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Original article

Virological investigation on the presence of CPV-2 in the middle black Sea Region of Turkey

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Abstract

Canine parvovirus (CPV) is a highly contagious agent prevalent worldwide, particularly in young dogs, and is associated with severe gastrointestinal symptoms. It is considered the leading cause of death due to haemorrhagic diarrhoea in puppies. As a result of antigenic and genetic changes in the virus, various variants such as CPV-2a, 2b, 2c, new 2a, and new 2b have emerged. This study aimed to investigate the presence of CPV-2 in the Black Sea region of Turkey and perform molecular characterisation. Samples were collected from dogs presented to the clinics of Samsun Ondokuz Mayıs University Faculty of Veterinary Medicine Animal Hospital with complaints of vomiting and diarrhoea. Blood, faeces, rectal swabs and tissue samples from 45 dogs suspected of CPV-2 infection were analysed using the real-time polymerase chain reaction method, which detected viral nucleic acid in 24 samples. Virus isolation was performed on the African green monkey kidney (VERO) cell line for eight positive samples. Whole-genome sequence analyses were conducted on six isolates using next-generation sequencing, classifying these isolates as CPV-2a variants. Among our isolates, three different amino acid changes were detected in the VP2 protein, the major capsid protein of CPV-2. The fact that two animals testing positive in this study had been previously vaccinated raises questions regarding vaccine effectiveness. In conclusion, our study provides insights into the current status of CPV-2 in the Black Sea region of Turkey and underscores the importance of conducting regular epidemiological studies and implementing an effective vaccination policy.

Keywords: CPV-2, dog, isolation, Next-Generation Sequencing, Real-Time PCR

Introduction

Canine parvoviruses (CPVs) are divided into two types: canine parvovirus type 1 (CPV-1) and canine parvovirus type 2 (CPV-2). CPV-1, commonly known as canine minute virus, is not genetically related to CPV-2. CPV-2 is a major cause of haemorrhagic enteritis and death worldwide, particularly in puppies, with morbidity rates of up to 10% and high mortality (Mazzaferro 2020, Tuteja et al. 2022, Ammar et al. 2024). Through genetic evolution, the host range of CPV-2 has gradually expanded to include not only dogs and cats but also grey wolves and raccoons, with some reports suggesting that CPVs can also infect pigs (Temeeyasen et al. 2022).

CPV-2 is classified within the genus *Protoparvovirus*, subfamily *Parvovirinae*, of the family *Parvoviridae* (Pénzes 2020). Its genome comprises linear, single-stranded DNA with negative-sense icosahedral symmetry, approximately 5 kb in length (Dema et al. 2021). The virus encodes two nonstructural proteins (NS1 and NS2) and three structural proteins (VP1, VP2, and VP3) across two open reading frames. VP1 and VP2 are the major structural proteins that form the viral capsid through selective mRNA splicing, with VP2 comprising approximately 90% of the capsid (Mira et al. 2019, Hartmann et al. 2023).

CPV-2 was distinguished from feline panleukopenia virus (FPV) through amino acid changes at Arg80Lys, Lys93Asn, Val103Ala, Asp323Asn, Asn564Ser and Ala568Gly positions (Doan et al. 2021). Since its emergence in 1978, CPV-2 has undergone significant antigenic and genetic changes, leading to the appearance of a new variant, CPV-2a. This variant spread rapidly and soon replaced the original virus (Hao et al. 2022). Subsequent variants, CPV-2b and CPV-2c, are distinguished by mutations at the 426th amino acid residue, while the Ser297Ala mutation is considered characteristic of the new CPV-2a and CPV-2b variants (Li et al. 2022).

The most common route of CPV-2 transmission is the faecal – oral route or through contact with contaminated surfaces. Once inside the body, the virus targets rapidly dividing cells, primarily intestinal epithelial cells and lymphocytes. Proliferation of infected intestinal epithelial cells leads to intestinal hyperdynamism, inflammation and tissue necrosis, which can lead to haemorrhagic enteritis. This process may trigger systemic inflammatory response syndrome (SIRS), septicaemia, and endotoxaemia. Additionally, viral replication in bone marrow and lymphoid tissues can cause leukopenia, further compromising the host's immune function (Tuteja et al. 2022, Zhou et al. 2024). Myocardial cells in young puppies are also actively dividing, rendering them susceptible to CPV infection, which can result in myocarditis (Ford et al. 2017).

