

## Evaluation of *Canine parvovirus* – 2 case observed in a Toy Poodle breed dog

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### Case Report

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### ABSTRACT

*Canine parvovirus* (CPV-2) is a severe disease in puppies, characterized by high morbidity and mortality, often causing hemorrhagic enteritis and death. This case involved a three-month-old female toy poodle brought to the clinic with anorexia, diarrhea, lethargy, and vomiting. The dog was clinically diagnosed with CPV-2, and PCR analysis of blood and fecal samples at the Department of Virology, Faculty of Veterinary Medicine, Selcuk University, confirmed viral DNA presence. After treatment, the dog fully recovered. This study underscores CPV-2 infection risks in vaccinated populations, highlighting the significance of monitoring VP2 region nucleotide changes and antigenic variants to ensure vaccine efficacy and effective disease control.

**Keywords:** *Canine parvovirus*, case report, viral enteritis, PCR

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## Introduction

*Canine Parvovirus* type 2 (CPV-2), worldwide commonly encountered in dogs and particularly seen in puppies under 6 months of age due to the decline of maternal antibodies, is a dangerous enteric pathogen that causes pathogenic diarrhea in dogs, associated with high morbidity and significant mortality. It can also lead to fatal complications related to myocarditis development. (de Oliveira et al., 2019; Hoang et al., 2019; Dik and Şimşek, 2021; Abayli et al., 2022). This pathogen belongs to the Protoparvovirus genus of the Parvoviridae family and contains a non-enveloped virion of approximately 25 nm in diameter, with a single-stranded DNA of about 5 kb in size. It has a genetic organization that encodes three structural proteins, VP1, VP2, and VP3, and two non-structural polypeptides, NS1 and NS2 (de Oliveira et al., 2019; Hoang et al., 2019).

For the successful control of the disease in animals, attenuated modified live virus (MLV) vaccines and inactivated vaccines are available. Despite proper vaccination, the presence of maternal antibodies in the animal to be vaccinated, the emergence of new variants, and possible mutational changes in the VP2

region, as mentioned earlier, have been reported to negatively affect the vaccine's efficacy. This situation can result in vaccinated dogs being exposed to CPV infection (Nandi and Kumar, 2010; Decaro et al., 2020). Clinical signs (foul-smelling mucoid or bloody diarrhea, dehydration, fever, vomiting) usually appear after an incubation period of 3-7 days (Decaro and Buonavoglia, 2012; Hasib et al., 2021). In dogs suspected of CPV-2 enteritis, clinical cases should always be confirmed by laboratory tests. Particularly, leukopenia, lymphopenia, and thrombocytopenia are more commonly observed in animals infected with CPV-2. A decrease in the white blood cell (WBC) count to below 2000–3000 cells/mL (leukopenia) is a consistent finding in symptomatic animals (Decaro and Buonavoglia, 2012; Hasib et al., 2021). It should also be considered that these blood parameters could be an important tool in determining the prognosis of the infection in puppies (Castro et al., 2013).

This study aims to investigate *Canine Parvovirus* (CPV-2) infections that persist even in vaccinated populations, contribute to clinical practices related to diagnosis and treatment, evaluate the causes of vaccine

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failures, and analyze the effectiveness of vaccination strategies in veterinary medicine. Conducted with ethical approval, this study could provide valuable contributions to both local and international veterinary and virology fields.

### Case

The Selcuk University Animal Experiments Local Ethics Committee approved this study (ethics approval number 2023/025 dated 30.03.2023). Additionally, an informed consent form from the dog owner is also available. The case material involved a 3-month-old female Toy Poodle dog that was brought to the veterinary clinic with complaints of loss of appetite, lethargy, and vomiting (Fig. 1). According to the anamnesis, the dog had been adopted two days earlier, vomited 3-4 times a day, and the vomit was a yellow foamy liquid. Additionally, it was noted that the environment where the dog was kept before adoption was a setting where animals were housed together, making it susceptible to infections. When the animal's vaccination history was questioned, it was learned that inactive CCoV vaccine; modified live vaccine containing CDV, CPV, Adenovirus type-2, Parainfluenza agents were administered at 5-6 weeks of age and the second dose of these vaccines was repeated 3 weeks later.

