



## Research article

# Genetic characterization of canine distemper virus from infected dogs in Can Tho City, Mekong Delta, Vietnam

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## Abstract

The study aimed to evaluate the endemicity and determine the genetic characteristics of canine distemper virus (CDV) in Can Tho City. A study was carried out on 1,120 dogs raised in Can Tho City, and there were 150 dogs showed clinical signs of canine distemper. The result showed 68/150 (45.33%) dogs were positive for CDV, which was determined based on the test kit of Rapid Test Immuno provided by Asan company (Korea). Dogs under 6 months-age had the highest infection rate (68.00%), significantly higher than other age groups ( $P<0.05$ ). Male dogs (50%) were more affected than females (41.86%). Exotic dogs showed a higher CDV infection rate compared to domestic dogs, with statistical significance ( $P<0.05$ ). All dogs displayed clinical signs such as anorectic, moodiness, fever, sneezing, and eye conjunctivitis. Vaccinated dogs had a lower infection rate (6.25%) compared to unvaccinated or under-vaccinated dogs (80% and 69.57% respectively). The study on genetic characteristics of 7 representative Distemper virus strains isolated from the infected dogs was conducted via sequencing the H gene. Genetic analysis of seven representative CDV strains revealed a high similarity (99.82-99.96%) among isolated strains, indicating a common origin and frequent circulation in the study area. Nucleotide similarity with strains in the GenBank ranged from 93.26% to 99.56%, and with vaccine strains from 96.72% to 97.08%. The isolated CDV strains belonged to the Asia 1 lineage, commonly found in Vietnam and other Asian countries.

**Keywords:** Can Tho city, Distemper virus, Dogs, lineage Asia 1, H gene

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## INTRODUCTION

Canine distemper virus (CDV) is the etiological agent of a multisystemic infection that affects different species of carnivores. CDV affects nearly all body systems (Beineke et al. 2015). Puppies from 3–6 months old are particularly susceptible (Greene and Vandervelde, 2012). On a clinical level, canine distemper (CD) is characterized by signs of fever, leukopenia, gastrointestinal and respiratory manifestations, as well as neurological signs (Luo et al., 2016; Rendon-Marin et al., 2019). The prevalence rate of CDV in females is higher than in males (McCaw et al., 1998). According to Yang et al. (2020), although the current rapid immunochromatographic test (RICT) kits have limited sensitivity and should be enhanced by incorporating antibodies capable of detecting all CDV lineages, RICT kits without cross-reactivity offer several advantages compared to other diagnostic methods. They are simple, fast, and can be effectively utilized by both pet owners and veterinarians.

CDV belongs to the genus *Morbillivirus*, family *Paramyxoviridae*, which includes the measles virus, phocine distemper virus, and rinderpest virus (Greene and Appel, 2006). *Morbilliviruses* have enveloped virions and a negative-sense, single-stranded RNA genome. Viruses have a nucleocapsid structure containing a single strand of unsegmented RNA consisting of about 1,600 nucleotides encoding six structural and one nonstructural proteins (Diallo, 1990; Murphy et al., 1999). Although considered to have only one serotype, there are differences in antigenic structure among CDV strains. The study of Gámiz et al. (2011) showed that the Hemagglutinin (H) gene is one of the six crucial structural proteins (N, M, F, H, P, and L) in the virus, which undergoes numerous mutations that alter the antigenic properties of the virus. Two glycoproteins (F and H) are responsible for viral attachment, entry, and membrane fusion (Cha et al., 2012). Among viral proteins, the H protein of CDV has the most significant genetic variation. Therefore, the H gene is widely utilized in studying the genetic diversity of CDV strains in the natural environment (Duque-Valencia et al., 2019). Based on the nucleotide sequence of the gene coding for the H protein, there are 7 major virus lineages isolated from different geographical areas: America 1 (including most vaccine strains), America 2 (circulating in North America), Asia 1 and Asia 2, Europe, Arctic (circulating in the Arctic and Europe), and Africa (Bolt et al., 1997; Iwatsuki et al., 1997; Pardo et al., 2005; Martella et al., 2007; Calderon et al., 2007; Woma et al., 2010; Zhao et al., 2010; Gámiz et al., 2011).