Although CPV-2 can affect dogs of all ages, severe infection is most common in puppies between six weeks and six months of age. In a study conducted in Egypt, CPV prevalence was 85.2% in dogs under three months, 79.6% in dogs between three and six months, and 25% in dogs over six months (Mekky et al. 2024). Similarly, Elbaz et al. (2021) reported a positivity rate of 76% in dogs under six months, 68% in the three- to six-month age group, and 8% in dogs older than six months. Another case-control study indicated that the risk of infection was higher in puppies younger than three months and in those aged three to six months (Ammar et al. 2024).

Current treatment of CPV-2 infection is primarily based on symptomatic and supportive therapy. The main approach focuses on alleviating the symptoms of gastroenteritis and preventing secondary infections. Fluid therapy, antiemetics and antibiotics form the core components of treatment (Gerlach et al. 2020).

Although controlling CPV-2 infections remains a global challenge, vaccination is the most important measure for minimising infection (Decaro et al. 2020). Most vaccines developed against CPV-2 are modified live virus vaccines, with inactivated vaccines being less common. Modified live vaccines are generally preferred because they replicate within the host, providing long-term immunity without causing tissue damage or clinical symptoms. Some vaccines available today contain the original CPV-2 type, while others contain CPV-2b strains (Mia and Hasan 2021). Factors such as age, nutrition and environmental conditions, along with the emergence of different viral variants in various regions, significantly influence vaccine efficacy. Therefore, regular molecular screening of field CPV strains is crucial for effective prevention (Zhou et al. 2024).

The aim of this study was to investigate the presence of CPV-2 in dogs in Samsun Province, located in the Black Sea region of Turkey, to isolate the virus, perform its genetic characterisation, identify the circulating variant in the region, and determine its relationship to CPV-2 genotypes in Turkey and globally.

Materials and Methods

Ethical statement

This study was approved by the Ondokuz Mayıs University Animal Experiments Local Ethics Committee, which determined that additional ethical clearance was not required (reference number: 68489742-604. 01.03-E.15648).

Table 1. Information on primers and probe used in the diagnosis of CPV-2.

Method	Primer	5' → 3' Sequence	Amplicon Size	Reference
Real-Time PCR	CPV-2 Forward	AAACAGGAATTAACTATACTAATATATTTA	93 bp	(Decaro et al. 2005)
	CPV-2 Reverse	AAATTTGACCATTTGGATAAACT		
	CPV-2 Prob	FAM–TGGTCCTTTAACTGCATTAAATAATGTACC- TAMRA		
Conventional PCR	CPV-2Pabs (Forward)	GAAGAGTGGTTGTAAATAATT	1500 bp	(Pereira et al. 2000)
	CPV-2555rev (Reverse)	GGTGCTAGTTGATATGTAATAAACA		(Buonavoglia et al. 2001)

Sampling

In our study, sampling was carried out on both vaccinated and unvaccinated dogs of various ages and breeds, presented to the clinics of Samsun Ondokuz Mayıs University Veterinary Faculty Animal Hospital with complaints of vomiting and diarrhoea, between August 2020 and August 2022. Samples were collected from 45 suspected dogs, including blood, faeces and rectal swabs, as well as organ samples (jejunum and ileum from the small intestine, mesenteric lymph nodes, spleen and heart) obtained from necropsies of animals that had died from similar causes. Of the dogs, 17 were female and 28 were male; two had been vaccinated, while the remainder were unvaccinated. Forty-two dogs were aged 0-6 months and three were aged 6-12 months.

DNA isolation

For DNA isolation, viral nucleic acid was extracted from blood, faecal, swab and organ samples using a commercially available extraction kit (Invitrogen Genomic DNA Extraction Kit, Cat. No: K1820-02) according to the manufacturer's instructions. The extracted samples were stored at -20°C until polymerase chain reaction (PCR) analysis.

Real-time PCR assay

For real-time PCR, the commercially available Bio-Rad iTaq Universal Probes One-Step Kit (Cat. No: 1725141) was used, and PCR was performed on the Bio-Rad CFX Connect Real-Time Machine (Bio-Rad, USA). Primers and probe targeting the VP2 gene of CPV-2, designed by Decaro et al. (2005), were used (Table 1). The reaction mix was prepared in a total volume of 25 µL, consisting of 10 µL 2X iTaq Buffer, 1 µL (10 µM) each of forward and reverse primers, 0.5 µL (10 µM) probe, 5 µL template DNA, and 2.5 µL RNase-free water per sample. Thermal cycling conditions comprised initial denaturation at 95°C for 5 min, followed by 40 cycles of 95°C for 15 s, 52°C for 30 s (this temperature was selected as it provided the highest amplification efficiency and specificity based on optimisation studies considering the Tm values of the primer pair) and 72°C for 1 min.