During the clinical examination of the dog, no notable case of diarrhea was initially observed. However, lethargy and abdominal pain symptoms were prominent. The body temperature (38.2 °C), heart rate (124 bpm), and respiratory rate (28 breaths/min) were within normal limits. For a complete blood count, 1 ml of blood was drawn into a heparinized tube. The hemogram results showed leukopenia, neutropenia, and lymphopenia (Table 1). The stool sample collected with a swab was found to be mucous, loose and foul-smelling and it was determined that bloody diarrhea symptoms developed after a short time. It was determined that there was a decline in general body condition as dehydration occurred and symptomatic treatment was started immediately. Based on the clinical examination and evaluation of the hemogram results, parvoviral enteritis was suspected in the dog. Fecal and whole blood samples from the case were sent to the Virology Laboratory at Selcuk University Veterinary Faculty for definitive diagnosis and PCR analysis was conducted for CPV-2.

### PCR Analysis

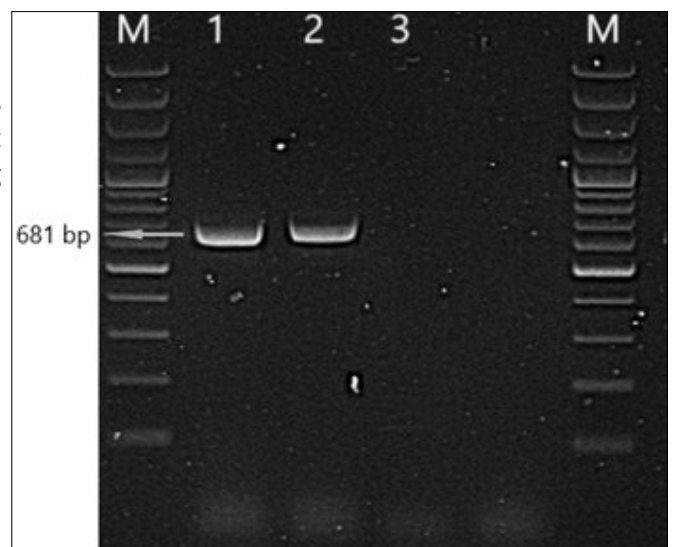
Viral DNA extraction from the fecal and whole blood samples delivered to the laboratory was performed using the "DNeasy Blood & Tissue Kit (50)" (QIAGEN, 69504, Germany) according to the manufacturer's instructions. The extracted viral DNA products from the samples were tested using the commercial PCR kit

FIREPol® Master Mix with 12.5 mM MgCl<sub>2</sub>, 5x (SolisBiodyne). The fecal samples, diluted 1:10 with blood and PBS, were centrifuged at 4000 rpm for 5 minutes. After centrifugation, the leukocyte layer from the whole blood sample and the supernatant from the fecal sample were used. In light of the information that the dominant CPV strain circulating in three different regions of Turkey is CPV-2b (Abaylı et al., 2022), CPV-2ab primers were chosen for the PCR process on the test samples. The primer pairs used in the analysis are specific to the VP2 region of the virus and can detect CPV-2a, CPV-2b, and CPV-2c variants. The primer pairs used in the analysis are as follows:

