Original Article

Molecular Detection of Canine Distemper Virus Among Dogs Showing Neurologic and Non-neurologic Forms of Disease

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ABSTRACT

Background: Canine distemper (CD) is the dogs' most contagious and lethal viral disease. Despite the widespread use of vaccines to control CD, the prevalence of the CD virus (CDV) has increased at an alarming rate in recent years.

Objectives: To identify the genotypes responsible for the neurological and non-neurological clinical forms of CD and to investigate the presence of the virus in the neurological and non-neurological forms of the disease.

Methods: In this descriptive-analytical study, the samples were collected from 70 CD suspected unvaccinated dogs with clinical signs of distemper. All cases were tested with rapid tests and separated into 3 groups based on clinical signs and symptoms. Cerebral spinal fluid (CSF), respiratory secretion, and fecal samples of allall 70 cases were examined for reverse transcription polymerase chain reaction (RT-PCR). After sequencing the hemagglutinin gene (*H* gene), phylogenetic analysis of the gene isolated from CDVs was carried out using MEGA software, version 7.

Results: The RT-PCR results showed that the respiratory secretion sample in the non-neurological CDV group (85%) and the neurological CDV group (80%) had the highest level of virus contamination. However, in the non-neurological CDV group, the CSF sample (40%) had a high level of infection. In neurotic groups, cases older than 12 months showed the highest percentage of distemper contamination, and in the non-neurologic CDV group, those between 3 and 6 months were more involved. Sequencing and phylogenetic analysis of the *H* gene revealed the CDV as a member of the endemic Arctic-like genetic lineage.

Conclusion: The genotypic examination of the hemagglutinin gene of the distemper virus reveals that the recent isolates of neurologic and non-neurologic clinical forms of CDV in Iran are similar. In positive rapid test cases, the PCR test of respiratory secretions for virus detection ranks with the highest sensitivity. In neurologic cases with negative rapid test results, PCR of CSF had the highest sensitivity, so it may serve as a diagnostic criterion.

Keywords: Distemper, Dog, Hemagglutinin, Cerebrospinal fluid, Neurologic, Non-neurologic

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Introduction

Distemper is a fatal contagious viral disease in dogs and other species, such as raccoons, ferrets, otters, pandas, and skunks (Namroodi et al., 2014). The canine distemper virus (CDV) is a single-stranded RNA virus of the Morbillivirus genus and the Paramyxoviridae family. This enveloped CDV is highly susceptible to chemical disinfectants (Bi et al., 2015). Although CDV only has one serotype, it possesses 17 major genotypes based on genetic variability of membrane glycoproteins, including America-1 to -5, South America-1 to -3, Asia-1 to 4, Europe Wildlife, Arctic, South Africa, America-1/Europe, and Rockborn-like (Loots et al., 2017).

Disease severity and clinical symptoms depend on multiple factors, such as virus strain, immunity, and host age (Hornsey et al., 2019). In general, CDV infection disease could be classified into two primary clinical forms: Non-neurologic CDV whose clinical symptoms include fever, yellow or green discharge from the eyes and nose, cough, dyspnea, depression, lethargy and anorexia, diarrhea, and vomiting without any neurologic symptoms (Zhao & Yanrong, 2022; Hornsey et al., 2019); neurologic CDV, with neurological disorders, including abnormal behaviors, chewing gum, seizures, blindness, paresis and paralysis, imbalance and rotation (Hornsey et al., 2019).

CDV genome is composed of 6 genes encoding eight proteins: 2 non-structural proteins (C and V) and 6 structural proteins (nucleocapsid protein, matrix protein, phosphoprotein, large protein, and 2 membrane glycoproteins known as hemagglutinin (H) protein) (da Fontoura Budaszewski & Von Messling, 2016; Duque-Valencia et al., 2019). H protein binds the virus to the host cell (Rendon-Marin et al., 2019). It also stimulates the host's immune system, protecting it from the virus (Rendon-Marin et al., 2019). The highest level of mutation occurs in the H gene (Wang et al., 2020). Thus, designing specific primers targeting the *H* gene makes it possible to differentiate the field CDV lineages (Rendon-Marin et al., 2019).

The diagnosis of canine distemper is primarily based on clinical suspicion. Furthermore, clinicians prefer the rapid CDV antigen test kit due to its simple, prompt, and feasible application in most laboratories. The test can detect distemper virus in the conjunctiva, urine, serum, or plasma with high accuracy. CDV antigen rapid test has shown excellent sensitivity (98%) and specificity (98.5%) compared to other methods (Costa et al., 2019; Saaed & Alsarhan, 2022). In our study, we used a Bionote (Woodley equipment) rapid test kit with a sensitivity of 100% and specificity of 98.5% compared to the nested PCR. However, the definitive method for diagnosing distemper is amplifying conserved specific genes, such as the neuraminidase (*NA*) gene, by PCR (Ricci et al., 2021). In this case, the researchers reported that the conserved nucleoprotein (*NP*) gene is considered a better target for amplifying specific fragments from all strains of CDV (Namroodi et al., 2013; Wang et al., 2020).

Most studies have focused on the prevalence of CDV among rural dogs in Iran (Avizeh et al., 2007; Namroodi et al., 2015; Sarchahi & Arbabi, 2022; Tavakoli Zaniani et al., 2021). Unfortunately, little epidemiological research has been conducted on the CDV lineages in Iran. Our understanding of the molecular prevalence of CDV in different forms of the disease and the genetic variability of CDVs inducing neurologic and non-neurologic forms of the disease are limited. Therefore, this study was designed to investigate the difference between the molecular prevalence of CDV in dogs with non-neurologic and neurologic forms of distemper. We also performed a phylogenetic analysis to evaluate the genetic similarities between non-neurologic and neurologic CDV.

Material and Methods

Target population

The target population consisted of dogs referred to veterinary hospitals in Tehran City, Iran, from October 2020 to September 2021. A total of 70 dogs with clinical signs of CDV without vaccination history were included in this study. Based on the manufacturer's instructions, a CDV rapid test was conducted for all animals using a CDV quick antigen kit (Woodly Equipment Company LTD., UK). Concerning the initial clinical examination and rapid kit results, 40 animals were grouped into 3. Group 1 included animals with positive kit results but without neurological clinical signs. They were classified as the "non-neurologic CDV" group. These animals mainly showed gastrointestinal and respiratory symptoms, including anorexia, diarrhea, vomiting, coughing, dyspnea, pneumonia, and nasal and ocular discharge (n=20). Group 2 included animals with neurologic symptoms and positive kit results. They were classified as the "neurologic CDV" group (n=10). Neurological signs included chewing gum, muscle tics, seizures, paralysis, circling, and blindness. Group 3 included animals with neurologic symptoms and negative kit results. They were classified as the "neurologic non-CDV" group (n=10).