Cell culture, virus isolation and confirmation with PCR assays

The African green monkey kidney (VERO) cell line was used for virus isolation. VERO cells were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 20% foetal bovine serum (FBS, Sigma, United Kingdom) and 1% antibiotic solution (10,000 U/mL penicillin–streptomycin, Gibco, USA) at 37°C in a 5% CO_2 atmosphere. Homogenates were prepared from samples that tested positive for CPV-2 by PCR. For stool samples, 10% suspensions in phosphate-buffered saline (PBS) were vigorously shaken with sterile beads using a TissueLyser LT (Qiagen, Germany) and centrifuged at 3,000 rpm for 30 min at 4°C. The supernatant was sterilised through a 0.22 µm disposable filter (Millipore, USA) prior to inoculation into the cell line. Approximately 1 g of organ samples (jejunum, ileum, mesenteric lymph nodes, spleen and heart) was homogenised with 5 mL DMEM containing 1% penicillin–streptomycin for 1 min at 6,000 rpm using a tissue homogeniser (Qiagen, Hilden, Germany). The homogenate was then centrifuged at 3,000 rpm for 10 min, and the supernatant was filtered through a sterile 0.22 µm filter. Rectal swabs were placed in 2 mL PBS containing 1% penicillin–streptomycin, vortexed, and centrifuged at 3,000 rpm for 10 min at 4°C. After centrifugation, the supernatant was passed through a sterile filter. A 500 µL aliquot of the supernatants obtained from the homogenates was inoculated into a monolayer VERO cells and incubated at 37°C with 5% CO_2 for 60 min. Following incubation, the supernatant was removed, and 5 mL of DMEM supplemented with 2% FBS was added, with cultures

maintained in a 37°C, 5% CO_2 incubator. Cells were examined daily under an inverted microscope (Olympus, Japan) for the presence of cytopathic effect (CPE). Real-time PCR and conventional PCR were used to confirm the presence of CPVs in infected cell lysates.

Conventional PCR

Conventional PCR was performed using the commercially available Thermo Scientific Taq DNA Polymerase Kit (Thermo, Cat. No: EP0402) on a Cleaver Scientific GTC96S Thermal Cycler. Viral DNA was amplified with primers targeting a 1,500 bp region of the VP2 gene; detailed information is provided in Table 1. The PCR was prepared in a final volume of 50 µL, comprising 5 µL template DNA, 1 µL (10 mM) dNTP mix, 5 µL 10X Taq Buffer, 2 µL (10 µM) each of forward and reverse primers, 1 µL Taq DNA polymerase, and 34 µl RNase-free water. Thermal cycling conditions included an initial denaturation at 94°C for 6 min, followed by 40 cycles at 94°C for 45 s, 50°C for 45 s, and 72°C for 45 s, with a final extension at 72°C for 10 min. Following amplification, 10 µL of each PCR product was loaded into 1% agarose gel wells stained with ethidium bromide and subjected to electrophoresis (Cleaver Scientific CS-300V, England) at 100 V for 40 min. DNA bands corresponding to 1,500 bp were visualised under UV light using a transilluminator gel imaging system (Vilber Lourmat, Sigma, Germany).

Sequencing and phylogenetic analysis

PCR amplicons were subjected to whole-genome sequencing using the next-generation sequencing (NGS) ılumina/WGS method (ENIGMA Biotechnology, Ankara, Turkey). The sequences obtained were compared with reference sequences and similar entries in GenBank. Multiple-sequence alignment was performed using MEGA 11 (Molecular Evolutionary Genetics Analysis) with the ClustalW algorithm. The Tamura 3-parameter model with gamma distribution (TN93+G) was selected as the best-fit model for nucleotide and protein analyses. A phylogenetic tree was constructed using the neighbour-joining (NJ) method with 1,000 bootstrap replicates in MEGA 11. Protein and nucleotide differences between the sequences obtained and reference sequences from GenBank were analysed.

Data accessibility

All data from this study are available for sharing, and the sequencing data of our isolates have been deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) under the following accession numbers: OR066200, OR066201, OR066202, OR066203, OR066204 and OR066205.

