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RESEARCH ARTICLE

Pathological and molecular investigation of canine distemper virus: Phylogenetic analysis of co-circulating genetic lineages in Türkiye

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ABSTRACT

Canine distemper virus (CDV) is a highly contagious virus that infects a wide variety of animals of carnivore species and may cause manifestations from subclinical infection to fatal disease. In this study, dogs clinically suspected having distemper were examined by reverse transcriptase-polymerase chain reaction (RT-PCR), histopathology and immuno-histochemistry. By histopathological examination, characteristic intracytoplasmic and/or intranuclear inclusion bodies were observed in the lung, stomach, small intestine, liver, kidney, spleen and central nervous system. Interstitial and broncho-interstitial pneumonia, gastroenteritis and encephalitis were revealed. CDV antigens were detected in all tissues with characteristic histopathological findings. The antigens were more abundant in the bronchial and bronchiolar epithelium and in the syntitial cells. Phylogenetic analyses were performed using the PCR-amplified partial sequences of the genes encoding the viral heamagglutinin and fusion proteins. The phylogenetic trees showed that the newly determined sequences were diverse and clustered within different lineages of the European or the Arctic strains.

KEYWORDS

canine distemper, immune-histochemistry, histopathology, PCR, hemagglutinin, fusion, phylogeny

INTRODUCTION

Canine distemper virus (CDV) is widespread all over the world and causes a highly contagious, multisystemic disease (Deem et al., 2000; Martella et al., 2006). The infection is characterized by respiratory, gastrointestinal and neurological signs including fever, nasal discharge, respiratory distress, cough, conjunctivitis, anorexia, vomiting, diarrhea, lymphopenia, convulsion, seizures and muscle spasms. Besides domestic and wild canids, the virus can infect hosts from the families Procyonidae, Mustelidae, Hyaenidae, Ursidae, Viveridae and Felidae (Deem et al., 2000; Nikolin et al., 2017).

Multisystemic disease occurs as a result of replication of the virus in the epithelial cells of the respiratory and gastrointestinal tract and in the urogenital system. The outcome of the disease can be fatal especially if the immune response of the infected animal is inadequate. The most important transmission route is through the respiratory secretions discharged by infected animals. CDV can be spread by direct contact or inhalation of aerosol (Deem et al., 2000; Carvalho et al., 2012).

CDV is a member of the genus *Morbillivirus* in the subfamily *Orthoparamyxovirinae* of the family *Paramyxoviridae* (Rima et al., 2019). The pleomorphic, enveloped virions are

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sensitive to disinfectants and contain a single-stranded, negative sense RNA genome, which can be divided into six gene regions. These gene regions encode the following proteins: the nucleocapsid (N) protein that is associated with the RNA in the capsid; the phosphoprotein (P) and the polymerase (L) proteins that together form the replicase-transcriptase complex; the matrix protein (M); the fusion (F) and hemagglutinin (H) proteins that are on the virions' surface in the lipid envelope and facilitate the entry into the cell (Sidhu et al., 1993; Martella and Buonavoglia, 2008). In addition, the H protein participates in the attachment of the virus to cellular receptors and is important in determining viral tropism and cytopathogenicity (Haas et al., 1999; von Messling et al., 2001). Phylogenetic studies, based on the *H* gene sequences of numerous strains from various geographic regions around the world, have been performed to reveal the genetic diversity of CDV. Based on these sequences, the CDV strains have been classified into at least 18 major genetic lineages: America-1, America-2, North America-3, South America/North America-4, America-5 (a sub-genotype of America-2), Asia 1–5, Europe Wildlife, Arctic, Africa-1, Africa-2, Europe-1/South America-1, South America 2–3 and Rock born-like (Anis et al., 2018; Bhatt et al., 2019; Duque Valencia et al., 2019; Weckworth et al., 2020).

In this study, we aimed to determine the presence of the virus in the tissues of dogs that had died as a result of CDV infection. We used molecular virological and immunohistochemical methods to update the information about CDV infection and the circulating strains in our country based on partial sequences, obtained by PCR from the *H* and *F* genes.

MATERIALS AND METHODS

Samples

The study was approved by the Animal Ethics Committee at Balıkesir University, Türkiye (No: 2019/12-2). The material of the study consisted of tissue samples taken from 11 unvaccinated, under one year old domestic dogs that died with a clinical history of neurological, respiratory and/or gastrointestinal signs. The dogs were submitted for necropsy at the Department of Pathology (Faculty of Veterinary Medicine, Balikesir University) between December 2019 and November 2020. Tissue samples from the relevant internal organs were collected for histopathologic and RT-PCR investigation. The tissues were stored frozen at −80 °C until tested by PCR.

RNA extraction and cDNA synthesis

For virological laboratory testing, tissue samples were used. Tissues was prepared as a 10% homogenate in phosphate-buffered saline (PBS) solution. After centrifugation at 3,000×*g* for 15 min, extraction of viral RNA was performed from the supernatant using the Viral RNA+DNA Preparation Kit (Jena Bioscience, Germany) in accordance with the manufacturer's instructions.

The cDNA synthesis was made according to the description supplied with the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA). The first mixture contained 3 µL sterile distilled water, 0.5 µL random hexamer primer and 3 µL RNA. The tubes were placed in a thermocycler set for 70 °C. After 5 min incubation, the tubes were immediately placed on ice. Then, the second mixture was prepared with 2.0 µL 5× reaction buffer, 1.0 µL 10 mM dNTP mix and 0.5 µL M-MuLV reverse transcriptase. This was added to the tubes containing the first mixture, which were then incubated at 48 °C for 45 min.

Reverse transcriptase-polymerase chain reaction (RT-PCR)

The sequence of the primers used in the RT-PCRs for the detection of the *H* and *F* genes along with the size of the amplicons are summarized in Table 1.

The 30 µl final volume of the reaction mixtures contained 3 µl cDNA, 75 mM Tris-HCl (pH 8.8), 20 mM $NH_4(SO_4)2$, 1.5 mM $MgCl_2$, primers 10 pmol, 0.2 mM dNTP and 0.5 U Taq DNA polymerase (MBI, Fermentas, Lithuania). For the amplification of the *H* gene, the thermal program consisted of a denaturation step at 95 °C for 5 min followed by 35 cycles of 94 °C for 60 s, 48 °C for 60 s, and 72 °C for 60 s. The final extension was performed at 72 °C for 10 min. In case of the *F* gene, the program consisted of the initial step at 95 °C for 5 min, followed by 35 cycles of 94 °C for 60 s, 45 °C for 60 s, for 72 °C for 60 s, with a final extension at 72 °C for 10 min. The minor differences in the annealing temperature and other conditions of the RT-PCR were determined by optimization tests. RNase free water was included as the negative control in all RT-PCR runs.

