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The Research of Effectiveness of Parvulyte Gel[®] in Dogs with Parvoviral Enteritis

Parvoviral Enteritisli Köpeklerde Parvulyte Jelin[®] Etkinliğinin Araştırılması

ABSTRACT

This study aimed to demonstrate the efficacy of Parvulyte[®] in dogs with parvoviral enteritis. The animal material of the study consisted of 14 dogs diagnosed with parvoviral enteritis due to clinical examination and immunochromatographic rapid test kits brought to XX University Veterinary Health Application and Research Center Internal Diseases Clinic and private veterinary clinics in Izmir. After the diagnosis of parvoviral enteritis, the dogs in the first group received fluid therapy along with vitamin-mineral-electrolyte-amino acid supplements, pantoprazole, cefazolin sodium and maropitant citrate (Group I, n=7). The dogs in the second group received Parvulyte[®] in addition to the same treatment protocol (Group II, n=7). Both groups were treated for 7 days. It was observed that the dogs in Group II had an increase in the lymphocyte count, a faster increase in antibody titers, and a faster clinical recovery compared to the stool scoring table created. As a result, Parvulyte[®] accelerated the clinical recovery and shortened the hospitalization time in dogs with parvoviral enteritis.

Keywords: CPV, Dog, Parvoviral enteritis, Parvulyte.

ÖΖ

Bu çalışma Parvulyte[®]'nin parvoviral enteritisli köpeklerdeki etkinliğini göstermeyi amaçlamıştır. Çalışmanın hayvan materyalini, XX Üniversitesi Veteriner Sağlık Uygulama ve Araştırma Merkezi İç Hastalıkları Kliniği ve İzmir'deki özel veteriner kliniklerine getirilen, klinik muayene ve immunokromatografik hızlı test kitleri ile parvoviral enteritis tanısı konulan 14 köpek oluşturdu. Parvoviral enteritis tanısı konulduktan sonra birinci gruptaki köpeklere sıvı tedavisi ile birlikte vitamin-mineral-elektrolit-amino asit takviyesi, pantoprazol, sefazolin sodyum ve maropitant sitrat verildi (Grup I, n=7). İkinci gruptaki köpekler aynı tedavi protokolüne ek olarak Parvulyte[®] almıştır (Grup II, n=7). Her iki grup da 7 gün boyunca tedavi edilmiştir. Grup II'deki köpeklerin lenfosit sayısında artış, antikor titrelerinde daha hızlı bir artış ve oluşturulan dışkı skorlama tablosuna kıyasla daha hızlı bir klinik iyileşme olduğu gözlemlenmiştir. Sonuç olarak, Parvulyte[®] parvoviral enteritisli köpeklerde klinik iyileşmeyi hızlandırmış ve hastanede kalış süresini kısaltmıştır.

Anahtar Kelimeler: CPV, Köpek, Parvoviral enterit, Parvulyte.

INTRODUCTION

Parvoviral enteritis is considered one of the most important reasons for morbidity and mortality in puppies worldwide. Canine parvovirus is a single-stranded DNA virus that belongs to the *Protoparvovirus* genus, and *Parvoviridae* family and infects rapidly dividing cells of the gastrointestinal tract, bone marrow, lymphoid tissue and cardiac myocytes. Although the origin of canine parvovirus is not fully known, there is a theory that it may have emerged as a variant of the feline panleukopenia virus that can infect dogs, as it shares 98% structural homology.

