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

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Running title

Molecular Detection of Canine Distemper Virus among Dogs

Abstract

Background: Canine distemper (CD) is one of the most contagious and lethal viral diseases in dogs. 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. **Objective**: To identify the genotypes responsible for the neurological and non-neurological clinical forms of canine distemper and to investigate the presence of the virus in the neurological and non-neurological forms of the disease**. Materials and methods**: In this descriptive-analytical study, samples were collected from 70 CD suspected unvaccinated dogs with clinical symptoms of distemper. All cases were first tested with rapid tests and then separated into 3

different groups based on clinical symptoms. CSF, respiratory secretion, and fecal samples of all 40 cases were examined for reverse transcription polymerase chain reaction (RT-PCR). After sequencing the hemagglutinin gene (H gene), phylogenetic analysis of the H gene of isolated CDVs was carried out using MEGA™ 7 software. **Results**: The analysis of 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, but in the non-neurological CDV group, the CSF sample (40%) had a high level of infection. In neurotic groups, ages over 12 months showed the highest percentage of distemper contamination, and in the Non-neurologic CDV group, ages 3 to 6 months were more involved. Sequencing and phylogenetic analysis of the H gene revealed the CDV to be a member of the endemic Arctic-like genetic lineage. **Conclusion**: The results of this study indicated that genotypic examination of the hemagglutinin gene of the distemper virus revealed that the recent isolates are closely similar in both neurologic and non-neurologic clinical forms of CDV in Iran. In positive rapid test cases, the PCR test of Respiratory secretions for detection of the virus is the most sensitive. In neurologic cases which has negative rapid test results, PCR of CSF had the highest sensitivity, so can be a diagnostic solution.

Key word: Distemper, Dog, Hemagglutinin, CSF, Neurologic, Non-Neurologic

Introduction

Distemper is a fatal contagious viral disease in dogs and other species, such as raccoons, ferrets, otters, pandas, and skunks (Namroodi, Rostami, Ardebili, & Langroudi, 2014). The canine distemper virus

(CDV) is a single-stranded RNA virus of the Morbillivirus genus and the Paramyxoviridae family. CDV is an enveloped virus that is highly susceptible to chemical disinfectants (Bi *et al*., 2015). CDV only has one serotype; however, based on genetic variability of membrane glycoproteins, 17 major genotypes have been reported, 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).

The severity of disease and clinical symptoms depends on multiple factors, including virus strain, immunity, and host age (Hornsey *et al*., 2019). In general, CDV infection disease could be classified into two major clinical forms: (a) non-neurologic CDV in which 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 and Yanrong, 2022; Hornsey *et al*., 2019); (b) 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 six genes encoding eight proteins: 2 non-structural proteins (C and V) and six structural proteins (nucleocapsid protein, matrix protein, phosphoprotein, large protein, and two membrane glycoproteins known as hemagglutinin (H) protein) (da Fontoura Budaszewski & Von Messling, 2016; Valencia *et al*., 2019). H protein is responsible for binding the virus to the host cell (Rendon-Marin *et al*., 2019). It also stimulates the host's immune system, leading to a protective response against 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 make it possible to differentiate the field CDV lineages (Rendon-Marin *et al*., 2019).

74 Practical diagnosis of canine distemper is primarily based on clinical suspicion. Also, the rapid CDV Ag
75 test kit is highly preferred by clinicians due to it is a simple, rapid, and feasible method in most
76 laboratories and can detect distemper virus in the conjunctiva, urine, serum, or plasma with a high degree
77 of accuracy. CDV antigen rapid test has shown excellent sensitivity (98%) and specificity (98.5%)
78 compared to other methods (Costa *et al*., 2019; Saaed & Alsarhan, 2022). In our study we used bionote
79 (Woodley equipment) rapid test kit with sensitivity of 100 % and specificity of 98.5% in comparison to
80 the nested PCR. However, the most tdefinitive method for diagnosing distemper is amplifying conserved
81 specific genes, such as the neuraminidase (NP) gene by PCR (Ricci *et al*., 2021). In this case, the
82 researchers reported that the conserved nucleoprotein (NP) gene is considered to be a better target for
83 amplification of specific fragments from all strains of CDV (Namroodi *et al*., 2013; Wang et al., 2020).