The PCR products were analyzed by gel electrophoresis using a standard 100 bp ladder in 1% agarose gels, stained with GelRed. UV light and gel imaging device were used.

Sequencing and phylogenetic analyses

The PCR products were purified using a commercial purification kit and then sequenced commercially by the Sanger

Table 1. The sequence of the oligonucleotide primers used for the detection of CDV

Primer name	Nucleotide sequence 3′ → 5′	Genomic position	Product size	Reference
CDV-Fus-6F	TGTGTATTCGTCTCAGA	6270–6287		Romanutti et al. (2016)
CDV-Fus-Rev	TAAGACGTGTGACCAGAGTGCTTTAG	7042–7067	797 bp	
1F-Zhao2010fwd	TTAGGGCTCAGGTAGTCCA	7057–7075		Zhao et al. (2010)
2R-7711rev	TGAGATCAAAGACATGGA	7694–7711	654 bp	Trebbien et al. (2014)

method. The raw sequence data were aligned with the Clustal W algorithm using BioEdit version 7.0.5 (Hall, 1999). Nucleotide (nt) sequences were compared with those of different reference canine distemper virus isolates using the BLAST software available at the NCBI website. Phylogenetic analysis was performed with the MEGA v6.0 program. For this purpose, the neighbor joining method was used. Bootstrap values were calculated using 1,000 iterations (Tamura et al., 2013).

Histopathology and immunohistochemistry

Histopathological and immuno-histochemical (IHC) examinations were performed only on the PCR positives cases. The tissues (lung, stomach, small intestine, mesenteric lymph nodes, liver, kidney, spleen and brain) were fixed in 10% neutral buffered formalin and further processed using routine protocols for histopathological examination. The tissues were embedded in paraffin, cut into 4 µm sections and stained with hematoxylin and eosin (HE).

Immuno-histochemical examination for the detection CDV antigens was performed using the avidin–biotin immunoperoxidase complex method. Sections on adhesive slides were deparaffinized and dehydrated. Peroxidase activity was blocked in 3% hydrogen peroxide–methanol solution. The sections were washed with PBS, and nonspecific proteins were blocked by incubating with protein-blocking a sera for 5 min. The sections were incubated with mouse monoclonal CDV antibody (CDV nucleoprotein, 1:400 dilution, VMRD, Pulman, USA) at room temperature for 1 h. After washing with PBS, the sections were incubated with a biotinylated goat anti-mouse antibody at room temperature for 20 min. After another PBS rinse, the sections were treated with horseradish peroxidase–conjugated streptavidin for 20 min. After washing in PBS, diaminobenzidine (Cell Signaling Technology, 8090S) was used as a chromogen, and Mayer's hematoxylin was used for counterstaining. Tissue sections from a previous case of distemper were used as positive controls. For negative controls, PBS solution was substituted for the primary antibody.

Results

The presence of the genome of CDV was successfully detected by PCR in the tissues of 5 out of the 11 dogs studied. All PCR products, from both (*F* and *H*) gene regions were subjected to sequence analyses. It was determined that four novel CDV sequences (TR/BAL/CDV/53, TR/BAL/CDV/54, TR/BAL/CDV/55, TR/BAL/CDV/59) shared high identity values (94.7%–98.5%) among each other, and formed a separate branch within the Arctic lineage. When the corresponding sequences from CDVs, previously reported, were compared, 83.3%–99.8% nt sequence identity was revealed. The fifth sequence, named TR/BAL/CDV/60, was found to be located in a separate branch within the European lineage (Table 2). As a result of the phylogenetic analysis from the partial *F* gene sequences, four of our sequences (TR/BAL/CDV/53, TR/BAL/CDV/54, TR/BAL/CDV/55, TR/BAL/CDV/59) were closest to CDVs detected in, Brazil, Germany and Türkiye previously (Fig. 1). According to the analysis, based on the partial *H* gene sequences, the four similar strains were most closely related to strains reported from Türkiye (with 95.8%–97.7% nt identity) and Italy (95.0%–97.6%). It is noteworthy that our fifth sequence (TR/BAL/CDV/60) seemed to be close to the South African strain (Fig. 2).

The amino acid (aa) substitutions identified in the sequences of the H protein are presented in Fig. 3. When the partial viral aa sequences were examined, we found that our newly-detected viruses shared 90.9 %–97.6% similarity among each other, and 81.4%–99.5% with the reference strains and other previously sequenced viruses.

By histopathological examination, demyelination, focal gliosis, intracytoplasmic and intranuclear inclusion bodies were observed in the brain (Fig. 4a). Mononuclear cell infiltration was observed around the vessels. CDV antigen was detected in the astrocytes, glia, neuron and Purkinje cells by IHC staining (Fig. 4b). The reaction was more intense in areas where demyelination was observed.

In all cases, interstitial pneumonia was detected in the lungs. The alveolar septa were thickened. Broncho-interstitial pneumonia was observed in three of the cases. Purulent bronchopneumonia was present in addition to interstitial pneumonia. Characteristic intracytoplasmic and intranuclear inclusion bodies were frequently detected in the bronchial/bronchiolar epithelial cells and, in one case, in syncytial cells as well (Fig. 4c). In only one case, syncytial cells in the lung were significantly numerous. CDV antigen was detected in the bronchial and bronchiolar epithelial cells, alveolar macrophages, inclusion bodies as well as in the syncytial cells (Fig. 4d). Lymphoid depletion and necrosis in the follicles of the spleen were observed in the case with syncytial cells in the lung. Syncytial cells (Fig. 5a) and eosinophilic intranuclear inclusions (Fig. 5b) were visible in

Table 2. Label, tissue origin, GenBank accession number and genetic lineage of the partial sequences of canine distemper viruses detected in this study

No	ID	Tissue	Hemaglutinin gene	Fusion gene	Genetic lineage
1	TR/BAL/CDV/53	lung	OK184443	OK244693	Arctic
2	TR/BAL/CDV/54	liver	OK184444	OK244694	Arctic
3	TR/BAL/CDV/55	intestine	OK184445	OK244695	Arctic
4	TR/BAL/CDV/59	brain	OK184446	OK244696	Arctic
5	TR/BAL/CDV/60	stomach	OK184447	OK244692	Europe

