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Research Article

Efficacy of Filgrastim in Canine Parvoviral Enteritis Accompanied by Severe Leukopenia

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Abstract

Canine parvoviral enteritis (CPE) is a common, highly contagious viral disease characterized by severe hemorrhagic gastroenteritis in dogs. Leukopenia and neutropenia in dogs with parvoviral enteritis are considered negative prognostic indicators. This study aimed to investigate the effect of filgrastim [recombinant methionyl human granulocyte colony-stimulating factor (r-metHuG-CSF)] on leukocyte counts in dogs with parvoviral enteritis. The animal material consisted of thirty-seven owned dogs with parvoviral enteritis from various breeds, which were brought to the Small Animal Clinic of the Department of Internal Medicine, Faculty of Veterinary Medicine, Erciyes University. The dogs included in the study were divided into two groups. The first group received standard treatment (ST=18) and the second group received Filgrastim (ST+Filg=19) in addition to the standard treatment. For the assessment of leukocyte counts, blood samples were taken on day 0 (before starting treatment), day 3, and day 5 after treatment. It was observed that 16 out of the nineteen dogs treated with Filgrastim in addition to standard treatment (84.21%) showed improvement. In the ST+Filg group, the median WBC and neutrophil values on the 5th day after treatment were significantly higher than on the 0th day (P=0.001, P=0.006, respectively). In addition, the median WBC, lymphocyte and neutrophil values of the dogs in the ST+Filg group on the 3rd and 5th days after treatment were found to be significantly higher than the same day measurements of the ST group (P<0.001). As a result, this study determined that filgrastim (r-metHuG-CSF) contributed positively to the improvement of leukopenia in dogs with CPE, in conjunction with clinical recovery. It was concluded that filgrastim (r-metHuG-CSF) may be included in treatment protocols as one of the immunostimulant drugs to increase leukocyte counts in the treatment of dogs with parvoviral enteritis.

Keywords: Canine parvoviral enteritis, Dog, Filgrastim, Leukocyte, Treatment

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Introduction

Canine parvoviral enteritis (CPE) is a viral disease in dogs that is common, highly contagious, and characterized by severe hemorrhagic gastroenteritis [1]. *Canine parvovirus* (CPV) was first identified in the late 1970s and has since become a panzootic condition affecting dogs of all ages, primarily affecting puppies [2]. In 2022, the International Committee on Taxonomy of Viruses (ICTV) classified CPV in the *Parvoviridae* family, *Parvovirinae* subfamily, and *Protoparvovirus* genus [3]. CPV is an enveloped virus with a single-stranded DNA. *Canine parvovirus* type 2 (CPV, *Proparvovirus carnivoran* 1) and its variants, CPV-2a, 2b, and 2c, are responsible for the disease [4]. Although dogs of all ages can be affected, the disease is more common in puppies with underdeveloped immune systems and defense mechanisms (6 weeks to 6 months) [5]. The rapid replication of the virus targets cells with the ability to divide since viral replication occurs during the S phase of cell division. Therefore, puppies are more severely affected than older dogs [6]. If the animals are left untreated, CPE has an approximate mortality rate of about 91% [7]. In CPE cases, death is often associated with severe sepsis and endotoxemia and electrolyte abnormalities [5,8,9].

Typically, the prognosis of an illness is determined by the severity of clinical and laboratory data at the onset of treatment. With an appropriate treatment regimen, high mortality rates can significantly be reduced [5,10]. Clinical symptoms of CPE include lethargy, vomiting, fever, diarrhea, and anorexia. As the disease progresses, diarrhea becomes foul-smelling and bloody. High fever, severe vomiting, and bloody diarrhea result in a significant loss of fluids and blood from the body. Especially in puppies, this fluid loss can lead to death in a short time [1].

Abnormal laboratory findings in CPE include leukopenia, lymphopenia, neutropenia, and anemia [1,4,11,12]. This hematological outcome contributes significantly to the impairment of immune function. As a result of viral infection, the thymic cortex collapses and disappears. This causes substantial leukopenia in infected animals, along with the death of leukocyte precursors in the bone marrow [1,8]. Dogs that die from CPE generally have a total leukocyte count equal to or less than 1030 cells/μL, and it has been reported that persistent lymphocytopenia, monocytopoenia, and eosinopenia are observed within the first 3 days after hospitalization [4]. According to Goddard et al.[13] after admission and the first 48 h of hospitalization, total leukocyte counts above 4500/μl and lymphocyte counts exceeding 1000/μl have a substantial impact on survival.

In addition to acid-base and electrolyte imbalances, hydration and balancing oncotic pressure are important aspects in the treatment of CPE. Antiemetics, broad-spectrum antibiotics, probiotics, vitamins, minerals, amino acids, antiviral drugs, and immunostimulants are among the other drugs used in treatment of CPE [14,15]. Symptomatic treatment, supported by immunomodulators, cytokines, interferons, and antioxidant substances, significantly reduces mortality rate resulting from this disease [1,16,17]. The hematopoietic regulatory glycoproteins known as granulocyte colony-stimulating factors (G-CSF) facilitate the growth, maturation, and stimulation of neutrophils within the bone marrow [18]. The use of human granulocyte colony-stimulating factor (hG-CSF) in dogs has been reported to stimulate bone marrow and neutrophil release [16-18]. One form of colony stimulating factor is filgrastim, which belongs to a class of drugs that promote blood cell formation and function [19]. White blood cell production can be enhanced by the naturally occurring protein called G-CSF. Filgrastim is a man-made version of G-CSF that stimulates white blood cell production, particularly neutrophil production. Filgrastim prevents neutropenia associated with cancer treatment in human medicine [20]. Moreover, it can be utilized to increase white blood cell counts prior to stem cell extraction for transplantation [20,21]. Filgrastim is also used in dogs and cats to treat severe neutropenia [22].

In recent years, Punia et al.[23] in dogs with hemorrhagic gastro-enteritis, Areshkumar et al.[24] in 1 CPE positive dog, and Gülersoy et al.[17] in CPE-positive dogs without taking leukopenia levels into account, used human-specific granulocyte-colony stimulating factor (rcG-CSF) and provided useful information about its effectiveness. Additionally, Duffy et al.[16] in parvovirus induced neutropenia and Armenise et al.[18] in CPE-positive animals with leukocyte counts below 3000 (cell/μL) investigated the effectiveness of dog-specific granulocyte-colony stimulating factor (rcG-CSF). In the current study, unlike similar studies conducted to date, only dogs with leukopenia were used and it was aimed to investigate the effect of human-specific granulocyte-colony stimulating factor (rcG-CSF, Filgrastim) on total leukocyte, lymphocyte and granulocyte levels in a total of 19 CPE-positive dogs.

Materials and Methods

Ethics Statement

The Erciyes University Animal Experiments Local Ethics Committee accepted this work, and a certificate of approval (Decision date and no: 07.09.2023/169) was acquired.