84 There are few epidemiological data about the CDV lineages in Iran; most studies have focused on the
85 prevalence of CDV among rural dogs in Iran (Avizeh, Shapouri, & Akhlaghi, 2007; Somayeh Namroodi
86 *et al*., 2015; Sarchahi & Arbabi, 2022; Tavakoli Zaniani *et al*., 2021). However, our understanding of the
87 molecular prevalence of CDV in different forms of the disease and the genetic variability of CDVs
88 inducing neurologic and non-neurologic forms of the disease are limited. Therefore, this study was
89 designed to investigate the difference between the molecular prevalence of CDV in dogs with non-
90 neurologic and neurologic forms of Distemper. We also performed phylogenetic analysis to evaluate the
91 genetic similarities between non-neurologic and neurologic CDV.

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Material and methods

Target population

The target population consisted of dogs referred to veterinary hospitals in Tehran from October 2020 to September 2021. A total of 70 dogs with clinical signs of CDV without vaccination history were included in this study. CDV rapid test was conducted for all animals using CDV rapid antigen kit (Woodly equipment company LTD., UK) based on the kit manufacturer's instruction. With respect to the initial clinical examination and rapid kit results, 40 animals were grouped into three classes: (Group 1) animals with positive kit results but without neurological clinical signs 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) animals with neurologic symptoms and positive kit results were classified as the "neurologic CDV" group (N = 10). Neurological signs included chewing gum, muscle tics, seizure, paralysis, circling, and blindness; (Group 3) animals with neurologic symptoms and negative kit results were classified as the "neurologic non-CDV" group (N = 10).

Sampling

Samples were collected from CSF fluid, nasal-conjunctive secretion, and stool of all animals. Samples were obtained using a sterile swab from the nasal canal and conjunctive secretions. Also, stool samples

were collected using a sterile swab. Cerebrospinal fluid (CSF) samples were collected based on the cisternal puncture method (Suzuki & Ferrario, 1984). Therefore, CSF samples were collected using a sterile syringe (gage22) from the cerebellomedullary cistern under complete anesthesia. Then, they were immediately transferred to -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). RNA was extracted from clinical samples using an RNA extraction kit (Bioneer Co, Korea) following the manufacturer's instruction and was stored at −80 °C. 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 (10mM), 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 1min, followed by denaturation at 95°C for 1 min, annealing at 47°C for 1min and elongation at 72°C for 1min (35 cycles), and a final extension at 72°C for 10 min. After amplification, 5µl 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 (http://www.ncbi.nlm.nih.gov/). Eventually, nucleotide sequences were submitted to the GenBank database (http://www.ncbi.nlm.nih.gov). H gene sequences of 13 CDV isolates obtained from this analysis along with 37 collected sequences from the Genbank were used for phylogenetic analysis using MEGA software v.11 (Sohpal, Dey, & Singh, 2010). Hemagglutinin gene sequences of four 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. Internal node uncertainty was assessed through 500 bootstrap replications. Subsequently, the aligned H gene sequences were then used to construct the phylogenetic tree based on neighbor-joining and maximum likelihood methods(Nikbakht, Jamshidi, & Mohyedini, 2018).

Statistical analysis

The statistical analyses were performed using SPSS software (version 19). A chi-square test was used to 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 Spearman's test. A *P*-value < 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 the neurologic non-CDV (Group 3), based on the results of the CDV Ag rapid test and clinical signs (Table 2). Overall, out of 40 dogs, 29 (72.5%) dogs carried the distemper virus-specific gene (Fig 1). Among different groups, group 1 had the most positive samples (85.0%), followed by group 2 (80.0%) and group 3 (40.0%). In group1 and group 2, among the positive samples, respiratory secretions had significantly higher virus infection with frequencies of 75% (*P*-value = 0.021) and 70% (*P*-value =0.033), respectively. In contrast, in group 3, CSF sample had significantly higher frequency (40.0%) among positive samples (*P*-value = 0.041) (Table 2). In group 1, the infection being more in the age of 3-6 months (*P*-value = 0.031), but in group 2 (*P*-value = 0.022) and group 3 (*P*-value = 0.036), ages over 12 months had the highest rate of distemper infection (Table 3).

