Phylogenetic Analysis and Biological Characteristics Identification of a Canine Distemper Virus Strain Isolated from a vaccinated Domestic Dog in the Northeast China

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Research Article

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Abstract

Canine distemper virus (CDV) is currently circulating in domestic and wild animals. The host range of CDV comprises all families within the order Carnivora, and it has recently expanded to nonhuman primates, moreover the host range still has the potential for further expansion. In this research, a CDV strain named BT was isolated from a vaccinated domestic dog in Changchun, Northeast of China, and identified by Electron microscope (EM) and Immunofluorescence Assay (IFA). The whole genome sequence of the virus was obtained by RT-PCR and Sanger sequencing. Phylogenetic analysis was performed based on the Coding sequence (CDS) of hemagglutinin (H) protein and the complete genome sequence of the virus respectively, the results showed that the CDV-BT strain still classified into the Asia-1 lineage. This research shows that CDV-BT strain's antigenicity of the epitope ^{444}GDKYPIHFNDER^{455} in nucleocapsid (N) protein and the epitope ^{178}ARGDIFPPY^{186} in H protein were significantly different from vaccine strain by amino acid substitutions, and suggests that the characterization of genetic diversity among the circulating CDVs is essential for future CDV's research and disease monitoring.

Introduction

Canine distemper virus (CDV) is a nonsegmented, minus-stranded and enveloped RNA virus, which is belongs to the *genus Morbillivirus*, family *Paramyxoviridae* [1]. CDV infection causes mutiple systemic disease involving respiratory as well as the gastrointestinal tracts and the central nervous system. Clinically, it is characterized by bipolar fever, respiratory symptoms, gastroenteritis, as well as neurological symptoms [2].With the continuous evolution of CDV, its host range has been extended from the traditional families Canidae, Mustelidae, and Procyonidae to the families Felidae, Hyaenidae, Ursidae, Ailuridae, Viverridae, Cercopithecidae and Phocidae [3]. Besides, some cases of canine distemper (CD) have also been reported in collared peccary (order Artiodactyla) in Arizona in the United States, captive marmot (order Rodentia) in Switzerland and anteater (order Pilosa) in Brazil [4–6], the true host range of CDV remains to be determined. As a highly contagious febrile viral disease in carnivores, CDV posing a substantial threat to the wildlife conservation worldwide at the moment.

The CDV genome consists of 15 690 nucleotides, encoding six structural proteins and two non-structural proteins. The six structural proteins are nucleocaspid (N), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin protein (H) and large protein (L). The two non-structural proteins, C and V, are encoded either by mRNA editing or alternate Open Reading Frame (ORF) in the gene which encoding P protein. Multiple studies have shown that genetic variability of the H gene is higher than that of other CDV genes [7, 8]. Currently, although all of the CDV isolates belong to a single serotype, there was significant genetic diversity among the different CDV isolates, and CDV can be classified into different geographical lineages based on the diversity of H gene. Over the past few decades, CDV has been divided into the following major geographic lineages: America-1 (vaccine) and − 2, Asia-1 and − 2, Europe-1/SouthAmerica-1, European wildlife (Europe-2), Arctic like (Europe-3), South Africa (Africa-1) and East Africa (Africa-2) [9]. Nevertheless, with the improvement of epidemiological investigation of canine distemper virus in recent years, new geographical lineages have been identified continuously, including

America-3, -4, and − 5 [10 − 12], Asia-3, -4 and − 5 [13–15], South America-2, -3 and − 4 [16, 17]. With the development of molecular biology techniques, it has become easier to obtain the genome sequence information of a CDV strain. It will facilitate to further insights into the virus evolution and the identification of molecular determinants underlying CDV cross the species barrier.

Due to the high fatality rate of canine distemper and the lack of specific drugs to treat the disease, vaccination is the most effective and economical measure to control CDV at present [18]. Commercial attenuated live canine distemper vaccines were introduced on the market in the 1950s [19]. The major vaccine strains contain: Onderstpoort, Lederle, Convac, Snyder Hill, Rockborn and CDV3, all vaccine strains except the Rockborn strain are classified as America-1 lineage, Rockborn strain and other field strains can be classified into a single lineage, which we called Rockborn like lineage. The Onderstepoort strain was isolated from a North American fox farm during a canine distemper outbreak in the 1930s. The strain has been weakened by 50 successive passes in ferrets and 130 successive passes in the chorio-allantoic membrane of hen's eggs. Beginning in the 1950s, the live attenuated strain of Onderstepoort dominated the market, especially in Europe, and is still included in most currently available vaccines. In 1950, the Rockborn strain was isolated from a Swedish dog with canine distemper and was "completely weakened" in canine kidney cells after 56 successive passes. The Rockborn strain of vaccine was distributed worldwide after 1962 and has been shown to provide solid immunity and protection in vaccinated animals. However, there have been reports of post-vaccination encephalitis in dogs immunized with the Rockborn strain of vaccine in the 1970s. As a result, the Rockborn strain was withdrawn from several markets after the mid-1990s. Although the widespread use of commercial vaccines has greatly controlled the disease, several canine distemper cases in vaccinated animals have been reported worldwide in recent years [20, 21], and unique genetic diversity has been reported in these CDV isolates [22, 23]. Since most commercial CDV vaccines are produced by the America-1 lineage strain, the difference in antigen may explain the CD cases has increased in recent decades worldwide even in vaccinated animals. Therefore, monitoring the prevalence of CDV may help us to further understand the evolution of canine distemper virus and thus providing assistance for the prevention and control of canine distemper virus. In the current research, we have isolated a CDV from a vaccinated dog with canine distemper in the northeast of China, obtained the genomic sequence information and analyzed the phylogeny of this virus. Moreover, we analyzed the antigenic differences between this isolate and the vaccine strain by using two monoclonal antibodies against CDV.

Materials And Methods

Viruses, cell lines, and clinical samples

The vaccine strain CDV-3 (GenBank accession number: EU726268.1), wild-type strain CDV-PS (GenBank accession number: JN896331.1), and the Vero-dslam cell line expressing canine Signaling lymphocyte activation molecule (SLAM) was preserved in our laboratory.

The CDV-positive clinical specimens were collected from the Idog pet hospitals in Changchun, Jilin, China. Diseased dogs show clinical signs of respiratory, nervous and digestive, systems. After a natural death, an autopsy is performed at the hospital. Fresh tissues including lung, spleen, liver, and lymph nodes were collected for laboratory diagnosis and virus isolation.