Animals

Canine parvoviral enteritis (CPE) was diagnosed in sixty dogs brought to the Erciyes University Faculty of Veterinary Medicine, Department of Internal Medicine, Small Animal Clinic, Infectious Diseases Unit during the study period. Thirty-seven of these 60 dogs with CPE had significant leukopenia (including lymphopenia and neutropenia) on complete blood count analysis. Eighteen out of the thirty-seven dogs were included in the Standard Treatment (ST) group. Nineteen out of thirty-seven dogs were included in the Standard Treatment + Filgrastim (ST+Filg) group. Filgrastim (r-metHuG-CSF) was administered to the dogs in the ST+Filg group as an immune stimulant drug in addition to the standard parvoviral enteritis treatment in order to increase their leukocyte counts. Dogs with CPE included in the study were pursued for 5 days. After the 5th day, these dogs were continued with an additional treatment protocol if deemed necessary. This additional protocol included supportive treatment practices such as dietary supplements, probiotics, anti-anemic medications and vitamin supplements. Blood samples were taken from these dogs on day 0 (before treatment) and on days 3 and 5 after treatment *(Fig. 1)*. During the treatment, these dogs were brought to the treatment daily by the pet owners

Owned dogs between the ages of six weeks and six months, with or without a history of vaccinations, exhibiting the typical clinical symptoms of canine parvoviral enteritis (vomiting and either hemorrhagic or non-haemorrhagic

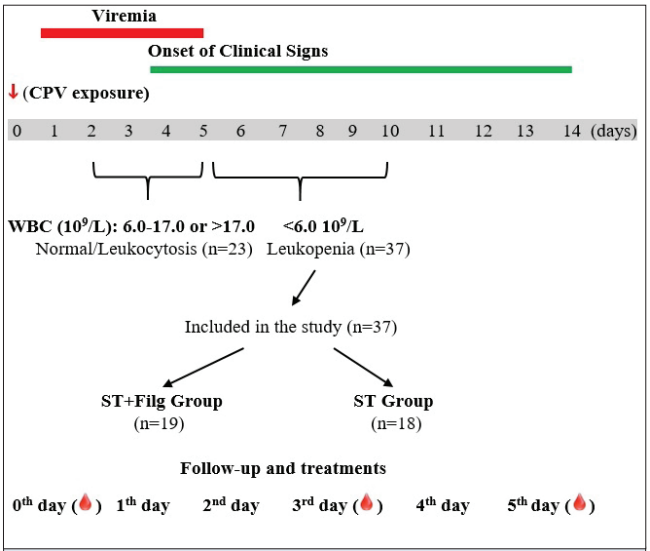

Fig 1. Study design and number of dogs included in the study. ST: Standard treatment, ST+Filg: Standard Treatment + Filgrastim

diarrhea), testing positive for CPV antigen in feces using a test kit and positive PCR test results, and having marked leukopenia (<6.000x10⁹/L) in a complete blood count analysis were the criteria for inclusion in the study.

Physical Examination

Every dog involved in the research was given a thorough physical examination. These examinations were performed by a single individual. Parameters such as body temperature, heart rate, respiratory rate, and blood pressure were measured. The degree of dehydration (<5% [subclinical], 5% [mild], 6%-8% [moderate], 8%-10% [severe], ~12% [hypovolemia]) was estimated using parameters such as skin elasticity, capillary refill time, dryness of mucous membranes [25].

Using the Rapid Diagnostic Kit for Antigen Detection

In suspected cases of canine parvoviral enteritis based on the clinical examination, lateral flow immuno-chromatographic (LFI) rapid diagnostic kits for CPV antigen (Anigen Rapid CPV antigen test kit Bionote®, BIONOTE Inc., South Korea) were employed. Before analysis, all kit materials were brought to room temperature, and the test platform was placed on a flat surface. Rectal fecal samples were obtained from the dogs using sterile swabs. These swab samples were then immersed in a tube containing sample buffer and mixed for 10 sec to ensure homogenization. Subsequently, the tube was placed on a flat surface and left for 3 min. After the particles settled at the bottom of the tube, the content was aspirated from the upper part of the tube. Then, three drops of the sample were dispensed into the sample well. The results were observed within 5 min. If a single line was visible in the observation window, the result was considered negative. If two lines were visible, the result was considered positive.

Polymerase Chain Reaction (PCR) Analyses

After collecting fecal samples from dogs showing clinical symptoms and testing positive with the LFI, the samples were processed at the Erciyes University, Faculty of Veterinary Medicine Virology Laboratory. To prepare the samples for DNA extraction, a portion of the feces was mixed with sterile 1x phosphate-buffered saline (PBS) solution containing 1% penicillin-streptomycin inside a biosafety level 2 (BSL-2) cabinet. The mixture was then passed through 0.22 µm syringe filters.

For DNA extraction, an optimized phenol-chloroform-based method was employed in the laboratory. In brief, 500 µL of the sample was mixed with 250 µL of phenol and 250 µL of chloroform/isoamyl alcohol (24/1) and vortexed. After centrifugation at 10.000 rpm for 10 min, the upper phase (400 µL) was transferred to a new microcentrifuge tube. Then, 3M sodium acetate (pH 4.2) at 1/10 volume and absolute ethanol at 2/10 volume were added, followed by vortexing.

The sample was incubated at -80ºC for 1 h and then centrifuged at 10.000 rpm for 10 min. The supernatant was removed, and 500 µL of 70% ethanol was added, followed by vortexing. The supernatant was disposed of following a second centrifugation cycle at 10.000 rpm for 10 min. The remaining DNA pellet was air-dried on filter paper. Finally, 20 µL of nuclease-free water was added, and the DNA was stored at +4ºC for use in the PCR process.

For PCR, a DNA extraction mix was prepared with 5 µL of DNA template, 1 µL each of forward and reverse primers (10 pmol), 1 µL of 10 mM dNTPs, 5 µL of 10x PCR Taq buffer (Transgen Biotech, AP111-01), 0.5 µL of Taq DNA polymerase enzyme (2.5 U), and 36.5 µL of nuclease-free water as the final volume. The primers used were specific to the VP2 gene of the virus: F: 5'-GCTGAGGTTGGTTATAGTGCR-3' and R: 5'-TGGATTCCAAGTATGAGAKGCT-3'. After preparing the samples for PCR, they were placed in a thermal cycler. The PCR program consisted of an initial denaturation at 95ºC for 5 min, followed by 35 cycles of denaturation at 95ºC for 1 min, annealing at 52ºC for 1 min, and extension at 72ºC for 1 min. A final extension step was carried out at 72ºC for 10 min. The PCR amplification products were run on a 1.7% agarose gel at 120V for 30 min in an electrophoresis system. The results were visualized under UV light.

Blood Pressure Measuring

Non-invasive blood pressure measurements were obtained using the PetTrust oscillometer (PetTrust, BioCARE, Taiwan). To minimize stress during blood pressure measurements in dogs and help them acclimate to unfamiliar personnel, a 5-10 min waiting period was

observed. During the blood pressure measurements, the animal owner, assisting staff, and the veterinarian conducting the examination were present. With the use of the measuring tape that came with the PetTrust, the cuff size was established. To select the appropriate cuff size, the circumference of the dog's right forelimb (proximal) was measured with the assistance of a flexible measuring tape. Approximately 30% of the circumference of the right forelimb was used as the standard for selecting the appropriate cuff size. The available cuff sizes were 2.05, 2.55, 3.05, 3.55, 4.05, 4.55, and 5.55 cm, respectively.

To measure blood pressure in the proximal part of the right forelimb, the cuff's bladder was placed over the arteria radialis in the middle of the antebrachium, ensuring that it was neither too tight nor too loose. When the device was activated, the cuff automatically inflated. The oscillometric measurement method was used to measure the mean arterial pressure (MAP), diastolic blood pressure (DBP), and systolic blood pressure (SBP). The results were then recorded. All measurements were conducted by an experienced veterinarian. The initial blood pressure measurements were disregarded. Subsequently, a total of three measurements were taken with a 15 sec interval between consecutive readings. The arithmetic average of these three readings was used for data analysis.