In Vietnam, a number of studies on the genetic characteristics of the strains of CDV circulating in infected dogs in the field have shown that there is a very high similarity between CDV strains and those recorded in the world (Dai and Dat, 2016). In addition, Nen et al. (2017) identified some genetic characteristics of CDV isolated in some northern provinces of Vietnam belonging to 3 lineages Asia 1, Asia 2, and Classic. However, in the Mekong Delta, studies on the genetic characteristics of CDV in dogs are still limited. Therefore, this study was carried out to identify and compare the genetic characteristics of CDV strains isolated in sick dogs at the Veterinary Clinic, Can Tho University, as well as the basis for research on preventive vaccines in the region.

There have been some case reports of CDV in the Mekong Delta region of Vietnam. However, the information is still very limited. As a result, this study provides additional valuable data for adapting and selecting suitable vaccines to combat Distemper disease in Vietnam. Additionally, it offers valuable insights for future vaccine research endeavors.

## MATERIALS AND METHODS

### Animals and sample collection Canine distemper virus-positive samples

The experimental procedure adhered to the Animal Welfare Assessment (DT2020-02/KNV), and consent was obtained from all owners of the dogs involved. Out of the 1,120 dogs examined in Can Tho City, 150 exhibited clinical manifestations of Canine Distemper (CD) based on a combination of historical data and clinical examinations. These signs included fever, purulent ocular and nasal discharge, tonsillitis, bronchitis, gastroenteritis, as well as neurological disturbances.

Conjunctival swabs were collected from 150 dogs to determine CDV antigen with Rapid Test Immuno CDV Ag by the Korean Asan Company. The study of [Yang et al. \(2020\)](#) showed that Korean RICT kits could be useful for the rapid detection of CDV strains. RICT kits are without cross-reactivity and have advantages over other diagnostic methods in that they are straightforward and rapid. These cases of positive dogs for CDV Rapid test noted some characteristics such as breed, sex, age, and vaccination schedule.

### Molecular study

A total of 7 positive dogs via CDV Rapid test were occupied to collect the tracheal and conjunctival exudates samples with a sterile cotton swab. These samples were placed into a tube containing a transport medium to ensure its stability. They were labeled as CT01, CT03, CT04, CT06, CT07, CT08, CT09. These virus strains represent strains isolated from domestic (CT01, CT06, CT08) and foreign (CT03, CT04, CT07, CT09) dogs from three to six years of age. Viruses were extracted RNA according to the instructions for use of the QIAamp Viral RNA Mini kit (Qiagen, Germany); RNA samples were stored at -70°C. Amplification of the entire H gene region of CDV was performed using RT-PCR (reverse transcription-polymerase chain reaction). The primer sequences for this segment are presented in [Table 1](#).

**Table 1** Nucleotide sequences of primers that amplify the H gene ([Gámiz et al., 2011](#))

Primer	Oligonucleotide Sequence (5'-3')	Nucleotide position	Fragment size (bp)
DHI-F	TGGTTCACAAGATGGTATTC	8,005	613
DHI-R	CAACACCACTAAATTGGACT	8,617	

The thermal cycle for the RT-PCR reaction was as follows: reverse transcription: 45 °C for 45 minutes; pre-denaturation stage: 94 °C for 5 minutes; 30 cycles: 94 °C for 30 seconds, 51 °C for 30 seconds, 72 °C for 30 seconds; and 72 °C for 10 minutes (Gámiz et al., 2011). Ingredients for one RT - PCR reaction: 10 µL Go taq® green master mix 2X (Promega, USA); 1 µL MgCl<sub>2</sub> (25 mM); 0.5 µL M - MLV (Moloney Murine Leukemia Virus); 0.5 µL forward primer; 0.5 µL reverse primer; 4 µL of extracted RNA sample and 8.5 µL of purified water. The RT-PCR product was sent to the laboratory of Phu Sa Biochemistry Co., Ltd. (Can Tho) for sequencing the H gene region.