Results

Real-Time PCR

Of the 45 samples collected in the study, 24 (53.3%) tested positive by real-time PCR. Among the positive samples, 17 (100%) were from female dogs and 7 (25%) from male dogs. Regarding age groups, 23 (54.1%) were aged 0-6 months, and 1 (33.3%) was aged 6-12 months. In terms of vaccination status, 22 (51.1%) were from unvaccinated dogs, while 2 (100%) were from vaccinated animals.

Virus isolation

As a result of the virus isolation study on samples that tested positive by real-time PCR, eight samples successfully grew in cell culture. CPEs, including cell rounding and lysis, were observed in three samples, while no CPE was detected in the remaining five samples (Fig. 1). All eight cell culture isolates were further confirmed by conventional PCR using primers targeting the 1,500 bp region of the VP2 gene (Fig. 2).

Sequencing and phylogenetic analyses

Six samples that tested positive and were successfully isolated in cell culture (three cytopathogenic and three non-cytopathogenic) were selected for whole-genome sequencing using the NGS method. The sequence data were analysed and organised using MEGA 11. A phylogenetic tree was constructed including 55 CPV-2 sequences from GenBank, representing strains reported both in Turkey and worldwide, alongside the six isolates from this study (Fig. 3). Multiple-sequence alignment was performed, and poorly aligned regions were trimmed for accurate comparison. The isolates were designated TR.CPV.Samsun.01-TR.CPV.Samsun.06 and uploaded to GenBank under accession numbers OR066200–OR066205. Similarity with CPV-2 sequences in GenBank was assessed using the NCBI BLAST tool. The presence of Asn at amino acid position 426 of the VP2 protein indicated that all samples belonged to the CPV-2a variant. Analysis of amino acid substitutions revealed changes in the NS1–NS2 proteins: Y544F and E545V in OR066201; L582S in OR066200, OR066201, OR066202 and OR066203; and K583E and L597P in OR066204, OR066200, OR066202 and OR066205. In the VP1 protein, A2P was observed in OR066202 and OR066203,

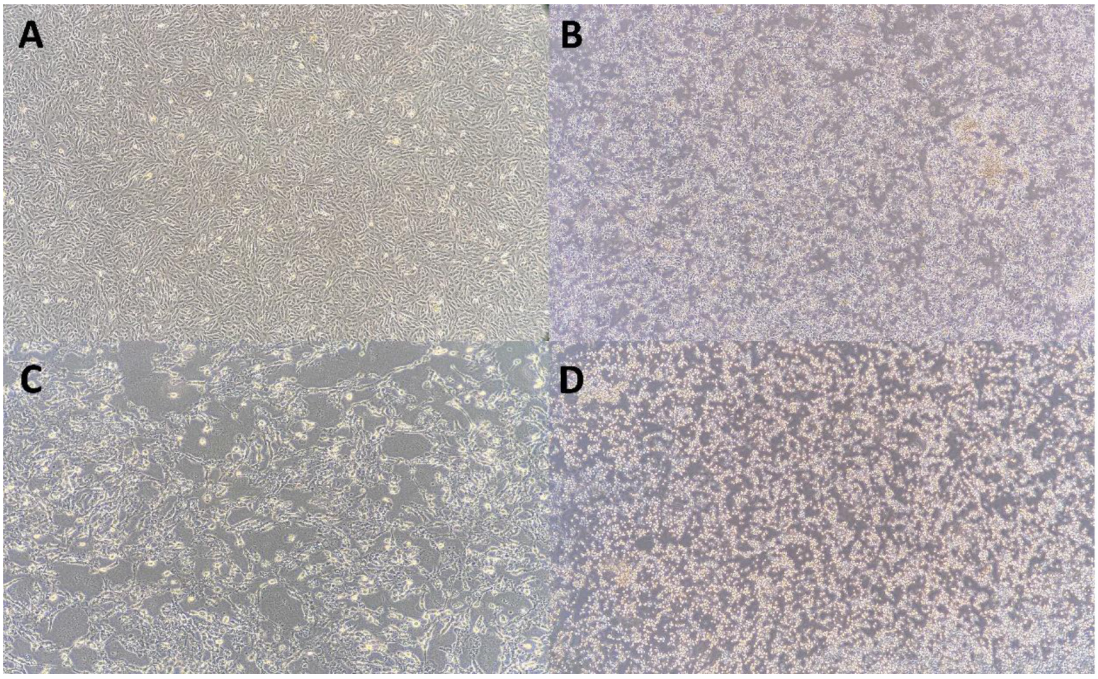

Fig. 1. Photos taken from an inverted microscope show the CPV-2 growth in the VERO cell line 4 days post-inoculation. (A: Cell control of VERO cell line (x40), B: CPE on cells due to TR.CPV.Samsun01 isolate growth, C: CPE on cells due to TR.CPV.Samsun03 isolate growth, D: CPE on cells due to TR.CPV.Samsun05 isolate growth)