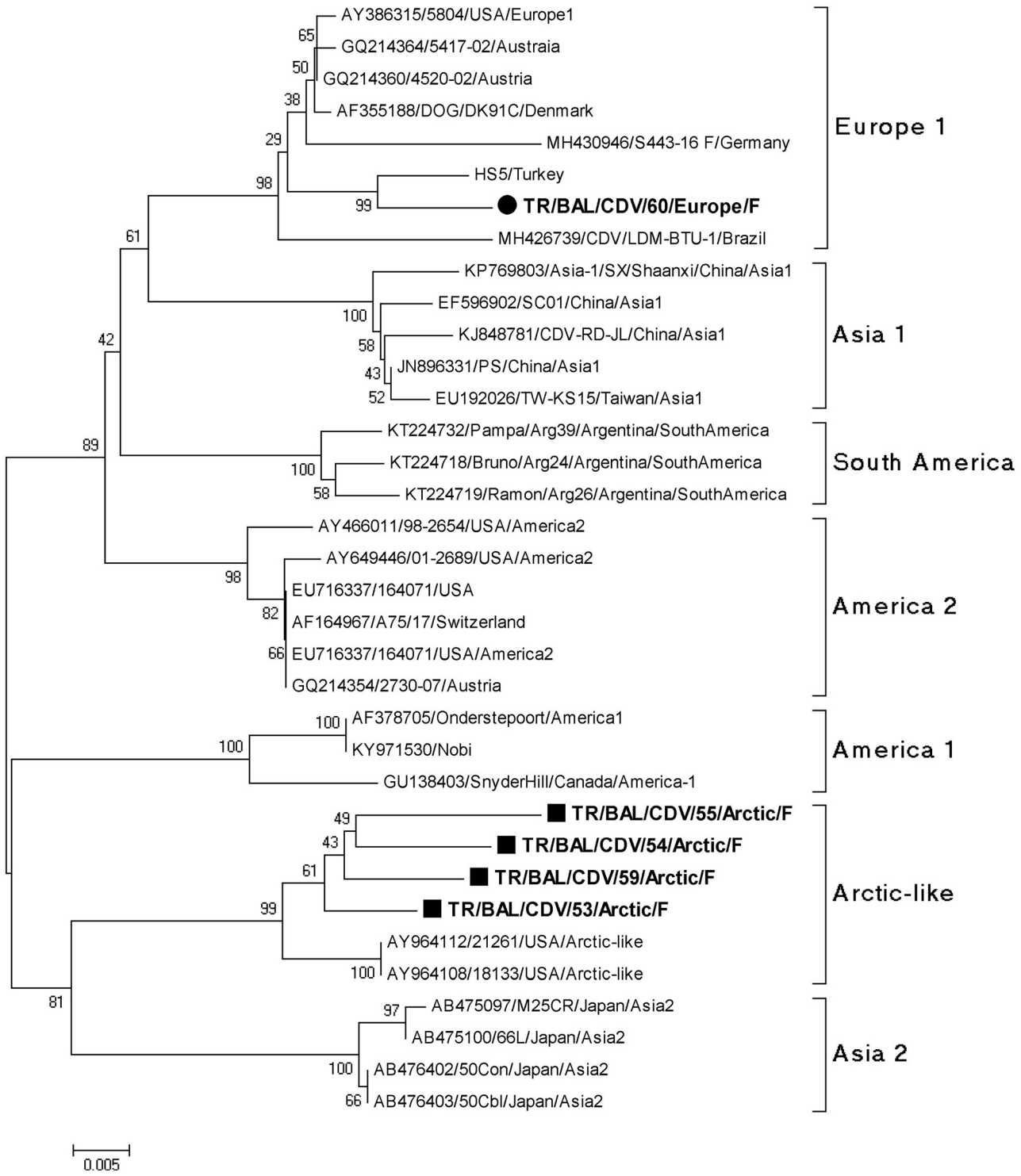

Fig. 1. Neighbor joining phylogenetic tree based on the partial nucleotide sequences of the *fusion* gene of canine distemper viruses. The novel consensus sequences obtained in this study are indicated by solid black square and circle. The scale bar represents 0.5% nucleotide divergence

the white pulp, especially in the necrotic areas. In the gastric, intestinal and pancreatic epithelial cells, intracytoplasmic and intranuclear inclusion bodies were detected (Fig. 6a). The presence of CDV antigens was confirmed. In addition, necrosis in the crypt epithelium of the small intestines was observed. CDV antigens were detected in all tissues with histopathological findings, consistent with the severity of the lesions.

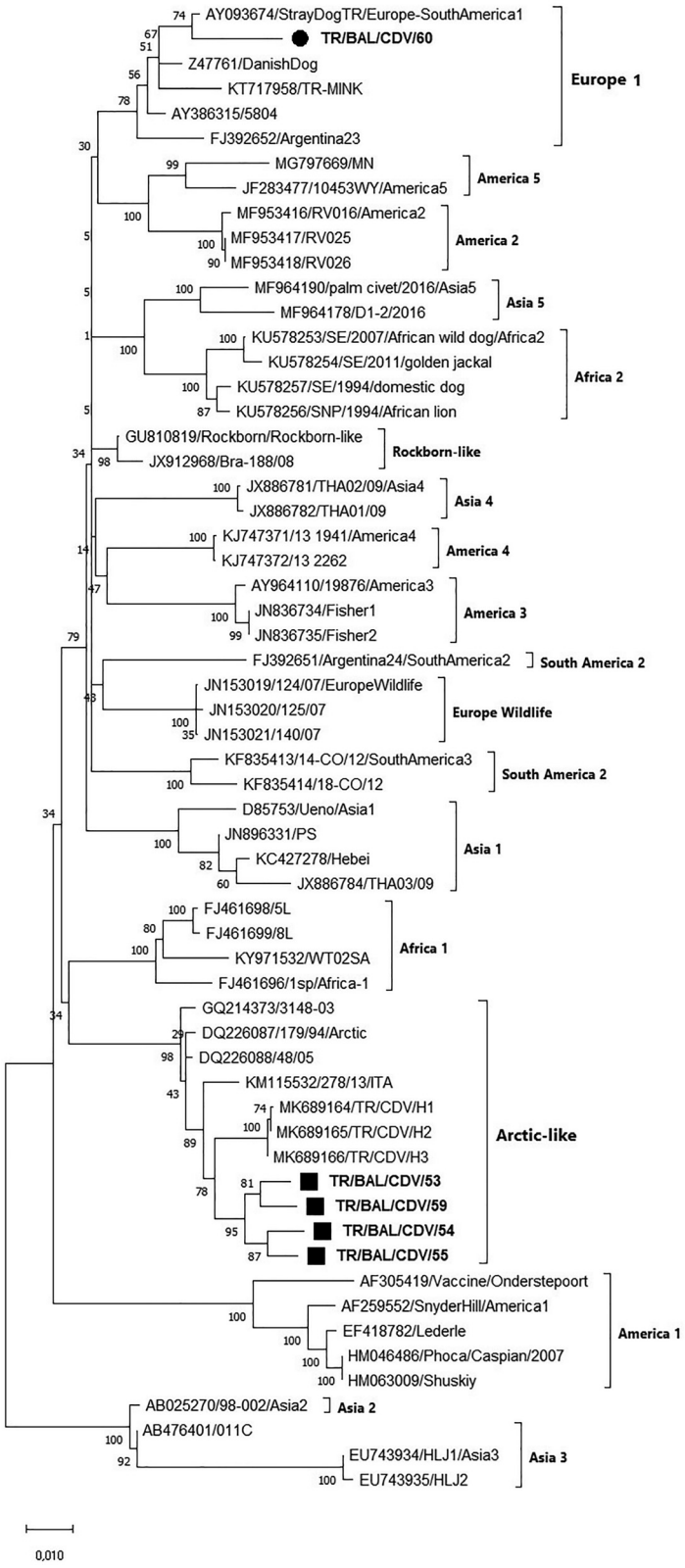

Fig. 2. Neighbour joining phylogenetic tree based on the partial nucleotide sequences of the *hemagglutinin* gene of canine distemper viruses. Consensus sequences obtained in this study are indicated by solid black square and circle. The scale bar represents 1% nucleotide divergence

