphoid tissues, and cell precursors in bone marrow, resulting in a dysfunction of innate host defense. The destruction of leukocyte precursors may worsen sepsis and the patient's systemic inflammatory response. Leukocytes may be a therapeutic target because NEUs and LYMs are the primary mediators against invading pathogens in CPE [13]. Moreover, severe dehydration causes organ dysfunction (e.g., acute renal failure) in which the hemodynamics and hemostasis are vital to cope with the inflammatory changes, even though fluid resuscitation may alter the hemostatic status of dogs with CPE, leading to exacerbated variables [38]. The improvement in clinical scores and treatment efficacy may correspond to leukocyte recovery during treatment [23,39] because of the presence of leukopenia, known to be a suggestive hallmark of CPE [24]. Similarly, the total leukocyte count (i.e., WBC) recovered on day seven in the antiviral treatment groups compared to the ST group. However, the level remained low on day three in all treatment groups.

The PLT level was low in the CPE dogs on days zero and three, while the PLT level was higher in the ST+IFN groups on day seven than its baseline. The PLR (i.e., PLT-to-LYM ratio) values were high on days zero and three in the treatment groups, while PLR was lower in the ST+OSEL and ST+IFN groups on day seven than in the healthy control, which is consistent with previous reports [40]. PLR may be reduced by OSEL and rFeIFN-ω antiviral treatment groups, which have important improvements in clinical scores and survival rates on day seven. Therefore, the PLR levels may be higher in the non-survivors than in the survivors in septic patients [41]. The PLR level changed with time in the ST group, which has high mortality, and in the ST+FAM group, which has relatively high clinical scores. A recent report indicated a U-shaped relationship between the baseline PLR and in-hospital mortality of septic patients in the change in PLR over time [42]. The NLR (i.e., NEU-to-LYM ratio) levels were higher in CPE dogs than the healthy dogs in the present study. The increase in NLR may be associated with high mortality. In contrast, the decrease in NLR may be associated with an adequate balance in the inflammatory response of CPV infection [43] and immune system diseases [44,45]. The finding of the lowest NLR value in the ST+IFN group on day 3 suggests a good prognosis, which has further resulted in a higher survival rate in this group at the end of the study. On the other hand, there may be other effects to limit the use of the NLR value, which is influenced by cortisol secretion that may cause neutrophilia and lymphopenia because the cortisol level has not been measured in these dogs.

Inflammatory biomarkers, such as acute phase proteins, cytokines, cholesterol, and pancreatic lipase levels, as well as coagulation, are altered in response to a CPV infection. CRP and haptoglobin are associated with the severity of inflammatory response and the extent of specific humoral immunity after vaccination with modified live virus against distemper and parvovirus [46]. In this study, the high CRP and haptoglobin levels

demonstrated a positive acute phase reaction concerning the systemic inflammatory response against a CPV infection that can result in death without treatment. On the other hand, the increase in CRP concentration in CPE has not been proven to be a good predictor of survival when used alone [47], even though the high serum level of CRP is positively associated with mortality [48]. Therefore, the clinicopathologic changes in serum markers might not be used alone in predicting morbidity or mortality [3]. Accordingly, the high CRP and haptoglobin levels continued until day seven in the affected dogs.

The acute inflammatory response is typically initiated by the overproduction of cytokines strongly associated with systemic inflammatory response syndrome caused by bacterial or viral agents [49-52]. Cytokine and acute phase protein concentrations appear to vary depending on the time point of the CPV infection. Some cytokine responses have been previously evaluated in dogs [53-55]. The TNF-α, IL-1β, IFN-α, and IFN-γ levels were high in the dogs with CPE regarding cytokine overproduction. The elevated state of TNF-α, IFN-α, CRP, and haptoglobin have not been changed significantly in all groups by the treatments. Early cytokine biomarkers, such as TNF-α and IL-1β, could not be useful indicators for the prognosis and survival rate of the disease. Cytokine overproduction is consistent with the increased levels of acute phase proteins during the study in the ST and ST plus antiviral addition groups. One of the most important reasons for prolonging the inflammatory response could be the depression of adaptive immunity, resulting in a reduction of LYMs and their secreted products, such as antibodies in unvaccinated puppies because of the parvoviral affinity with lymphoid tissues and cell precursors. No direct antiviral drug against the CPV has been discovered, despite OSEL and rFeIFN-ω being used as possible antiviral therapy [3,19]. Moreover, viral replication must be prevented by the innate and adaptive immunity of viral diseases. Thus, a possible antiviral influence of the cytokine drug rFeIFN-ω could be the stimulation of inflammatory response against the infection by binding to IFN receptors, resulting in a prolongation of the inflammatory state and the activation of acute protein reactants, as shown in this study.