Virus isolation

The CDV positive lung sample was inoculated into the Vero-dslam cell line for virus isolation. First, CDV positive lung tissue homogenate was prepared in Dulbecco Modified Eagle medium (DMEM) serum-free medium. After ultrasonic treatment and centrifugation of the samples,the viral suspension was inoculated into 90% monolayer Vero-dslam cells in 6-well plates. The Vero-dslam cells inoculated viral suspension were maintained in DMEM containing 5% fetal calf serum (FCS), 100 μg/ml streptomycin and 100 units/ml penicillin. Replacement fresh medium in the culture wells, after 30 min incubation. The Vero-dslam cells were placed in a constant and humidified 35°C incubator with 5% CO_2. The cell cultures were observed and recorded daily for the appearance of cytopathic effect (CPE) for 6 days. When obvious CPE apparent in the Vero-dslam cells were observed under an inverted microscope, the viral cultures was collected. The titer of the CDV was determined by 50% tissue culture infective dose ($TCID_{50}$) assay and calculated by Reed and Muench method. The remaining viruses were stored at −80°C for use.

Immunofluorescence Assay (IFA)

Monoclonal antibodies (mAbs) C8 against CDV N protein and mAbs 1A5 against CDV H protein were preserved in our laboratory [2, 24]. The Vero-dslam cells were seeded in the 96-well plates, and the cells infected by the CDV isolate were used as experimental group, and the cells without CDV infection were used as the negative control group. At 36 h after infection, all groups of Vero-dslam cells were fixed with 100 μL ice-cold cell fixation solution (4% paraformaldehyde and 0.1% Triton X-100 in PBS) for 60 min; then the fixation solution was removed, the fixed cells were washed twice by using PBS. The fixed Vero-dslam cells were blocked with 5% skim milk in TBST at 37°C for 60-120 min. After blocking, all groups of cells were incubated with the primary antibodies mAbs C8 and 1A5 at 37°C for 120 min, respectively. Then, all cells were washed by using PBST three times and incubated with secondary antibodise Dylight594–conjugated goat antimouse IgG (Abbkine, China) at 37°C for 60-90 min. After the incubation, all groups of cells were washed three times by using PBST. Finally, all groups of Vero-dslam cells were observed by fluorescence microscope (Nikon TS100, Japan). Since the Vero-dslam cell line used in this research expressed the Enhanced Green Fluorescent Protein (EGFP) and could emit green fluorescence, we selected Dylight594-conjugated goat anti mouse IgG which could emit orange-red fluorescence for the IFA.

RNA extraction and reverse-transcription (RT)

The viral genome RNA was extracted by using Easypure® Viral RNA Kit (TransGen, Beijing, China). RT of viral genomic RNA was performed by using PrimeScript™ 1st Strand cDNA Synthesis Kit (TaKaRa, Dalian, China). Reverse-transcribed reaction system (20-μL volumes) containing Template RNA <1 μg, PrimeScript RTase (200 U/μL) 1.0 μL, RNase Inhibitor (40 U/μL) 0.5 μL, Random Primers (50 μM) 1.0 μL, dNTP Mixture (10 mM each) 1.0 μL, 5× PrimeScript Buffer 4.0 μL, RNase free dH_2O up to 20 μL. The reaction system was placed at 42°C for 30-60 min, and then at 95°C for 5 min.

Primers, amplification, cloning, and sequencing of the genome

Fourteen pairs of primers covering the whole CDV genome were designed by Primer 5.0 software based on the conserved region of CDV genome (Table 1). The amplification conditions for the PCR reactions were performed by three steps, First, initial denaturation at 95°C for 5 min. Second, the target fragment was amplified by 35 cycles of denaturation at 95°C for 30 s, annealing at 50–58°C for 60–120 s and extension at 72°C for 30 s. Finally, the final extension is performed at 72°C for 8 min. The PCR products with the expected size were purified and ligated into the pMD 18-T vector (TaKaRa, Dalian, China). For each fragment, 3 positive recombinant plasmids were sequenced using M13 universal primers by Comate Bioscience Company (Changchun, China). Sequences are processed and assembled by using the Lasergene program package (DNAStar, Madison, WI, USA).

Phylogenetic assay

The entire H protein Coding sequence (CDS) and full genome sequence were aligned with the sequences of other CDV strains in the GenBank collected from different places and years worldwide respectively. Subsequently, the Maximum-likelihood analyses were performed by general time reversible (GTR+G+I) model with 1000 bootstraps in MEGA 6.0 [25].

Western Blotting (WB)

The antigenic differences between the CDV isolate, CDV-PS and CDV3 were analyzed by WB assay using two mAbs C8 and 1A5. Virus samples was separated by 12% Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). And then the virus proteins were transferred to 0.45 μm PVDF membranes (Merck Millipore, Germany) by wet-blotting.

The PVDF membranes were blocked at 37°C for 120 min in 5% skim milk in TBST, and then incubated with the mAbs C8 and 1A5 (diluted 1:2,000 in 5% skim milk in TBST) at 37°C for 90-120 min, respectively. Subsequently,all PVDF membranes were washed three times by using TBST and then incubated with a 1:2,000 dilution of horseradish peroxidase (HRP)–conjugated goat anti-mouse IgG (CWBIO, China) at 37°C for 60 min. After incubating secondary antibodies, the membranes were washed there times once

again using TBST. Finally, the color rendering was performed by using BeyoECL Star kit (Beyotime, China).

Analysis of epitope information

To determine the homology of the epitopes between different CDV strains, the amino acid sequences of the region encompassing the mAbs C8 and 1A5 truncated B-cell epitopes were aligned with other geographic lineages CDV strains which were available in the NCBI database (https://www.ncbi.nlm.nih.gov/protein/). The sequences of CDV genome containing the coding region information of all structural proteins of CDV were selected. Sequences are processed and edited by using the Lasergene program package (DNAStar, Madison, WI, USA).

Results

Virus isolation

A wild-type CDV isolate, named BT, was isolated from the lung tissue of the CDV infection dog and examined by EM for identified the morphology of virus particles. CDV particles with typical morphology were detected by Electron microscope (EM) (Figure 1). A virion of approximate 100-150 nm in diameter was observed in negative-stain preparations of Vero-dslam cell inoculated with the BT isolate.

IFA

The immunefluorescence assay results showed that both of the infected cells and uninfected cells can fluoresce green under 490 nm wavelength excitation light. The mAbs 1A5 could recognize with the isolate CDV-BT, while the C8 could not. Neither 1A5 nor C8 binds to uninfected cells (Figure 2).

Genomic amplification

The genome of CDV-BT strain could be amplified by 14 pairs of primers (Table 1). The fragment length of each product was in line with forecast, by 1% agarose gel electrophoresis assay (Figure 3). TA cloning and sequencing were performed for each fragment, and DNA Star Lasergene program was used to assemble the measured sequences. Finally, the whole genome sequence information of CDV-BT strain with a length of 15,690 bp was obtained.