Sampling

Samples were collected from all animals' cerebrospinal fluid (CSF), nasal-conjunctive secretion, and stool. Samples were obtained using a sterile swab from the nasal canal and conjunctive secretions. Also, stool samples were collected using a sterile swab. CSF samples were collected using the cisternal puncture method (Suzuki & Ferrario, 1984). Therefore, CSF samples were collected using a sterile syringe (gage 22) from the cerebellomedullary cistern under complete anesthesia. Then, they were immediately kept at -20˚C (Pouramini et al., 2017).

RT-PCR

To detect CDV, the *N* gene was targeted using CDV-N primers (Table 1). Moreover, in positive samples, the *H* gene segment of the virus was amplified for phylogenetic analysis using CDV-H primers (Table 1). Following the manufacturer's instruction, RNA was extracted from clinical samples using an RNA extraction kit (Bioneer Co, Korea) and was stored at -80˚C. The extracted RNA was reverse-transcribed into cDNA using a two-step RT-PCR kit (Vi1vantis, Malaysia), providing the manufacturer's recommended reaction conditions. PCR reaction was performed in a final volume of 20 µL, including 10 µL of Mastermix (Vivantis, Malaysia), 0.5 µL of each primer (10 mM), 2 µL of template DNA, and 7 µL of deionized water. The PCR amplification was performed under the following conditions: Initial denaturation at 95˚C for 1 min, followed by denaturation at 95˚C for 1 min, annealing at 47˚C for 1 min, and elongation at 72˚C for 1 min (35 cycles), and a final extension at 72˚C for 10 min. After amplification, 5 µL of the reaction mixture was transferred to 1% agarose gel.

Sequencing and phylogenetic analysis

PCR products of the *H* gene were purified using a PCR purification kit (Bioneer, Korea). Purified amplicons were sequenced using ABI 313 DNA sequencing instruments (Seq Lab Co, Germany). Editing and analysis of the raw DNA sequence was performed using BioEdit software (a free software sequence analysis program developed by Tom Hall at North Carolina State University). Sequences were compared with CDV sequences deposited in the GenBank using BLAST software. Eventually, nucleotide sequences were submitted to the GenBank database. *H* gene sequences of 13 CDV isolates obtained from this analysis and 37 collected sequences from the Genbank were used for phylogenetic analysis using MEGA software, version 11 (Sohpal et al., 2010). Hemagglutinin gene sequences of 4 distemper virus vaccines, including vaccine a (accession: FJ461701.1, GI: 239949421), vaccine B (accession: FJ461709.1, GI: 239949437), vaccine C (accession: FJ461708.1, GI: 239949435), and vaccine E (accession: FJ461710.1, GI: 239949439) were also added to the data. The internal node uncertainty was assessed through 500 bootstrap replications. Subsequently, the aligned *H* gene sequences were used to construct the phylogenetic tree based on neighbor-joining and maximum likelihood methods (Nikbakht et al., 2018).

Statistical analysis

The statistical analyses were performed using SPSS software, version 19). The chi-square test was used to

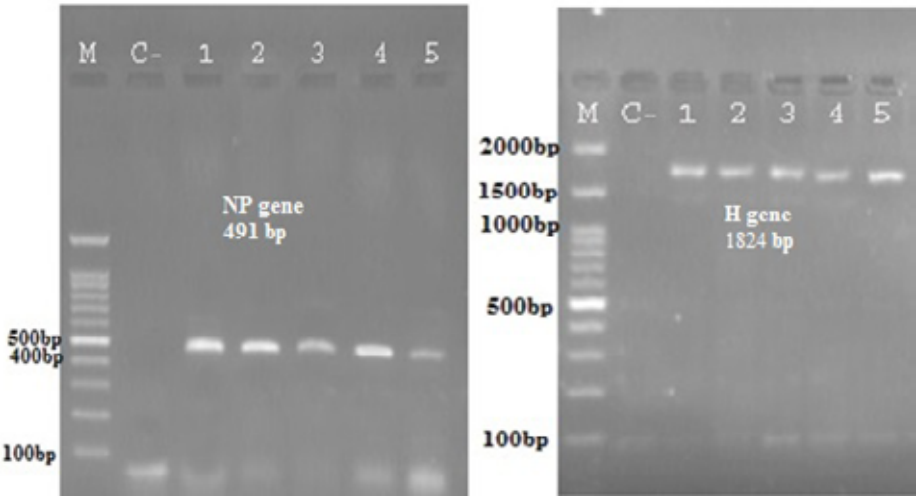


Figure 1. RT-PCR results on agarose gel (1%) electrophoresis of different samples by primer of N and H genes

Note: Lanes M: Molecular marker (100-bp ladder); Lane C-: Negative control; Lanes 1 to 6: Suspected sample; Lane C+: Positive control.

investigate the difference between the molecular prevalence of CDV between non-neurologic CDV and neurologic CDV, as well as between nervous CDV and neurologic non-CDV groups. We also assessed the effects of gender and sex on the molecular prevalence in different groups using the Spearman test. A P<0.05 was considered to be statistically significant.

Results

RT-PCR

The population of dogs was divided into three different groups: Non-neurologic CDV (group 1), neurologic CDV (group 2), and neurologic non-CDV (group 3), based on the results of the CDV antigen rapid test and clinical signs (Table 2). Overall, out of 40 dogs, 29(72.5%) carried the distemper virus-specific gene (Figure 1). Among different groups, the first group had the most positive samples (85.0%), followed by group 2(80.0%), and group 3(40.0%). In group 1 and group 2, respiratory secretions had significantly higher virus infection among the positive samples, with frequencies of 75% (P=0.021) and 70% (P=0.033), respectively. In contrast, in group 3, the CSF sample had a significantly higher frequency (40.0%) among positive samples (P=0.041) (Table 2). In group 1, the infection was more in the age of 3-6 months (P=0.031), but in group 2 (P=0.022) and group 3 (P=0.036), ages over 12 months had the highest rate of distemper infection (Table 3).