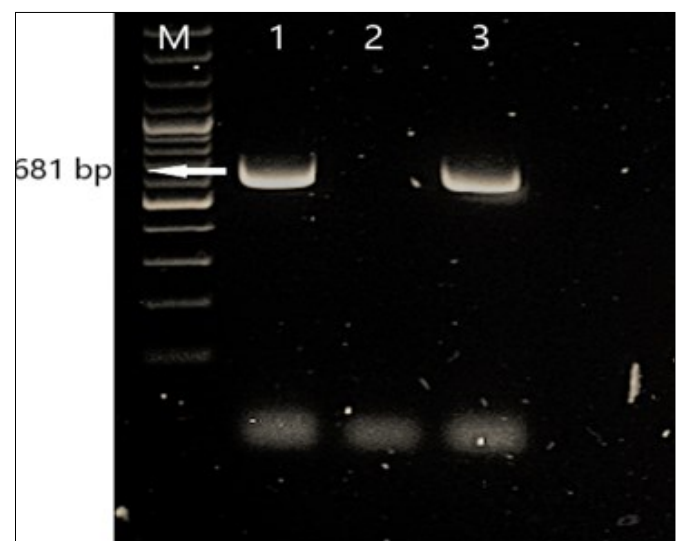
CPV-2ab (F) GAA GAG TGG TTG TAA ATA ATT

CPV- 2ab (R) CCT ATA TAA CCA AAG TTA GTA

The PCR test results from the patient animal confirmed the presence of CPV-2 (Figure 1 and Figure 2).



**Figure 1.** 1. Sampling Day: M:100bp Marker. 1: Blood 2: Stool. 3: (-) Control



**Figure 2:** 4. Sampling Day: M:100bp Marker. 1: Blood 2: (-) Control 3: (+) Control.

### Medical Treatment

Intravenous rehydration with 0.9% NaCl solution (60 ml/kg) (POLIFLEKS, Polifarma®, Turkey) and bicarbonate solution 1 ml CARBOTER (Teknovet®, Turkey) to address dehydration and electrolyte loss. Vitamin B12 1 ml (Dodeksvetaş®, Turkey) was administered. Ceftriaxone UNACEFİN (AVIS®, Turkey) 25 mg/kg was given due to the risk of bacterial translocation in the degenerative intestinal epithelium and concurrent neutropenia. Maropitant CERENIA (ZOETIS®, ABD), 1 mg/kg SC, was used to manage vomiting, which could cause dehydration and electrolyte loss and limit oral feeding support. Filgrastim 5 mg/kg IV (NEUPOGEN AMGEN®, ABD) was administered due to the presence of neutropenia. Hyperimmune serum 1 kg/0.4 ml (POLYGLOB BIOVETA®, Czech Republic) was given for 5 days to increase specific antibody levels and mitigate the disease progression.



**Figure 3.** CPV-2 positive Toy Poodle breed dog and bloody stool.

**Table 1.** Hemogram Results

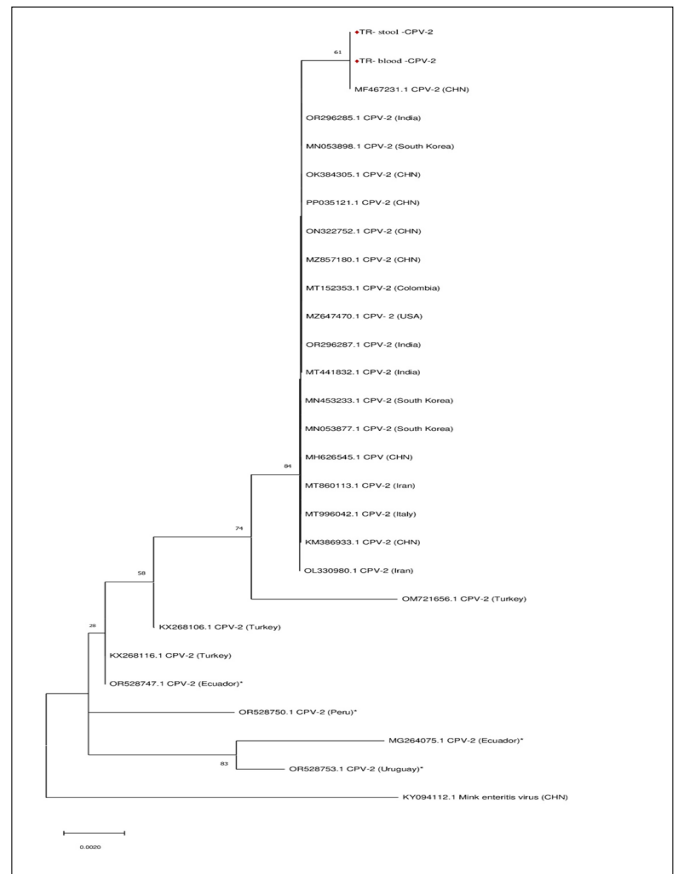
Parameters	Results 1	Results 2	Results 3	Reference Range
WBC ( $10^9/L$ )	0.89	0.81	6.53	6.00-17.00
NEU ( $10^9/L$ )	0.29	0.04	3.12	3.20-12.30
LYM ( $10^9/L$ )	0.44	0.6	2.62	0.80- 5.30
MON ( $10^9/L$ )	0.11	0.06	0.69	0.00-1.50
EOS ( $10^9/L$ )	0.05	0.11	0.1	0.0-10.0
RBC ( $10^{12}/L$ )	6.19	5.38	5.29	5.10-8.50
HGB (g/dL)	13.9	12.5	12.3	11.0-19.5
HCT (%)	41	34.8	34.3	32.5-58.0
MCV (fL)	66.2	64.6	64.7	60.0-76.0
MCHC (g/L)	339	360	360	300-380
PLT ( $10^9/L$ )	323	37	286	117- 490