Phylogenetic analysis

Firstly, 249 CDV H protein CDS representative of all the CDV lineages were selected from GenBank (data are given in Online Resource 1) for the phylogenetic analysis based on the CDV H protein CDS with the H

protein CDS of CDV-BT strain. Phocine distemper virus (PDV) H protein CDS (GenBank accession number: KU342692) was included for outgroup rooting. The analysis showed that CDV-BT classified into Asia-1 lineage (Figure 4), showing 97.3–99.3% similarity with other 38 sequences of the same lineage. Secondly, 84 CDV complete genome sequences, each of which was 15690 bp in length, were selected from GenBank (data are given in Online Resource 2), for the phylogenetic analysis based on the CDV complete genome with CDV-BT strain. The analysis showed that CDV-BT complete classified into Asia-1 lineage (Figure 5), showing 97.1–99.2% similarity with other 33 sequences of the same lineage.

WB

The WB results showed that the mAbs 1A5 reacted with CDV-BT and CDV-PS, while did not react with CDV3 (Figure 6A), the approximate size of the CDV H protein recognized by mAbs 1A5 is the same as the expected 78 kDa. The mAbs C8 reacted with CDV-PS and CDV3, while did not react with CDV-BT (Figure 6B), the approximate size of the CDV N protein recognized by mAbs C8 is the same as the expected 58 kDa. Both of the mAbs did not recognize uninfected Vero-dslam (Figure 6).

Epitope information analysis

In our previous studies, it has been confirmed that the epitope recognized by 1A5 is located between the amino acids ^{178}ARGDIFPPY^{186} in the H protein [2], and the epitope recognized by C8 is located between the amino acids ^{444}GDKYPIHFNDER^{455} in the N protein [24]. We selected 126 sequences of CDV genome containing the CDS of all structural proteins of CDV from GenBank (data are given in Online Resource 3) for the analysis. The statistical results showed that the peptide epitope recognized by mAb C8 is not conserved among Asia-1 lineage and different lineages, which has several substitutions in lineages Asia-1, Asia-2, Asia-4, Asia-5, America-1, America-2, America4, Arctic like, Caspian, East Africa, South Africa, Europe-1/South America-1 and European wildlife (Figure 7). The CDV-BT strain displayed substitutions at amino acid position 451 in N protein, which makes CDV-BT strain unable to be recognized by mAbs C8. The same amino acid substitution was found in three CDV strains (GenBank accession number: KP769803, KP793921, KP677502) isolated from giant pandas in 2014. But there is a P□S substitutions at amino acid position 457 in the three CDV strains isolated from giant pandas (Figure 7). The peptide epitope recognized by mAbs 1A5 is more conservative among Asia-1 lineage there are 94.6% (35/37) sequences of Asia-1 lineage have the same peptide epitope, but not conserved in all lineages. Besides, both of the epitopes recognized by mAbs 1A5 and C8 in CDV-BT are different from the Vaccine strains.

Discussion

Since the first isolation of CDV by Carré in 1905, CDV still has occured a challenge to domestic dogs, furry animals, and wild animals [3]. The host range of CDV includes all families in the order Carnivora and has recently been extended to non-human primates [26], moreover the host range still has the potential for

further expansion. CDV H protein is the main protein to stimulate the body to produce neutralizing antibodies. Meanwhile, as the glycoprotein acting on SLAM and nectin-4 receptors, H protein is also related to host tropism and pathogenicity of the virus [27]. Therefore, lots of researches and phylogenetic analysis on CDV are based on the analysis of H protein. With the development of sequencing technology, it is easier and cheaper to obtain the complete gene sequence information of a CDV strain at present. More information about the virus can be obtained from the full genome sequence, which enables us to obtain more accurate and comprehensive results in the phylogenetic and pathogenicity analysis of the virus.

In this research, a CDV strain named BT was isolated from a vaccinated domestic dog in Changchun, Northeast China, and identified by EM and IFA. The whole genome sequence of the virus was obtained by RT-PCR and Sanger sequencing. Phylogenetic analysis was performed based on the CDS of H protein and the complete genome sequence of the virus respectively. Both results showed that the CDV-BT strain classified into the Asia-1 lineage, The CDV-BT strain had the highest similarity with CDV-PS, and the H protein CDS and complete genome similarity of these two virus strains were 99.3 % and 99.2%, respectively. The CDV-PS strain was isolated from a domestic dog in Northeast China in our laboratory in 2010, which indicated that the sequence of CDV-BT strain is not significantly different from the PS strain that was prevalent in dogs in the region a decade ago. Then, two mAbs prepared in previous experiment in our laboratory were used to identify the reactivity between these two mAbs and the CDV strains BT,PS and CDV3 ,respectively. The results showed that mAbs 1A5 recognized CDV-BT and CDV-PS, but did not recognize CDV3. However, mAbs C8 recognized CDV-PS and CDV3, but did not recognize CDV-BT. CDV-BT and CDV3 showed completely opposite antigenicity in these two epitopes. This may be the antigenic drift of the BT strain in response to the immune stress of the vaccine, which causes the vaccinated dog to develop distemper. There are significant differences in the determination of neutralizing titers between CDV vaccine strain" America-1" lineage and "South America -4" lineage strain as reported recently[28]. Given those results and the fact that multiple recognized CDV cases have been recorded in vaccinated animals [10, 22, 29, 30]. For better understanding the antigenic differences between the multiple worldwide circulating lineages, performing the wider, updated antigenic analyses of CDV is necessary.

When researching on the CDV, we should not only focus on H protein, but also consider other structural proteins, such as N protein, F protein and so on.

In conclusion, we isolated a CDV strain named BT which is currently circulating in Northeast China, and sequenced the complete genome sequence. Phylogenetic analysis showed that the CDV-BT strain still classified into the Asia-1 lineage, and its antigenicity of the epitope ^{444}GDKYPIHFNDER^{455} in N protein and the epitope ^{178}ARGDIFPPY^{186} in H protein were significantly different from vaccine strain by amino acid substitutions. The characterization of genetic diversity among the circulating CDVs is essential for future CDV's research and disease monitoring. Investigation on the immunogenicity and pathogenicity of the CDV should be further explored.

Declarations

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Conflicts of interest/Competing interests:

No conflict of interest exists in the submission of this manuscript, and the manuscript has been approved by all authors for publication.

Availability of data and material:

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request

Code availability:

Not applicable

Authors' contributions:

Shipeng Cheng designed the study. Zhigang Cao, Yuening Cheng, performed the research. Zhigang Cao, Li Yi, Yuening Cheng and Jianke Wang analyzed data. Yuening Cheng, Pengfei Shi and Erkai Feng prepared the figures, tables and supplementary materials. Zhigang Cao wrote the manuscript.