Regarding the comparison of groups 1 and 2, the frequency of CDV was not significantly different (P>0.05) (Table 2). Among the positive samples, the frequency of CSF-positive samples in group 2(40.0%) was significantly higher than that in group 1(20.0%) (P=0.038). There was no significant difference in other types of samples (P>0.05) (Table 2). The frequency of CDV-positive samples among genders in groups 1 and 2 was comparable (Table 2), without significant differences between the two groups (P>0.05). Age-wise prevalence study between the two groups revealed that group 1, aged 3-6 months (P=0.031), and group 2, aged over 12 months (P=0.018), had the highest rate of distemper infection (Table 3).

In comparison between groups 2 and 3, the number of positive cases in group 2(80.0%) was higher than that in group 3(40.0%) (Table 2). Among positive samples, the frequency rates of positive fecal (P=0.031) and respiratory secretion samples (P=0.034) were significantly lower in group 3 than in group 2. However, the frequency of CSF-positive samples was not significantly different (P>0.05). The frequency of CDV-positive samples

Table 1. Primers used to amplify the NP and *H* genes

Gene	Primers	Sequence (5' to 3')	Amplicon Size (bp)
H	H-F H-R	F-TGGTTCACAAGATGGTATTC R-CAACACCACTAAATTGGACT	613
NP	N-F N-R	F-GTAGACGAAGGGTCGAAAG R-GAATCGCCTCAAAGATAGG	297

Table 2. Frequency of distemper virus among different study groups based on PCR test

Groups	No. (%)			
	Dogs Carrying the Virus	PCR⁺		
		Feces¥	Respiratory Secretions¥	CSF$
Non-neurologic CDV (n=20)	17(85.0)	9(45.0)	15(75.0)*	4(20.0)
Neurologic CDV (n=10)	8(80.0)	6(60.0)	7(70.0)*	4(40.0)
Neurologic non-CDV (n=10)	4(40.0)	0(0.0)	2(20.0)	4(40.0)*
Total (n=40)	29(72.5)	15(51.7)	24(82.9)	12(41.4)

*Significantly different within the group (P<0.05), $Significantly different between non-neurologic CDV and neurologic CDV groups (P<0.05), ¥Significantly different between neurologic CDV and neurologic non-CDV groups (P<0.05).

Table 3. Frequency of CDV positive animals based on PCR test among different genders and age classes

Dogs Carrying the Virus	PCR⁺ (n=29)					
	No. (%)					
	Gender		Age Class (mo)			
	Male	Female	1-3	3-6$	6-12	12≤$
Non- neurologic CDV (n=17)	9(53.0)	8(47.0)	3(17.6)	10(58.8)*	3(17.6)	1(5.9)
Neurologic CDV (n=8)	4(50.0)	4(50.0)	1(12.5)	2(25)	0(0.0)	5(62.5)*
Neurologic non-CDV (n=4)	2(50.0)	2(50.0)	0(0.0)	0(0.0)	1(25.0)	3(75.0)*
Total (%)	15(52.8)	14(48.2)	4(13.8)	12(41.4)	4(13.8)	9(31.0)

*Significantly different within the group (P<0.05), $Significantly different between non- neurologic CDV and neurologic CDV groups (P<0.05), ¥Significantly different between neurologic CDV and neurologic non-CDV groups (P<0.05).

among genders between groups 1 and 2 was similar (Table 2), and no significant difference was observed between the groups (P>0.05). Also, there was no significant difference in the prevalence of the virus between different age groups (P>0.05).

Sequencing and phylogenetic analysis

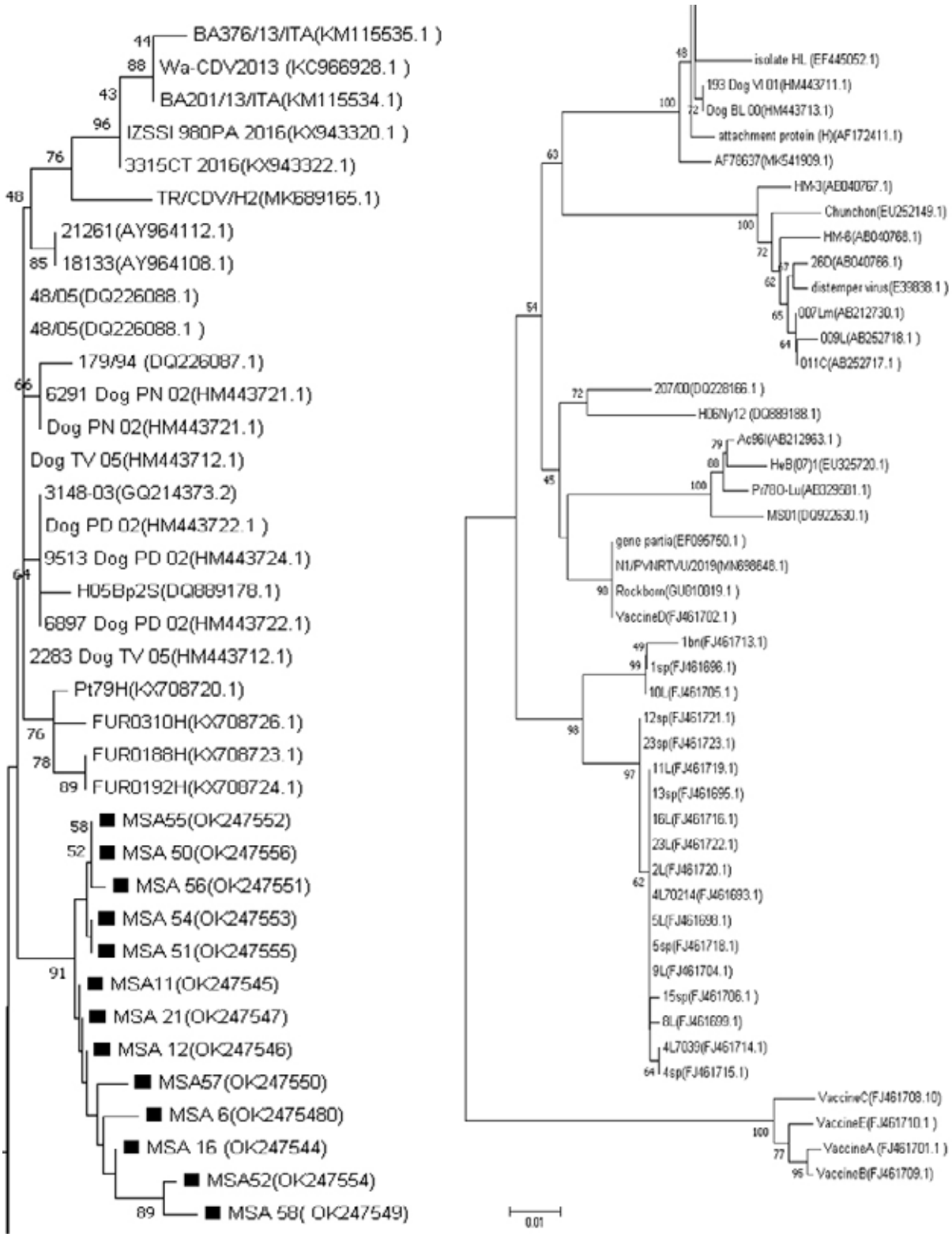
The *H* gene of 13 samples was amplified (Figure 1) and sequenced to perform the phylogenetic analysis. Sequences were analyzed using the BLAST search program and partial *H* gene sequences were submitted to GenBank (accession numbers: Ok247544, Ok247545, Ok247546, Ok247547, Ok247548, Ok247549, Ok247550, Ok247551, Ok247552, Ok247553, Ok247554, Ok247555, Ok247556). Phylogenetic analysis showed that all strains of CDV isolated belonged to the Arctic virus-like genetic lineage. Sequence analysis detected 92.1% similarity between Iranian H sequences gene, and all of them were located in the same cluster (Figure 2). Disregarding Iranian isolates, a high similarity was observed between our samples and those from Russia (FURO310, FURO188, FURO192, and Pt79H). Also, all 4 vaccines (A, B, C, and E) were very different from recent isolates regarding the *H* gene sequence and were placed in a separate cluster.