In the comparison of groups 1 and 2, the frequency of CDV was not significantly different (*P*-value > 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*-value = 0.038). There was no significant difference in other types of samples (*P*-value > 0.05) (Table 2). The frequency of CDV positive samples among genders in groups 1 and 2 was comparable (Table 2) and no significant differences were observed between the CDV frequencies between the two groups (*P*-value > 0.05). Age-wise prevalence study between the two groups revealed that group 1, ages 3-6 months (*P*-value = 0.031), and group 2, ages over 12 months (*P*-value = 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 in group 3 (40.0%) (Table 2). Among positive samples, the frequency of positive fecal (*P*-value = 0.031) and respiratory secretion samples (*P*-value = 0.034) was significantly lower in group 3 than those in group 2. However, the frequency of CSF-positive samples was not significantly different (*P*-value > 0.05). The frequency of CDV-positive samples among genders between groups 1 and 2 was similar (Table 2) and no significant difference was observed between the groups (*P*-value > 0.05). Also, there was no significant difference in the prevalence of the virus between different ages (*P*-value > 0.05).

Sequencing and Phylogenetic analysis

To perform the phylogenetic analysis, the H gene of 13 samples was amplified (Fig 1) and sequenced. Sequences were analyzed using the BLAST search program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) 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 belong 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 (Fig 2). Disregarding Iranian isolates, a high similarity was observed between our samples and those from Russia (FURO310, FURO188, FURO192, and Pt79H). Also, all four vaccines A, B, C, and E were very different from recent isolates in terms of 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 group of carnivores that can contract distemper (Martinez-Gutierrez & Ruiz-Saenz, 2016). Moreover, they are the main reservoir of distemper virus (Costa *et al*., 2019; Martinez-Gutierrez & Ruiz-Saenz, 2016). Considering that this virus has different genotypes that can cause different clinical symptoms in dogs and since the relationship between virus genotypes in causing different clinical symptoms in dogs has not been studied

so far (Chen *et al*., 2018). For this purpose, in the present study it has been tried to compare the prevalence of the virus in different samples in non-neurologic and neurologic CDV forms, and also the effect of the type of virus in causing the disease form has been investigated using genotyping.

Within groups analyses

Examining the presence of the CDV 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, and this is probably due to the tissue affinity of the CDV to the respiratory system (Nicholls *et al*., 2007; Pratakpiriya *et al*., 2017). In this case, Pratakpiriya *et al* (2017) reported that CDV propagates in the respiratory tract epithelium, using nectin-4 (also known as poliovirus-like receptor protein-4 (PVRL4) as a receptor (Pratakpiriya *et al*., 2017). Also, the results of 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, and the presence of the virus in the CSF samples of both groups was 40%. Since in the neurologic non-CDV group, the CSF sample had the highest level of virus contamination, it can be inferred that the presence of the virus is related to the occurrence of neurological symptoms, and 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 the virus in specific tissues, and this issue is raised for the first time. In this case alone, the study by Gabriella *et al*. (2006) reported that urine, tonsils, conjunctival swabs, and whole blood contained high viral loads in the acute form of distemper (Martella *et al*., 2007).

Examining the presence of the virus in different ages of dogs showed that in the non- neurologic CDV group, ages 3 to 6 months are at risk of distemper (*P*-value = 0.031). Studies have shown that an immature immune system in puppies is a possible reason for their high rate of infection, especially between 3 and 6 months of age when maternal antibodies in puppies declines (Baumgärtner *et al*., 1995). On the other hand, Kauffman *et al* (1982) reported that 3-6-month-old dogs are more susceptible to distemper than other age dogs due to the reduced replacement and repair ability of lymphocytes (Kauffman *et al*., 1982). In this regard, the researchers showed that especially 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 were far more than in old dogs, and this led to their high infection (Tatsuo, Ono, & Yanagi, 2001; Volkan Yilmaz *et al*., 2022). In agreement with our study, Jóżwik & Frymus (2002) showed that 72% of dogs tested for distemper virus were younger than 1 year of age (Jóżwik & Frymus, 2002). We found that the neurological form was more pronounced at older ages, which is consistent with the findings of previous studies (Hall, Imagawa, & Choppin, 1979; Headley *et al*., 2009). Studies have shown that young dogs are more susceptible to distemper virus and after the apparent recovery, the agent remains in a series of organs such as the iris, central nervous system, and plantar fascia, and in 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 of the detection of CDV in Non-neurologic CDV and neurologic CDV were significantly higher than in the neurologic non-CDV group. This finding points to the