## Data analysis

Survey of related factors to CDV including ages, breeds, gender, and vaccination schedule CDV by  $\chi^2$  test of Minitab (version 16.0) software.

Accessing GenBank to collect and determine the nucleotide similarity of the published CDV gene sequences with the gene fragment obtained in the study through the Blast program on GenBank. Phylogenetic origin based on H gene sequences from virus strains obtained by MEGA (Molecular Evolutionary Genetics Analysis, version 7.0) software. Using the Maximum Likelihood test method with a bootstrap value of 1,000 repetitions.

## RESULTS

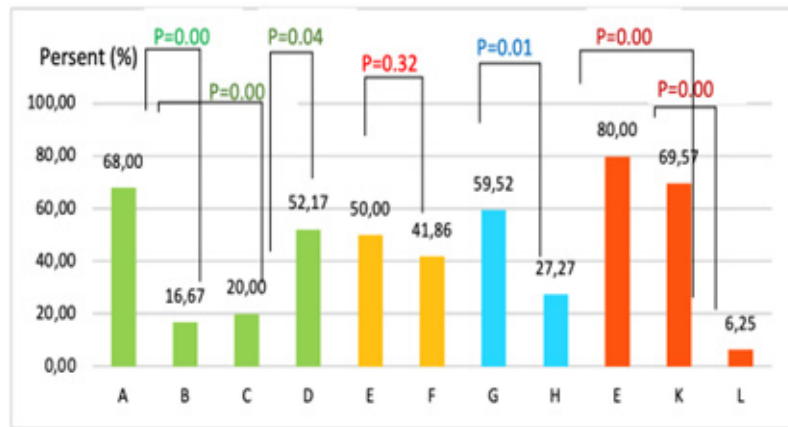
### Canine distemper in dogs at Can Tho City

A survey of 1,120 dogs raised in Can Tho City revealed that 150 dogs showed clinical signs of CD. The CD was determined to base on the test kit of Rapid Test Immumo supplied by Asan company (Korea). Of 150 dogs examined with the test kit, there were that 68 dogs were positive for CDV. Table 2 showed the results of the frequency of clinical signs in CDV-infected dogs, which were fever, sneezing, eye conjunctivitis, cough, internal runny nose (100%); skin nodules (36.76%); keratosis hyperkeratosis of paw pad and nose skin (41.18%); neurological signs (41.18%).

**Table 2** Frequency of clinical signs in CDV-infected dogs

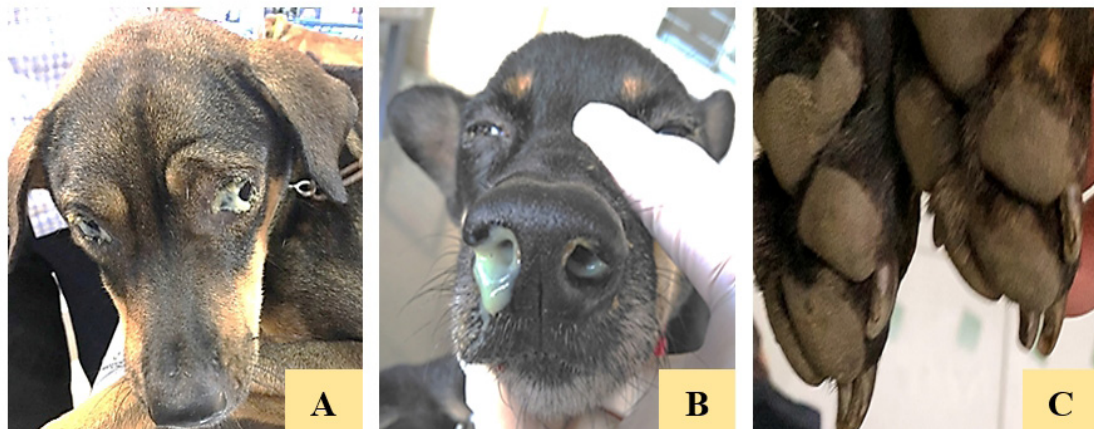
Clinical signs	Amount (Dogs)	Percent (%)
Fever, sneezing, eye conjunctivitis, cough, internal runny nose	68	100.00
Skin nodules, abdominal pustules	25	36.76
Keratosis hyperkeratosis of paw pad and nose skin	28	41.18
Neurological signs	28	41.18