Discussion

Distemper is a deadly and contagious disease in dogs and other species, including raccoons, ferrets, otters, pandas, and big cats (Martinez-Gutierrez & Ruiz-Saenz, 2016). Dogs are the largest carnivores that can contract distemper (Martinez-Gutierrez & Ruiz-Saenz, 2016). Moreover, they are the main reservoir of the distemper virus (Costa et al., 2019; Martinez-Gutierrez & Ruiz-Saenz, 2016). This virus has different genotypes that can cause various clinical symptoms in dogs. However, the relationship between virus genotypes causing different clinical signs in dogs has not been studied (Chen et al., 2018). For this purpose, the present study compared the prevalence of the virus in different samples in non-neurologic and neurologic CDV forms. Also, the effect of the type of virus in causing the disease form has been investigated using genotyping.

Within group analyses

Examining the CDV presence in the samples of the groups showed that in both neurologic CDV and non-neurologic CDV groups, the presence of distemper-specific genes in respiratory secretions was much higher than in stool and CSF samples, probably due to the CDV tissue affinity to the respiratory system (Nicholls et al., 2007; Pratakpiriya et al., 2017). In this case, Pratakpiriya et al. reported that CDV propagates in the respiratory tract epithelium, using nectin-4 (also known as poliovirus-like receptor protein-4 (PVRL4) as a receptor. Furthermore, the PCR test showed that the CSF samples in the dogs of the neurologic non-CDV group were similar to the samples of the neurologic CDV group regarding the presence of the virus in the CSF samples (both 40%). In the neurologic non-CDV group, the CSF samples had the highest level of virus contamination related to neurological symptoms. Hence, the neurologic forms of the CSF sample are suitable for virus detection. To the best of our knowledge, no studies have been yet performed on the relationship between neural form and the presence of


BA376/13/ITA(KM115535.1)
Wa-CDV2013 (KC966928.1)
BA201/13/ITA(KM115534.1)
IZSSI 980PA 2016(KX943320.1)
3315CT 2016(KX943322.1)
TR/CDV/H2(MK589165.1)
21261(AY964112.1)
18133(AY964108.1)
48/05(DQ226088.1)
48/05(DQ226088.1)
179/94 (DQ226087.1)
6291 Dog PN 02(HM443721.1)
Dog PN 02(HM443721.1)
Dog TV 05(HM443712.1)
3148-03(GQ214373.2)
Dog PD 02(HM443722.1)
9513 Dog PD 02(HM443724.1)
H05Bp2S(DQ889178.1)
6897 Dog PD 02(HM443722.1)
2283 Dog TV 05(HM443712.1)
Pt79H(KX708720.1)
FUR0310H(KX708726.1)
FUR0188H(KX708723.1)
FUR0192H(KX708724.1)
MSA55(OK247552)
MSA 50(OK247556)
MSA 56(OK247551)
MSA 54(OK247553)
MSA 51(OK247555)
MSA11(OK247545)
MSA 21(OK247547)
MSA 12(OK247546)
MSA57(OK247550)
MSA 6(OK2475480)
MSA 16 (OK247544)
MSA52(OK247554)
MSA 58(OK247549)
isolate HL (EF445052.1)
193 Dog VI 01(HM443711.1)
Dog BL 00(HM443713.1)
attachment protein (H)(AF172411.1)
AF78637(MK541909.1)
HM-3(AB040767.1)
Chunchon(EU252149.1)
HM-6(AB040768.1)
26D(AB040766.1)
distemper virus(E39838.1)
007Lm(AB212730.1)
009L(AB252718.1)
011C(AB252717.1)
207/00(DQ228166.1)
H06Ny12 (DQ889188.1)
Ac96I(AB212963.1)
HeB(07)1(EU325720.1)
Pt78O-Lu(AB029681.1)
MS01(DQ922630.1)
gene partia(EF095750.1)
NI/PVNRTVU/2019(MN698648.1)
Rockborn(GU810819.1)
VaccineD(FJ461702.1)
1bn(FJ461713.1)
1sp(FJ461696.1)
10L(FJ461705.1)
12sp(FJ461721.1)
23sp(FJ461723.1)
11L(FJ461719.1)
13sp(FJ461695.1)
16L(FJ461716.1)
23L(FJ461722.1)
2L(FJ461720.1)
4L70214(FJ461693.1)
5L(FJ461698.1)
5sp(FJ461718.1)
9L(FJ461704.1)
15sp(FJ461706.1)
8L(FJ461699.1)
4L7039(FJ461714.1)
4sp(FJ461715.1)
VaccineC(FJ461708.10)
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VaccineA (FJ461701.1)
VaccineB(FJ461709.1)
0.01