Since the Parvoviridae family is also found in wild mammals, it is thought that genetic variations from wildlife may have played a role in the evolution of CPV-1 and CPV-2.^{1,2} CPV-1, also known as canine minut virus, was first discovered as a cause of gastrointestinal and respiratory tract infections in dogs in the late 1960s. Mutation of CPV-1 resulted in a decade later in a markedly different variant, CPV-2, causing the first pandemic outbreaks in adult and young dogs not previously exposed to CPV. Since the first isolation of CPV-1 and CPV-2, three variants have emerged: CPV-2a, CPV-2b and CPV-2c. The disease still maintains its importance despite vaccines developed and administered against CPV-2 strains.^{3,4} CPV-2 strains are highly resistant to infection strategies because their ability to infect mammalian hosts other than domestic dogs, such as raccoons, cats, coyotes, and wolves, can be found in many places in the environment, and can survive for more than a year under favorable conditions. Transmission of parvovirus occurs via the faecal-oral route after exposure to the virus in faeces, vomit, and fomites.^{5,6} Since there is no specific antiviral drug, treatment of parvoviral enteritis largely involves supportive treatments. One of the primary challenges and burdens for patients in the treatment of parvoviral enteritis is the cost of hospitalization and treatment. A study conducted in Australia reported that most parvovirus cases occur in socioeconomically underprivileged areas. Possible lack of training and lack of financial opportunity for vaccination put dogs at higher risk of contracting the disease in disadvantaged areas. The decision whether to hospitalize an animal with parvoviral enteritis to receive standard treatment rather than outpatient treatment or euthanasia largely depends on the owner's ability and willingness to pay the costs of care.⁵⁻⁹

IgY can be found in birds, reptiles, amphibians, and lungbreathing fish. IgY is also the evolutionary precursor of IgG and IgE, which are found only in mammals.¹⁰ Antibodies are protein molecules that are produced in response to an antigen. They are widely used in research, diagnosis and treatment due to their ability to bind to specific targets. Most antibodies available today are produced in mammals, especially in small rodents. However, antibody production in mammals can be challenging because some antigens can elicit weak immune responses or they are not even immunogenic. Additionally, the process of producing antibodies in mammals involves painful procedures such as immunization, blood sample collection, and sacrifice. The ongoing search for more efficient and economical techniques, as well as the reduction of animal use, has led to increased interest in egg yolk antibodies. Obtaining antibodies from egg yolk is a non-invasive method that eliminates the need for blood collection. The use of

polyclonal IgY against infectious diseases minimizes the risk of antimicrobial resistance because it can target different antigens in the same microorganism. Therefore, specific IgY antibodies are a suitable alternative for antimicrobial use in human and veterinary medicine in the recent emergence of resistant bacteria. Due to its potential to prevent bacterial infections in animals, IgY technology is thought to be useful in strategies to reduce the use of antibiotics in animal husbandry, which has an important role in the emergence and spread of resistant bacterial strains.¹¹⁻¹³ Various studies have been conducted on the therapeutic effectiveness of IgY in viral, fungal and protozoon infections. It has been observed that the duration of diarrhea and hyperthermia was shortened in animals treated with oral administration of IgY-rich egg yolk powder against rotavirus infection in calves, and other findings such

not observed in calves without the application.¹⁴⁻¹⁹ The aim of this study was to evaluate the efficacy of Parvulyte[®], a commercial product containing IgY, in dogs

as calves with anorexia, dehydration and depression were

MATERIALS AND METHODS

with parvoviral enteritis.

Animal Material

Ethical committee approval was received from the Ethics Committee of Afyon Kocatepe University (Date: 24/02/2020, Decision No:49533702/219). The animal material of the study consisted of 14 dogs which are different breeds, 1-8 months of age, different genders, who applied to the Afyon Kocatepe University Veterinary Health Application and Research Center Internal Medicine Clinic with complaints of acute enteritis, and were diagnosed with parvoviral enteritis as a result of the clinical examination and immunochromatographic test, and also test results were excluded canine coronavirus and giardia infections (Asan Easy Test[®], Korea). The dogs included in the study were numbered, then physical examination findings and laboratory findings were recorded.

Study Groups

14 dogs were randomly grouped in this study.