Ethics approval:

This article does not contain any studies with animals performed by any of the authors.

Consent to participate:

Not applicable

Consent for publication:

Not applicable

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Table

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

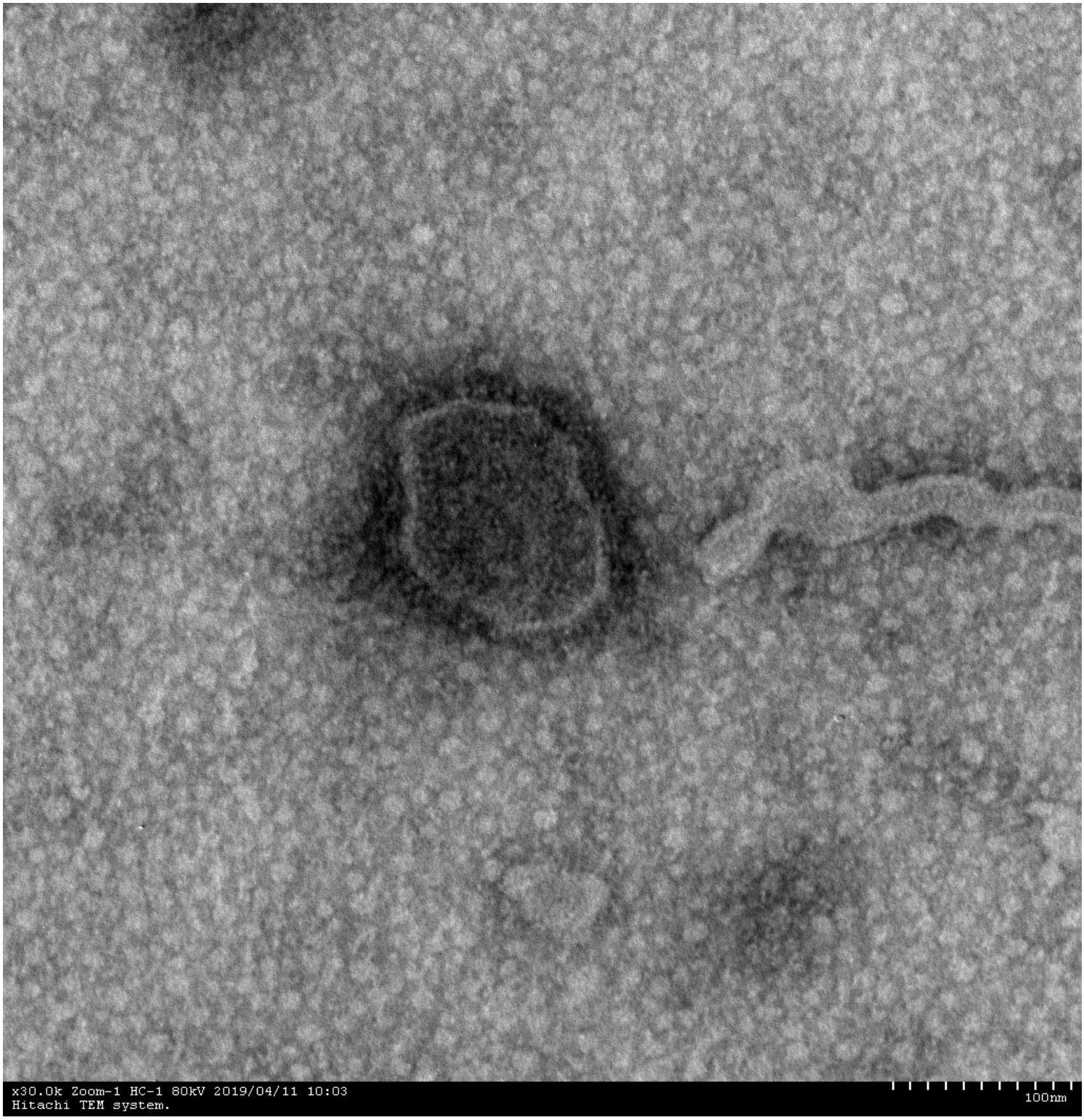


Figure 1

Identification of BT isolate by electron microscope The morphology of BT isolate under electron microscope (negative staining), the virion of approximate 100-150 nm in diameter was observed