Blood Sampling and Complete Blood Count Analysis

After the clinical examination of the dogs (before starting the treatment), blood samples were collected from the cephalic veins of the dogs for hematological examinations to determine the infection status and leukocyte counts within the standard practices of the clinic. Subsequently, blood samples were collected again on the 3rd and 5th days after the initial treatment. Blood samples were duly taken into BD Vacutainer® K_2 EDTA tubes (Becton Dickinson, USA) for hematology analyses. Blood samples were delivered to the lab (Erciyes University, Faculty of Veterinary Medicine, Animal Hospital, clinical hematology, and biochemistry laboratory). Hematological analyses of the dogs with parvoviral enteritis were performed using a complete blood count device (Exigo EosVet, Boule Medical AB, Stockholm, Sweden) in the laboratory. Blood samples taken into BD Vacutainer® K_2 EDTA (Becton Dickinson, USA) tubes were homogenized for 3 min at 40 rpm/min using a roller blood mixer before analysis.

Treatment

In order to administer fluid-electrolyte therapy, the ill dogs' level of dehydration was assessed. For the purpose of fluid-electrolyte therapy, the following were administered: lactated ringer (Polifleks, Polifarma), 5% Dextrose (Polifleks, Polifarma), isotonic 0.9% NaCl (Polifleks®, Polifarma), Dextran 40 (Poliflex 100 mg/mL Dextran 40 + 9 mg/mL Isotonic Sodium Chloride solution for IV infusion, Polifarma) alone or in combination. Maintenance fluids were supplemented with potassium chloride (0.05-0.4 mEq/kg/h) for sustaining normokalemia or restoring hypokalemia. The total amount of fluid to be given was calculated according to the formulas [25]: Fluid deficit (L) (body weight [kg] X % dehydration/100) + maintenance needs (40-60 mL/kg per day) + ongoing losses (1 mL/kg per h).

Ampicillin-sulbactam (SULCID® 0.5 g IM/IV, İbrahim Etem Ulagay İlaç Sanayi Türk A.Ş.) was administered at a dose of 10-30 mg/kg, IV, q 6-8 h. An intravenous injection of vitamin C (Vitce®, Sanovel) at a dose of 200 mg/kg was given for five days. Amino acids, vitamins and electrolytes for extra energy (Duphalayte®, Zoetis) was administered in a practical dose of 10 mL/kg for 5-7 days. Metoclopramide (Metpamid®, Sifar İlaçları Tic. ve San. A.Ş.) was used at a dose of 0.5 mg/kg. In persistent vomiting cases, maropitant citrate (Cerenia®, Zoetis) was also injected subcutaneously once a day at a dose of 1 mg/kg. *Bacillus clausii* (Enterogermina, Sanofi-Aventis SpA) was administered orally at a dose of 2 mL for 3-5 days as a probiotic. Hyoscine butylbromide (Buscopan® 20 mg/mL Injectable Solution, Sanofi İlaç San. ve Tic. A.Ş.) was administered by intramuscular injection daily twice at a dose of 0.5 mg/kg as an antispasmodic drug. In addition to standard treatment in the ST+Filg group, Filgrastim (recombinant methionyl human granulocyte colony-stimulating factor [r-metHuG-CSF] [Fraven 30 MIU/0.5 mL, IV infusion/SC Injection, Arven İlaç San.]) was administered subcutaneously at a dosage of 10 µg/kg once a day for 5 days. This dose of filgrastim (r-metHuG-CSF) was chosen in accordance to the study performed by Punia et al.[23] and Areshkumar et al.[24].

Statistical Analysis

Commercial software (SPSS for Windows Release 25.0 Program, SPSS Inc, Chicago, IL, USA) was used to conduct statistical analyses. All of the data were visually examined, the Shapiro-Wilk test was used to check for normality, and descriptive statistics were conducted. Every set of data that passes the normality test was shown with its mean and standard deviation. The variables that did not pass the normality test are indicated with a "#" and displayed as the median and interquartile range (IQR). Independent Samples t test (alternative; Mann-Whitney U test) was used for comparisons between groups. In repeated measures, ANOVA was used to compare between measurements. The Bonferroni and Tukey HSD tests were used in post hoc comparisons. The relationship between categorical variables was evaluated using Pearson's χ^2 test (and Fisher's exact test). For every analysis, a P-value of less than 0.05 was considered statistically significant.

Results

During the study period, a total of 60 dogs brought to Erciyes University, Faculty of Veterinary Medicine, Department of Internal Medicine, Small Animal Clinic were diagnosed with canine parvoviral enteritis. Thirty-seven out of the sixty dogs diagnosed with parvoviral enteritis met the inclusion criteria for the study. Of these sixty dogs with CPE, 23 were excluded from the study because they did not meet the inclusion criteria.

In the ST+Filg group, 19 owned dogs with parvoviral enteritis, consisting of various breeds were included. Among these 19 dogs, 11 (57.9%) were male, and 8 (42.1%) were female. The average age of these dogs was 136 (80-155) days. The dogs included in the ST+Filg group were of various breeds, including Akbash (n=1), German Hunting Terrier (n=1), Belgian Malinois (n=2), Border Collie (n=1), Doberman Pinscher (n=2), Kangal Shepherd Dog (n=3), Crossbreed (n=3), Pointer (n=1), Bolonka (n=3), Siberian Husky (n=1), and Terrier (n=1). Their median body weight was 9.50 (5.50-17.75) kg.

In the ST group, 18 owned dogs with parvoviral enteritis, consisting of various breeds were included. Among these 18 dogs, 10 (55.6%) were male, and 8 (44.4%) were female. The median age of these dogs were 79 (69-120) days. The dogs included in the ST group were of various breeds, including German Shepherd Dog (n=4), Belgian Groenendael (n=1), Akbash (n=2), Doberman Pinscher (n=1), Kangal Shepherd Dog (n=4), Crossbreed (n=2), Rottweiler (n=3), and Bolonka (n=1). Their median body weight was 6.90 (4.23-10.0) kg.

Vaccine and Antiparasitic Drug History Information

According to the history obtained from the pet owners, it was determined that out of these 37 dogs, 17 had received both internal and external parasite treatments regularly and on time, 8 had not received any, and 11 had an unknown anti-parasitic treatment status. Among these dogs, 15 had received a single combined (canine distemper, canine infectious hepatitis, canine infectious laryngotracheitis, canine parvovirus, canine parainfluenza, and canine leptospirosis) vaccine, and 11 had received twice the combined vaccines. The vaccination status of the rest of the 11 dogs were unknown.

Feces Antigen Testing

In cases of suspected canine parvoviral enteritis based on clinical examination, 37 dogs were evaluated as CPV antigen positive in analysis with lateral flow immunochromatography (LFI) rapid diagnostic kits for CPV antigen. PCR was also performed on the same fecal samples. Following the use of specific primers, PCR amplification was carried out on 1.7% agarose gel, and the results were evaluated under UV light. The samples from all dogs were evaluated as positive by PCR as well. Only 7 samples of PCR images are shown *(Fig. 2)*.