Group I

As part of the supportive treatment for the dogs in the first group, 0.9% NaCl solution (Bioflex[®], Osel İlaç, Türkiye), which was clinically calculated according to the patient's dehydration status and maintenance needs; lactated ringer's solution (Bioflex[®], Osel La., Türkiye); 5% dextrose solution (Bioflex[®], Osel İlaç, Türkiye); solution (Duphalyte[®], Zoetis, Türkiye) containing vitamins, minerals, electrolytes and amino acids at a dose of 10 ml/kg; pantoprazole (Protaz[®], MTA İlaç, Türkiye) at a dose of 1 mg/kg every 24 hours; cefazolin sodium (Cefozin[®], Bilim İlaç, Türkiye) at a dose of 25 mg/kg every 12 hours intravenously and; and in cases where vomiting was observed, maropitant citrate (Cerenia[®], Zoetis, Türkiye) was administered subcutaneously at a dose of 1 mg/kg every 24 hours were administered for 7 days.

Group II

For supportive treatment for the dogs in the second group, 0.9% NaCl solution (Bioflex®, Osel İlaç, Türkiye), which clinically calculated according to the patient's dehydration status and maintenance needs; lactated ringer's solution (Bioflex[®], Osel İlaç, Türkiye); 5% dextrose solution (Bioflex[®], Osel İlaç, Türkiye); solution (Duphalyte[®], Zoetis, Türkiye) containing vitamins, minerals, electrolytes and amino acids at a dose of 10 ml/kg; pantoprazole (Protaz[®]) at a dose of 1 mg/kg every 24 hours; cefazolin sodium (Cefozin[®], Bilim Ilaç, Türkiye) at a dose of 25 mg/kg every 12 hours intravenously and; and in cases where vomiting was observed, maropitant citrate (Cerenia[®], Zoetis, Türkiye) was administered subcutaneously at a dose of 1 mg/kg every 24 hours were administered for 7 days. In addition to these applications, the dose of 2.8 grams/dog of a product (Parvulyte[®], Uranovet, Spain) containing IgY, essential fatty acids, starch, B-complex vitamins (B1, B2, B6, B12, D1), vitamin E, folic acid, biotin, niacin, oligoelements and amino acids were administered orally.

Samples and Measurements

Vascular access through the vena cephalic antebrachia using a 24G or 22G branul was obtained from the dogs in both study groups, and 4 ml blood samples were taken into EDTA-containing tubes using this vascular access on the 0th, 3rd and 7th days of treatment. WBC, RBC, HGB, NEU, LYM, MCV, MCH, MCHC, HCT measurements were done using a fully automatic hemogram device and the results were recorded without waiting for the blood samples taken (HumaCount 80TS, Vet Mode, Germany).

4 ml blood samples were taken into EDTA-containing tubes using a 24G or 22G branul was obtained from the dogs in both study groups on the 0th, 7th and 14th days of treatment. Canine parvovirus antibodies were detected at low titre (below 1:40), medium titre (1:80) and high titre (1:160) according to the intensity of the band lines in the result window was determined qualitatively and recorded using a commercial immunochromatographic test kit with 5 μ L of the whole blood samples taken (Uranotest Parvo Immune Status[®], Spain).

Faecal Scoring

A stool scoring table, which was based on the Nestle-Purina stool scoring system, was created and numbered 1-6 according to stool consistency, to be evaluated on days 0, 3 and 7 in dogs in both study groups (Table 1).

Table 1. Faecal scoring table.		
Faeces score	Result	
	- Solid, not rigid, flexible	
1	- Segmented view	
	 No residue on the ground when collected 	
	 Log-shaped, moist surface 	
2	 No visible segmentation 	
Z	- Leaves residue on the floor when removed	
	but retains its shape	
	 Very moist, mud consistency 	
3	- Log shaped	
5	 When removed, it leaves residue on the 	
	ground and loses its shape.	
	 Very moist but with a definite shape 	
4	 Found in piles rather than logs 	
4	 When removed, it leaves a residue and 	
	loses its shape, light brown.	
	 Has texture but no distinct shape 	
5	 Brown in clumps or spots 	
	 Leaves residue on the floor when collected 	
	- Aqueous	
6	- No texture	
	- Puddle shaped, bloody	

Statistical Analysis

The Kruskal-Wallis test at 5% significance level was used to test whether there was a significant difference in hematological findings and fecal scoring on days 0, 3 and 7 and antibody titers on days 0, 7 and 14. SPSS package program was used to analyze the data collected in the study.