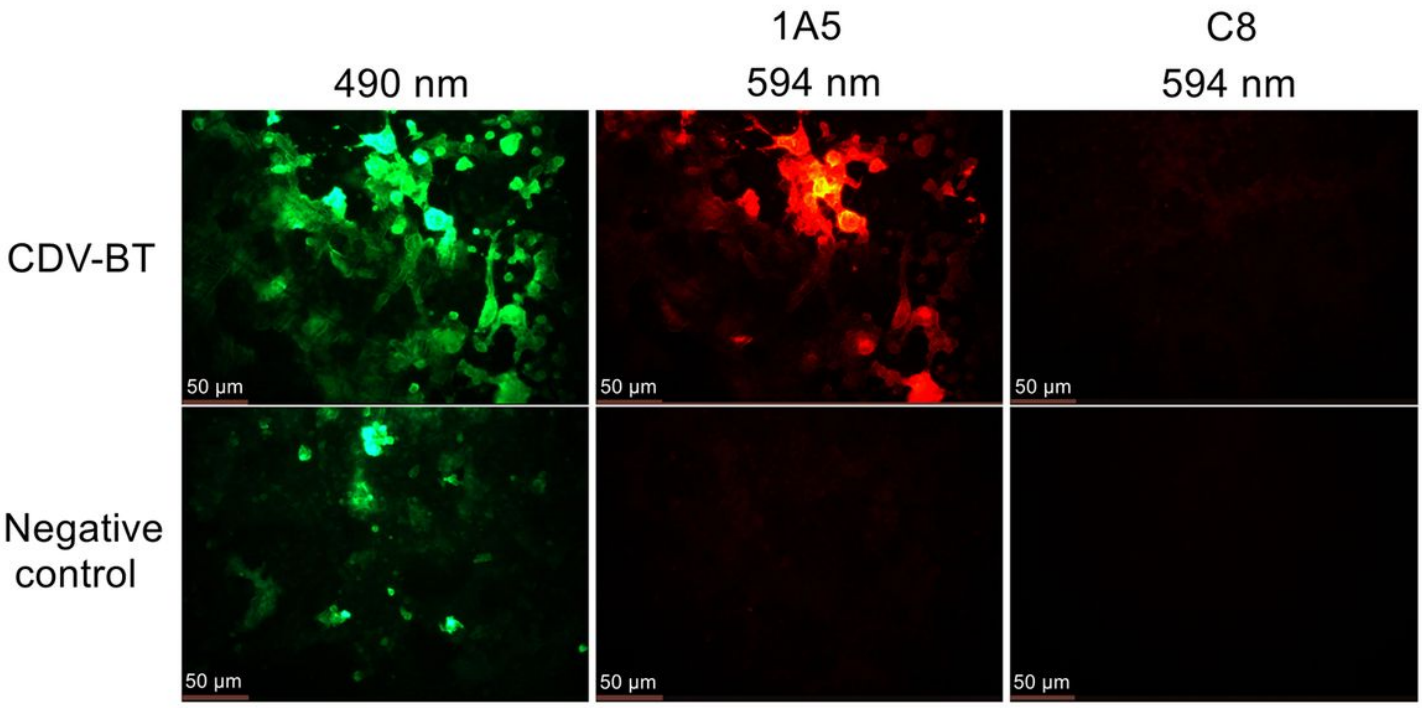


Figure 2

The IFA results Both of the infected cells and uninfected cells can fluoresce green under 490 nm wavelength excitation light. The mAbs 1A5 could recognize with the isolate CDV-BT, the infected cells can fluoresce red under 594 nm wavelength excitation light, while the C8 could not. Neither 1A5 nor C8 binds to uninfected cells.

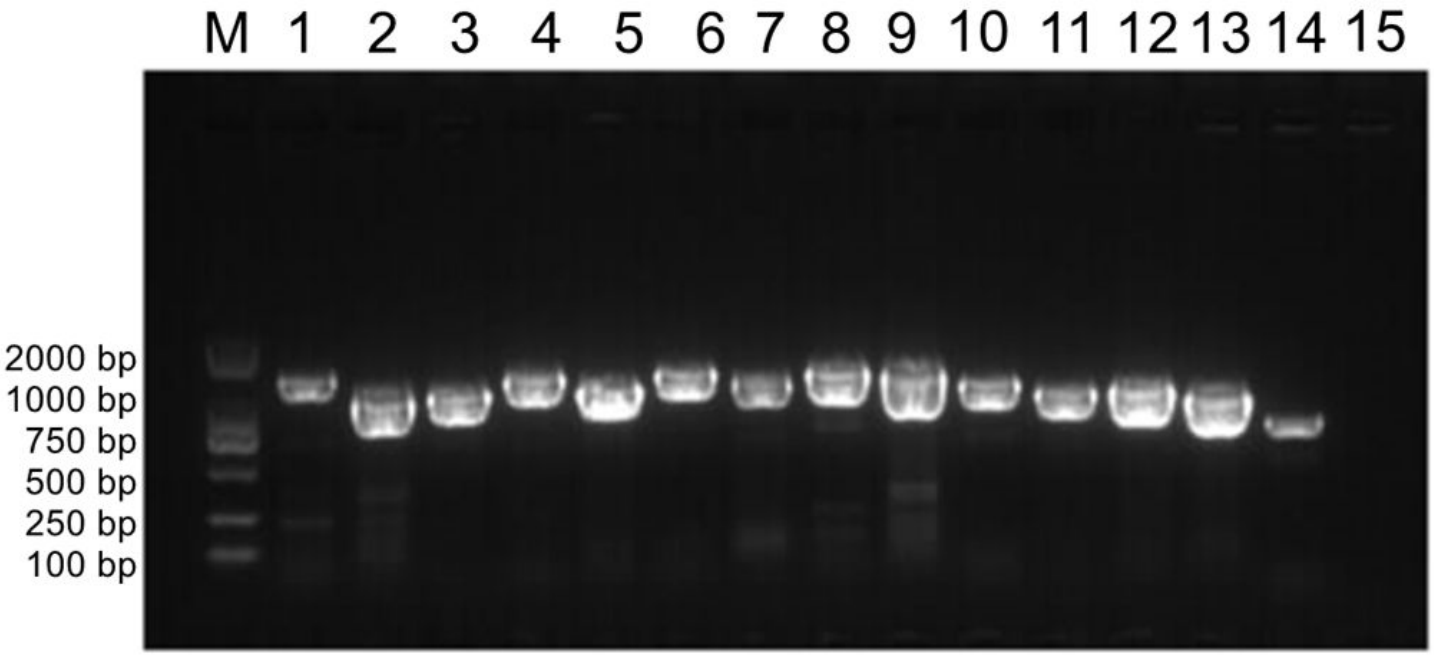


Figure 3

The Genomic amplification result Agarose gel electrophoresis (1.0%) of the CDV-BT Genomic amplified by 14 pairs of primers. Lane M: DNA marker; lane 1-14 represent the products of 1-14 pairs of primers respectively; lane 1: 1306 bp; lane 2: 1151 bp; lane 3: 1147 bp; lane 4: 1350 bp; lane 5: 1225 bp; lane 6: 1392 bp; lane 7: 1300 bp; lane 8: 1467 bp; lane 9: 1482 bp; lane 10: 1235 bp; lane 11: 1136 bp; lane 12: 1210 bp; lane 13: 1133 bp; lane 14: 797 bp; lane 15: Negative control.