Results 1 = Hemogram results of January 9, 2023, Results 2 = Hemogram results of January 11, 2023, Results 3 = Hemogram results of January 12, 2023 .

### Phylogenetic analysis

Samples identified as CPV-2 positive by PCR were subjected to sequence analysis by a commercial

company. The data obtained from the sequence analysis were identified using the GenBank service provided by the National Center for Biotechnology Information (NCBI) through the BLAST web page. Nucleotide sequences were compared using AliView software. The data, converted to FASTA format, were analyzed using the Maximum Likelihood method with 1000 bootstrap replicates within the MEGA-X software, and a phylogenetic tree was constructed using the Tamura 3-parameter method. The sequences used in the phylogenetic tree were selected from foreign isolates in the GenBank database that showed the highest similarity to our local isolates. This selection was made by evaluating sequence similarity percentages through BLAST analysis. Care was also taken to include reference sequences that reflect phylogenetic diversity. Based on these results, the presence of CPV-2 infection was confirmed, and the identified local isolate was compared with isolates from different countries around the world to create a phylogenetic tree, allowing for the molecular differentiation of the virus.



**Figure 4.** Phylogenetic analysis of the VP2 gene region of CPV-2. Strains from the GenBank database and from Konya. The phylogenetic tree was constructed using the Maximum Likelihood method (1000 bootstrap) and the Tamura 3-parameter model within the MEGA-X program. The branches marked in red (■) represent local strains specific to the single dog described in our case report. The scale bar indicates 0.0020 nucleotide substitutions per site.