Physical Examination Findings

In the clinical examination of these 37 dogs with CPE in the ST + Filg (n=19) and ST (n=18) groups when they were admitted to the hospital, diarrhea (haemorrhagic diarrhea=22, non-haemorrhagic diarrhea=15) in 100% (37/37), vomiting in 86.49% (32/37), anorexia in 86.49% (32/37), lethargy in 78.38% (29/37), depression in 94.59% (35/37), dehydration (mild=7, moderate=9, severe=18) in 91.89% (34/37), enlargement of retropharyngeal lymph nodes in 75.68% (28/37), poor pulse quality in 59.46% (22/37), tachycardia in 62.16% (23/37) and fever in 43.24% (16/37) were detected.

The mean/median values of body temperature (°C), heart rate (bpm), and respiratory rate (breaths/min) for the dogs with CPE in the ST+Filg group when they were admitted to the hospital were measured as follows: 38.9 (38.1-39.9), 131.61±34.82, 32.00 (29.50-45.00), respectively. There was a statistically significant difference between the body

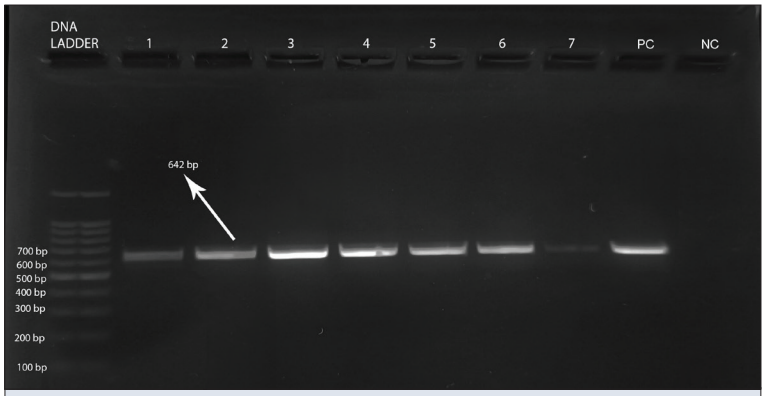

Fig 2. *Canine parvovirus* VP2 gene PCR results. Lines 1-7 represent the samples, where PC stands for the positive control and NK stands for the negative control (nuclease-free water). The specific primers used targeted a 642 bp PCR amplicon length, and all samples, including PC (positive control) and samples 1-7, were evaluated as positive

temperature measurements of dogs in the ST+Filg group (P<0.001). The mean body temperature on the 3rd and 5th days after treatment was significantly lower than on the 0th day (P=0.001, P=0.023). However, there was no statistically significant difference in respiratory rate and heart rate measurements of dogs in the ST+Filg group. The mean/median values of body temperature (°C), heart rate (bpm), and respiratory rate (breaths/min) for the dogs with CPE in the ST group when they were admitted to the hospital were measured as follows: 38.2 (38.0-38.7), 168.88±11.74, 30.00 (25.0-35.0), respectively. There was no significant difference between body temperature (°C), heart rate (bpm), and respiratory rate (breaths/min) measurements of dogs in the ST group (*Table 1*).

The mean CRT (sec) values of dogs with CPE in the ST+Filg group on the 3rd (2.24±0.75 sec) and 5th days (2.41±0.80 sec) after treatment were found to be significantly lower than the mean CRT (3.41±0.87 sec) values obtained on day 0 (P=0.001, P=0.002; respectively). There was no significant difference between the 3rd and 5th days in the ST + Filg group in terms of the CRT variable (P = 0.188). The average CRT (2.24±0.75 sec) values of dogs in the ST+Filg group on the 3rd day was found to be significantly lower than the same day measurement (3.29±0.29 sec) of the ST group (P=0.002) *(Table 1)*.

In the ST+Filg group, it was determined that 16 out of the 19 dogs treated with filgrastim (r-metHuG-CSF) (84.2%) showed improvement, while three of them (15.8%) did not survive. In the ST group, deaths were higher and most of the dogs died within the first 5 days. Of the 18 dogs included in the ST group, 12 recovered and 8 died *(Table 1)*. There was no statistically significant relationship between survival status and group categories of dogs with CPE in the study (χ^2=3.633, P=0.060). Leukocyte and neutrophil values of dogs with CPE that died were lower than those that survived (P<0.05) *(Table 1)*.

Blood Pressure Measurement Findings

The median SBP, DBP and MAP values of the dogs with parvoviral enteritis included in the ST+Filg and ST group when they were brought to the hospital were at the upper limit of the reference ranges specified for dogs [28]. There was no significant difference between the blood pressure measurements (SBP, DBP, MAP) of dogs with parvoviral enteritis in the ST+Filg and ST groups on the 3rd and 5th

Table 1. *Comparison of physical examination findings and survival status in dogs with parvoviral enteritis*

Variables		0th day (n=19)	3rd days (n=16)	5th days (n=16)	P Values	Ref. Ranges [26,27]
T (°C)#	ST + Filg.	38.9 (38.1-39.9)^{a}	38.2 (37.3-39.0)^{b}	38.4 (38.2-38.8)^{ab}	**<0.001**	37.5-39.2
	ST	38.2 (38.0-38.7)	38.2 (38.1-38.9)	38.6 (38.1-39.0)	0.574	
	P values	0.353	0.933	0.854		
RR (min)#	ST + Filg.	32.00 (29.50-45.00)	34.00 (26.00-44.00)	44.00 (30.00-52.00)	0.075	18.0-34.0
	ST	30.00 (25.00-35.00)	38.00 (29.00-51.00)	39.00 (32.00-51.00)	0.173	
	P values	0.990	0.116	0.320		
HR (bpm)	ST + Filg.	131.61±34.32^{A}	110.00±14.14	124.00±34.13	0.275	70.0-120.0
	ST	168.88±11.74^{B}	159.00±11.76	151.00±14.63	0.335	
	P values	**0.007**	0.059	0.867		
CRT (sec)	ST + Filg.	3.41±0.87^{a}	2.24±0.75^{b,A}	2.41±0.80^{b}	**<0.001**	<3.0 sec
	ST	3.79±0.42	3.29±0.29^{B}	2.86±0.34	0.065	
	P values	0.761	**0.002**	0.581		
Survival Status*						
Dead	ST + Filg.	0.0% (0/19)	15.79% (3/19)	15.79% (3/19)	χ^2=0.060 3.633	
	ST	0.0% (0/18)	33.33% (6/18)	44.44% (8/18)		
Survived	ST + Filg	100% (19/19)	84.21% (16/19)	84.21% (16/19)		
	ST	0.0% (0/18)	66.66% (12/18)	55.55% (10/18)		

*ST: Standard treatment, ST+Filg.: Standard Treatment + Filgrastim, CRT: Capillary refill time, HR: heart rate, T: body temperature, RT: Respiration rate. For each set of data that passes the normality test, the mean and standard deviation (SD) are displayed. The variables designated with a # and displayed as the median (25th-75th percentile) are those that did not pass the normality test. *The data were expressed as % (n /total). ^{a,b} Values within a row with different superscripts differ significantly at P<0.05*

Table 2. *Comparison of blood pressure before (day 0) and after (days 3 and 5) treatment*