importance of the rapid distemper test method and 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, and on the other hand, the CSF sample is a suitable target for the diagnosis of CDV in neurological form. In this case, Amude *et al* (2007) also reported that the assessment of cerebrospinal fluid can be more appropriate in the diagnosis of distemper disease in its neurological form (Amude, Alfieri, & Alfieri, 2007). Interestingly, we found a significantly higher CDV detection in the respiratory secretions of the neurologic CDV group compared to the neurologic non-CDV group. This finding indicates that respiratory secretions are highly reliable when even rapid distemper test results are negative. It is noteworthy that inhibitors sometimes reduce the sensitivity of the rapid test or even lead to false negative results (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 (V Yilmaz *et al*., 2022). In their study, the samples were taken from dogs under one year old, and CDV was detected mostly in the respiratory secretion samples, which was in concordance with our results. Sarchahi *et al*. (2022) also showed that for the diagnosis of CDV by RT-PCR in dogs with neurological symptoms, whole blood and mucosal swabs are not suitable samples while CSF is much more suitable (Sarchahi & Arbabi, 2022). There was no significant difference in CDV prevalence between genders which is in agreement with previous studies (Cattet *et al*., 2004).

Phylogenetic study

Virus typing is important in many aspects, to identify different genotypes that lead to neurologic or non-neurologic forms, identify the virus location and its dominant type in the region, and also show the origin of the expanded genes to some extent in the microbial population (Namroodi, Rezaie, & Milanlou, 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 Sequence analysis of the H gene, and dendrogram mapping showed that there was no difference between viruses isolated from the non-neurologic form and the neurologic form and that they were all in 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 strains previously reported from Russia, the latter strains are likely to have originated in Russia. In this case, in a study conducted by Namroodi *et al* (2015) in Iran, it was shown that distemper virus isolates are located in the Arctic and European lineages and were probably transferred from Turkey to Iran (Somayeh Namroodi *et al*., 2015).

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 degree of compatibility and similarity between the four vaccines A, B, C, and E was 20 percent 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 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 (Mochizuki *et al*., 1999). These findings led to the development of 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 the high genotypic difference between the recent strains and the mentioned vaccines. Therefore, studies with larger samples size covering more geographical regions are suggested.

Conclusion

In this study, we showed that molecular techniques could be used to detect CDV in both neurologic and non- neurologic forms of Distemper. However, respiratory secretions in both neurologic and non-neurologic forms are of great importance for the diagnosis. 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.

299 Since all 13 isolates are located in polar clusters and are very similar to the strains obtained in Russia,
300 there is a possibility of its transfer from Russia to Iran

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Table 1: Primers used to amplify the nucleoprotein (NP) and hemagglutinin (H) genes.

Gene	Primers	Sequence (5' to 3')	Amplicon size (bp)	Reference

H	H-F H-R	F-ATGCTCTCTTACCAAGACAA R-GGCACGCAAGACCTCAACCT	1824	(Chen et al., 2018)
NP	N-F N-R	F-TCCCCTGGACAGTTGATCCA R-TTCCCTGGGGATCGTTTGAT	491	

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

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Groups	Number of 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 group (p-value < 0.05).

[$] Significant different between non- neurologic CDV and neurologic CDV groups (p-value < 0.05).

[¥] Significant different between neurologic CDV and neurologic non-CDV groups (p-value < 0.05).

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

Number of dogs carrying the virus	PCR + (n = 29)					
	Gender		Age class (month)			
	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 group (p-value < 0.05).

[$] Significant different between non- neurologic CDV and neurologic CDV groups (p-value < 0.05).

[¥]Significant different between neurologic CDV and neurologic non-CDV groups (p-value < 0.05).

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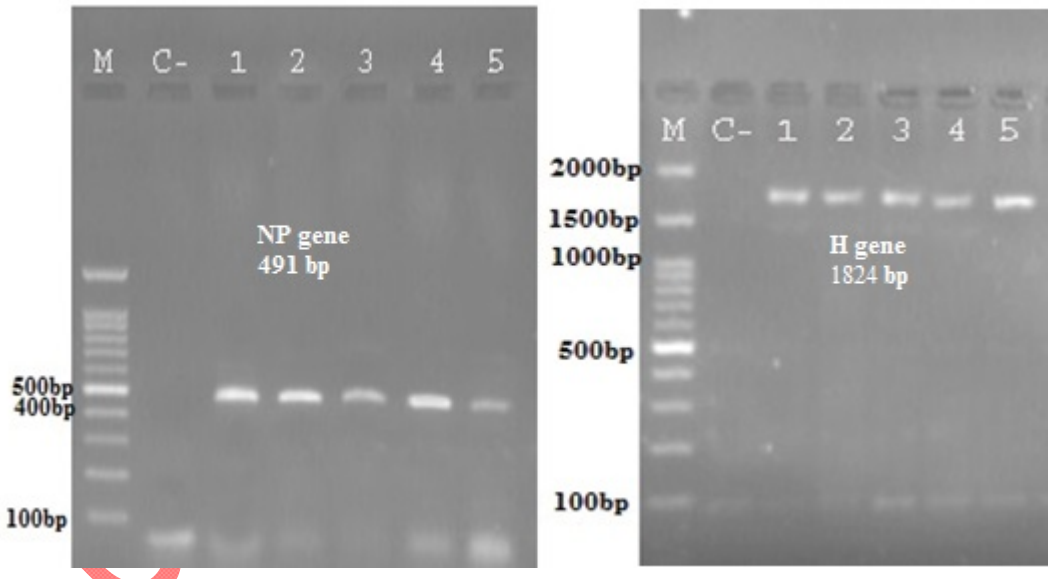
490 **Figure legends**