	KY094112.1 Mink_enteritis_virus_(CHN)	OM721656.1_CPV-2_(Turkey)	MF467231.1_CPV-2_(CHN)	TR-600-CPV-2	TR-600-CPV-2	OM727252.1_CPV-2_(CHN)	MT441832.1_CPV-2_(India)	OK384305.1_CPV-2_(CHN)	PP035121.1_CPV-2_(CHN)	MZ857180.1_CPV-2_(CHN)	MT152353.1_CPV-2_(Colombia)	MN053898.1_CPV-2_(South_Korea)	MZ647470.1_CPV-2_(USA)	OR296285.1_CPV-2_(India)	OR296287.1_CPV-2_(India)	OL330980.1_CPV-2_(Iran)	OK386933.1_CPV-2_(CHN)	MN453233.1_CPV-2_(South_Korea)	MN53877.1_CPV-2_(South_Korea)	MH626545.1_CPV-2_(CHN)	MT860113.1_CPV-2_(Iran)	MT996042.1_CPV-2_(Italy)	OR528750.1_CPV-2_(Peru)*	OR528747.1_CPV-2_(Ecuador)*	MG264075.1_CPV-2_(Ecuador)*	KX268106.1_CPV-2_(Turkey)	MG264075.1_CPV-2_(Ecuador)*	OR528753.1_CPV-2_(Uruguay)*
KY094112.1																												
Mink_enteritis_virus_(CHN)																												
OM721656.1_CPV-2_(Turkey)	0.0220																											
MF467231.1_CPV-2_(CHN)	0.0222	0.0008																										
TR-600-CPV-2	0.0222	0.0008	0.0003																									
TR-600-CPV-2	0.0222	0.0008	0.0003	0.0000																								
OM727252.1_CPV-2_(CHN)	0.0220	0.0017	0.0012	0.0002	0.0002																							
MT441832.1_CPV-2_(India)	0.0220	0.0017	0.0012	0.0002	0.0002	0.0000																						
OK384305.1_CPV-2_(CHN)	0.0220	0.0017	0.0012	0.0002	0.0002	0.0000	0.0000																					
PP035121.1_CPV-2_(CHN)	0.0220	0.0017	0.0012	0.0002	0.0002	0.0000	0.0000	0.0000																				
MZ857180.1_CPV-2_(CHN)	0.0220	0.0017	0.0012	0.0002	0.0002	0.0000	0.0000	0.0000	0.0000																			
MT152353.1_CPV-2_(Colombia)	0.0220	0.0017	0.0012	0.0002	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000																		
MN053898.1_CPV-2_(South_Korea)	0.0220	0.0017	0.0012	0.0002	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000																	
MZ647470.1_CPV-2_(USA)	0.0220	0.0017	0.0012	0.0002	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000																
OR296285.1_CPV-2_(India)	0.0220	0.0017	0.0012	0.0002	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000															
OR296287.1_CPV-2_(India)	0.0220	0.0017	0.0012	0.0002	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000														
OL330980.1_CPV-2_(Iran)	0.0220	0.0017	0.0012	0.0002	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000													
OK386933.1_CPV-2_(CHN)	0.0220	0.0017	0.0012	0.0002	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000												
MN453233.1_CPV-2_(South_Korea)	0.0220	0.0017	0.0012	0.0002	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000											
MN53877.1_CPV-2_(South_Korea)	0.0220	0.0017	0.0012	0.0002	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000										
MH626545.1_CPV-2_(CHN)	0.0220	0.0017	0.0012	0.0002	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000									
MT860113.1_CPV-2_(Iran)	0.0220	0.0017	0.0012	0.0002	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000								
MT996042.1_CPV-2_(Italy)	0.0220	0.0017	0.0012	0.0002	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000							
OR528750.1_CPV-2_(Peru)*	0.0218	0.0111	0.0113	0.0113	0.0111	0.0111	0.0111	0.0111	0.0111	0.0111	0.0111	0.0111	0.0111	0.0111	0.0111	0.0111	0.0111	0.0111	0.0111	0.0111	0.0111	0.0111						
OR528747.1_CPV-2_(Ecuador)*	0.0213	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008					
MG264075.1_CPV-2_(Ecuador)*	0.0213	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008				
KX268106.1_CPV-2_(Turkey)	0.0215	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008			
MG264075.1_CPV-2_(Ecuador)*	0.0222	0.0117	0.0118	0.0118	0.0117	0.0117	0.0117	0.0117	0.0117	0.0117	0.0117	0.0117	0.0117	0.0117	0.0117	0.0117	0.0117	0.0117	0.0117	0.0117	0.0117	0.0117	0.0117	0.0117	0.0117	0.0117		
OR528753.1_CPV-2_(Uruguay)*	0.0218	0.0113	0.0115	0.0115	0.0113	0.0113	0.0113	0.0113	0.0113	0.0113	0.0113	0.0113	0.0113	0.0113	0.0113	0.0113	0.0113	0.0113	0.0113	0.0113	0.0113	0.0113	0.0113	0.0113	0.0113	0.0113	0.0113	