RESULTS

In this study, the effects of standard treatment and an IgYcontaining product (Parvulyte[®]) applied in addition to standard treatment were evaluated within the framework of hematological findings, stool scoring and antibody titres, and the findings are presented after applying the treatment protocols established for both groups. While a significant difference was observed between days in terms of stool score and antibody titre values in Group I, a significant difference was observed between days in terms of LYM, stool score and antibody titre values in Group II (P<.05).

Haematological Findings

Although an improvement parallel to the response to treatment was observed in all the WBC, NEU, RBC, HCT, PLT, LYM, MCV, MCH and HGB parameters measured from the blood samples taken from the dogs in Group I on days 0, 3 and 7, there were no statistically significant difference observed between days (*P*>.05).

An improvement parallel to the response to treatment was observed in all WBC, NEU, RBC, HCT, PLT, LYM, MCV, MCH and HGB parameters measured from the blood samples taken on days 0, 3 and 7 from the dogs in Group II. Unlike Group I, a statistically significant difference was observed in Group II in terms of LYM values between day 0 and day 3 and day 0 and day 7 (P<.05) (Table 2).

Table 2. Statistical analysis of lymphocyte data of dogs in	
group II.	

Day	Significance (p value)
0-3	0.023
3 – 7	1.000
0 – 7	0.003

Faecal Score

The faecal score in the dogs included in the study was scored between 1 and 6. In dogs in both groups, the faecal score on day 0 was determined as 6 in five dogs and 5 in two dogs (Table 3, Table 4).

On the 3rd day of treatment, the faecal score in the dogs in Group I was determined as 5 in two dogs, 4 in four dogs and 3 in one dog. The faecal score in the dogs in Group II was determined as 3 in five dogs and 2 in two dogs (Table 3, Table 4).

On the 7th day of treatment, the faecal score in dogs in Group I was determined as 3 in one dog, 2 in three dogs, and 1 in three dogs. The faecal score in the dogs in Group II was determined as 2 in one dog, while it was determined as 1 in the other six dogs (Table 3, Table 4).

A significant difference was observed in the dogs in both groups in terms of stool score values between day 0 and day 7 (P<.05) (Table 5, Table 6). Although no statistically significant difference was detected, when evaluated clinically, it was observed that the stool scores in dogs in Group II improved faster compared to Group I (Table 3, Table 4).

A significant difference was observed in the dogs in both groups in terms of stool score values between day 0 and day 7 (P<.05) (Table 5, Table 6). Although no statistically significant difference was detected, when evaluated

clinically, it was observed that the stool scores in dogs in Group II improved faster compared to Group I (Table 3, Table 4).

Table 3. Faecal scores of dogs in Group I on days 0, 3 and 7.			
Number	Day 0	Day 3	Day 7
1	6	5	2
2	6	5	3
3	6	4	1
4	5	3	1
5	6	4	2
6	5	4	1
7	6	4	2

Table 4. Faecal scores of dogs in group II on days 0, 3 and 7.			
Number	Day 0	Day 3	Day 7
1	6	3	1
2	6	3	1
3	6	2	1
4	5	2	1
5	6	3	1
6	5	3	1
7	6	3	2

Table 5. Statistical analysis of faecal score data of dogs in Group I.

Day	Significance (p value)		
0 – 3	0.139		
3 – 7	0.86		
0-7	0.000		

Table 6. Statistical analysis of faecal score data of dogs in Group II.

Day	Significance (p value)		
0-3	0.081		
3 – 7	0.113		
0-7	0.000		

Antibody Titres

Antibody titres were determined to be low (below 1:40) on day 0 in all dogs in both groups (Table 7, Table 8).

The antibody titres in dogs in Group I were determined as medium titre (1:80) in five dogs, and high titre (1:160) in two dogs on the 7th day of treatment. Antibody titres in the dogs in Group II were determined as medium titre (1:80) in two dogs and high titre (1:160) in five dogs (Table 7, Table 8).

Antibody titres were determined to be high (1:160) on the 14^{th} day in all dogs in both groups (Table 7, Table 8).