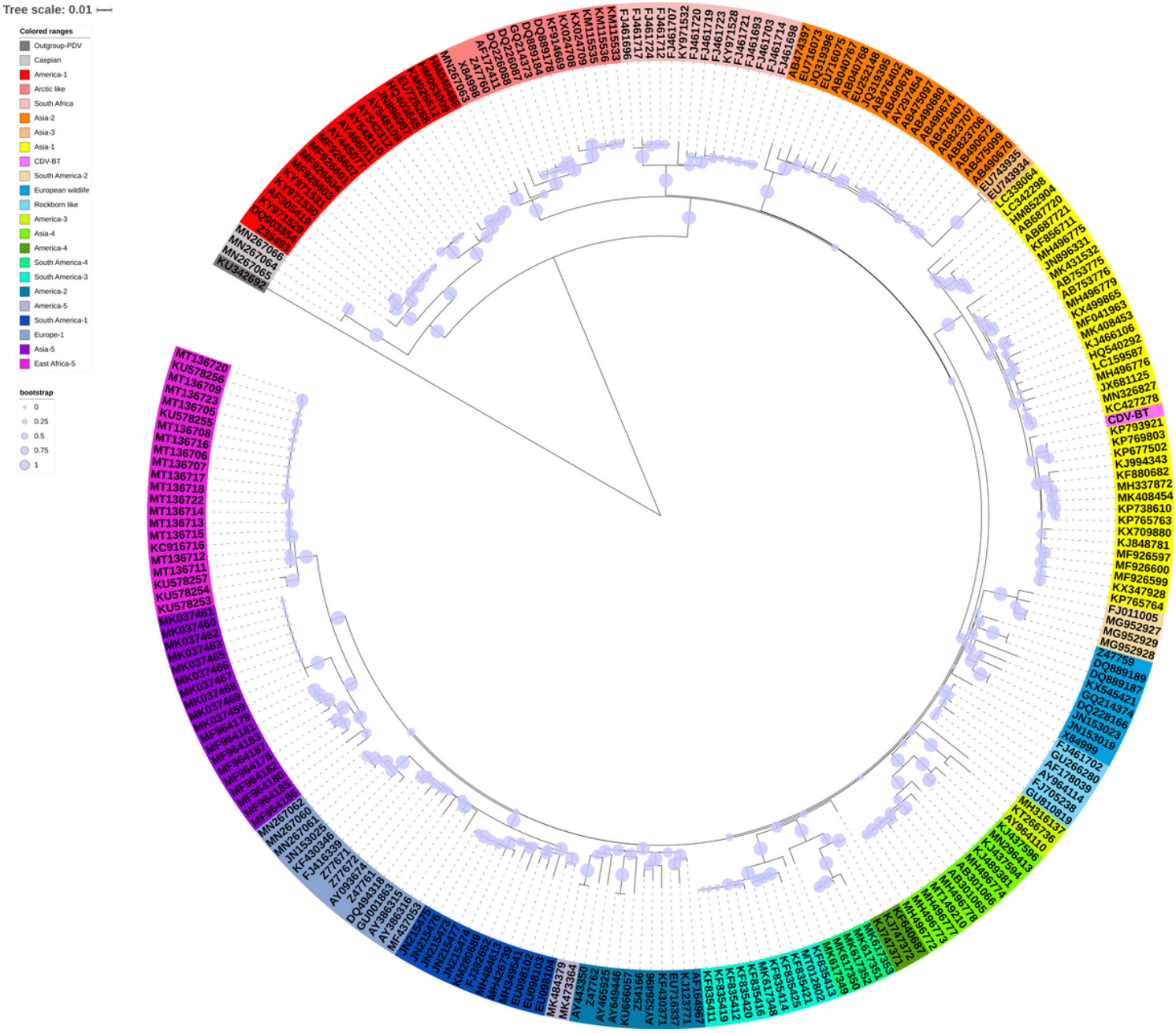


Figure 4

The Maximum-likelihood tree of CDV-H protein CDS. Maximum-likelihood tree of 250 complete CDV-H protein Coding sequences. H gene sequence of phocine distemper virus (GenBank accession no. KU_342692.1) was used as outgroup. Detailed information on sequences used can be found in Online Resource 1. Maximum-likelihood analyses were performed by general time reversible (GTR+G+I) model with 1000 bootstraps in MEGA 6.0

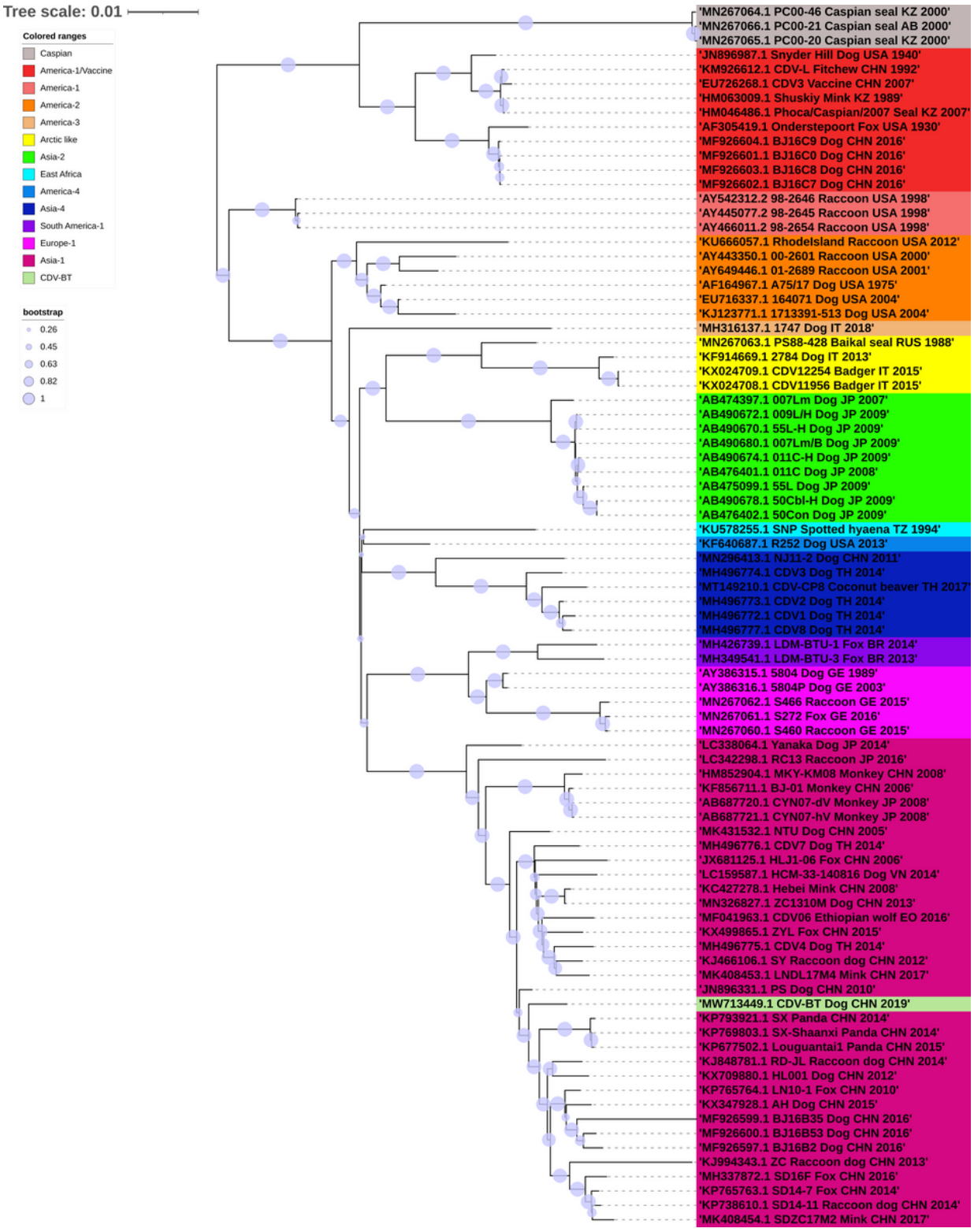


Figure 5

The Maximum-likelihood tree of CDV complete genonme sequence. Maximum-likelihood tree of 85 complete CDV complete genonme sequences. Detailed information on sequences used can be found in Online Resource 2. Maximum-likelihood analyses were performed by general time reversible (GTR+G+I) model with 1000 bootstraps in MEGA 6.0