**Figure 5:** Genetic distance analysis results with CPV-2 sequences. CPV-2 VP2 isolates obtained from NCBI Blast.; MG264075.1\_CPV-2\_(Ecuador), OR528753.1\_CPV-2\_(Uruguay), MF467231.1\_CPV-2\_(CHN), MT996042.1\_CPV-2\_(Italy), MT860113.1\_CPV-2\_(Iran), MH626545.1\_CPV-2\_(CHN), MN053877.1\_CPV-2\_(South\_Korea), MN453233.1\_CPV-2\_(South\_Korea), KM386933.1\_CPV-2\_(CHN), OL330980.1\_CPV-2\_(Iran), OR296287.1\_CPV-2\_(India), OR296285.1\_CPV-2\_(India), MZ647470.1\_CPV-2\_(USA), MN053898.1\_CPV-2\_(South\_Korea), MT152353.1\_CPV-2\_(Colombia), OR528747.1\_CPV-2\_(Ecuador), OR528750.1\_CPV-2\_(Peru), ON322752.1\_CPV-2\_(CHN), MT441832.1\_CPV-2\_(India), OK384305.1\_CPV-2\_(CHN), PP035121.1\_CPV-2\_(CHN), MZ857180.1\_CPV-2\_(CHN), OM721656.1\_CPV-2\_(Turkey), KX268106.1\_CPV-2\_(Turkey), KX268116.1\_CPV-2\_(Turkey), KY094112.1\_Mink\_enteritis\_virus\_(CHN)

## Discussion

In this case, a Toy Poodle suspected of being infected with parvovirus exhibited clinical signs such as loss of appetite, lethargy, diarrhea and vomiting. Immediately after the diagnosis of the infection, symptomatic treatment was initiated, including intravenous fluids, antibiotics, and medication. With the implementation of the treatment protocol, it was observed that within 3-4 days, the hemogram parameters significantly improved (Tables 1), and the clinical symptoms of the animal rapidly subsided. During this period, it was noted that the dog, which had begun medical treatment, returned to its normal life completely within a week.

Considering the treatment protocols mentioned above, studies have proven that the treatment of parvoviral enteritis (CPV) is largely supportive and symptomatic. The main components of treatment include 1) fluid therapy, 2) antibiotic therapy, 3) antiemetic therapy, and 4) nutritional support. 1) Maintaining hydration and oncotic support, as well as correcting acid-base and electrolyte imbalances in CPV cases, is extremely important. Since subcutaneous fluid absorption is impaired in dehydrated animals, intravenous fluid therapy is the cornerstone of treatment. In puppies with severe hypovolemia, circulation volume must be restored within 1-2 hours. Balanced isotonic crystalloid solutions (e.g., Lactated Ringer's) are the preferred fluids to improve perfusion

parameters such as capillary refill time, mucosal color, and pulse quality. 2) Due to the high risk of septicemia associated with mucosal barrier disruption and concurrent deep neutropenia, parenteral administration of broad-spectrum bactericidal antibiotics is recommended in dogs with severe CPV infections. 3) For antiemetic therapy, metoclopramide—a dopaminergic antagonist that blocks the chemoreceptor trigger zone and exerts a prokinetic effect in the upper gastrointestinal tract—has been suggested for infusion in dogs with severe vomiting. 4) Enteral nutrition promotes improved mucosal integrity, faster recovery, and, consequently, a reduced likelihood of bacterial translocation (Mylonakis et al., 2016). The above-mentioned protocol was also emphasized in a study by Mohr et al. (2003), in which early enteral feeding via a nasoesophageal catheter, initiated 12 hours after diagnosis, was associated with earlier clinical recovery, significant weight gain, and better intestinal barrier function compared to dogs subjected to enteral feeding restrictions. In this case, following the treatment protocol described by Mylonakis et al. (2016) and Mohr et al. (2003), medical therapy was administered to the affected dog, including intravenous (IV) NaCl and bicarbonate solutions to restore hydration and electrolyte balance, vitamin B12, and ceftriaxone (25 mg/kg IV) to address the risk of bacterial translocation in degenerative intestinal epithelium and concurrent neutropenia.