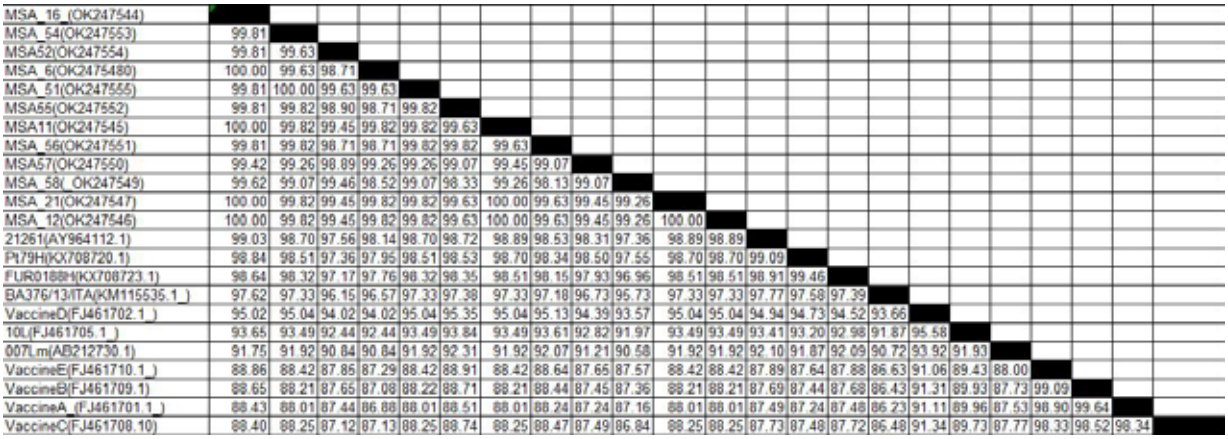

Figure 2. Phylogenetic relationship of the 13 CDV isolates from the study (solid squares) to other reported viruses based on the full-length *H* gene

Note: The phylogenetic tree was constructed using the maximum-likelihood method in MEGA6.

the virus in specific tissues, and this issue is raised for the first time. In this context Martella et al. (2008) reported that urine, tonsils, conjunctival swabs, and whole blood contained high viral loads in the acute form of distemper.

Examining the presence of the virus in different age groups of dogs showed that in the non-neurologic CDV group, those aged 3 to 6 months are at risk of distemper (P=0.031). Studies have shown that an immature immune system in puppies may contribute to their high infection rate, especially between 3 and 6 months of age when maternal antibodies in puppies decline (Baumgärtner et al., 1995). However, Kauffman et al. (1982) believed that 3-6 months old dogs are more susceptible to distemper than other age dogs due to the reduced replacement and repair ability of lymphocytes. In this regard, the researchers suggest that the infection of young animals may be related to the virus-specific receptors in the immune system. In other words, the distemper virus receptors, such as signaling lymphocyte activation molecule (SLAM) in young dogs are far more than those in old dogs, leading to their high infection (Tatsuo et al., 2001; Yilmaz et al., 2022). In agreement with our study, Jóźwik & Frymus (2002) showed that 72% of dogs tested for distemper virus were younger than 1 year. We found that the neurological form was more pronounced at older ages, which is consistent with the findings of previous studies (Hall et al., 1979; Headley et al., 2009). Studies have shown that young dogs are more susceptible to the distemper virus, and after the apparent recovery, the virus remains in a series of organs such as the iris, central nervous system, and plantar fascia, and old age when the immune system is weakened, nervous involvement appears (Galán et al., 2014; Headley et al., 2009).

Between groups analyses

As depicted in Table 1, the frequency rates of CDV detection in non-neurologic CDV and neurologic CDV were significantly higher than those in the neurologic non-CDV group. This finding points to the importance of the rapid distemper test method. It shows that the probability of the molecular distemper test being negative is high if the results of other tests are negative. The prevalence of distemper virus based on PCR test in the CSF sample was higher in the neurologic CDV and neurologic non-CDV groups than in the non-neurologic CDV group. This finding shows that neurological symptoms had a significant relationship with the presence of the virus in the CSF samples.

Accordingly, the CSF is a suitable sample for diagnosing CDV in neurological form. In this context, Amude et al. (2007) also reported that assessing cerebrospinal fluid can be more appropriate in diagnosing distemper disease in its neurological form. Interestingly, we found a significantly higher CDV detection in the respiratory secretions of the neurologic CDV group than in the neurologic non-CDV group. This finding indicates that respiratory secretions are highly reliable when even rapid distemper test results are negative. Notably, inhibitors sometimes reduce the sensitivity of the quick test or even lead to false negative effects (Wilkes et al., 2014). In the study of Yilmaz et al. (2022), the contamination of different dog samples was investigated using the RT-PCR method, and viral nucleic acid was detected at higher rates in the nasal swabs compared to the other samples. In their study, the samples were taken from dogs under 1 year old, and CDV was detected mainly in the respiratory

secretion samples, consistent with our results. Sarchahi et al. (2022) also reported that whole blood and mucosal swabs are unsuitable samples for diagnosing CDV by RT-PCR in dogs with neurological symptoms, while CSF is much more suitable. There was no significant difference in CDV prevalence between genders, which agrees with previous studies (Cattet et al., 2004).

Phylogenetic study

Virus typing is essential in many aspects, such as identifying different genotypes that lead to neurologic or non-neurologic forms, detecting the virus location and its dominant type in the region, and showing the origin of the expanded genes to some extent in the microbial population (Namroodi et al., 2017). The sequencing-based method is one of the molecular methods used to classify microorganisms. In the SLST method, a target gene is usually sequenced among the isolates, and the isolates are classified based on the nucleotide sequence (Ahmed & Alp, 2015; Zhang et al., 2021). In our study, epidemiological investigations on isolates were performed based on the *H* gene and its sequence analysis. The dendrogram mapping showed no difference between viruses isolated from the non-neurologic and the neurologic forms; all belonged to the same cluster. Phylogenetic analysis of 14 distemper viruses showed that all isolates were embedded in the Arctic cluster. Since the closest strains recorded in the NCBI Database were previously reported from Russia, the latter strains likely originated in Russia. Similarly, a study conducted by Namroodi et al. (2015) in Iran showed that distemper virus isolates are located in the Arctic and European lineages and were probably transferred from Turkey to Iran.