Figure 1 showed that the highest CDV-infected rate was dogs at <6 months old (68%), which is statistically significant with other groups with  $P < 0.05$ . Dogs over 5 years old had a rate 52.17%. The dogs that were not vaccinated and insufficient-dose vaccinated were high CDV-infected ratio if 80% and 69.57%, respectively.



**Figure 1** The bar chart show the prevalence of CDV dogs based on breeds, ages, gender, and vaccination schedule. A: Age  $\leq 6$  months, B: 6 months  $<$  Age  $\leq 2$  years, C: 2 years  $<$  Age  $\leq 5$  years, D: Age  $> 5$  years, E: male, F: female, G: Wild dogs, H: Domestic dogs, E: No vaccination, K: Insufficient-dose vaccination, L: Vaccination.

Clinical signs of CD are often unapparent or initially mild during the initial phase of fever and are characterized by mucopurulent oculonasal discharges, conjunctivitis, respiratory distress, anorexia, vomiting, dehydration due to diarrhea, and cutaneous rash (Appel et al., 1987). The findings of the above author are well in accordance with the clinical signs of CDV-infected dogs in this study, including purulent ocular release (Figure 2A), purulent nasal release (Figure 2B), and hyperkeratinization of the foot pad (Figure 2C).



**Figure 1** Some Clinical signs of CD. (A) Purulent ocular discharge in a dog with CDV infection; (B) Purulent nasal discharge - Canine distemper in dog; (C) Hyperkeratinization of foot pad.



## The similarity of nucleotide and amino acid sequences between the strains of CDV at the Can Tho City and the published strains

The H gene sequences of 7 strains of CDV were sequenced with a length of 613 (bp) nucleotides. The results of comparing the H gene sequences between the CDV strains that cause disease in dogs in Can Tho City with the strains of GenBank showed that there was a very high similarity rate (from 93.17% to 99.96%) (Table 3).

**Table 3** Similarities in nucleotide sequences between CDV strains isolated in Can Tho City and reference strains on GenBank

Strains	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1																				
2	99,96																			
3	99,96	99,96																		
4	99,96	99,96	99,96																	
5	99,82	99,86	99,86	99,86																
6	99,89	99,86	99,86	99,86	99,71															
7	99,89	99,86	99,86	99,86	99,71	99,71														
8	93,26	93,31	93,31	93,31	93,48	93,17	93,17													
9	96,03	96,07	96,07	96,07	96,24	95,91	95,91	94,35												
10	96,84	96,88	96,88	96,88	97,04	96,72	96,72	94,74	98,25											
11	96,88	96,92	96,92	96,92	97,08	96,76	96,76	94,79	98,29	99,82										
12	96,84	96,88	96,88	96,88	97,04	96,72	96,72	94,75	98,25	99,78	99,96									
13	94,65	94,69	94,69	94,69	94,86	94,52	94,52	93,32	95,70	96,55	96,52	96,48								
14	95,36	95,40	95,40	95,40	95,57	95,23	95,23	94,06	96,34	97,27	97,31	97,27	95,82							
15	95,45	95,49	95,49	95,49	95,65	95,36	95,36	93,99	96,27	97,08	97,12	97,08	95,54	96,39						
16	93,47	93,51	93,51	93,51	93,69	93,46	93,46	97,55	94,42	94,94	94,98	94,94	93,61	94,43	94,18					
17	93,67	93,71	93,71	93,71	93,88	93,58	93,58	99,30	94,79	95,17	95,22	95,17	93,76	94,50	94,43	98,02				
18	95,68	95,72	95,72	95,72	95,88	95,55	95,55	93,68	96,96	97,64	97,67	97,64	95,33	95,91	95,75	93,92	94,08			
19	95,56	95,60	95,60	95,60	95,76	95,51	95,51	93,93	96,92	97,68	97,72	97,68	95,42	95,99	95,84	94,17	94,37	96,50		
20	99,31	99,34	99,34	99,34	99,49	99,20	99,20	93,36	96,19	97,08	97,12	97,08	95,03	95,69	95,78	93,65	93,76	95,92	95,88	
21	99,38	99,42	99,42	99,42	99,56	99,27	99,27	93,35	96,07	96,80	96,84	96,80	94,65	95,40	95,41	93,51	93,75	95,63	95,51	99,27