Variables		0th day (n=19)	3rd days (n=16)	5th days (n=16)	*P* values	Ref. Ranges [28]
SBP (mmHg)	ST + Filg.	133.00 (101.00-139.00)^{A}	125.00 (114.75-128.50)	120.50 (109.00-126.50)	0.207	90-140 mmHg
	ST	128.00 (114.75-156.75)^{B}	118.00 (108.75-139.50)	128.00 (118.50-154.75)	0.639	
	P values	**0.045**	0.429	0.102		
DBP (mmHg)	ST + Filg.	125.00 (114.75-128.50)^{a}	80.00 (70.00-93.25)^{b}	77.50 (67.50-101.75)^{b}	**<0.001**	50-80 mmHg
	ST	91.50 (78.25-104.50)^{a}	74.00 (62.00-95.50)^{b}	101.00 (91.25-104.00)^{b}	**0.004**	
	P values	0.580	0.755	0.095		
MAP (mmHg)	ST + Filg.	90.00 (77.00-107.00)	96.00 (86.25-106.75)	91.00 (85.00-103.50)	0.220	60-100 mmHg
	ST	101.00 (92.50-119.75)	95.50 (81.25-139.25)	110.00 (99.00-120.25)	0.843	
	P values	0.141	0.495	0.150		

ST: Standard Treatment, ST+Filg.: Standard Treatment + Filgrastim, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure. Data were espresse as the median (25th-75th percentile). ^{a,b} *Values within a row with different superscripts differ significantly at P<0.05*

Table 3. *Comparison of hematological parameters before (day 0) and after (days 3 and 5) treatment*

Variables		0th day	3rd days	5th days	P Values	Ref. Ranges [29,30]
WBC (10^9/L) #	ST + Filg.	1.90 (1.30-4.95)^{a}	6.60 (5.10-16.10)^{ab,A}	13.40 (11.78-18.33)^{b,A}	**0.013**	6.00-17.00
	ST	2.75 (1.00-4.30)^{a}	3.30 (3.00-5.10)^{b,B}	7.98 (2.30-12.90)^{c,B}	**<0.001**	
	P Values	0.654	**0.004**	**0.005**		
Lymph (10^9/L) #	ST + Filg.	0.80 (0.35-1.15)^{a}	2.20 (1.40-2.80)^{b,A}	3.20 (2.05-5.13)^{b,A}	**0.017**	0.90-5.00
	ST	0.86 (0.40-1.10)^{a}	1.20 (0.60-1.59)^{b,B}	2.14 (2.10-5.10)^{c,B}	**<0.001**	
	P Values	0.918	**0.007**	**0.041**		
Mono (10^9/L) #	ST + Filg.	0.20 (0.20-0.40)	0.70 (0.40-1.20)^{A}	1.15 (0.90-2.35)	0.112	0.30-1.50
	ST	0.27 (0.18-0.38)^{a}	0.44 (0.20-0.54)^{b,B}	0.56 (0.30-0.80)^{b}	**0.001**	
	P Values	0.975	**0.042**	0.052		
Neut (10^9/L) #	ST + Filg.	1.00 (0.50-3.35)^{a}	4.00 (2.30-12.70)^{ab,A}	9.40 (6.00-13.25)^{b,A}	**0.007**	3.50-12.00
	ST	1.61 (0.50-3.10)^{a}	2.50 (2.00-3.22)^{b,B}	5.06 (3.18-6.10)^{c,B}	**0.001**	
	P Values	0.557	**0.008**	**0.005**		
Eos (%) #	ST + Filg.	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.996	1.00-18.00
	ST	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.992	
	P Values	0.990	0.996	0.980		
RBC (10^{12}/L)	ST + Filg.	6.92±1.13^{a,A}	6.18±0.88^{b}	5.56±0.62^{b,A}	**0.004**	5.50-8.50
	ST	6.07±0.59^{a,B}	5.91±0.48^{a}	5.69±0.55^{b,B}	**<0.001**	
	P Values	**0.002**	0.152	**0.026**		
Hgb (g/dL)	ST + Filg.	14.38±3.03^{a,A}	12.46±2.91^{b,A}	10.17±1.74^{b}	**0.004**	12.00-18.00
	ST	11.40±1.70^{a,B}	10.80±1.01^{a,B}	9.84±1.56^{b}	**0.002**	
	P Values	**<0.001**	**0.045**	0.064		
Hct (%)	ST + Filg.	40.92±8.69^{a,A}	35.20±7.73^{ab,A}	29.03±3.84^{b}	**0.002**	37.00-55.00
	ST	34.06±4.41^{a,B}	32.84±2.95^{a,B}	28.26±2.85^{b}	**<0.001**	
	P Values	**<0.001**	**0.044**	0.069		
MCV (fL)	ST + Filg.	57.96±7.69	56.63±8.44	53.30±4.70	0.203	60.00-72.00
	ST	55.69±4.96	55.19±4.39	53.67±2.89	0.240	
	P Values	0.301	0.467	0.926		

MCH (pg)	ST + Filg.	20.36±2.50[A]	20.06±3.23	18.30±2.42	0.276	19.50-25.50
	ST	18.57±1.39[B]	18.24±0.87	18.87±1.44	0.365	
	P Values	**0.008**	0.088	0.728		
MCHC (g/dL)	ST + Filg.	35.23±1.49	35.40±1.25	34.90±1.86	0.651	32.00-38.50
	ST	33.55±2.58	33.24±1.84	35.33±1.23	0.419	
	P Values	0.056	0.068	0.554		
RDWa (fl)	ST + Filg.	49.36±3.09	48.00±3.12	49.50±5.46	0.276	35.00-65.00
	ST	48.90±1.92	47.08±3.01	46.90±3.63	0.202	
	P Values	0.638	0.100	0.358		
RDW (%)[#]	ST + Filg.	15.40 (14.55-19.70)	15.10 (14.90-20.10)	18.10 (15.50-24.83)	0.449	12.00-17.50
	ST	17.21 (14.80-21.80)	17.54 (15.10-21.40)	17.11 (14.60-24.50)	0.176	
	P Values	0.592	0.929	0.494		
PLT (10^9/L)	ST + Filg.	301.18±91.39	297.57±170.70	261.00±91.33	0.156	200.00-500.00
	ST	366.10±182.52[a]	263.70±133.00[a]	180.02±44.94[b]	**0.005**	
	P Values	0.273	0.884	0.449		
MPV (fL)	ST + Filg.	6.45 ± 0.66	6.13 ± 0.66	6.40±1.41	0.513	5.50-10.50
	ST	6.61±0.98[a]	5.93±0.54[b]	6.04±0.41[b]	**0.022**	
	P Values	0.706	0.667	0.724		

ST: Standard treatment, ST+Filg.: Standard Treatment+Filgrastim, RBC: Red Blood Cell, Hct: hematocrit, Hgb: hemoglobin concentration, Lymph: lymphocyte, Neut: Neutrophil, Mono: monocyte, Eos: Eosinophil, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin volume, MCHC: mean corpuscular hemoglobin concentration, WBC: White Blood Cell, PLT: platelet, RDW: Red cell distribution, RDWa: absolute value of the width of the distribution of red blood cells, MPV: mean platelet volume. For each set of data that passes the normality test, the mean and standard deviation (SD) are displayed. The variables designated with a [#] and displayed as the median (25th-75th percentile) are those that did not pass the normality test. [a,b,c] Values within a row with different superscripts differ significantly at P<0.05

days. DBP values of dogs with parvoviral enteritis in the ST+Filg and ST groupS on days 3 and 5 were found to be significantly lower than day 0 (P<0.05) *(Table 2)*.