which contributes to dehydration, electrolyte loss, and limits oral nutritional support, while filgrastim (5 mg/kg IV) was administered due to the presence of neutropenia. Additionally, hyperimmune serum was given for five days to enhance specific antibody levels and mitigate disease severity. During the course of medical treatment, the dog showed significant improvement and returned to a completely normal state within one week.

Determining the virus source responsible for viral shedding in dogs during the post-vaccination period is quite limited (Decaro et al., 2020). Generally, it is believed that the attenuated pathogens in MLV (modified live vaccines) do not cause disease due to their low virulence and limited replication (Lin et al., 2015; Freisl et al., 2017). However, in the present case, the infection occurred despite the dog being vaccinated. The occurrence of infection post-vaccination has been attributed to several factors, including the dog's exposure to a circulating field isolate before its transfer, the stress of travel after vaccination, which may have suppressed the immune system (immunosuppression) and rendered the animal vulnerable to infection. Based on this information, it is suggested that the strain causing the disease likely originated from a field isolate circulating at the time of the dog's transfer, exacerbated by immunosuppression and leading to vaccination failure. Furthermore, Decaro et al. (2020) noted that vaccination failures have been linked to the presence of maternal antibodies, the circulation of different antigenic variants of the virus, or mistakes in vaccine storage or administration. Additionally, the successful treatment of the case and the prevention of death may be attributed to the protective effects of a single or double dose of a multivalent vaccine. This possibility should not be overlooked, as vaccines are generally considered effective in alleviating clinical symptoms or preventing fatal outcomes. In addition, the successful treatment of the case and the prevention of death may be attributed to the protective effects of a single or double dose of a highly effective vaccine. This possibility should not be overlooked, as vaccines are generally considered effective in alleviating clinical signs or preventing fatal outcomes (Decaro et al., 2014). Moreover, puppies are often infected with field strains shortly before or after vaccination; however, diarrhea can also be caused by other viral or bacterial infections, parasitism, or poor management. Nevertheless, many veterinarians and dog owners mistakenly believe that enteric illness occurring after the administration of the CPV vaccine is due to the reversion to virulence of the modified live vaccine (MLV) virus. In one study, molecular tests on 29

fecal samples from dogs that developed diarrhea after CPV vaccination revealed that most of these animals were infected with CPV field strains or other canine pathogens. However, while CPV vaccine strains were detected in the feces of 11 dogs alongside field strains or other pathogens, three samples tested positive only for the vaccine strain with no other canine pathogens detected. This confirmed that the observed diarrhea was most commonly associated with infection by CPV-2 field strains. As a result, since no reversion to virulence of CPV MLV vaccine strains has been demonstrated, this study shows that most cases of gastroenteritis following vaccination are related to infection with CPV field strains shortly before or after vaccine administration (Decaro et al., 2007). In another study, two commercial vaccines containing CPV-2 or CPV-2b strains were administered to 26 dogs to evaluate the duration and extent of viremia and viral shedding through feces caused by the CPV vaccine. The analysis using real-time PCR showed that live vaccines could remain in the feces for up to 28 days after vaccination. All these data indicate that MLV CPV strains replicate in the dogs' bloodstream and intestinal mucosa, leading to viremia and viral shedding through feces. Such a situation, where CPV or its nucleic acid is detected in the feces of vaccinated dogs, could lead to false-positive results that may cause misdiagnosis of the disease (Decaro et al., 2014). This factor, in addition to the possibility of infection, must also be taken into account.