(1) CT01; (2) CT03; (3) CT04; (4) CT06; (5) CT07; (6) CT08; (7) CT09; (8) CDV/AB472690/Vietnam/2016; (9) CDV/Z54166/The Netherland/1996; (10) CDV/FJ461702/South Africa/2010; (11) CDV/EF095750/Hungary/2016; (12) CDV/FJ705238/Taiwan/2010; (13) CDV/AB040767/Japan/2002; (14) CDV/DQ226087/Italy/2006; (15) CDV/FJ461715/South Africa/2010; (16) CDV/HM063009/Kazakhstan/2010; (17) CDV/AF378705/USA/2012; (18) CDV/FJ416339/Germany/2016; (19) CDV/DQ228166/Italy/2006; (20) CDV/JX681125/China/2013; (21) CDV/LC159587/Vietnam/2019

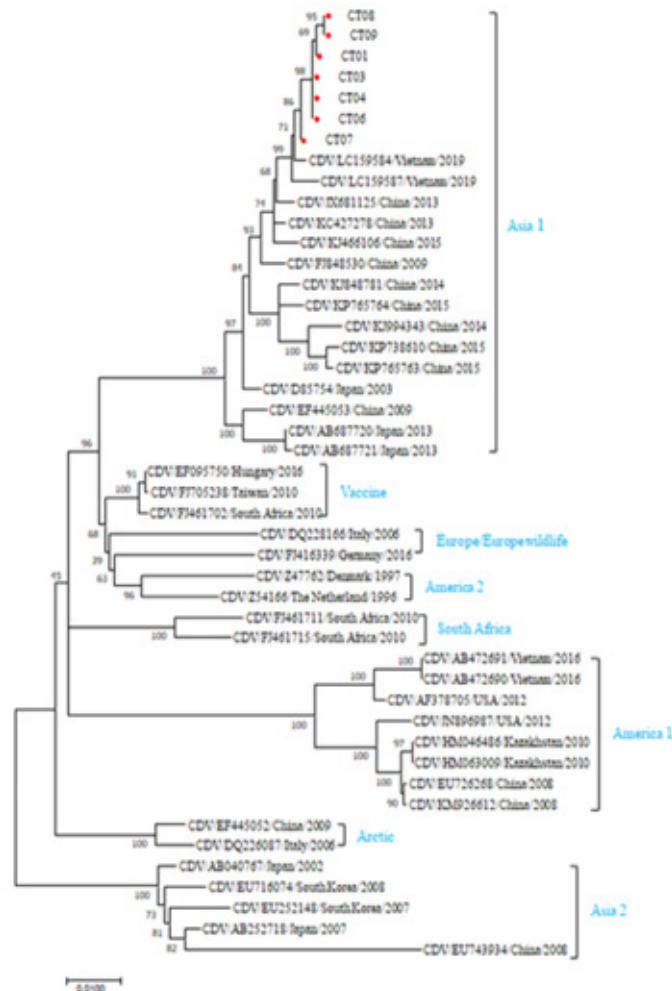
The CDV strains isolated in the study exhibited a high degree of closeness to each other, with a similarity rate ranging from 99.82% to 99.96%. This indicated that the isolated CDV strains causing disease in dogs may have originated from a common source and were prevalent in the study area. The results also showed that the nucleotide similarity between the strains in the study and other strains in the GenBank database ranged from 93.26% to 99.56%. Furthermore, the vaccine strains available in the GenBank database exhibited a nucleotide similarity of 96.72% to 97.08% with the strains in the study.