Hematological Findings

The mean WBC, lymphocyte, monocyte and neutrophil values of dogs in both groups (ST+Filg and ST) were significantly lower than the reference ranges determined for dogs at time 0 *(Table 3)*. In the ST + Filg group, there was a significant difference between pre-treatment (0th day) and post-treatment measurements (3rd and 5th days) in terms of WBC, lymphocyte and neutrophil variables (P=0.006, P=0.004 and P=0.007, respectively. The median WBC value on the 5th day after treatment was significantly higher than on the 0th day (P=0.001). The median lymphocyte value on the 3rd and 5th days after treatment was significantly higher than on the 0th day (P=0.048, P=0.004, respectively). The median neutrophil value on the 5th day after treatment was significantly higher than on the 0th day (P=0.006) *(Table 3)*.

Median WBC, lymphocyte, monocyte and neutrophil values of dogs with parvoviral enteritis in the ST group on the 5th day after treatment were significantly higher than before treatment (0th day) (P<0.001, P<0.001, P=0.001, P<0.001, respectively). Median WBC, lymphocyte, monocyte and neutrophil values on the 3th day after treatment were significantly higher than on the 0rd day (P=0.005, P=0.023, P<0.001, P=0.004, respectively). Median WBC, lymphocyte and neutrophil values on the 5th day after treatment were significantly higher than on the 3rd day (P=0.006, P=0.005, P=0.031, respectively) *(Table 3)*.

The median WBC, lymphocyte and neutrophil values of the dogs in the ST+Filg group on the 3rd and 5th days were significantly higher than the same day measurements of the ST group (P<0.05) *(Table 3)*.

The measurements were taken before treatment (day 0) and after filgrastim (r-metHuG-CSF) therapy (days 3 and 5) in the ST+Filg group showed a statistically significant difference in the RBC, Hgb, and Hct variables (P=0.004, P=0.004, and P=0.002, respectively). Furthermore, the mean RBC value on the 3rd and 5th days after treatment was significantly lower than on the 0th day (P=0.016, P=0.001, recpectively). In addition, the mean Hgb value on the 3rd and 5th days after treatment was significantly lower than on the 0th day (P=0.011, P=0.002; recpectively) and also the mean Hct value on the 5th day after treatment was significantly lower than on the 0th day (P=0.001). Other hematological variables were not statistically significant *(Table 3)*.

In the ST group, the mean RBC, Hgb and Hct values on days 5 treatment was significantly lower than on day 3 (P<0.001, P=0.001, P<0.001, recpectively) and on day 0

(P<0.001, P=0.009, P<0.001, recpectively). The median Hgb and Hct values of the dogs in the ST+Filg group on the 3rd days were significantly higher than the same day measurements of the ST group (P=0.045, P=0.044, recpectively) *(Table 2)*. The median RBC value of the dogs in the ST+Filg group on the 5rd days were significantly lower than the same day measurements of the ST group (P=0.026). Other significant differences between groups and measurements are given in *Table 3*.

Discussion

Canine parvovirus (CPV) and canine distemper virus (CDV) are two important pathogens that can infect both domestic and wild animals [1,4,31-33]. Leukopenia and neutropenia in dogs with CPE are regarded as negative prognostic indicators [4,13,32,34]. Upon evaluating the data obtained from the present study, it was determined that out of these nineteen dogs treated with filgrastim [recombinant methionyl human granulocyte colony-stimulating factor (r-metHuG-CSF)] in addition to standard treatment, sixteen of the CPV infected dogs (84.21%) showed improvement while the rest of three (15.8%) did not survive. Additionally, the blood parameters of WBC, lymphocyte, and neutrophil values significantly increased starting from the third day of filgrastim (r-metHuG-CSF) treatment.

Gülersoy et al.[17] in a study, concluded that in CPE only granulocytes were effected and filgrastim was effective on the granulocyte count in the groups they formed without taking into account leukopenia levels, but when their findings were examined, a decrease was observed in the Filgrastim group on days 1 and 3 compared to day 0. In the current study, dogs that did not show signs of leukopenia were not included to the study. Therefore, the effectiveness of Filgrastim (r-metHuG-CSF) on leukocytes was more clearly demonstrated.

Punia et al.[23] reported that all 11 dogs with hemorrhagic gastroenteritis treated with Filgrastim in addition to supportive treatment recovered. Similarly, all 31 CPE positive dogs treated with recombinant canine granulocyte-colony stimulating factor (rcG-CSF) were reported to recover [18]. In our study, the mortality rate on the 5th day was calculated as 44% (8/18) in the ST group receiving standard treatment. The higher mortality rate observed in this group compared to the ST+Flig group suggests that it may be related to the fact that the dogs in this group did not receive any drugs that increase leukocyte levels. The mortality rate (15.8%) in the ST+Filg group was lower than the ST group. This rate was found to be close to the mortality rate reported by Güleryüz et al.[17]. However, in the current study, dogs with significantly lower total leukocyte count and its subgroups were included in the study. Therefore, it was believed that present study was better designed to demonstrate the effectiveness of the drug. Several researchers have expressed that leukopenia and neutropenia are negative prognostic indicators in dogs with parvoviral enteritis [4,13,32,34]. Therefore, monitoring complete blood count parameters in dogs with parvoviral enteritis at the time of admission and post-treatment can provide valuable information for the prediction of prognosis. According to the literature, although it is stated that the success of treatment in dogs using dog-specific granulocyte-colony stimulating factor (rcG-CSF) is 100%, the important disadvantages of this agent should be taken into consideration since it is difficult to obtain from the market and its price is quite high.

In this study, it was observed that the median WBC, lymphocyte, monocyte and neutrophil values of the dogs included in the study were significantly lower than the reference values of healthy dogs [29]. In a study, in which the pathogenesis of canine parvoviral enteritis was investigated experimentally, four days after the virus inoculation, five of the eight infected dogs showed signs of lymphopenia; five days later, all of the infected dogs showed signs of lymphopenia. The mean total leukocyte of virus-infected dogs was lower five and six days after virus inoculation compared to dogs that were passively vaccinated and control dogs, although the total leukocyte counts of individual dogs varied greatly. Five days following the inoculation, one infected dog was panleukopenic [35]. A possible explanation for our findings may be related to the fact that dogs with CPE, included in the present study, were brought to the hospital at an advanced stage of the disease, that is, after at least 4 days had passed since the onset of the disease. Indeed, as described by Mazzaferro et al.[1] bone marrow depression and CPV's attack on the bone marrow, thymus, and other lymphoid tissues are observed to cause leukopenia, lymphopenia, and neutropenia in the advanced stage.

CPV infection is associated with non-specific symptoms such as pyrexia, lethargy, anorexia, and increased respiratory rate within 2-7 days after the onset of infection [35-37]. Gülersoy and Naseri [9] reported higher body temperature, heart, and respiratory rate values in dogs with CPE compared to healthy dogs. When the dogs with CPE were admitted to the current investigation, their mean body temperature was almost at the top limit of the reference values [26]. The CPE-positive dogs body temperature dropped on the 3rd and 5th days following treatment, and this drop was statistically significant (P<0.001) compared to the pre-treatment level. This could be connected to the dogs' improved general health upon admission and the way that sepsis or systemic inflammatory response syndrome (SIRS) cleared up after receiving medication [9]. The dogs in the present study were found to be dehydrated during a clinical examination conducted at the time of

their hospital admission, and their RBC, Hgb, and Hct levels were in close proximity to the upper limits of the reference values [29]. However, on the 3rd and 5th days after treatment, these values began to decrease. Actually, dogs with parvoviral enteritis experience considerable fluid and protein loss in their gastrointestinal tracts [38]. Because dogs with parvoviral enteritis that vomit and have diarrhea lose fluid, which causes hypovolemia and dehydration. These conditions impair tissue perfusion, which can cause changes in mucosal color, tachycardia, and delayed capillary refill time [1]. In our current study, the decrease in RBC, Hgb and Hct values may be related to the improvement of dehydration and general condition with fluid and electrolyte therapy, as well as the start of fluid intake and the decrease in diarrhea. Another possible reason could be related to anemia resulting from hemorrhagic gastroenteritis that occurs concurrently with diarrhea. Because, hemorrhagic gastroenteritis is a common clinical manifestation of CPE [1].