One of the objectives of genetic studies is to evaluate the changes in the strains of this virus in the region compared to the vaccines used to compare the overlap of the vaccine and the dominant strain in the region because, in the case of high changes in the strains, new vaccines with high similarity and overlap should be produced and used. In the present study, the compatibility and similarity between the 4 vaccines (A, B, C, and E) was 20% with the recent strains. In a case study by Mochizuki et al. (1999) in Japan, hemagglutinin (*H*) genes obtained from current vaccines and clinical isolates of the distemper virus were genetically analyzed, and the results revealed that two genotypes of distemper virus are circulating among dogs in Japan, which are highly genetically different from vaccine strains. These findings will lead to the developing new high-performance vaccines to include all new genotypes. In contrast, in a study by Zhao et al. (2010), three genotypes of distemper virus were detected in China, of which the Asia-1 genotype was the most common, and all three genotypes were 90% similar to the vaccines used in that country. Our results suggest that the low similarity between wild and vaccine isolates might affect the efficacy of the applied vaccines. In other words, the probable reason for the high number of positive cases of the distemper virus in Iran might be due to the vast genotypic difference between the recent strains and the mentioned vaccines. Therefore, studies with larger sample sizes covering more geographical regions are suggested.

Conclusion

This study showed that molecular techniques could detect CDV in neurologic and non-neurologic forms of distemper. However, respiratory secretions in both neurologic and non-neurologic forms are crucial for the diagnosis of the disease. In neurologic cases with negative rapid test results, PCR of CSF samples had the highest sensitivity; therefore, it could be a diagnostic solution for suspected cases. Genotypic analysis of the hemagglutinin gene of the distemper virus showed that the recent isolates are very similar in both neurological and non-neurological clinical forms of CDV in Iran. Since all 13 isolates are located in polar clusters and are very similar to the strains obtained in Russia, there is a possibility of its transfer from Russia to Iran.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

Conceptualization and supervision: Shahram Jamshidi and Arash ghalyanchi Langeroudi; Methodology: Shahram Jamshidi and Hamidreza Moosavian; Investigation: Seyed Mohammad Mojtahedzadeh, Iradj Ashrafi Seyed Milad Vahedi; Data collection: Seyed Mohammad Mojtahedzadeh; Data analysis: Hesameddin Akbarein; Writing: Seyed Mohammad Mojtahedzadeh.

Conflict of interest

The authors declared no conflict of interest.

References

Ahmed, S. S., & Alp, E. (2015). Genotyping methods for monitoring the epidemic evolution of A. baumannii strains. *Journal of Infection in Developing Countries, 9*(4), 347–354. [DOI:10.3855/jidc.6201] [PMID]

Amude, A. M., Alfieri, A. A., & Alfieri, A. F. (2007). Clinicopathological findings in dogs with distemper encephalomyelitis presented without characteristic signs of the disease. *Research in Veterinary Science, 82*(3), 416–422. [DOI:10.1016/j.rvsc.2006.08.008] [PMID]

Avizeh, R., Shapouri, M. R., & Akhlaghi, N. (2007). Antibody titers against canine distemper virus in unvaccinated rural dogs from Ahvaz, Iran. Pakistan Journal of Biological Sciences: PJBS, 10(21), 3970–3972. [DOI:10.3923/pjbs.2007.3970.3972] [PMID]

Baumgärtner, W., Boyce, R. W., Alldinger, S., Axthelm, M. K., Weisbrode, S. E., Krakowka, S., & Gaedke, K. (1995). Metaphyseal bone lesions in young dogs with systemic canine distemper virus infection. *Veterinary Microbiology, 44*(2-4), 201–209. [DOI:10.1016/0378-1135(95)00013-Z] [PMID]

Bi, Z., Xia, X., Wang, Y., & Mei, Y. (2015). Development and characterization of neutralizing monoclonal antibodies against canine distemper virus hemagglutinin protein. *Microbiology and Immunology, 59*(4), 202–208. [DOI:10.1111/1348-0421.12238] [PMID]

Cattet, M. R., Duignan, P. J., House, C. A., & Aubin, D. J. (2004). Antibodies to canine distemper and phocine distemper viruses in polar bears from the Canadian arctic. *Journal of Wildlife Diseases, 40*(2), 338–342. [DOI:10.7589/0090-3558-40.2.338] [PMID]

Chen, M., Xin, T., Hou, S., Lin, W., Song, W., & Zhu, H., et al. (2018). Genotyping and pathogenic characterization of canine distemper virus based on mutations in the hemagglutinin gene in Chinese domestic dogs. Polish Journal of Veterinary Sciences, 21(3), 623–629. [DOI:10.24425/124301]

Costa, V. G. D., Saivish, M. V., Rodrigues, R. L., Lima Silva, R. F., Moreli, M. L., & Krüger, R. H. (2019). Molecular and serological surveys of canine distemper virus: A meta-analysis of cross-sectional studies. *PloS One, 14*(5), e0217594. [DOI:10.1371/journal.pone.0217594] [PMID] [PMCID]

da Fontoura Budaszewski, R., & von Messling, V. (2016). Morbillivirus experimental animal models: Measles virus pathogenesis insights from canine distemper virus. *Viruses, 8*(10), 274. [DOI:10.3390/v8100274] [PMID] [PMCID]

Duque-Valencia, J., Sarute, N., Olarte-Castillo, X. A., & Ruíz-Sáenz, J. (2019). Evolution and interspecies transmission of canine distemper virus: An Outlook of the Diverse Evolutionary Landscapes of a Multi-Host Virus. *Viruses, 11*(7), 582. [DOI:10.3390/v11070582] [PMID] [PMCID]

Galán, A., Gamito, A., Carletti, B. E., Guisado, A., de las Mulas, J. M., & Pérez, J., et al. (2014). Uncommon acute neurologic presentation of canine distemper in 4 adult dogs. *The Canadian Veterinary Journal=La revue ve□te□rinaire canadienne, 55*(4), 373–378. [PMID] [PMCID]

Hall, W. W., Imagawa, D. T., & Choppin, P. W. (1979). Immunological evidence for the synthesis of all canine distemper virus polypeptides in chronic neurological diseases in dogs. Chronic distemper and old dog encephalitis differ from SSPE in man. *Virology, 98*(1), 283–287. [DOI:10.1016/0042-6822(79)90550-6] [PMID]

Headley, S. A., Amude, A. M., Alfieri, A. F., Bracarense, A. P., Alfieri, A. A., & Summers, B. A. (2009). Molecular detection of Canine distemper virus and the immunohistochemical characterization of the neurologic lesions in naturally occurring old dog encephalitis. *Journal of Veterinary Diagnostic Investigation: Official Publication of the American Association of Veterinary Laboratory Diagnosticians, Inc, 21*(5), 588–597. [DOI:10.1177/104063870902100502] [PMID]