**Table 4** Similarities in amino acid sequences between CDV strains isolated in Can Tho City and reference strains on GenBank

Strains	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1																				
2	99,96																			
3	99,96	100																		
4	99,96	99,96	100																	
5	99,82	99,86	99,86	99,86																
6	99,89	99,86	99,86	99,86	99,71															
7	99,89	99,86	99,86	99,86	99,71	100														
8	93,26	93,31	93,31	93,31	93,48	93,17	93,17													
9	96,03	96,07	96,07	96,07	96,24	95,91	95,91	94,35												
10	96,84	96,88	96,88	96,88	97,04	96,72	96,72	94,74	98,25											
11	96,88	96,92	96,92	96,92	97,08	96,76	96,76	94,79	98,29	99,82										
12	96,84	96,88	96,88	96,88	97,04	96,72	96,72	94,75	98,25	99,78	99,96									
13	94,65	94,69	94,69	94,69	94,86	94,52	94,52	93,32	95,70	96,55	96,52	96,48								
14	95,36	95,40	95,40	95,40	95,57	95,23	95,23	94,06	96,34	97,27	97,31	97,27	95,82							
15	95,45	95,49	95,49	95,49	95,65	95,36	95,36	93,99	96,27	97,08	97,12	97,08	95,54	96,39						
16	93,47	93,51	93,51	93,51	93,69	93,46	93,46	97,55	94,42	94,94	94,98	94,94	93,61	94,43	94,18					
17	93,67	93,71	93,71	93,71	93,88	93,58	93,58	99,30	94,79	95,17	95,22	95,17	93,76	94,50	94,43	98,02				
18	95,68	95,72	95,72	95,72	95,88	95,55	95,55	93,68	96,96	97,64	97,67	97,64	95,33	95,91	95,75	93,92	94,08			
19	95,56	95,60	95,60	95,60	95,76	95,51	95,51	93,93	96,92	97,68	97,72	97,68	95,42	95,99	95,84	94,17	94,37	96,50		
20	99,31	99,34	99,34	99,34	99,49	99,20	99,20	93,36	96,19	97,08	97,12	97,08	95,03	95,69	95,78	93,65	93,76	95,92	95,88	
21	99,38	99,42	99,42	99,42	99,56	99,27	99,27	93,35	96,07	96,80	96,84	96,80	94,65	95,40	95,41	93,51	93,75	95,63	95,51	99,27

(1) CT01; (2) CT03; (3) CT04; (4) CT06; (5) CT07; (6) CT08; (7) CT09; (8) CDV/AB472690/Vietnam/2016; (9) CDV/Z54166/The Netherland/1996; (10) CDV/FJ461702/South Africa/2010; (11) CDV/EF095750/Hungary/2016; (12) CDV/FJ705238/Taiwan/2010; (13) CDV/AB040767/Japan/2002; (14) CDV/DQ226087/Italy/2006; (15) CDV/FJ461715/South Africa/2010; (16) CDV/HM063009/Kazakhstan/2010; (17) CDV/AF378705/USA/2012; (18) CDV/FJ416339/Germany/2016; (19) CDV/DQ228166/Italy/2006; (20) CDV/JX681125/China/2013; (21) CDV/LC159587/Vietnam/2019

Due to the high nucleotide sequence similarity in the H gene between the isolated CDV strains and the reference strains, these viral strains also exhibited a high degree of amino acid sequence similarity ranging from 93.17% to 100% (Table 3). The amino acid similarity between the studied strains and other strains in the GenBank database was found to be 99.03% to 99.56%, while the vaccine strains in the GenBank database displayed an amino acid similarity of 96.72% to 97.08%.