A significant difference was detected in terms of antibody titre values between day 0 and day 7 and day 0 and day 14 for both Group I and Group II (P<.05) (Table 9, Table 10). However, antibodies in dogs in Group II reached high titres faster than in Group I (Table 7, Table 8).

Table 7. Antibody titres of dogs in Group I on days 0, 7			
and 14.			
Number	Day 0	Day 7	Day 14
1	<1:40	1:80	1:160
2	<1:40	1:80	1:160
3	<1:40	1:80	1:160
4	<1:40	1:160	1:160
5	<1:40	1:80	1:160
6	<1:40	1:160	1:160
7	<1:40	1:80	1:160

Table 8. Antibody titres of dogs in Group II on days 0, 7			
and 14.			
Number	Day 0	Day 7	Day 14
1	<1:40	1:80	1:160
2	<1:40	1:160	1:160
3	<1:40	1:160	1:160
4	<1:40	1:160	1:160
5	<1:40	1:160	1:160
6	<1:40	1:80	1:160
7	<1:40	1:160	1:160

Table 9. Significance level of antibody titres of dogs in Group I on days between 0, 7 and 14.

Day	Significance (p value)		
0-7	0.029		
7 – 10	0.320		
10-14	0.000		

Table 10. Significance level of antibody titres of dogs in Group II on days between 0, 7 and 14.

Day	Significance (p value)		
0 – 7	0.003		
7 – 10	1.000		
10-14	0.000		

The antibody titres in dogs in Group I were determined as medium titre (1:80) in five dogs, and high titre (1:160) in two dogs on the 7^{th} day of treatment. Antibody titres in the

dogs in Group II were determined as medium titre (1:80) in two dogs and high titre (1:160) in five dogs (Table 7, Table 8).

Antibody titres were determined to be high (1:160) on the 14th day in all dogs in both groups (Table 7, Table 8).

A significant difference was detected in terms of antibody titre values between day 0 and day 7 and day 0 and day 14 for both Group I and Group II (P<.05) (Table 9, Table 10). However, antibodies in dogs in Group II reached high titres faster than in Group I (Table 7, Table 8).

DISCUSSION

The most effective method of preventing CPV infection and disease is a careful and strategic vaccination program aimed at producing protective antibodies. Although dogs aged 6-16 weeks are more sensitive, CPV infection can be seen in dogs of all ages and breeds. Puppies born to vaccinated mothers who have received colostrum have passive immunity provided by maternal antibodies. Since circulating maternal antibody levels begin to decline at 8-12 weeks of age, the risk of infection is higher in offspring during this period. If the maternal antibody concentration from the mother is low, circulating maternal antibody levels may decline earlier than 8-12 weeks. Therefore, vaccination strategies are implemented to stimulate innate immunity with a series of vaccinations during the period when maternal antibodies decrease. Maternal antibodies, especially in puppies aged 49-69 days, can affect the antibodies produced by vaccine administration. For this reason, vaccination time has an important place in protocols for preventing infection in puppies.^{5,6,20,21} All the dogs included in this study were between the ages of 2-6 months and no breed predisposition could be detected, but it was found that 57.14% (8/14) shared the same environment with at least one dog. According to the anamnesis information obtained from the owners of the dogs included in the study, it was determined that 35.7% (5/14) of the dogs were two-dose vaccinated, 21.42% (3/14) were single-dose vaccinated and 42.85% (6/14) were unvaccinated. Again, according to the anamnesis information obtained, it was determined that all vaccinated dogs were included in the vaccination program at the age of 8 weeks and the second application was made 14-21 days after the first application. These data obtained, consistent with other studies, have shown that the incidence of parvoviral enteritis may increase in crowded dogs, the effectiveness of early vaccinations may be impaired by maternal antibodies, and two doses of vaccination may be insufficient to prevent parvoviral enteritis. However, as a result of the information received

from dog owners, it is thought that the main reasons for not complying with appropriate vaccination programs are due to dog owners' concerns about vaccination costs and their insufficient knowledge about vaccination programs.