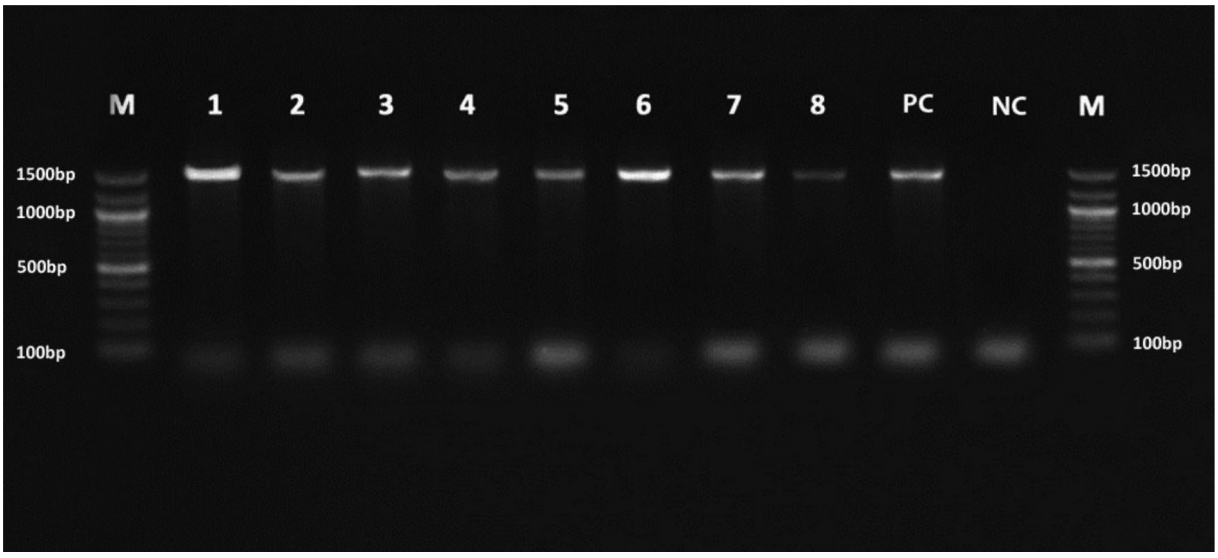

Fig. 2. PCR amplification of samples isolated in cell culture according to the gene encoding the capsid protein of CPV-2 (Lane M contains 100-base pair (bp) DNA ladder, PC: Positive Control, NC: Negative Control)

and D23N was observed in OR066201 and OR066204. In the VP2 protein, G11C was observed in OR066205, F267Y in all isolates, and Y367D in OR066201 and OR066205 (Tables 2-4). BLAST analysis indicated that our isolates share 99.37% identity with American CPV-2b (M38245.1), 99.15% with Turkish CPV-2b, 99.12% with Iraq CPV-2a, 98.99% with Japanese CPV-2b (LC270891.1), and 98.87% with Brazilian CPV-2c (OP985292.1). Additional comparisons showed 98.43% identity with Chinese CPV-2a (OP985292.1), 98.43% with Brazilian CPV-2c (KY073269.1), and 98.93% with Italian FPV (KX434461.1). The six isolates were 99% identical to each other.

Discussion

CPV-2 was first reported in dogs in the mid-1970s and soon underwent genetic changes, leading to the identification of CPV-2a. Subsequently, antigenically and genetically distinct subtypes emerged, including CPV-2b, CPV-2c, new 2a and new 2b (Buonavoglia