Furthermore, IL-1β, a potent pro-inflammatory cytokine produced by the innate immune system, is decreased in the ST+IFN group from a high level to the normal level of the healthy animals on day seven, which may support the anti-inflammatory efficacy of rFeIFN-ω. Therefore, the increase in cytokines and acute phase proteins compared with the healthy group suggests that these markers may be used as prognostic factors to evaluate the inflammatory response in CPE patients after treatment. On the other hand, the small number of animals participating in the groups and the seven-day duration may reflect some characteristics of the inflammatory response in naturally infected dogs with CPE. The biomarkers (i.e., cytokines) on the sampling time points may provide evidence during early inflammatory changes but may not reflect the prognostic value in CPV infection.

In conclusion, supportive treatment of CPE is necessary to correct the clinical signs and increase survival, even though the failure of immunity in susceptible dogs may limit the treatment success. These results show that the therapeutic efficacies of antiviral drugs accompanied by a supportive treatment may be a valuable part of supportive and symptomatic treatment of CPE that has been shown by improving the clinical scores and survival rates. Adding rFeIFN-ω (a cytokine drug) to the ST (symptomatic and supportive treatment) may improve clinical severity and survival. Leucocytes, cytokines, and acute phase protein response may give prognostic information after day three in CPE. In addition, the decrease in NLR in favor of LYM on day three may indicate the prognostic value before the improvement of the leucocyte counts and inflammatory markers in CPE.