Because CPV attacks actively proliferating bone marrow, thymus and other lymphoid tissue cells, the total leukocyte count, and the presence of leukoneutropenia are considered important features in patients with CPV. Additionally, approximately 21% of dogs with parvoviral enteritis had anaemia, 31% had leukopenia, 28% had leucocytosis, 55% had neutropenia, 17% had neutrophilia, 27.6% had eosinopenia, 4% had eosinophilia, also lymphopenia was observed in 28%, lymphocytosis in 4%, monocytosis in 66%, and thrombocytopenia in 4%.^{6,22} In this study, it was observed that 21.42% (3/14) of dogs with parvoviral enteritis had anaemia, 35.7% (5/14) had leukopenia, 14.2% (2/14) had thrombocytopenia, and 50% (7/14) had lymphopenia. The observation that the complete blood count parameters measured in the remaining dogs were within reference ranges suggests that the changes in the complete blood count associated with parvoviral enteritis occur mostly on lymphocytes.

The presence of cytopenia while the disease may be useful in predicting survival. It has been reported that there is no significant difference in survival in terms of neutropenia, but a total leukocyte count higher than 4500/ml and a lymphocyte count higher than 1000/ml at the time of admission and during the 48-hour hospitalization period strongly predict survival.^{23,24} Lymphopenia was detected on day 0 in 50% of the fourteen dogs included in this study, and it was observed that the lymphocyte count was above 1000/ml on day 3 and later in all these dogs, consistent with the literature. However, the increase in the number of lymphocytes between days in dogs in Group II was also found to be statistically significant.

It was reported that niacin has a protective effect on intestinal health. Niacin increases intestinal immune function by regulating down-regulation of TNF- α , IL-1 β , IFN- γ and IL-8 expression and up-regulation of IL-10 and TGF- β expression. In addition, niacin also reduces colonic myeloperoxidase activity. Niacin supplementation has been reported to increase the number of beneficial bacteria in the colon and alleviate the inflammatory response in the intestinal mucosa in piglets.²⁵ Biotin is a water-soluble vitamin and an essential micronutrient that must be obtained from exogenous sources and commensal bacteria. Biotin has a role in preventing the production of inflammatory cytokines and maintaining the integrity of the intestinal barrier.²⁶ Amylum escapes duodenal-ileal digestion and affects stool quality by preventing colonic

bacterial fermentation.²⁷ In this study, Parvulyte[®] IgY applied to dogs in Group II contains niacin, biotin, and amylum in addition to oligoelements and amino acids. In niacin, Group II, where biotin and amylum supplementation were applied, the improvement in stool score was much faster compared to Group I considering the intestinal damage that occurs in parvoviral enteritis, we think that this improvement may be due to the antiinflammatory and nutritional effects of niacin, biotin and amylum supplements on the intestinal mucosa and enterocytes.

IgY application can modulate the immune response at the mucosal level in viral diarrhoeas. It was also reported that IgY application is effective in the prophylaxis and treatment of both viral and bacterial diarrhoea.²⁸ It has been reported that intravenous administration of IgY is effective in the treatment of the severe clinical form of parvoviral enteritis without causing any side effects.²⁹ In this study, the number of lymphocytes in dogs in Group II, where IgY was applied, increased statistically significantly compared to Group I, and the improvement in the stool score was much faster, and the increase in antibody titres to high titres in a shorter period demonstrates the effectiveness of oral administration of IgY in parvoviral enteritis.

As a conclusion, parvoviral enteritis is one of the most important causes of mortality in puppies. The severity of damage caused by the virus in affected offspring and the application of appropriate supportive treatment have a significant impact on survival. This study revealed that oral IgY administration in addition to standard supportive treatment in dogs with parvoviral enteritis accelerates clinical recovery, shortens hospitalization time, and accelerates the immune response to the virus, without causing side effects compared to supportive treatment alone. Therefore, since IgY application is applied together with standard treatment, the study can be repeated by increasing the sample group and the effectiveness of IgY application alone can be investigated.

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