Hornsey, S. J., Philibert, H., Godson, D. L., & Snead, E. C. R. (2019). Canine adenovirus type 1 causing neurological signs in a 5-week-old puppy. *BMC Veterinary Research, 15*(1), 418. [DOI:10.1186/s12917-019-2173-5] [PMID] [PMCID]

Józwik, A., & Frymus, T. (2002). Natural distemper in vaccinated and unvaccinated dogs in Warsaw. *Journal of Veterinary Medicine. B, Infectious Diseases and Veterinary Public Health, 49*(9), 413–414. [DOI:10.1046/j.1439-0450.2002.00549.x] [PMID]

Kauffman, C. A., Bergman, A. G., & O'Connor, R. P. (1982). Distemper virus infection in ferrets: An animal model of measles-induced immunosuppression. *Clinical and Experimental Immunology, 47*(3), 617–625. [PMID] [PMCID]

Loots, A. K., Mitchell, E., Dalton, D. L., Kotzé, A., & Venter, E. H. (2017). Advances in canine distemper virus pathogenesis research: A wildlife perspective. *The Journal of General Virology, 98*(3), 311–321. [DOI:10.1099/jgv.0.000666] [PMID]

Martella, V., Elia, G., & Buonavoglia, C. (2008). Canine distemper virus. The Veterinary clinics of North America. *Small animal Practice, 38*(4), 787-797, vii-viii. [DOI:10.1016/j.cvsm.2008.02.007] [PMID]

Martella, V., Elia, G., Lucente, M. S., Decaro, N., Lorusso, E., & Banyai, K., et al. (2007). Genotyping canine distemper virus (CDV) by a hemi-nested multiplex PCR provides a rapid approach for investigation of CDV outbreaks. *Veterinary Microbiology, 122*(1-2), 32–42. [DOI:10.1016/j.vetmic.2007.01.005] [PMID]

Martinez-Gutierrez, M., & Ruiz-Saenz, J. (2016). Diversity of susceptible hosts in canine distemper virus infection: A systematic review and data synthesis. *BMC Veterinary Research, 12*, 78. [DOI:10.1186/s12917-016-0702-z] [PMID] [PMCID]

Mochizuki, M., Hashimoto, M., Hagiwara, S., Yoshida, Y., & Ishiguro, S. (1999). Genotypes of canine distemper virus determined by analysis of the hemagglutinin genes of recent isolates from dogs in Japan. *Journal of Clinical Microbiology, 37*(9), 2936–2942. [DOI:10.1128/JCM.37.9.2936-2942.1999] [PMID] [PMCID]

Namroodi, S., Rostami, A., Majidzadeh-Ardebili, K., Ghalyanchi-Langroudi, A., & Morovvati, A. (2013). Molecular and serological detection of canine distemper virus (CDV) in rural dogs, Iran. *Iranian Journal of Virology, 7*(1 and 2), 37-43. [DOI:10.21859/isv.7.1.2.37]

Namroodi, S., Rostami, A., Majidzadeh Ardebili, K., & Ghalyanchi Langroudi, A. (2014). Aphylogenetic study on the NP gene of detected canine distemper virus in (2008-2011) Iran. *Iranian Journal of Veterinary Medicine, 8*(3), 163-168. [DOI:10.22059/ijvm.2014.51887]

Namroodi, S., Rostami, A., Majidzadeh-Ardebili, K., Ghalyanchi Langroudi, A., & Morovvati, A. (2015). Detection of Arctic and European cluster of canine distemper virus in north and center of Iran. *Veterinary Research Forum: An International Quarterly Journal, 6*(3), 199–204. [PMID] [PMCID]

Namroodi, S., Milanlou, D., & Rezaei, H. (2017). Heavy metal bioaccumulation and its potential relation with incidence of canine parvovirus infection in Golden Jackals, North Iran. *Iranian Journal of Veterinary Medicine, 11*(2), 113-125. [DOI:10.22059/ijvm.2017.62079]

Nikbakhat, G., Shahram, S., & Mohyedidini, S. (2018). Detection of a new canine parvovirus mutant in Iran. *Iranian Journal of Veterinary Medicine, 12*(1), 1-7. [DOI:10.22059/ijvm.2018.231479.1004805]

Nicholls, J. M., Bourne, A. J., Chen, H., Guan, Y., & Peiris, J. S. (2007). Sialic acid receptor detection in the human respiratory tract: Evidence for widespread distribution of potential binding sites for human and avian influenza viruses. *Respiratory Research, 8*(1), 73. [DOI:10.1186/1465-9921-8-73] [PMID] [PMCID]

Pratakpiriya, W., Ping Teh, A. P., Radtanakatikanon, A., Pirarat, N., Thi Lan, N., & Takeda, M., et al. (2017). Expression of canine distemper virus receptor nectin-4 in the central nervous system of dogs. *Scientific Reports, 7*(1), 349. [DOI:10.1038/s41598-017-00375-6] [PMID] [PMCID]

Pouramini, A., Jamshidi, S., Shayan, P., Ebrahimzadeh, E., Namavari, M., & Shirian, S. (2017). Molecular and serological detection of neospora caninum in multiple tissues and CSF in asymptomatic infected stray dogs. *Iranian Journal of Veterinary Medicine, 11*(2), 105-112. [DOI:10.22059/ijvm.2017.61602]

Rendon-Marin, S., da Fontoura Budaszewski, R., Canal, C. W., & Ruiz-Saenz, J. (2019). Tropism and molecular pathogenesis of canine distemper virus. *Virology Journal, 16*(1), 30. [DOI:10.1186/s12985-019-1136-6] [PMID] [PMCID]

Ricci, I., Cersini, A., Manna, G., Marcario, G. A., Conti, R., & Brocherel, G., et al. (2021). A canine distemper virus retrospective study conducted from 2011 to 2019 in Central Italy (Latium and Tuscany Regions). *Viruses, 13*(2), 272. [DOI:10.3390/v13020272] [PMID] [PMCID]

Saaed, M. M., & Alsarhan, Q. T. (2022). Detection of canine distemper virus in stray and pet dogs in Mosul City, Iraq. *Iraqi Journal of Veterinary Sciences, 36*(2), 315-319. [DOI:10.33899/ijvs.2021.130127.1739]

Sarchahi, A. A., & Arbabi, M. (2022). Status epilepticus caused by canine distemper virus in a striped hyena (Hyaena hyaena). *Veterinary Record Case Reports, 10*(2), e353. [DOI:10.1002/vrc2.353]