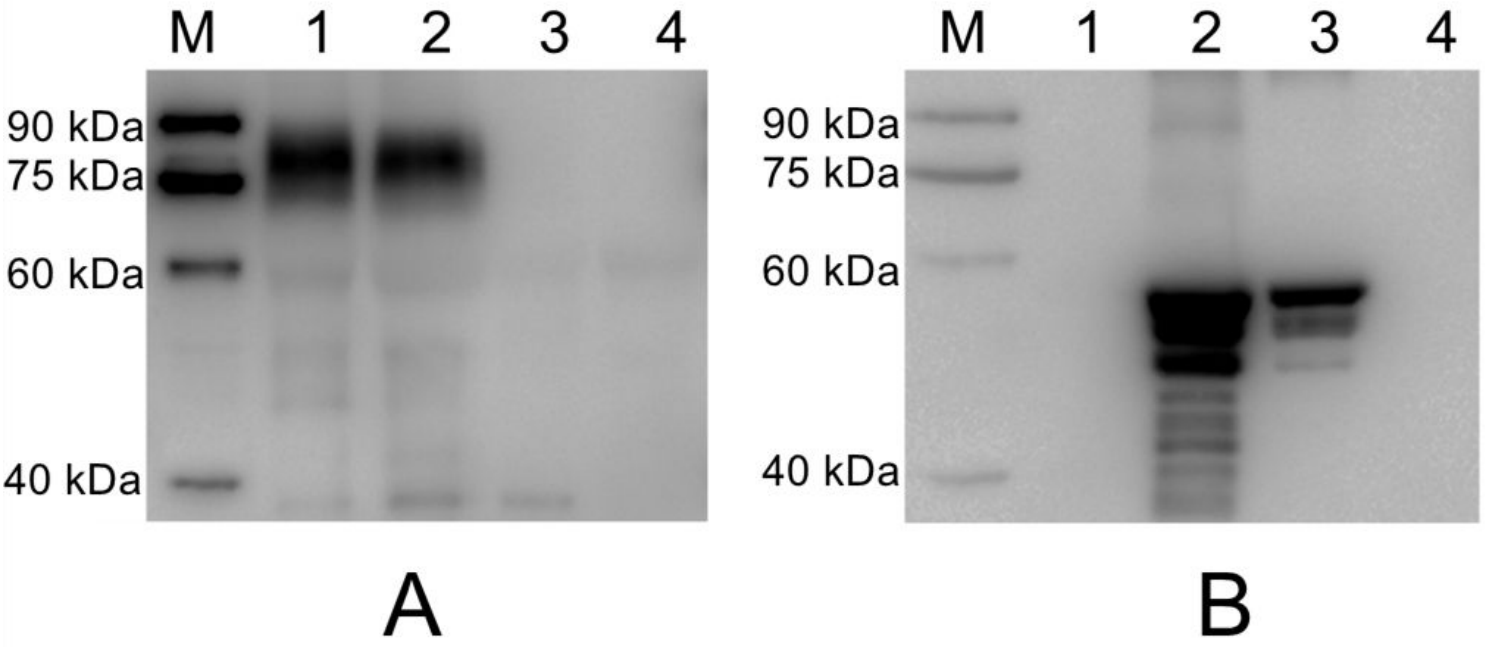
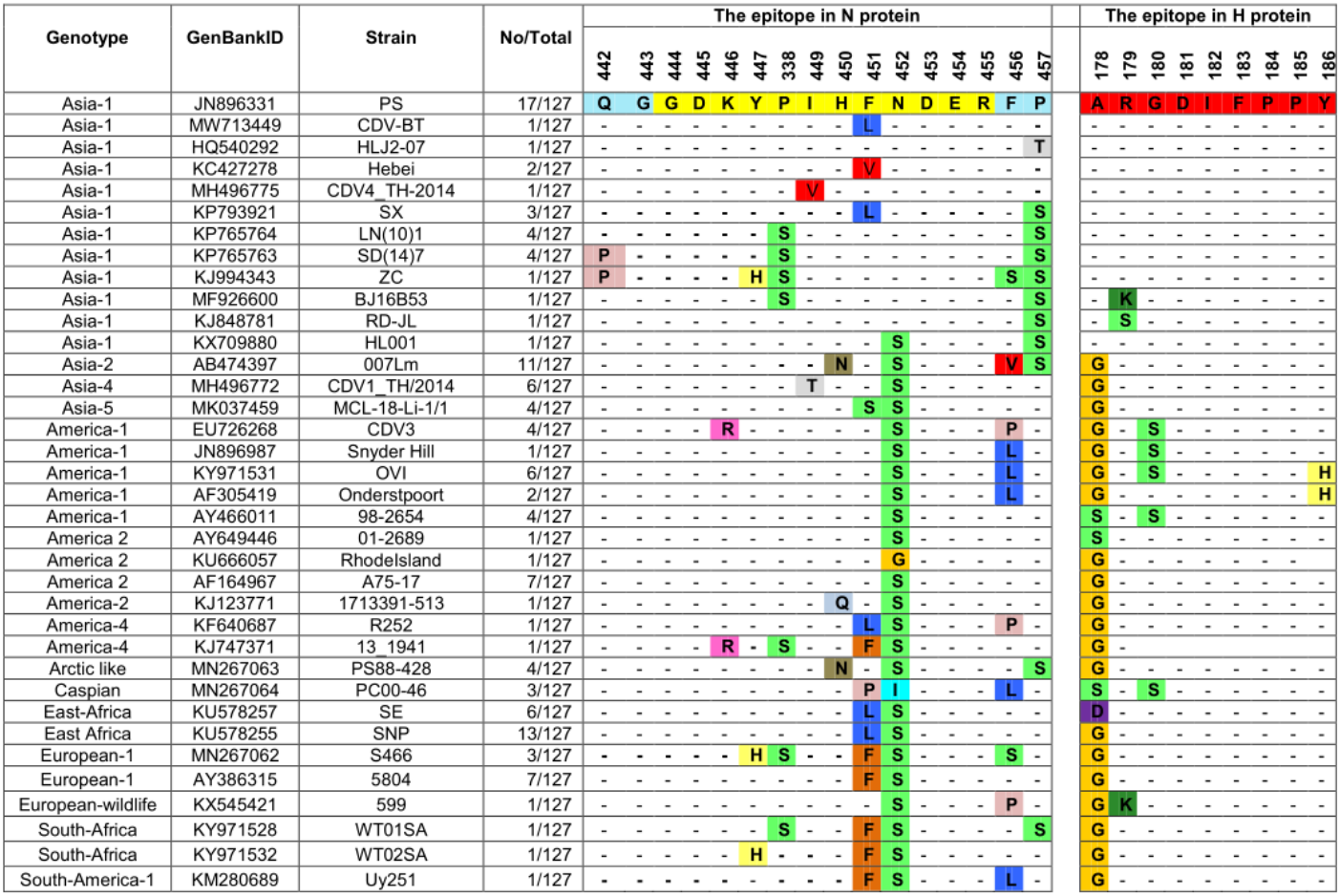