**Figure 3** Genetic relationships between CDV strains isolated in dogs at Cantho city (CT01, CT03, CT04, CT06, CT07, CT08, and CT09: Indicate in red dots.) and strains published on Genbank.

### Genetic relationship between studied CDV strains and CDV strains circulating in Vietnam and the world

The results of the CDV strain analysis indicate that they belong to the same branch within the Asia 1 lineage, and they exhibit significant similarity to strains originating from China and Japan. Furthermore, the analysis of the origin of the CDV strains, based on nucleotide differences in the H gene segment of the 7 CDV strains in the study, revealed that all 7 CDV strains were distinct from the branches identified in reference strains worldwide, which belonged to different lineages such as Europe, America 1, America 2, Africa, Arctic, and Asia 2. This result indicates that despite belonging to the same group, the analyzed CDV strains were subdivided into smaller subgroups based on the individual dogs from which they were collected. CDV/Dog/CT07/2020 strain differed from the remaining six virus strains, showing 86% similarity (Figure 3).



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## DISCUSSION

The CD is an infectious disease that spreads easily and is highly fatal. It primarily affects various systems in the body, including the respiratory, gastrointestinal, and neurological systems (Woma et al., 2009; Greene and Vandervelde, 2012). In our study, the frequency of clinical signs observed in CDV-positive dogs was fever, sneezing, eye conjunctivitis, cough, and internal runny nose. All these observed signs are non-specific for CD because we can misdiagnose other diseases with similar signs, such as respiratory tract infections, pneumonia, and encephalitis. Although, CDV cannot be easily distinguished based on clinical signs. In contrast, some clinical signs, such as skin nodules, abdominal pustules, keratosis hyperkeratosis of paw pad and nose skin, and neurological signs, are highly specific to the CD. According to Greene and Vandervelde (2012), clinical signs of CD include nausea, vomiting, diarrhea, nasal and ocular discharges, sneezing, coughing, difficulty breathing (dyspnea) and rapid breathing (tachypnea), myoclonus, seizures, partial or complete paralysis (paresis/plegia) and lameness, impetigo, as well as hyperkeratosis of the nasal or foot pads. The results of this survey were similar to those of Dang et al. (2021), who showed that dogs infected with CD have typical clinical signs such as anorectic, moody, fever, vomiting, green nasal discharge, and black color diarrhea.

Puppies that get sufficient maternally determined antibodies (MDA) are typically protected against CDV until they reach 3 months of age. Therefore, the infection essentially impacts puppies between the ages of 3 and 6 months, whereas those who do not get adequate MDA at birth are vulnerable to CDV (Greene and Vandervelde, 2012). CD is an immunosuppressive sickness that can make dogs of any age more vulnerable to secondary viral and bacterial infections (Blancou, 2004). In our results, 68% of CDV-positive dogs were under 6 months of age, and 52,17% of dogs were over 5 years old. Dogs under 6 months of age have a higher rate of CDV infection than other age groups, and there was a significant difference. Similar results were found in other research by Greene and Vandervelde (2012), which showed that dogs aged 2-6 months old had the highest CDV-positive rate.

According to our results, dogs that were not vaccinated against CDV or received an insufficient vaccination dose have corresponding high infection rates of 80% and 69.57%, respectively. This result demonstrates the significance of immunization in disease prevention. Other studies have also highlighted the importance of immunization. Mousafarkhani et al. (2023) reported that 91.84% of dogs positive for CDV had not been vaccinated. The unvaccinated and insufficient-doses vaccinated dogs were a high CDV-infected ratio. In addition, the lacking of complete or partial immunity against CDV may be responsible for the variant clinical signs of CD in dogs due to the pathophysiology mechanisms associated with the disease occurrence (Geetha, 2019).