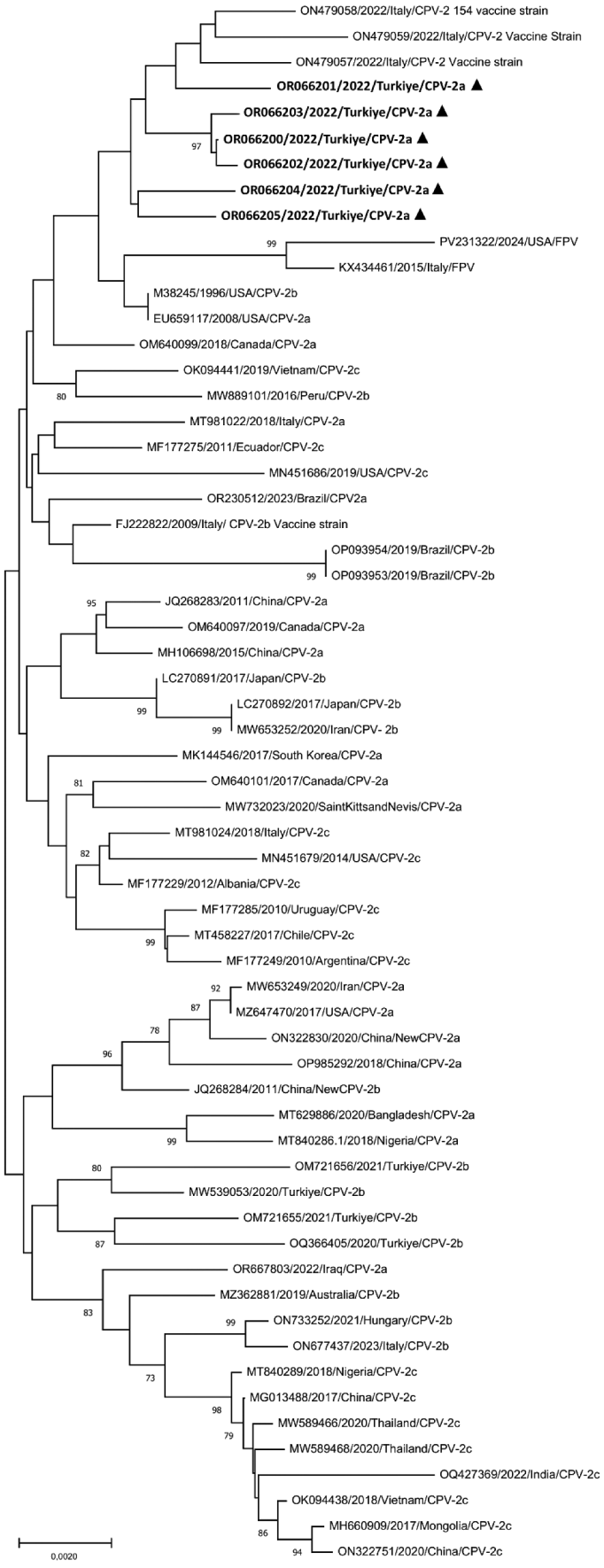

Fig. 3. Phylogenetic tree constructed from complete genome of CPV-2 nucleotide sequences and assessed using 1000 bootstrap replications. The black triangles indicate the CPV-2a isolates from our study.

Table 2. NS1-NS2 gene amino acid variations of CPV-2.

Protein	NS1-NS2				
Nucleotide Positions	1631	1634	1745	1747	1790
Amino acid Positions	544	545	582	583	597
Reference Sequence	A	A	T	G	T
TR.CPV.SAMSUN.01	A	A	**C**	**A**	T
TR.CPV.SAMSUN.02	**T**	**T**	T	**A**	T
TR.CPV.SAMSUN.03	A	A	**C**	**A**	T
TR.CPV.SAMSUN.04	A	A	**C**	**A**	T
TR.CPV.SAMSUN.05	A	A	T	G	**C**
TR.CPV.SAMSUN.06	A	A	T	G	T
Aminoacid Variations	**Y→F**	**E→V**	**L→S**	**K→E**	**L→P**

Table 3. VP1 gene amino acid variations of CPV-2.

Protein	VP1	
Nucleotide Positions	4	67
Amino acid Positions	2	23
Reference Sequence	G	A
TR.CPV.SAMSUN.01	G	A
TR.CPV.SAMSUN.02	G	**G**
TR.CPV.SAMSUN.03	**C**	A
TR.CPV.SAMSUN.04	**C**	A
TR.CPV.SAMSUN.05	G	**G**
TR.CPV.SAMSUN.06	G	**G**
Amino acid Variations	**A→P**	**D→N**

Table 4. VP2 gene amino acid variations of CPV-2.