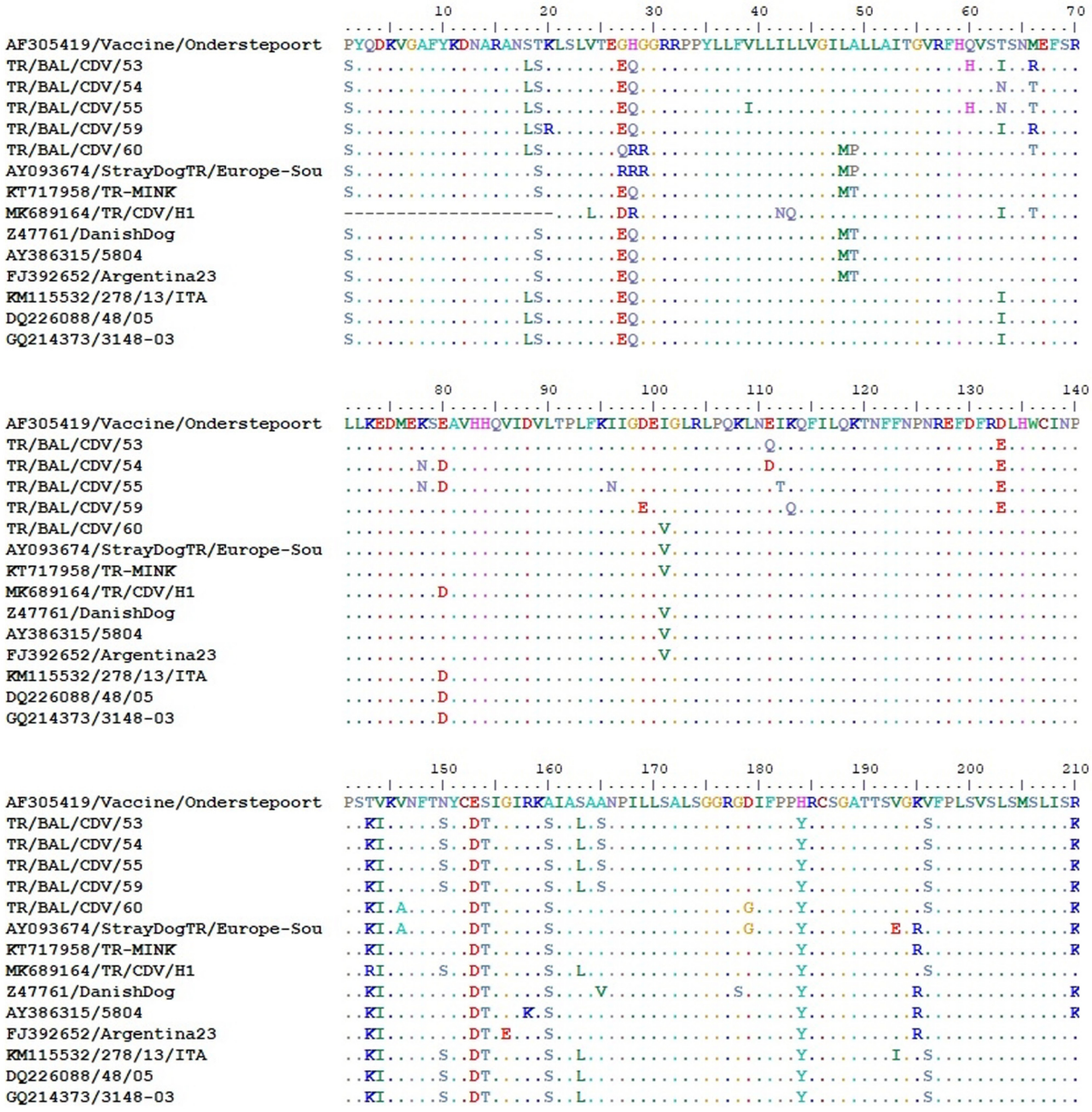

Fig. 3. Non-synonymous amino acid substitutions identified in the partial sequences of the hemagglutinin proteins determined in this study as compared with the corresponding sequence from selected CDV strains (marked by the GenBank accession numbers and labels), including vaccine strains

DISCUSSION

There are many viral agents that cause multisystemic infections in dogs (Dinçer et al., 2020; Timurkan et al., 2021). CDV infection is one of the most important of these, which is a lethal, contagious infection of many carnivore species all over the world. The virus has the ability to infect three distinct types of host cells, including epithelial, lymphoid and neurological cells, and creates a generalized disease manifestation by affecting the respiratory and digestive tracts, and the central nervous system (CNS) (Rendon Marin et al., 2019).

Several studies have been conducted all over the world on the genetic characterisation of CDVs, based on various gene regions. In a study, which included 208 CDV sequences obtained from sixteen countries from all continents between 1975 and 2011, the evolution and global distribution of the virus have been examined based on the *H* gene. It has been revealed that CDV, which diversified into two ancestral clades in the 1880s and spread worldwide to form the eight