The results of the CDV phylogenetic analysis indicated high similarity in nucleotide and amino acid sequences. The vaccine strain of the virus falls into a separate branch and belongs to the vaccine subgroup. These findings are consistent with previous studies on other research results, such as the report of Nen et al. (2017) also recorded a high degree of similarity between isolated strains of CDV (90.05-99.61%) and the published vaccine strain (89.99-99.34%). The analysis results of Dai and Dat (2016) also show that

the nucleotide similarity of the CDV samples in the field is very high (99.30-100%) and similar to the virus strains in the vaccine (96.10-96.60%). Guo et al. (2013) also recorded the nucleotide sequence in the H gene between the CDV strain in China compared with other strains worldwide, with the similarity rate ranging from 85.40-99.80%.

This study also indicated these strains were the same group belonging to the Asia 1 lineage and exhibited significant similarity to strains sourced from China and Japan. Despite having high similarity in nucleotide and amino acid sequences, CDV strains isolated exhibit distinct subgroup characteristics. This suggests the existence of various CDV strains circulating in Vietnam, underscoring the significance of carefully selecting a suitable vaccine for effective prevention. The results of this study were similar to those of Lan et al. (2005, 2006, 2009); Tan et al. (2011); Nen et al. (2017), who showed that virus isolates in Japan, China, and Asia are circulating 2 main lineages, Asia 1 and Asia 2. Another study by Guo et al. (2013) also showed that the CDV strains that cause disease in pandas and wild dogs in China belong to lineage Asia 1. The research results of Dai and Dat (2016) also showed that many CDV strains existing and samples collected at veterinary clinics in Ho Chi Minh City all belonged to lineage Asia 1. Although CDV was first detected in Vietnam in 1950, in 2009, the CDV strain in Vietnam was identified with lineage America 1 (Lan et al., 2009). However, the research findings of Dung et al. (2017) showed that the CDV strains collected from veterinary clinics in Ho Chi Minh City belong to the Asia 1 lineage and are similar to circulating virus strains in other Asian countries such as Taiwan, China, South Korea, Japan, and Thailand. These findings differ from previous studies conducted in Vietnam. Therefore, the results of this study have practical implications for adjusting and selecting appropriate virus strains for vaccine importation or developing vaccines from locally circulating CDV strains to prevent the disease. This result showed that the isolated virus was also highly similar to the strains used to make the vaccine; Therefore, if vaccinated with vaccines produced from these virus strains (CDV/FJ461702/South Africa/2010; CDV/EF095750/Hungary/2016; CDV/FJ705238/Taiwan/2010) will increase the protection effect for dogs in the study area.

## CONCLUSIONS

The typical clinical signs observed in dogs infected with CDV include skin nodules, abdominal pustules, keratosis, hyperkeratosis of paw pad and nose skin, and neurological signs. Puppies without enough maternal antibodies are vulnerable to CDV, which mainly affects those aged 3-6 months. Unvaccinated and incompletely vaccinated dogs have a high risk of CDV infection.

The CDV strains isolated in Can Tho City have the same origin, as proven by their high closeness (99.82-99.96%) and likeness to field viruses and vaccine strains reported in GenBank. These strains, belonging to the Asia 1 lineage, are prevalent not only in Vietnam but also in other Asian countries. The presence of multiple CDV subtypes in Vietnam underscores the need for careful consideration when selecting vaccines for effective disease prevention in dogs. These research findings provide valuable insights for future studies aimed at vaccine selection and preparation specifically targeted against CD in Vietnam.

## AUTHOR CONTRIBUTIONS

**Tran Thi Thao;** Conceptualization and design of the experiment, methodology, formal analysis, manuscript preparation, editing, and finalization

**Nguyen Khanh Thuan, Dang Thi Tham, Van My Tien, Dang Thi My Tu;** Manuscript preparation, investigation, supervision, formal analysis, editing, and finalization

## CONFLICT OF INTEREST

We have no conflict of interest.

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