Protein	VP2										
Amino acid Positions	**11**	80	87	101	**267**	300	305	324	**367**	426	440
Reference Sequence	G	R	M	I	F	A	D	Y	Y	N	T
TR.CPV.SAMSUN.01	G	R	M	I	**Y**	A	D	Y	Y	N	T
TR.CPV.SAMSUN.02	G	R	M	I	**Y**	A	D	Y	**D**	N	T
TR.CPV.SAMSUN.03	G	R	M	I	**Y**	A	D	Y	Y	N	T
TR.CPV.SAMSUN.04	G	R	M	I	**Y**	A	D	Y	Y	N	T
TR.CPV.SAMSUN.05	G	R	M	I	**Y**	A	D	Y	Y	N	T
TR.CPV.SAMSUN.06	**C**	R	M	I	**Y**	A	D	Y	**D**	N	T
OM721655	G	R	L	T	Y	G	Y	I	D	D	A
OM721656	G	R	L	T	Y	G	Y	I	D	D	A
MW539053	G	R	L	T	Y	G	Y	I	D	D	A
M38245.1	G	R	M	I	F	A	D	Y	D	N	T
JN033694.1	G	R	L	T	F	G	Y	Y	D	D	T
MW653252.1	G	R	L	T	F	V	Y	Y	D	D	T
OP985292.1	G	R	L	T	Y	G	D	I	Y	N	A
M19296.1	G	R	M	I	F	A	D	Y	Y	N	T
KY073269.1	G	R	L	T	F	G	Y	Y	D	E	T
LC270891.1	G	R	L	T	F	G	Y	Y	D	D	T
KX434461.1	G	K	M	T	F	A	D	Y	D	N	T

et al. 2001, Li et al. 2017). These antigenic and genetic changes have also been associated with alterations in host range. Shortly after its emergence, CPV-2 spread rapidly, reaching near-pandemic proportions. Today, the original CPV-2 is no longer detected worldwide, having been replaced by the 2a, 2b, and 2c variants (Battilani et al. 2019, Voorhees et al. 2019).

VP2, the major capsid protein of CPV-2, determines the host range and pathogenic potential of the virus, and molecular characterisation is based on amino acid changes in the VP2 gene (Decaro and Buonavoglia 2012). Molecular analyses have shown that mutations at amino acid positions 297 and 426 of VP2 play a key role in differentiating CPV-2 into distinct variants. Accordingly, CPV-2a possesses 426Asn, CPV-2b has 426Asp, and CPV-2c carries 426Glu, while the Ser297Ala mutation has given rise to the new CPV-2a and CPV-2b variants. Five amino acid changes have also been identified between the original CPV-2 and CPV-2a, occurring at positions Met87Leu, Ile101Thr, Ala300Gly, Asp305Tyr and Asn375Asp (Kwan et al. 2021, Li et al. 2022). Continuous viral evolution has led to further mutations in VP2 at Ala5Gly, Tyr324Ile, Gln370Arg, Thr440Ala, Arg481Lys and Val555Ile. It has been reported that the Ala5Gly, Thr440Ala and Arg481Lys substitutions may alter the antigenic structure and immunogenicity of the virus, while Tyr324Ile, Gln370Arg and Val555Ile may affect the host range by influencing receptor binding (Alexis et al. 2021, Liu et al. 2021, Jiang et al. 2021). Additionally, the Phe267Tyr, Tyr324Ile and Thr440Ala mutations have been associated with vaccine failures (Zhou et al. 2017).

This study aimed to investigate the molecular characterisation of CPV-2 detected in dogs from the Black Sea region of Turkey. Sequence analysis of the isolates obtained revealed Asn at position 426, indicating that the isolates belong to the CPV-2a variant. However, the mutations Met87Leu, Ile101Thr, Ala300Gly, Asp305Tyr and Thr440Ala were not observed, and these amino acid residues were identical to those of the original CPV-2. Given that the original CPV-2 is no longer in circulation and that CPV-2a, 2b, 2c, new 2a and new 2b variants currently circulate worldwide, it is possible that retrospective mutations have occurred in our isolates. Furthermore, phylogenetic analyses showed that our isolates clustered closely with the vaccine strain. Since the majority of vaccines currently in use are live attenuated, this close clustering indicates that the vaccine-derived virus may be shed into the environment via the faeces of vaccinated animals, indicating the potential presence of vaccine-like strains in the field.