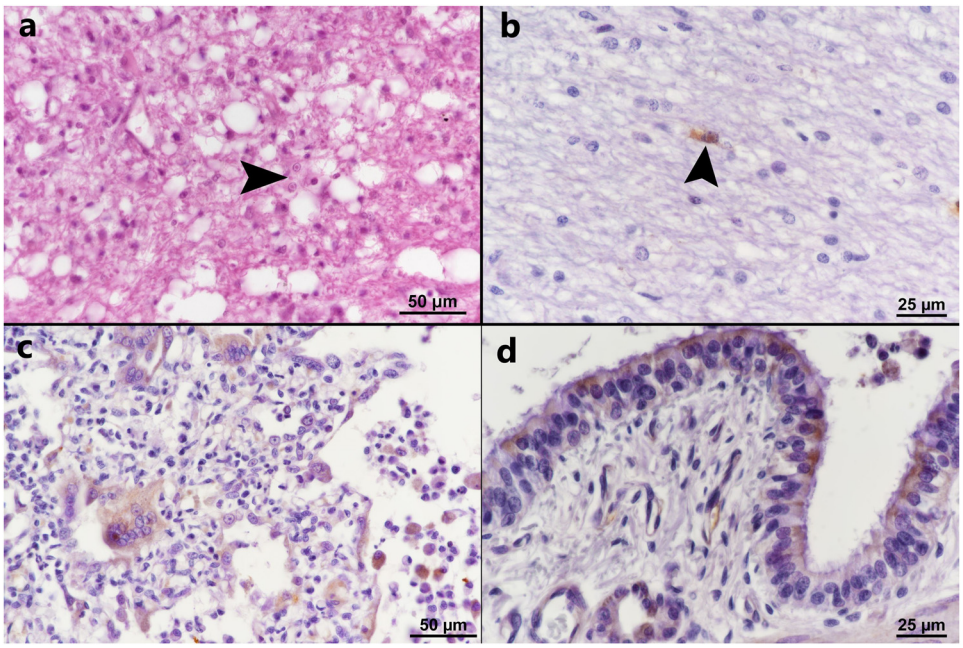

Fig. 4. Histopathological and immuno-histochemical findings in a dog that tested positive in PCRs for the detection of *F* and *H* genes. a: Inclusion bodies (arrowhead) in the brain (HE). b: Positive immunoreactivity with CDV antigen in the glial cytoplasm and intranuclear inclusion (arrowhead). c: Syncytial cells and inclusions with positive immunoreaction in the lung (IHC, HE). d: CDV antigen positive immunoreactivity in the bronchiolar epithelial cells (IHC)

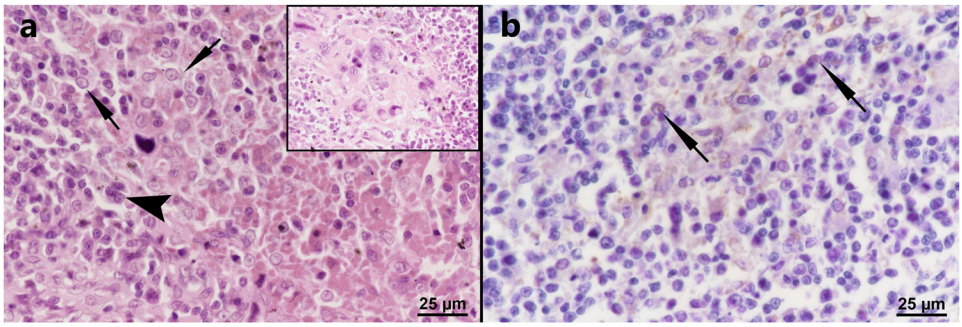

Fig. 5. a: Lymphoid depletion, necrosis in the follicular center, syncytium formation (arrowhead, inset), intracytoplasmic and intranuclear inclusion bodies (arrow) in some areas of the white pulp of the spleen (HE). b: CDV positive immunoreactivity of the cytoplasmic and intranuclear inclusion bodies (arrow) in necrotic areas in the follicles of the spleen (IHC)

of the nine current lineages. The other lineage was determined to form the North America-1 clade (Panzera et al., 2015). In different studies, conducted in Türkiye and other countries in the world, the *NP* gene was chosen because it is a well-conserved region and thus provides convenience for easy detecting the presence of the virus (Ricci et al., 2021; Yılmaz et al., 2022; Saltik and Kale, 2023). Although its PCR detection is less reliable, phylogenetic studies are conducted with the *H* gene. This gene shows high degree of heterogeneity. At the same time, it has been revealed that 549 amino acids of the protein encoded by the *H* gene may have a direct role in determining the host tropism of the virus (Bhatt et al., 2019). In addition, it is widely used to determine the relationship between CDV strains due to its key role in the formation of genetic diversity, cell tropism, host variability and protective immunity (Mira et al., 2018; Aziz et al., 2020). Therefore, the detection of both genes, the *F* and *H* was performed in the present study.

At least 18 major genetic lineages of CDV have been identified to date. Strains belonging to the group Asia-1 show a wide geographic distribution and are present in many countries including China, Thailand and Korea (Bae et al., 2013; Radtanakatikanon et al., 2013). A new genetically distinct CDV strain (India-1/Asia-5) has been identified in India more recently (Bhatt et al., 2019). On the other hand, the Asia-2 strains have been found to circulate in Pakistan (Aziz et al., 2020). It has been also reported that South American strains, which form four different lineages, show a wide variety (Panzera et al., 2014).

The lineage of Arctic strains was discovered in the late 1980s. At that time, the viruses belonging to this lineage have been found to be responsible for the deaths of seals in

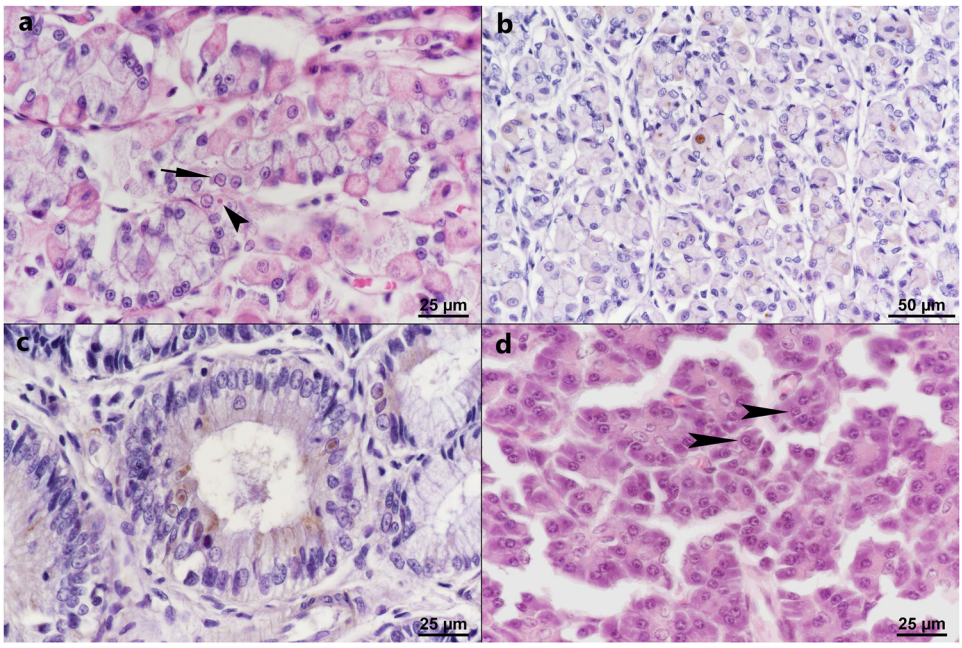

Fig. 6. a: Intracytoplasmic (arrowhead) and intranuclear (arrow) inclusion bodies in the stomach (HE). b: CDV immune-positive intranuclear inclusion in the stomach (IHC). c: Positive immunoreactivity to CDV antigen in the cytoplasmic and intranuclear inclusions in the intestine (IHC). d: Intranuclear inclusion bodies (arrowhead in the pancreas (HE)