REFERENCES

1. Mia M, Hasan M. Update on canine parvovirus infection: a review from the literature. Vet Sci: Res Rev. 2021;7:92-100.
2. Singh P, Kaur G, Chandra M, Dwivedi PN. Prevalence and molecular characterization of canine parvovirus. Vet World. 2021;14(3):603-606.
PUBMED | CROSSREF
3. Mazzaferro EM. Update on canine parvoviral enteritis. Vet Clin North Am Small Anim Pract. 2020;50(6):1307-1325.
PUBMED | CROSSREF
4. Decaro N, Buonavoglia C, Barrs VR. Canine parvovirus vaccination and immunisation failures: are we far from disease eradication? Vet Microbiol. 2020;247:108760.
PUBMED | CROSSREF
5. Polat PF, Şahan A, Aksoy G, Timurkan MO, Dinçer E. Molecular and restriction fragment length polymorphism analysis of canine parvovirus 2 (CPV-2) in dogs in southeast Anatolia, Turkey. Onderstepoort J Vet Res. 2019;86(1):e1-e8.
PUBMED | CROSSREF
6. Timurkan M, Oğuzoğlu T. Molecular characterization of canine parvovirus (CPV) infection in dogs in Turkey. Vet Ital. 2015;51(1):39-44.
PUBMED | CROSSREF
7. Favrot C, Olivry T, Dunston SM, Degorce-Rubiales F, Guy JS. Parvovirus infection of keratinocytes as a cause of canine erythema multiforme. Vet Pathol. 2000;37(6):647-649.
PUBMED | CROSSREF
8. Greene CE, Decaro N. Canine viral enteritis. In: Greene CE, editor. *Infectious Diseases of the Dog and Cat*. 4th ed. St. Louis: Elsevier; 2012, 67-76.
9. Goddard A, Leisewitz AL. Canine parvovirus. Vet Clin North Am Small Anim Pract. 2010;40(6):1041-1053.
PUBMED | CROSSREF
10. Acciacca RA, Sullivan LA, Webb TL, Johnson V, Dow SW. Clinical evaluation of hyperimmune plasma for treatment of dogs with naturally occurring parvoviral enteritis. J Vet Emerg Crit Care. 2020;30(5):525-533.
PUBMED | CROSSREF
11. Chalifoux NV, Parker SE, Cosford KL. Prognostic indicators at presentation for canine parvoviral enteritis: 322 cases (2001–2018). J Vet Emerg Crit Care. 2021;31(3):402-413.
PUBMED | CROSSREF
12. Eregowda CG, De UK, Singh M, Prasad H, Akhilesh , Sarma K, et al. Assessment of certain biomarkers for predicting survival in response to treatment in dogs naturally infected with canine parvovirus. Microb Pathog. 2020;149:104485.
PUBMED | CROSSREF
13. Muñoz AI, Vallejo-Castillo L, Fragozo A, Vázquez-Leyva S, Pavón L, Pérez-Sánchez G, et al. Increased survival in puppies affected by Canine Parvovirus type II using an immunomodulator as a therapeutic aid. Sci Rep. 2021;11(1):19864.
PUBMED | CROSSREF
14. de Mari K, Maynard L, Eun HM, Lebreux B. Treatment of canine parvoviral enteritis with interferon-omega in a placebo-controlled field trial. Vet Rec. 2003;152(4):105-108.
PUBMED | CROSSREF
15. Savigny MR, Macintire DK. Use of oseltamivir in the treatment of canine parvoviral enteritis. J Vet Emerg Crit Care. 2010;20(1):132-142.
PUBMED | CROSSREF
16. Fulton RW, Burge LJ. Susceptibility of feline herpesvirus 1 and a feline calicivirus to feline interferon and recombinant human leukocyte interferons. Antimicrob Agents Chemother. 1985;28(5):698-699.
PUBMED | CROSSREF
17. Weiss RC. Synergistic antiviral activities of acyclovir and recombinant human leukocyte (alpha) interferon on feline herpesvirus replication. Am J Vet Res. 1989;50(10):1672-1677.
PUBMED
18. Mueller RS, Hartmann K. Interferon therapies in small animals. Vet J. 2021;271:105648.
PUBMED | CROSSREF
19. Gerlach M, Proksch AL, Dörfelt R, Unterer S, Hartmann K. Therapy of canine parvovirus infection - review and current insights. Tierarztl Prax Ausg K Klientiere Heimtiere. 2020;48(1):26-37.
PUBMED | CROSSREF