Sohpal, V. K., Dey, A., & Singh, A. (2010). MEGA biocentric software for sequence and phylogenetic analysis: A review. *International Journal of Bioinformatics Research and Applications, 6*(3), 230–240. [DOI:10.1504/IJBRA.2010.034072] [PMID]

Suzuki, H., & Ferrario, C. M. (1984). New method for the collection of cerebrospinal fluid from the cisterna magna of conscious dogs. *The American Journal of Physiology, 246*(4 Pt 2), H551–H558. [DOI:10.1152/ajpheart.1984.246.4.H551] [PMID]

Tatsuo, H., Ono, N., & Yanagi, Y. (2001). Morbilliviruses use signaling lymphocyte activation molecules (CD150) as cellular receptors. *Journal of Virology, 75*(13), 5842–5850.[DOI:10.1128/JVI.75.13.5842-5850.2001] [PMID] [PMCID]

Tavakoli Zaniani, A., Mokhtari, A., & Esmailnejad, A. (2021). Molecular and immunological investigation of Canine Distemper Virus (CDV) and its co- infection with canine parainfluenza virus type 2. *Iranian Journal of Medical Microbiology, 15*(2), 212-226. [DOI:10.30699/ijmm.15.2.212]

Wang, X., Xu, W., Fan, K., Chiu, H. C., & Huang, C. (2020). Codon usage bias in the H gene of canine distemper virus. *Microbial Pathogenesis, 149*, 104511. [DOI:10.1016/j.micpath.2020.104511] [PMID]

Wilkes, R. P., Tsai, Y. L., Lee, P. Y., Lee, F. C., Chang, H. F., & Wang, H. T. (2014). Rapid and sensitive detection of canine distemper virus by one-tube reverse transcription-insulated isothermal polymerase chain reaction. *BMC Veterinary Research, 10*, 213. [DOI:10.1186/s12917-014-0213-8] [PMID] [PMCID]

Yilmaz, V., Coşkun, N., Timurkan, M. Ö., Karakurt, E., Nuhoğlu, H., & Erkılıc, E. E., et al. (2022). The Investigation of canine distemper virus in different diagnosis materials of dogs using molecular and pathological methods, Northeastern Turkey. *Indian Journal of Animal Research, 56*(3), 316-322. [DOI:10.18805/IJAR.B-1389]

Zhang, H., Meng, P., Song, X., Li, S., Yang, R., & Zhang, C., et al. (2021). Isolation and phylogenetic analysis of the canine distemper virus from a naturally infected dog in China. *Indian Journal of Animal Research. 55*(6), 629-635. [DOI:10.18805/IJAR.B-1298]

Zhao, J., & Ren, Y. (2022). Multiple receptors involved in invasion and neuropathogenicity of canine distemper virus: A Review. *Viruses, 14*(7), 1520. [DOI:10.3390/v14071520] [PMID] [PMCID]

مقاله پژوهشی

شناسایی مولکولی ویروس دیستمپر (CDV) در سگ‌های مبتلابه فرم‌های بالینی عصبی و غیرعصبی

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چکیده

زمینه مطالعه: دیستمپر سگ‌سانان یکی از واگیردارترین و کشنده‌ترین بیماری‌های ویروسی در سگ‌ها است. در سال‌های اخیر علی‌رغم واکسیناسیون گسترده برای کنترل دیستمپر سگ‌سانان، شیوع ویروس دیستمپر سگ‌سانان به نرخ هشداردهنده‌ای رسیده است.

هدف: این مطالعه باهدف شناسایی ژنوتیپ‌های مسئول فرم‌های بالینی عصبی و غیرعصبی دیستمر سگ‌ها و بررسی فراوانی حضور ویروس در فرم‌های بالینی عصبی و غیرعصبی بیماری انجام شد.

روش کار: در این مطالعه توصیفی‌تحقیقی از ۷۰ قلاده سگ واکسینه‌نشده مشکوک به ویروس دیستمپر با علائم بالینی این بیماری، نمونه‌برداری انجام شد. ابتدا همه موارد با کیت‌های تشخیصی سریع مورد آزمایش قرار گرفتند. سپس براساس علائم بالینی به ۳ گروه مختلف تقسیم شدند. نمونه‌های مایع مغزی نخاعی، ترشحات تنفسی و مدفوع هر ۴۰ سگ برای واکنش زنجیره‌ای پلیمراز-رونویسی معکوس (RT-PCR) مورد بررسی قرار گرفتند. پس از تعیین توالی ژن هماگلوتینین (ژن H)، تحلیل فیلوژنیکی ژن H جدایه‌های استخراج‌شده با استفاده از نرم‌افزار MEGA™ 7 انجام شد.

نتایج: تجزیه‌وتحلیل نتایج نشان داد ترشحات تنفسی در گروه دیستمپر-غیرعصبی (۸۵ درصد) و دیستمپر-عصبی (۸۰ درصد) دارای بیشترین نمونه مثبت برای تست RT-PCR گزارش شد، اما در گروه عصبی غیردیستمپر، نمونه مایع مغزی نخاعی بالاترین (۴۰ درصد) بود. در گروه‌های عصبی، سنین بالای ۱۲ ماه بیشترین درصد آلودگی دیستمپر را نشان دادند و در گروه دیستمپر-غیرعصبی، سنین ۳ تا ۶ ماه بیشتر درگیر بودند. توالی‌یابی و تجزیه‌وتحلیل فیلوژنیکی ژن H نشان داد که تمامی نمونه‌های موردبررسی متعلق به دودمان قطبی بودند.

نتیجه‌گیری نهایی: بررسی ژنوتیپی ژن هماگلوتینین ویروس دیستمپر نشان داد که جدایه‌های اخیر در هر دو شکل بالینی عصبی و غیرعصبی دیستمپر در ایران، مشابهت بسیار بالایی دارند. در سگ‌هایی که نتیجه تست سریع مثبت داشتند، تست PCR ترشحات تنفسی برای تشخیص ویروس، حساس‌ترین نمونه است. در موارد عصبی که نتایج تست سریع منفی داشتند، PCR مایع مغزی نخاعی بالاترین حساسیت را داشته است، بنابراین می‌تواند یک راه حل تشخیصی باشد.

کلیدواژه‌ها: دیستمپر، سگ، هماگلوتینین، مایع مغزی نخاعی، عصبی، غیرعصبی

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