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492 Figure 1: RT- PCR results on agarose gel (1%) electrophoresis of different samples by primer of NP and
493 H genes. Lanes M: molecular marker (100-bp ladder); lanes C-: negative control; lane 1 to 5: suspected
494 sample

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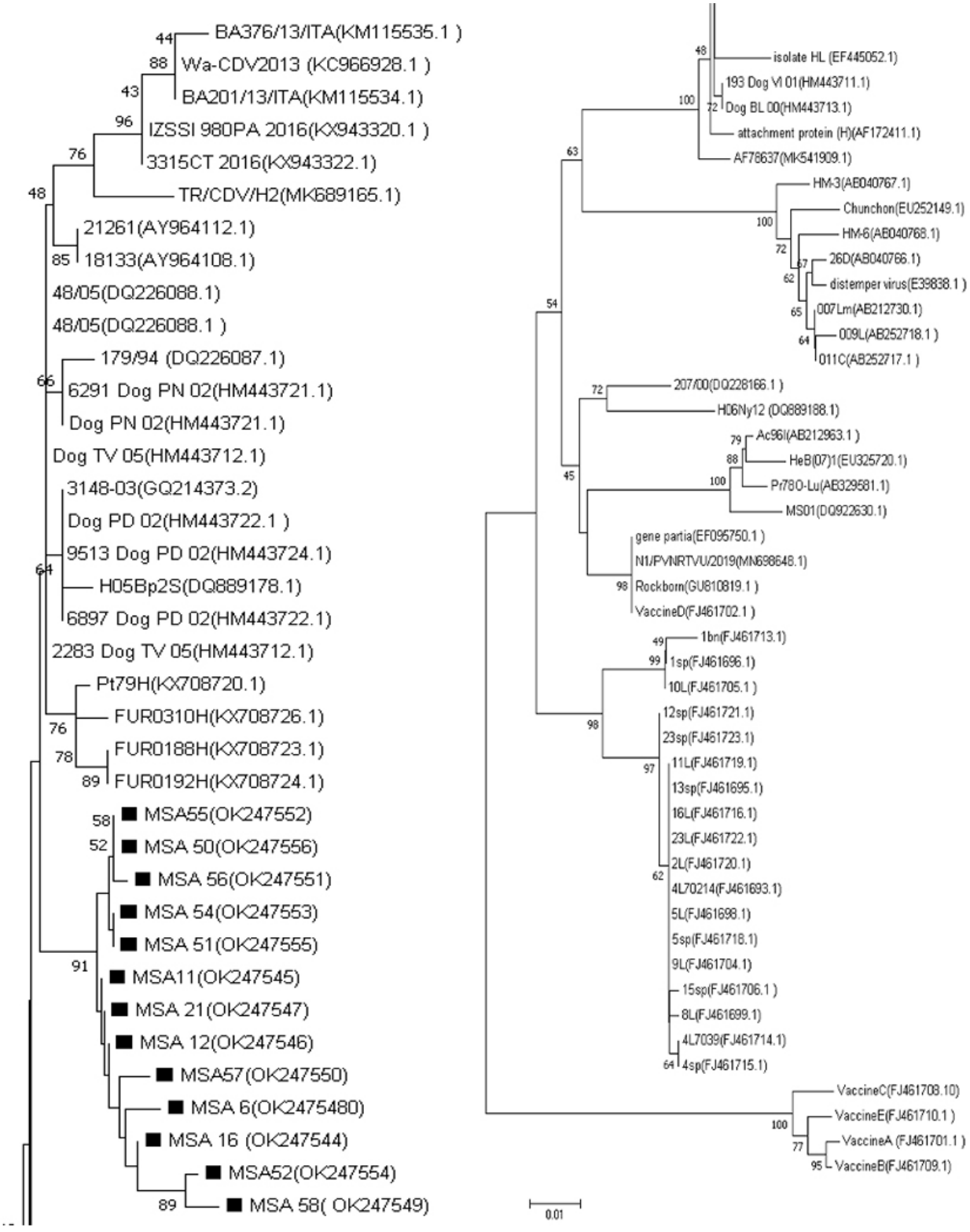
BA376/13/ITA(KM115535.1)
Wa-CDV2013 (KC966928.1)
BA201/13/ITA(KM115534.1)
IZSSI 980PA 2016(KX943320.1)
3315CT 2016(KX943322.1)
TR/CDV/H2(MK689165.1)
21261(AY964112.1)
18133(AY964108.1)
48/05(DQ226088.1)
48/05(DQ226088.1)
179/94 (DQ226087.1)
6291 Dog PN 02(HM443721.1)
Dog PN 02(HM443721.1)
Dog TV 05(HM443712.1)
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)
Pr780-Lu(AB329581.1)
MS01(DQ922630.1)
gene partia(EF095750.1)
N1/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)
VaccineE(FJ461710.1)
VaccineA (FJ461701.1)
VaccineB(FJ461709.1)
0.01