20. Thomasy SM, Shull O, Outerbridge CA, Lim CC, Freeman KS, Strom AR, et al. Oral administration of famciclovir for treatment of spontaneous ocular, respiratory, or dermatologic disease attributed to feline herpesvirus type 1: 59 cases (2006–2013). J Am Vet Med Assoc. 2016;249(5):526-538.
PUBMED | CROSSREF
21. Filer CW, Ramji JV, Allen GD, Brown TA, Fowles SE, Hollis FJ, et al. Metabolic and pharmacokinetic studies following oral administration of famciclovir to the rat and dog. Xenobiotica. 1995;25(5):477-490.
PUBMED | CROSSREF
22. Alves F, Prata S, Nunes T, Gomes J, Aguiar S, Aires da Silva F, et al. Canine parvovirus: a predicting canine model for sepsis. BMC Vet Res. 2020;16(1):199.
PUBMED | CROSSREF
23. Martin V, Najbar W, Gueguen S, Grousson D, Eun HM, Lebreux B, et al. Treatment of canine parvoviral enteritis with interferon-omega in a placebo-controlled challenge trial. Vet Microbiol. 2002;89(2-3):115-127.
PUBMED | CROSSREF
24. Decaro N, Desario C, Billi M, Lorusso E, Colaianni ML, Colao V, et al. Evaluation of an in-clinic assay for the diagnosis of canine parvovirus. Vet J. 2013;198(2):504-507.
PUBMED | CROSSREF
25. Buonavoglia C, Martella V, Pratelli A, Tempesta M, Cavalli A, Buonavoglia D, et al. Evidence for evolution of canine parvovirus type 2 in Italy. J Gen Virol. 2001;82(Pt 12):3021-3025.
PUBMED | CROSSREF
26. Butler JE, Peterman JH, Suter M, Dierks SE. The immunochemistry of solid-phase sandwich enzyme-linked immunosorbent assays. Fed Proc. 1987;46(8):2548-2556.
PUBMED
27. Hornbeck P, Winston SE, Fuller SA. Enzyme-linked immunosorbent assays (ELISA). Curr Protoc Mol Biol. 2001;Chapter 11:Unit11.2.
PUBMED | CROSSREF
28. Tang L, Zhang H, Zhang B. A note on error bars as a graphical representation of the variability of data in biomedical research: choosing between standard deviation and standard error of the mean. J Pancreatol. 2019;2(3):69-71.
PUBMED | CROSSREF
29. Aktaş M, Özkanlar Y, Kırbaş A. An investigation on risk factors that affect parvoviral enteritis in owned dogs referred to the clinic from Erzurum province. Atatürk Üniv Vet Bilim Derg. 2011;6:1-8.
30. Kelman M, Barrs VR, Norris JM, Ward MP. Canine parvovirus prevention and prevalence: veterinarian perceptions and behaviors. Prev Vet Med. 2020;174:104817.
PUBMED | CROSSREF
31. Abayli H, Aslan O, Tumer KC, Can-Sahna K, Tonbak S. Predominance and first complete genomic characterization of canine parvovirus 2b in Turkey. Arch Virol. 2022;167(9):1831-1840.
PUBMED | CROSSREF
32. Decaro N, Desario C, Beall MJ, Cavalli A, Campolo M, Dimarco AA, et al. Detection of canine parvovirus type 2c by a commercially available in-house rapid test. Vet J. 2010;184:373-375.
PUBMED | CROSSREF
33. Markovich JE, Stucker KM, Carr AH, Harbison CE, Scarlett JM, Parrish CR. Effects of canine parvovirus strain variations on diagnostic test results and clinical management of enteritis in dogs. J Am Vet Med Assoc. 2012;241(1):66-72.
PUBMED | CROSSREF
34. Ishiwata K, Minagawa T, Kajimoto T. Clinical effects of the recombinant feline interferon-omega on experimental parvovirus infection in beagle dogs. J Vet Med Sci. 1998;60(8):911-917.
PUBMED | CROSSREF
35. Minagawa T, Ishiwata K, Kajimoto T. Feline interferon-omega treatment on canine parvovirus infection. Vet Microbiol. 1999;69(1-2):51-53.
PUBMED | CROSSREF
36. Peltola VT, Murti KG, McCullers JA. Influenza virus neuraminidase contributes to secondary bacterial pneumonia. J Infect Dis. 2005;192(2):249-257.
PUBMED | CROSSREF
37. Zhou H, Su X, Lin L, Zhang J, Qi Q, Guo F, et al. Inhibitory effects of antiviral drug candidates on canine parvovirus in F81 cells. Viruses. 2019;11(8):742.
PUBMED | CROSSREF
38. Whitehead Z, Goddard A, Botha WJ, Pazzi P. Haemostatic changes associated with fluid resuscitation in canine parvoviral enteritis. J S Afr Vet Assoc. 2020;91(0):e1-e9.
PUBMED | CROSSREF

39. Armenise A, Trerotoli P, Cirone F, De Nitto A, De Sario C, Bertazzolo W, et al. Use of recombinant canine granulocyte-colony stimulating factor to increase leukocyte count in dogs naturally infected by canine parvovirus. Vet Microbiol. 2019;231:177-182.
PUBMED | CROSSREF

40. Pekmezci D, Çolak ZN. Determination of neutrophil/lymphocyte, monocyte/lymphocyte and platelet/lymphocyte ratios in dogs with occurring parvovirus infection. J Anatol Environ Anim Sci. 2021;6(4):585-591.
CROSSREF

41. Wang G, Mivefroshan A, Yaghoobpoor S, Khanzadeh S, Siri G, Rahmani F, et al. Prognostic value of platelet to lymphocyte ratio in sepsis: a systematic review and meta-analysis. BioMed Res Int. 2022;2022:9056363.
PUBMED | CROSSREF