In this study, two of the positive cases had been vaccinated. The detection of CPV-2 in vaccinated animals has also been reported in previous studies conducted in Turkey and worldwide (Mittal et al. 2014, Yip et al. 2020, Hasircioglu and Aslim 2022). Mutations such as Phe267Tyr, Tyr324Ile and Thr440Ala have been associated with vaccine failures (Zhou et al. 2017). The presence of Phe267Tyr in all our isolates, along with Thr367Asp substitutions in OR066201 and OR066205, supports the link to vaccine failure. In addition, the Gly11Cys mutation was detected in OR066205; the amino acid change at this position has never been mentioned before in the literature, and the evolutionary or functional significance of this position remains unclear. However, such novel variations in the CPV genome may provide potential clues regarding host adaptation and antigenic evolution of the virus. Glycine is an amino acid that provides high flexibility in the protein backbone due to its small structure (Charoenwongpaiboon et al. 2024). Different studies have reported that the thiol group of the cysteine stabilizes the three-dimensional structure of the protein via disulfide bonds, which play a critical role in conformational regulation (Wiedemann et al. 2020, Wong et al. 2020). In light of this information, the G→C conversion suggests an amino acid substitution that reduces structural flexibility and could eventually lead to the formation of new disulfide bonds. This change is believed to affect protein folding dynamics, structural stability, and antigenic properties. However, more comprehensive molecular epidemiological studies are needed to definitively determine the functional significance of this change.

Molecular epidemiological studies have revealed notable differences in the geographical distribution of CPV variants, with the predominant variant type varying by region (Hao et al. 2022). Most CPV-2 sequences submitted to the GenBank database to date originate from China, Uruguay, the United States, Peru, Canada and Brazil (Zhou et al. 2024). While CPV-2a remains the most commonly reported variant worldwide, rapid viral evolution has led to the emergence of different variants, with CPV-2c showing an increasing trend in many countries (Fu et al. 2022, Reddy et al. 2024). Currently, CPV-2a is still the dominant variant in Thailand (Jantafong et al. 2022), Nigeria (Adeyemo et al. 2024), Colombia (Galvis et al. 2021), Uruguay (Grecco et al. 2024) and Africa (Maganga et al. 2023), whereas CPV-2b predominates in Japan (Takano et al. 2021), Brazil (Silva et al. 2022) and Australia (Clark et al. 2018). Recent epidemiological data indicate that CPV-2c is predominant in China (Li et al. 2025), India (Reddy et al. 2024), New Zealand (Dunowska et al. 2025), Argentina (Grecco et al. 2024) and Sicily (Mira et al. 2024). Molecular analyses have suggested that the Ala5Gly and Gln370Arg mutations, detected specifically in CPV-2c but not in other genotypes, can contri-

bute to reduced vaccine efficacy against this variant (Li et al. 2025). In studies conducted so far in Turkey, all three CPV variants have been reported in circulation, with CPV-2b currently dominant, although CPV-2c is increasing in prevalence (Timurkan and Oguzoglu 2015, Karapinar et al. 2018, Polat et al. 2019, Akkutay-Yoldar and Koc 2020, Abayli et al. 2022, Temizkan and Temizkan 2023, Kurucay et al. 2023, Saltik and Koc 2024).

The findings of this study provide insights into the current status of CPV-2 infections in the Black Sea region of Turkey, as well as up-to-date information on the virus variants circulating in the field. Our data also highlight the similarities and differences between the CPV-2 isolates identified in this study and both regional and international variants.

CPV-2 infections can be controlled by developing effective vaccines and appropriate vaccination protocols. Both live attenuated and inactivated CPV-2 vaccines are currently available. However, the detection of CPV-2 in vaccinated animals has raised concerns regarding vaccine efficacy. Vaccine effectiveness may be influenced by several factors, including the level of maternal antibodies in the offspring, the specific vaccine strain used, and the vaccination protocol employed. Therefore, administering vaccines at the optimal time can minimise the interference of maternal antibodies, and selecting vaccine strains tailored to the circulating virus variants may help prevent vaccine failure.

Conclusions

Since their discovery, CPVs have posed a significant threat to both domestic and wild animals. The virus's resistance to environmental conditions, frequent genetic and antigenic changes, and its ability to cross species boundaries make infection control challenging. Rapid viral evolution may lead to the emergence of more virulent antigenic variants. Vaccination remains the safest method for disease control; however, ongoing viral mutations can reduce the efficacy of existing vaccines. Therefore, it is recommended to identify circulating variants, conduct regular epidemiological studies, update vaccine strains to match current variants, and revise vaccination policies to ensure effective control of CPV-2 infection.

Data Accessibility

All data from this study are available for sharing, and the sequencing data of our isolates have been deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) under the following accession numbers: OR066200, OR066201, OR066202, OR066203, OR066204 and OR066205.

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Author Declarations

Ethics approval

This study was approved by the Ondokuz Mayıs University Animal Experiments Local Ethics Committee, which determined that additional ethical clearance was not required (reference number: 68489742-604.01.03-E.15648).

Use of generative artificial intelligence

No artificial intelligence-assisted software or tools were used at any stage of this study, including the design, data analysis and article writing process.

Conflict of interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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