The median WBC, lymphocyte, and neutrophil counts were 1.90 (1.30-4.95), 0.80 (0.35-1.15) 10^9/L, and 1.00 (0.50-3.35) 10^9/L, respectively. To correct the leukopenia in these dogs, in addition to standard treatment, subcutaneous filgrastim (r-metHuG-CSF) was administered at a dose of 10 µg/kg once daily for 5 days. On the 3rd and 5th days after treatment, WBC, lymphocyte, and neutrophil counts increased to the normal reference range along with clinical improvement. These values were found to be significantly higher than the values on the 0th day. In addition, the median WBC, lymphocyte and neutrophil counts of dogs in the ST+Filg group on the 3rd and 5th days after treatment were found to be significantly higher than the same day measurements of dogs with ST group. Filgrastim (r-metHuG-CSF) primarily affects the bone marrow and stimulates the production of leukocytes. The vital molecules that regulate the differentiation, growth, and survival of blood cells are called granulocyte colony-stimulating factors, or G-CSF [16,18]. Additionally, a number of studies have shown that within 24 hours of dosing, G-CSF increases the generation and release of functional neutrophils in the bone marrow [17,39]. In contrast to the findings of Rewerts et al.[40] and Mischke et al.[41] the results obtained from the current study were consistent with the recent studies [17,23,24]. Punia et al.[23] administered a daily dose of 10 µg/kg of filgrastim for three days as supportive treatment to 11 dogs with severe leukopenia and neutropenia due to hemorrhagic gastroenteritis. They reported that within 48 h of the last treatment dose, the hematological profile improved, and all dogs showed rapid clinical improvement without any problems. On the contrary, Gülersoy et al.[17] did not report a significant increase in WBC, lymphocyte and granulocyte counts on the 1st and 3rd days in dogs with parvoviral enteritis treated with filgrastim in addition to standard treatment. This may be related to the inclusion of dogs with parvoviral enteritis in the study without considering their leukocyte levels. When the data in the study of Gülersoy et al.[17] is examined, lowest and highest values of the WBC, lymphocyte and granulocyte explain this situation. On the other hand, in dogs with parvoviral enteritis, impressive results have been reported with recombinant canine granulocyte-colony stimulating factor (rcG-CSF) by many other researchers as reported in the present study [16,18].

The main limitation of the present study is low sample size. The diversity of breed variation in dogs, especially the susceptibility of certain dog breeds to CPV, is a well-known factor [5]. Nevertheless, in this study, Filgrastim demonstrated no adverse effects, raised leukocyte levels to the target range from the third day, and proved to be more cost-effective than other medications.

In conclusion, this study found that filgrastim (r-metHuG-CSF) improved leukopenia in dogs with CPE in addition to aiding in their clinical recovery. The results imply that filgrastim, an immunostimulant medication, may be included in therapy regimens to raise leukocyte counts in the management of canine parvoviral enteritis.

Declarations

Availability of Data and Materials: The data given in this study may be obtained from the corresponding auther (G. Ekinci) on reasonable request.

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Conflict of Interest: The authors declare that they have no conflict of interest.

Author Contributions: İK, MÇ, VG, AGG and ACO supervised the study. GE, ET, SK, ES and AMAA collected the data. GE and ET made the statistics. The first draft of the manuscript was written by GE, ET, SK and ES and all authors contributed to the critical revision of the manuscript and have read and approved the final version.

References

1. Mazzaferro EM: Update on canine parvoviral enteritis. *Vet Clin North Am Small Anim Pract,* 50 (6): 1307-1325, 2020. DOI: 10.1016/j.cvsm.2020.07.008

2. Decaro N, Buonavoglia CB, Barrs VR: Canine parvovirus vaccination and immunisation failures: Are we far from disease eradication? *Vet Microbiol,* 247, 1-8, 2020. DOI: 10.1016/j.vetmic.2020.108760

3. International Committee on Taxonomy of Viruses (ICTV): Subfamily: Parvovirinae; Genus: Protoparvovirus. https://ictv.global/report/chapter/

parvoviridae/ parvoviridae/protoparvovirus. *Accessed:* 27.09.2023.

4. Greene CE, Decaro N: Canine viral enteritis. **In,** Greene CE (Ed): Infectious Diseases of the Dog and Cat. 4th ed., 67-80, St. Louis, Elsevier Saunders, 2012.

5. Mylonakis ME, Kalli I, Rallis TS: Canine Parvoviral Enteritis: An update on the clinical diagnosis, treatment, and prevention. *Vet Med (Auckl),* 7, 91-100, 2016. DOI: 10.2147/VMRR.S80971

6. Maclachlan NJ, Dubovi EJ: Fenner's Veterinary Virology. 5th ed., 245-257, Academic Press, 2016.

7. Prittie J: Canine parvoviral enteritis: A review of diagnosis, management, and prevention. *Vet Emerg Crit Care,* 14 (3): 167-176, 2004. DOI: 10.1111/j.1534-6935.2004.04020.x

8. Decaro N, Desario C, Campolo M, Elia G, Martella V, Ricci D, Lorusso E, Buonavoglia C: Clinical and virological findings in pups naturally infected by canine parvovirus type 2 Glu-426 mutant. *J Vet Diagn Invest*, 17 (2): 133-138, 2005. DOI: 10.1177/104063870501700206

9. Gülersoy E, Naseri A: Hematological status in septic or non septic dogs due to parvoviral enteritis. *Dicle Üniv Vet Fak Derg,* 15 (1): 45-52, 2022. DOI: 10.47027/duvetfd.1100794

10. Kalli I, Leontides LS, Mylonakis ME, Adamama-Moraitou K, Rallis T, Koutinas AF: Factors affecting the occurrence, duration of hospitalization and final outcome in canine parvovirus infection. *Res Vet Sci,* 89 (2): 174-178, 2010. DOI: 10.1016/j.rvsc.2010.02.013

11. Öcal N, Ünsüren H: Parvoviral hemorajik gastroenteritisli köpeklerin sağıltımında total parenteral beslemenin etkisi. *Kafkas Univ Vet Fak Derg,* 15 (2): 237-244, 2009. DOI: 10.9775/kvfd.2008.113-A

12. Ok M, Er C, Yıldız R, Çöl R, Aydoğdu U, Şen İ, Guzelbektes H: Evaluation of acute phase proteins, some cytokines and hemostatic parameters in dogs with sepsis. *Kafkas Univ Vet Fak Derg,* 21 (5): 761-766, 2015. DOI: 10.9775/kvfd.2015.13418

13. Goddard A, Leisewitz AL, Christopher MM, Duncan NM, Becker PJ: Prognostic usefulness of blood leukocyte changes in canine parvoviral enteritis. *J Vet Intern Med,* 22, 309-316, 2008. DOI: 10.1111/j.1939-1676.2008.0073.x

14. Venn EC, Preisner K, Boscan PL, Twedt DC, Sullivan LA: Evaluation of an outpatient protocol in the treatment of canine parvoviral enteritis. *J Vet Emerg Crit Care,* 27 (1): 52-65, 2017. DOI: 10.1111/vec.12561

15. Aydın O, Kırbaş A: Köpeklerin parvovirüs enfeksiyonunda tedavi uygulamarına güncel yaklaşım. *Bozok Vet Sci,* 2 (2): 62-72, 2021.