northwestern Europe and Siberia (Likhoshway et al., 1989; Visser et al., 1990). Since then, the occurrence of Arctic lineage strains has been reported from various parts of the world (America, China, Iran), as well as from European countries including Hungary, Italy and Türkiye (Demeter et al., 2007; Namroodi et al., 2015; Nguyen et al., 2017; Mira et al., 2018; Koç et al., 2021). Studies have also shown that both European and Arctic strains co-circulate in many countries including Hungary and Italy (Demeter et al., 2007; Ricci et al., 2021). Additionally, it has been revealed that European and Arctic strains occur simultaneously in Iran, where the vaccination is limited to certain regions (Namroodi et al., 2015). Considering the geographical proximity of our country to Iran, the circulation of two different strains in both countries may indicate that different strains of the virus may be exchanged between these ecosystems.

There are a number of studies on the molecular characterization of CDV in Türkiye. The first detected field CDVs have been found to belong to the European strains (Ozkul et al., 2004; Oğuzoğlu et al., 2018; Yılmaz et al., 2022). In phylogenetic analysis, based on the *H* gene sequences, the Turkish sequences have appeared in a branch close to the Italian lineage, including wild CDV strains under the Arctic CDV lineage (Koç et al., 2021). The presence of European and Arctic strains in Türkiye has been detected separately. However, our study is the first when the circulation of strains belonging to two different lineages is reported from the same geographical region.

Comparison of the novel H protein aa sequences, obtained in this study with their counterparts in other CDVs including vaccine strains, it was determined that at some points (namely at aa positions 18., 19., 133., 163., 165.) our Arctic type sequences were identical but differed from the fifth novel strain as well as from all the other strains (Fig. 3). It comes to mind that the aa changes may mark a different Arctic-like strain. These mutations may affect the characteristics of the virus, and may be important in the selection of the vaccine.

With the histopathological examination we revealed interstitial pneumonia in all cases, and broncho-interstitial pneumonia in three of these. In one case, syncytial cells were noted in the lumen of the alveoli. Intracytoplasmic and intranuclear inclusions were detected in bronchial epithelial cells and syncytial cells. These findings in the lungs are consistent with previous studies in different animal species, including dogs (Pratakpiriya, 2017; Wang et al., 2021b; Michelazzo et al., 2022).

CDV usually enters the organism by inhalation and it is firstly transported to the oropharyngeal lymph nodes. CDV primarily replicates in cells of the immune system. The virus spreads to the epithelial cells of the skin, respiratory, gastrointestinal, urinary system and the brain. Membrane fusion is necessary for the spread of morbillivirus from cell to cell. Membrane fusion results in the formation of a multinucleated cells i.e. syncytia, which is an important cytopathogenic feature of the virus (Rendon Marin et al., 2019). Two epithelial receptors are effective in the spread and aerosol transport of CDV. The first of these is signaling lymphocyte activation molecule (SLAM), which is expressed on B and T lymphocytes, dendritic cells, hematopoietic cells and macrophages. SLAM plays a key role in the affinity of the virus to distinct species, cell fusion, tropism, virulence and *in vitro* cytopathogenicity. Mutations in amino acids of the SLAM protein affect the form of infection. In studies,

conducted in distinct species, it has been shown that mutation of amino acids 74 and 129 of SLAM may affect the viral infection by influencing the formation and frequency of syncytia (Wang et al., 2021a). During a canine distemper epizooty in Switzerland in 2009, it has been determined that functional and structural differences in the H protein of CDV strains are responsible for the change in binding efficiency to SLAM, and as a result, for an increase in virus pathogenicity with high morbidity and mortality in a wide host spectrum (Origgi et al., 2012). The second epithelial receptor, the nectin cell adhesion molecule-4 (NECTIN-4), is a component of adherens junctions of epithelial cells. In various studies on the formation of syncytia, it has been reported that SLAM and NECTIN-4 receptors, which allow the virus to enter the cell, facilitate the cell to cell spread and are effective in the formation of syncytia (Rendon Marin et al., 2019; Shin et al., 2022). It has also been reported that morbilliviruses inhibit the JAK/STAT signaling pathway, affecting the natural immunity, and being particularly important in the entry of the virus into the respiratory tract epithelial cells, spreading from cell to cell, and syncytia formation (Shin et al., 2022). The presence of characteristic eosinophilic inclusions, frequently cytoplasmic but occasionally intranuclear, in the epithelial tissue, as well as intracytoplasmic inclusions less frequently in the CNS has also been documented (Maxie, 2015). In the present study, multiple syncytia were observed in the lungs and in necrotic centers in the spleen. Both cytoplasmic and intranuclear eosinophilic inclusion bodies were also detected in the spleen, stomach, pancreas and intestinal epithelial cells. Immunohistochemical analysis revealed CDV antigen in the lungs, stomach, intestines, pancreas, spleen and brain. CDV antigens were immune-stained more prominently in the demyelinated areas of the brain, in the bronchial/bronchiolar epithelial cells and in the syncytia. Considering that the viral sequence, from the case with the most intensive syncytium formation, indicated the presence of a European-like CDV strain, the relationship between the pathogenesis and the type of virus strain seems plausible.

Modified live vaccines, which have been used since the 1950s to combat CDV infection, have provided a great deal of efficacy. Most CDV vaccines are designed based on America-1 strains isolated between the 1940s and 1960s (Onderstepoort, Snyder Hill, Lederle). In recent years, it has been observed that the infection has increased significantly in wild carnivores as well as in domestic and stray dogs. Although an intensive vaccination program is implemented today, adequate immunity cannot be provided. This is explained by the fact that the virus can mutate and escape from the immune system. Outbreaks due to the mutations in the genome give the virus the ability to evade the immune response induced by current vaccines (Ricci et al., 2021). It has been reported that the reason for the detection of the virus in vaccinated animals may be the antigenic differences between the vaccine strain and the wild type virus, or the immune deficiency or lack of immunity resulting from the effect of maternal antibodies on the vaccine virus (Yeşilbağ et al., 2007). In addition, it is known that field viruses circulating in many countries originate from vaccine viruses that were genetically differentiated.

During the fight against infection, it is necessary to question the protective immunity level of the vaccine, the compatibility of the vaccine with the vaccination schedule, and the presence of maternal antibodies while establishing the vaccination schedule. Conducting genetic studies of circulating strains provides up-to-date information about the evolution of the virus and is also necessary for updating the vaccines that are going to be used.