Maternal antibodies are regarded as the main cause of vaccination failure against canine Parvovirus (CPV) in young dogs (Waner et al., 1996). These antibodies are vital for shielding puppies from CPV-2 infection (Mila et al., 2014). In a study where maternal antibody titers were measured in puppies at 2 days old, it was reported that antibody levels varied greatly, with titers ranging from 1:10 to 1:1280. Throughout the study, almost all puppies (96%) were experimentally infected with parvovirus. Viral infections predominantly occurred in puppies with maternal antibody titers of 1:80 or lower (Mila et al., 2014). Nandi and Kumar (2010) also found that antibody titers showing a strong correlation with CPV, such as titers of 1:80 or higher, are considered protective against infection, while a titer of 1:40 is not protective and may adversely affect the effectiveness of active CPV-2 vaccination in dogs. Based on these findings, it has been suggested that successful vaccination against CPV can only be safely achieved in seronegative puppies or those with very low antibody titers (e.g., below 1:10). Considering the half-life of maternal antibodies, the optimal time for vaccination is estimated to be around 10-11 weeks of

age, when maternal antibody titers have dropped to their lowest (below 1:20-1:40) (Decaro et al., 2020). Based on the data obtained from various studies, the fact that the dog mentioned in our case report contracted the infection despite being vaccinated with a modified live vaccine against parvovirus at 5-6 weeks of age suggests that there may be certain factors present that reduce the effectiveness or protective capability of the vaccine. When considering the factors that reduce vaccine efficacy, it is believed that vaccinating a dog with modified live vaccines during the early period of the first six weeks when maternal antibodies are present may weaken or inhibit the vaccine's effectiveness. This is likely due to the vaccine encountering maternal antibodies that interfere with its ability to prepare the immune system, as the dog may have a maternal antibody titer of 1:40 or higher.

In evaluating this case, it is possible that the underdeveloped immune system of Toy Poodle puppies, like other small breeds, increases their susceptibility to parvovirus infections (Headley et al., 2013). Therefore, this case is thought to be attributed to the general immune deficiency of puppies rather than a specific affinity of CPV for a particular breed. Additionally, to prevent CPV-2 infections, it is recommended to determine the maternal antibody levels of puppies before vaccination, particularly at six weeks of age. Based on the detected values, if a puppy has a maternal antibody level of 1:40 or higher, delaying the first dose of the CPV-2 vaccine by 2-3 weeks may improve vaccination success.

In this study, in addition to molecularly characterizing the partial VP2 gene of the parvoviral DNA isolated from stool and blood samples, the phylogenetic relationship of the field virus with reference strains obtained from GenBank was also determined.

For this purpose, multiple sequence alignment of CPV-2 VP2 gene sequences obtained from Konya revealed that both of our field isolates (TR-CPV-2\_blood and TR-CPV-2\_stool) were phylogenetically closely related to the Chinese strain (MF467231.1 CPV-2) and showed a high degree of homology (Figure 5). When the field isolates were compared with other Turkish isolates, they exhibited a particularly high similarity (99.9%) with the OM721656.1\_CPV-2\_(Turkey) strain. Similarly, our local isolates displayed maximum identity with other CPV-2 sequences.

In the sequence analysis conducted using the 620 bp partial VP2 region, when compared to the GenBank sequences, it was determined that at position 126, our local isolates encoded Adenine (A), whereas other sequences obtained from GenBank (including Turkish

isolates) encoded Guanine (G). According to the amino acid analysis results, the A → G nucleotide change at position 126 did not cause any amino acid substitution and was found to have no effect on the structure or antigenic properties of the VP2 protein. Therefore, as no major mutation that could reduce vaccine efficacy was detected, the occurrence of CPV infection in the vaccinated dog was not attributed to this genetic variation. Instead, the infection in the vaccinated dog was associated with other factors, such as immunosuppression (due to illness, stress, or medication) or the interference of maternal antibodies with vaccine efficacy. In conclusion, the data obtained in this case study are expected to contribute to future epidemiological studies and molecular characterization research related to CPV-2.

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