16. Duffy A, Dow S, Ogilvie G, Rao S, Hackett T: Hematologic improvement in dogs with parvovirus infection treated with recombinant canine granulocyte-colony stimulating factor. *J Vet Pharmacol Ther,* 33 (4): 352-356, 2010. DOI: 10.1111/j.1365-2885.2009.01153.x

17. Gülersoy E, Balıkçı C, Kısmet E, Şahan A, Günal I: The use of hG-CSF in canine parvoviral enteritis: Its effect on clinical and laboratory variables. *Veterinaria,* 72 (1): 71-87, 2023.

18. Armenise A, Trerotoli P, Cirone F, De Nitto A, De Sario C, Bertazzolo W, Pratelli A, Decaro N: Use of recombinant canine granulocyte-colony stimulating factor to increase leukocyte count in dogs naturally infected by canine parvovirus. *Vet Microbiol,* 231, 177-182, 2019. DOI: 10.1016/j.vetmic.2019.03.015

19. Rewerts JM, Henry CJ: Veterinary uses of recombinant human granulocyte colony-stimulating factor. II. Infectious diseases. *Comp Contin Educ Pract Vet,* 20 (7): 823-827, 1998.

20. Mehta HM, Malandra M, Corey SJ: G-CSF and GM-CSF in neutropenia. *J Immunol*, 195 (4): 1341-1349, 2015. DOI: 10.4049/jimmunol.1500861

21. Greenbaum AM, Link DC: Mechanisms of G-CSF-mediated hematopoietic stem and progenitor mobilization. *Leukemia,* 25 (2): 211-217, 2011. DOI: 10.1038/leu.2010.248

22. Kraft W, Kuffer M: Treatment of severe neutropenia in dogs and cats with filgrastim. *Tierarztl Prax,* 23, 609-613, 1995.

23. Punia S, Kumar T, Agnihotri D, Sharma M: A study on effect of filgrastim in severe leucopenia associated with hemorrhagic gastroenteritis in dogs. *J Pharm Innov,* 10 (Special Issue 11): 868-870, 2021.

24. Areshkumar M, Vijayalakshmi P, Perumal SV, Selvi D: Effect of filgrastim in a severe leucopenia associated parvoviral enteritis in Rottweiler. *Int J Curr Microbiol Appl Sci,* 6 (4): 1060-1065, 2017. DOI: 10.20546/ijcmas.2017.604.131

25. Tello L, Perez-Freytes R: Fluid and electrolyte therapy during vomiting and diarrhea. *Vet Clin North Am Small Anim Pract,* 47 (2): 505-519, 2017. DOI: 10.1016/j.cvsm.2016.09.013

26. Cugmas B, Šušterič P, Gorenjec NR, Plavec T: Comparison between rectal and body surface temperature in dogs by the calibrated infrared thermometer. *Vet Anim Sci,* 9:100120, 2020. DOI: 10.1016/j.vas.2020.100120

27. MSD Veterinary Manual: https://www.msdvetmanual.com/special-subjects/reference-guides, *Accessed:* 14.11.2023.

28. Acierno MJ, Brown S, Coleman AE, Jepson RE, Papich M, Stepien RL, Syme HM: ACVIM consensus statement: Guidelines for the identification, evaluation, and management of systemic hypertension in dogs and cats. *J Vet Intern Med,* 32 (6): 1803-1822, 2018. DOI: 10.1111/jvim.15331

29. Moritz A, Fickenscher Y, Meyer K, Failing K, Weiss DJ: Canine and feline hematology reference values for the ADVIA 120 hematology system. *Vet Clin Pathol,* 33 (1): 32-38, 2004. DOI: 10.1111/j.1939-165x.2004.tb00347.x

30. López I, Mesa I: Practical Guide to Laboratory Test Interpretation and Differential Diagnosis. Haematology and Biochemistry. 2-51, Grupo Asís Biomedia SL, Zaragoza, 2021.

31. Esin C, Cetiner H, Ozkilic GN, Pekmezci D: Canine distemper virus infection in two badgers *(Meles meles)* from the Black Sea Region of Türkiye. *Kafkas Univ Vet Fak Derg,* 28 (4): 533-534, 2022. DOI: 10.9775/kvfd.2022.27680

32. Dinler Ay C, Tuna GE, Ekren Asici GS, Ulutas B, Voyvoda H: Serum intestinal fatty acid-binding protein and calprotectin concentrations to assess clinical severity and prognosis of canine parvovirus enteritis. *Kafkas Univ Vet Fak Derg,* 28 (1): 105-114, 2022. DOI: 10.9775/kvfd.2021.26568

33. Zhang Y, Dong Y, Yu N, Xie H, Wang L: Development of multiple real-time fluorescent PCR for detection of *Porcine parvovirus* (PPV), *Porcine circovirus* Type 2 (PCV2) and *Haemophilus parasuis* (HPS). *Kafkas Univ Vet Fak Derg,* 29 (6): 611-617, 2023. DOI: 10.9775/kvfd.2023.27837

34. Geetha M, Selvaraju G: Prognostic indicator of canine parvoviral infection in dogs. *J Pharm Innov,* SP-10 (11): 2432-2434, 2021.

35. Meunier PC, Cooper BJ, Appel MJG, Lanieu ME, Slauson DO: Pathogenesis of canine parvovirus enteritis: Sequential virus distribution and passive immunization studies. *Vet Pathol,* 22 (6): 617-624, 1985. DOI: 10.1177/030098588502200

36. Meunier PC, Cooper BJ, Appel MJG, Slauson DO: Pathogenesis of canine parvovirus enteritis: The importance of viremia. *Vet Pathol,* 22 (1): 60-71, 1985. DOI: 10.1177/030098588502200110

37. Goddard A, Leisewitz A: Canine parvovirus. *Vet Clin North Am Small Anim Pract,* 40 (6): 1041-1053, 2010. DOI: 10.1016/j.cvsm.2010.07.007

38. Gülersoy E, Balıkçı C, Günal İ, Şahan A: Evaluation of the efficacy of blood gases and hemogram parameters in the diagnosis of non neurogenic distemper and parvoviral enteritis in dogs with acute gastroenteritis. *Rev Cient,* 32 (1): 1-8, 2022. DOI: 10.52973/rcfcv-e32091

39. Novotný J, Zvarová M, Prazáková L, Jandlová M, Konvicková L: G-CSF in the treatment of patients with chronic aplastic anemia with severe neutropenia. *Vnitr Lek,* 41 (10): 692-695, 1995.

40. Rewerts JM, McCaw DL, Cohn LA, Wagner-Mann C, Harrington D: Recombinant human granulocyte colony-stimulating factor for treatment of puppies with neutropenia secondary to canine parvovirus infection. *J Am Vet Med Assoc*, 213 (7): 991-992, 1998.

41. Mischke R, Barth T, Wohlsein P, Rohn K, Nolte I: Effect of recombinant human granulocyte colony-stimulating factor (rhG-CSF) on leukocyte count and survival rate of dogs with parvoviral enteritis. *Res Vet Sci,* 70 (3): 221-225, 2001. DOI: 10.1053/rvsc.2001.0464