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REFERENCES

Anis, E., Newell, T. K., Dyer, N. and Wilkes, R. P. (2018): Phylogenetic analysis of the wild-type strains of canine distemper virus circulating in the United States. Virol. J. **15,** 118.

Aziz, H., Altaaf, F., Bashir, A., Basit, A., Gardazi, S. A. M. and Haider, F. (2020): Phylogenetic analysis of canine distemper virus in a domestic dog in Pakistan. Hosts and Viruses **7,** 2329.

Bae, C. W., Lee, J. B., Park, S. Y., Song, C. S., Lee, N. H., Seo, K. H., Kang, Y. S., Park, C. K. and Choi, I. S. (2013): Deduced sequences of the membrane fusion and attachment proteins of canine distemper viruses isolated from dogs and wild animals in Korea. Virus Genes **47,** 56–65.

Bhatt, M., Rajak, K. K., Chakravarti, S., Yadav, A. K., Kumar, A., Gupta, V., Chander, V., Mathesh, K., Chandramohan, S., Sharma, A. K., Mahendran, K., Sankar, M., Muthuchelvan, D., Gandham, R. K., Baig, M., Singh, R. P. and Singh, R. K. (2019): Phylogenetic analysis of haemagglutinin gene deciphering a new genetically distinct lineage of canine distemper virus circulating among domestic dogs in India. Transbound. Emerg. Dis. **66,** 1252–1267.

Carvalho, O. V., Botelho, C. V., Ferreira, C. G., Scherer, P. O., Soares-Martins, J. A., Almeida, M. R. and Silva Júnior, A. (2012): Immunopathogenic and neurological mechanisms of canine distemper virus. Adv. Virol. **163860**.

Deem, S. L., Spelman, L. H., Yates, R. A. and Montali, R. J. (2000): Canine distemper in terrestrial carnivores: a review. J. Zoo. Wild. Med. **31,** 441–451.

Demeter, Z., Lakatos, B., Palade, E. A., Kozma, T., Forgách, P. and Rusvai, M. (2007): Genetic diversity of Hungarian canine distemper virus strains. Vet. Microbiol. **122,** 258–269.

Dinçer, E., Timurkan, M. Ö., Dinçer, P. F. P. and Aydın, H. (2020): Co-circulation of canine chaphamaparvovirus and canine parvovirus 2 in dogs with diarrhea in Turkey. Thai. J. Vet. Med. **50,** 495–501.

Duque-Valencia, J., Forero-Muñoz, N. R., Díaz, F. J., Martins, E., Barato, P. and Ruiz-Saenz, J. (2019): Phylogenetic evidence of the intercontinental circulation of a Canine distemper virus lineage in the Americas. Sci. Rep. **9,** 15747.

Hall, T. A. (1999): BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symp. Ser. **41,** 95–98.

Haas, L., Liermann, H., Harder, T. C., Barrett, T., Löchelt, M., von Messling, V., Baumgärtner, W. and Greiser-Wilke, I. (1999): Analysis of the H gene, the central untranslated region and the proximal coding part of the F gene of wild-type and vaccine canine distemper viruses. Vet. Microbiol. **69,** 15–18.

Koç, B. T., Akkutay-Yoldar, Z. and Oğuzoğlu, T. Ç. (2021): New members to Arctic-like lineage of canine distemper virus from Turkey. Comp. Immunol. Microbiol. Infect. Dis. **78,** 101678.

Likhoshway, Ye. V., Grachev, M. A., Kumarev, V. P., Solodun, Yu. V., Goldberg, O.A., Belykh, O. I., Nagieva, F. G., Nikulina, V. G. and Kolesnik, B. S. (1989): Baikal seal virus. Nature **339,** 266.

Martella, V., Cirone, F., Elia, G., Lorusso, E., Decaro, N., Campolo, M., Desario, C., Lucente, M.S., Bellacicco, A. L., Blixenkrone-Møller, M., Carmichael, L. E. and Buonavoglia, C. (2006): Heterogeneity within the hemagglutinin genes of canine distemper virus (CDV) strains detected in Italy. Vet. Microbiol. **116,** 301–309.

Martella, V., Elia, G. and Buonavoglia, C. (2008): Canine distemper virus. Vet. Clin. North Am. Small Anim. Pract. **38,** 787–797.

Maxie, M. G. (2015): Jubb, Kennedy & Palmer's Pathology of Domestic Animals: Volume 2 (**Vol. 2**). Elsevier Health Sciences.

Michelazzo, M. M. Z., Martinelli, T. M., de Amorim, V. R. G., Silva, L. E., Silva, F. H. P., Xavier, A. A. C., Cubas, Z. S., de Almeida, R. F., de Moraes, W. and Headley, S. A. (2022): Canine distemper virus and canine adenovirus type-2 infections in neotropical otters (*Lontra longicaudis*) from Southern Brazil. Braz. J. Microbiol. **53,** 369–375.

Mira, F., Purpari, G., Di Bella, S., Vicari, D., Schirò, G., Di Marco, P., Macaluso, G., Battilani, M. and Guercio, A. (2018): Update on canine distemper virus (CDV) strains of Arctic-like lineage detected in dogs in Italy. Vet. Ital. **54,** 225–236.

Namroodi, S., Rostami, A., Majidzadeh-Ardebili, K., Ghalyanchi Langroudi, A. and Morovvati, A. (2015): Detection of Arctic and European cluster of canine distemper virus in north and center of Iran. Vet. Res. Forum **6,** 199–204.

Nguyen, D. V., Suzuki, J., Minami, S., Yonemitsu, K., Nagata, N., Kuwata, R., Shimoda, H., Vu, C. K., Truong, T. Q. and Maeda, K. (2017): Isolation and phylogenetic analysis of canine distemper virus among domestic dogs in Vietnam. J. Vet. Med. Sci. **79,** 123–127.

Nikolin, V. M., Olarte-Castillo, X. A., Osterrieder, N., Hofer, H., Dubovi, E., Mazzoni, C. J., Brunner, E., Goller, K. V., Fyumagwa, R. D., Moehlman, P. D., Thierer, D. and East, M. L. (2017): Canine distemper virus in the Serengeti ecosystem: molecular adaptation to different carnivore species. Mol. Ecol. **26,** 2111–2130.

Oğuzoğlu, T. Ç., Yücel-Tenekeci, G., Çalışkan, M., Bozkurt, M. F. and De Massis, F. (2018): Detection and characterization of distemper virus in a mink (*Neovison vison*) in Turkey. Vet. Ital. **54,** 79–85.

Ozkul, A., Sancak, A. A., Güngör, E. and Burgu, I. (2004): Determination and phylogenetic analysis of canine distemper virus in dogs with nervous symptoms in Turkey. Acta Vet. Hung. **52,** 125–132.