42. Zheng R, Shi YY, Pan JY, Qian SZ. Decrease in the platelet-to-lymphocyte ratio in days after admission for sepsis correlates with in-hospital mortality. Shock. 2023;59(4):553-559.
PUBMED | CROSSREF

43. Muñoz AI, Maldonado-García JL, Fragozo A, Vallejo-Castillo L, Lucas-Gonzalez A, Trejo-Martínez I, et al. Altered neutrophil-to-lymphocyte ratio in sepsis secondary to canine parvoviral enteritis treated with and without an immunomodulator in puppies. Front Vet Sci. 2022;9:995443.
PUBMED | CROSSREF

44. Buonacera A, Stancanelli B, Colaci M, Malatino L. Neutrophil to lymphocyte ratio: an emerging marker of the relationships between the immune system and diseases. Int J Mol Sci. 2022;23(7):3636.
PUBMED | CROSSREF

45. Huang Z, Fu Z, Huang W, Huang K. Prognostic value of neutrophil-to-lymphocyte ratio in sepsis: a meta-analysis. Am J Emerg Med. 2020;38(3):641-647.
PUBMED | CROSSREF

46. Romiszewski P, Kostro K, Lisiecka U. Effects of subclinical inflammation on C-reactive protein and haptoglobin levels as well as specific humoral immunity in dogs vaccinated against canine distemper and parvovirus. BMC Vet Res. 2018;14(1):70.
PUBMED | CROSSREF

47. McClure V, van Schoor M, Thompson PN, Kjelgaard-Hansen M, Goddard A. Evaluation of the use of serum C-reactive protein concentration to predict outcome in puppies infected with canine parvovirus. J Am Vet Med Assoc. 2013;243(3):361-366.
PUBMED | CROSSREF

48. Kocaturk M, Martinez S, Eralp O, Tvarijonaviciute A, Ceron J, Yilmaz Z. Prognostic value of serum acute-phase proteins in dogs with parvoviral enteritis. J Small Anim Pract. 2010;51(9):478-483.
PUBMED | CROSSREF

49. Covin MA, Steiner JM. Measurement and clinical applications of C-reactive protein in gastrointestinal diseases of dogs. Vet Clin Pathol. 2022;50 Suppl 1(Suppl 1):29-36.
PUBMED | CROSSREF

50. Ozkanlar Y, Aktas M, Kaynar O, Ozkanlar S, Kırecci E, Yıldız L. Bovine respiratory disease in naturally infected calves: clinical signs, blood gases and cytokine response. Rev Med Vet (Toulouse). 2012;163:123-130.

51. Perez L. Acute phase protein response to viral infection and vaccination. Arch Biochem Biophys. 2019;671:196-202.
PUBMED | CROSSREF

52. Schoeman JP, Goddard A, Leisewitz AL. Biomarkers in canine parvovirus enteritis. N Z Vet J. 2013;61(4):217-222.
PUBMED | CROSSREF

53. Archer TM, Mulligan C, Narayanan L, Riggs C, Fellman C, Thomason JM, et al. Effects of oral administration of 5 immunosuppressive agents on activated T-cell cytokine expression in healthy dogs. J Vet Intern Med. 2020;34(3):1206-1213.
PUBMED | CROSSREF

54. Dandrieux JR, Narayanan L, Firestone S, Archer TM, Mansfield CS. Effect of immunosuppressive drugs on cytokine production in canine whole blood stimulated with lipopolysaccharide or a combination of ionomycin and phorbol 12-myristate 13-acetate. Vet Med Sci. 2019;5(2):199-205.
PUBMED | CROSSREF

55. Deng J, Li D, Huang X, Li W, Zhao F, Gu C, et al. Interferon-γ enhances the immunosuppressive ability of canine bone marrow-derived mesenchymal stem cells by activating the TLR3-dependent IDO/kynurenine pathway. Mol Biol Rep. 2022;49(9):8337-8347.
PUBMED | CROSSREF