Figure 6

The WB results Figure 6A: The WB results of mAbs 1A5. Lane M: Protein marker; lane 1: CDV-BT; lane 2: CDV-PS; lane 3: CDV3; lane 4: Vero-dslam. Figure 6B: The WB results of mAbs C8. Lane M: Protein marker; lane 1: CDV-BT; lane 2: CDV-PS; lane 3: CDV3; lane 4: Vero-dslam. The WB results showed that the mAbs 1A5 reacted with CDV-BT and CDV-PS, while did not react with CDV3, the approximate size of the CDV H protein recognized by mAbs 1A5 is the same as the expected 78 kDa (Figure 6A). The mAbs C8 reacted with CDV-PS and CDV3, while did not react with CDV-BT, the approximate size of the CDV N protein recognized by mAbs C8 is the same as the expected 58 kDa (Figure 6B). Both of the mAbs did not recognize uninfected Vero-dslam.



Genotype	GenBankID	Strain	No/Total	The epitope in N protein																The epitope in H protein									
				442	443	444	445	446	447	338	449	450	451	452	453	454	455	456	457	178	179	180	181	182	183	184	185	186
Asia-1	JN896331	PS	17/127	Q	G	G	D	K	Y	P	I	H	F	N	D	E	R	F	P	A	R	G	D	I	F	P	P	Y
Asia-1	MW713449	CDV-BT	1/127	-	-	-	-	-	-	-	-	-	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Asia-1	HQ540292	HLJ2-07	1/127	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-
Asia-1	KC427278	Hebei	2/127	-	-	-	-	-	-	-	-	-	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Asia-1	MH496775	CDV4_TH-2014	1/127	-	-	-	-	-	-	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Asia-1	KP793921	SX	3/127	-	-	-	-	-	-	-	-	L	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-
Asia-1	KP765764	LN(10)1	4/127	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-
Asia-1	KP765763	SD(14)7	4/127	P	-	-	-	-	S	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-
Asia-1	KJ994343	ZC	1/127	P	-	-	-	H	S	-	-	-	-	-	-	-	-	S	S	-	-	-	-	-	-	-	-	-
Asia-1	MF926600	BJ16B53	1/127	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	S	-	K	-	-	-	-	-	-	-
Asia-1	KJ848781	RD-JL	1/127	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	-	S	-	-	-	-	-	-	-
Asia-1	KX709880	HL001	1/127	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-
Asia-2	AB474397	007Lm	11/127	-	-	-	-	-	-	-	N	-	S	-	-	-	V	S	G	-	-	-	-	-	-	-	-	-
Asia-4	MH496772	CDV1_TH/2014	6/127	-	-	-	-	-	-	T	-	-	S	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-
Asia-5	MK037459	MCL-18-Li-1/1	4/127	-	-	-	-	-	-	-	-	S	S	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-
America-1	EU726268	CDV3	4/127	-	-	-	R	-	-	-	-	-	S	-	-	-	P	-	G	-	S	-	-	-	-	-	-	-
America-1	JN896987	Snyder Hill	1/127	-	-	-	-	-	-	-	-	-	S	-	-	-	L	-	G	-	S	-	-	-	-	-	-	-
America-1	KY971531	OVI	6/127	-	-	-	-	-	-	-	-	-	S	-	-	-	L	-	G	-	S	-	-	-	-	-	-	H
America-1	AF305419	Onderstpoort	2/127	-	-	-	-	-	-	-	-	-	S	-	-	-	L	-	G	-	-	-	-	-	-	-	-	H
America-1	AY466011	98-2654	4/127	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	S	-	S	-	-	-	-	-	-	-
America 2	AY649446	01-2689	1/127	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-
America 2	KU666057	Rhodelsland	1/127	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	G	-	-	-	-	-	-	-	-	
America 2	AF164967	A75-17	7/127	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-
America-2	KJ123771	1713391-513	1/127	-	-	-	-	-	-	-	Q	-	S	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-
America-4	KF640687	R252	1/127	-	-	-	-	-	-	-	-	L	S	-	-	-	P	-	G	-	-	-	-	-	-	-	-	-
America-4	KJ747371	13_1941	1/127	-	-	-	R	-	S	-	-	F	S	-	-	-	-	-	G	-								
Arctic like	MN267063	PS88-428	4/127	-	-	-	-	-	-	-	N	-	S	-	-	-	-	S	G	-	-	-	-	-	-	-	-	-
Caspian	MN267064	PC00-46	3/127	-	-	-	-	-	-	-	-	P	I	-	-	-	L	-	S	-	S	-	-	-	-	-	-	-
East-Africa	KU578257	SE	6/127	-	-	-	-	-	-	-	-	L	S	-	-	-	-	-	D	-	-	-	-	-	-	-	-	-
East Africa	KU578255	SNP	13/127	-	-	-	-	-	-	-	-	L	S	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-
European-1	MN267062	S466	3/127	-	-	-	-	-	H	S	-	-	F	S	-	-	-	S	G	-	-	-	-	-	-	-	-	-
European-1	AY386315	5804	7/127	-	-	-	-	-	-	-	-	-	F	S	-	-	-	-	G	-	-	-	-	-	-	-	-	-
European-wildlife	KX545421	599	1/127	-	-	-	-	-	-	-	-	-	-	S	-	-	-	P	-	G	K	-	-	-	-	-	-	-
South-Africa	KY971528	WT01SA	1/127	-	-	-	-	-	-	S	-	-	F	S	-	-	-	-	S	G	-	-	-	-	-	-	-	-
South-Africa	KY971532	WT02SA	1/127	-	-	-	-	-	H	-	-	-	F	S	-	-	-	-	-	G	-	-	-	-	-	-	-	-
South-America-1	KM280689	Uy251	1/127	-	-	-	-	-	-	-	-	-	F	S	-	-	-	L	-	G	-	-	-	-	-	-	-	-

Figure 7

The statistical results of peptide epitope information in 127 CDV strains Conservation analysis of 444GDKYPIHFNDER455 in the N protein and 178ARGDIFPPY186 in the H protein among different CDV strains and the highlight indicates the difference.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.pdf
- ESM1.pdf
- ESM2.pdf
- ESM3.pdf
- cdvbtsequence.fasta