Origgi, F. C., Plattet, P., Sattler, U., Robert, N., Casaubon, J., Mavrot, F., Pewsner, M., Wu, N., Giovannini, S., Oevermann, A., Stoffel, M. H., Gaschen, V., Segner, H. and Ryser-Degiorgis, M. P. (2012): Emergence of canine distemper virus strains with modified molecular signature and enhanced neuronal tropism leading to high mortality in wild carnivores. Vet. Pathol. **49,** 913–929.

Panzera, Y., Sarute, N., Carrau, L., Aldaz, J. and Perez, R. (2014): Genetic diversity of canine distemper virus in South America. Br. J. Virol. **1,** 48–53.

Panzera, Y., Sarute, N., Iraola, G., Hernández, M. and Pérez, R. (2015): Molecular phylogeography of canine distemper virus: geographic origin and global spreading. Mol. Phylogenet. Evol. **92**, 147–154. https://doi.org/10.1016/j.ympev.2015.06.015.

Pratakpiriya, W., Ping Teh, A. P., Radtanakatikanon, A., Pirarat, N., Thi Lan, N., Takeda, M., Techangamsuwan, S. and Yamaguchi, R. (2017): Expression of canine distemper virus receptor nectin-4 in the central nervous system of dogs. Sci. Rep. **7,** 349.

Radtanakatikanon, A., Keawcharoen, J., Charoenvisal, N. T., Poovorawan, Y., Prompetchara, E., Yamaguchi, R. and Techangamsuwan, S. (2013): Genotypic lineages and restriction fragment length polymorphism of canine distemper virus isolates in Thailand. Vet. Microbiol. **166,** 76–83.

Rendon Marin, S., da Fontoura Budaszewski, R., Canal, C. W. and Ruiz-Saenz, J. (2019): Tropism and molecular pathogenesis of canine distemper virus. Virol. J. **16,** 30.

Ricci, I., Cersini, A., Manna, G., Marcario, G. A., Conti, R., Brocherel, G., Grifoni, G., Eleni, C. and Scicluna, M. T. (2021): A canine distemper virus retrospective study conducted from 2011 to 2019 in Central Italy (Latium and Tuscany Regions). Viruses **13,** 272.

Rima, B., Balkema-Buschmann, A., Dundon, W. G., Duprex, P., Easton, A., Fouchier, R., Kurath, G., Lamb, R., Lee, B., Rota, P., Wang, L. and the ICTV Report Consortium (2019): ICTV virus taxonomy profile: *Paramyxoviridae*. J. Gen. Virol. **100,** 1593–1594.

Romanutti, C., Gallo Calderón, M., Keller, L., Mattion, N. and La Torre, J. (2016): RT-PCR and sequence analysis of the full-length fusion protein of Canine Distemper Virus from domestic dogs. J. Virol. Methods **228,** 79–83.

Saltik, H. S. and Kale, M. (2023): Rapid molecular detection and isolation of canine distemper virus in naturally infected dogs. Ankara Univ. Vet. Fak. Derg. **70,** 49–56. https://doi.org/10.33988/auvfd.846475.

Sidhu, M. S., Husar, W., Cook, S. D., Dowling, P. C. and Udem, S. A. (1993): Canine distemper terminal and intergenic non-protein coding nucleotide sequences: completion of the entire CDV genome sequence. Virology **193,** 66–72.

Shin, D. L., Chludzinski, E., Wu, N. H., Peng, J. Y., Ciurkiewicz, M., Sawatsky, B., Pfaller, C. K., Baechlein, C., von Messling, V., Haas, L., Beineke, A. and Herrler, G. (2022): Overcoming the barrier of the respiratory epithelium during canine distemper virus infection. mBio **13,** e0304321.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. (2013): MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. **30,** 2725–2729.

Timurkan, M. O., Aydin, H., Dincer, E. and Coskun, N. (2021): Molecular characterization of canine coronaviruses: an enteric and pantropic approach. Arch. Virol. **166,** 35–42.

Trebbien, R., Chriel, M., Struve, T., Hjulsager, C. K., Larsen, G. and Larsen, L. E. (2014): Wildlife reservoirs of canine distemper virus resulted in a major outbreak in Danish farmed mink (*Neovison vison*). PLoS One **9,** e85598.

Yeşilbağ, K., Yilmaz, Z., Ozkul, A. and Pratelli, A. (2007): Aetiological role of viruses in puppies with diarrhoea. Vet. Rec. **161,** 169–170.

Visser, I. K., Kumarev, V. P., Orvell, C., de Vries, P., Broeders, H. W., van de Bildt, M. W., Groen, J., Teppema, J. S., Burger, M. C., UytdeHaag, F. G. and Osterhaus, A. D. M. E. (1990): Comparison of two morbilliviruses isolated from seals during outbreaks of distemper in north west Europe and Siberia. Arch. Virol. **111,** 149–164.

von Messling, V., Zimmer, G., Herrler, G., Haas, L. and Cattaneo, R. (2001). The hemagglutinin of canine distemper virus determines tropism and cytopathogenicity. J. Virol. **75,** 6418–6427.

Wang, Y., Chen, J., Hu, B., Gong, C., Shi, N., Liu, M., Yan, X., Bai, X. and Zhao, J. (2021a): Mink SLAM V-region V74I substitutions contribute to the formation of syncytia induced by canine distemper virus. Front. Vet. Sci. **7,** 570283.

Wang, R., Wang, X., Zhai, J., Zhang, P., Irwin, D. M., Shen, X., Chen, W. and Shen, Y. (2021b): A new canine distemper virus lineage identified from red pandas in China. Transbound. Emerg. Dis. **2021,** 1–9.

Weckworth, J. K., Davis, B. W., Roelke-Parker, M. E., Wilkes, R. P., Packer, C., Eblate, E., Schwartz, M. K. and Mills, L. S. (2020): Identifying candidate genetic markers of CDV cross-species pathogenicity in African lions. Pathogens **9,** 872.

Yılmaz, V., Coşkun, N., Timurkan, M. O., Karakurt, E., Nuhoğlu, H., Erkılıç, E. E., Kırmızıgül, A. H. and Sezer, M. (2022): The investigation of canine distemper virus in different diagnosis materials of dogs using molecular and pathological methods, Northeastern Turkey. Indian J. Anim. Res. **56,** 316–322.

Zhao, J. J., Yan, X. J., Chai, X. L., Martella, V., Luo, G. L., Zhang, H. L., Gao, H., Liu, Y. X., Bai, X., Zhang, L., Chen, T., Xu, L., Zhao, C. F., Wang, F. X., Shao, X. Q., Wu, W. and Cheng, S. P. (2010): Phylogenetic analysis of the haemagglutinin gene of canine distemper virus strains detected from breeding foxes, raccoon dogs and minks in China. Vet. Microbiol. **140,** 34–42.