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

شناسایی مولکولی ویروس دیستمپر (CDV) در سگ های میتلا به فرم های بالینی عصبی و غیر عصبی

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چکیده فارسی

زمینه مطالعه : دیستمپر سگ سانان یکی از واگیردارترین و کشنده ترین بیماری های ویروسی در سگ ها است. علی رغم واکسیناسیون گسترده برای کنترل دیستمپر سگ سانان ، شیوع ویروس دیستمپر سگ سانان در سال های اخیر به نرخ هشداردهنده ای رسیده است. هدف:شناسایی ژنوتیپ های مسئول فرم های بالینی عصبی و غیر عصبی دیستمر سگ ها و بررسی فراوانی حضور ویروس در فرم های بالینی عصبی و غیر عصبی بیماری. روش کار: در این مطالعه توصیفی - تحقیقی از ۷۰ قلاده سگ واکسینه نشده مشکوک به ویروس دیستمپر با علایم بالینی این بیماری، نمونه برداری انجام شد. همه موارد ابتدا با کیت های تشخیصی سریع مورد آزمایش قرار گرفتند، سپس بر اساس علائم بالینی به 3 گروه مختلف تقسیم شدند. نمونه‌های مایع مغزی نخاعی، ترشحات تنفسی و مدفوع هر 40 سگ برای واکنش زنجیره‌ای پلیمراز-رونویسی معکوس (RT-PCR) مورد بررسی قرار گرفتند. پس از تعیین توالی ژن هماگلوتینین (ژن H) ، آنالیز فیلوژنیکی ژن H جدایه های استخراج شده با استفاده از نرم افزار MEGA™ 7 انجام شد.نتایج: تجزیه و تحلیل نتایج نشان داد که ترشحات تنفسی در گروه دیستمپر-غیر عصبی (85%) و دیستمپر-عصبی (80%) دارای بیشترین نمونه مثبت برای تست RT-PCR گزارش شد، اما در گروه عصبی غیر دیستمپر، نمونه مایع مغزی نخاعی بالاترین (40%) بود. در گروه های عصبی، سنین بالای 12 ماه بیشترین درصد آلودگی دیستمپر را نشان دادند و در گروه دیستمپر-غیر عصبی، سنین 3 تا 6 ماه بیشتر درگیر بودند. توالی یابی و تجزیه و تحلیل فیلوژنیکی ژن H نشان داد که تمامی نمونه های مورد بررسی متعلق به دودمان قطبی بودند. نتیجه گیری 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 سگ هایی که نتیجه تست سریع مثبت داشتند، تست PCR ترشحات تنفسی برای تشخیص ویروس حساس ترین نمونه است. در موارد عصبی که نتایج تست سریع منفی داشتند، PCR مایع مغزی نخاعی بالاترین حساسیت را داشته است، بنابراین می تواند یک راه حل تشخیصی باشد.

کلیدواژه: دیستمپر، سگ، هماگلوتینین، مایع مغزی نخاعی، عصبی

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