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Complete Genome Characterization of a Canine Distemper Virus Isolated from Chinese Raccoon Dog in China

Mingwei Tong, Li Yi and Shipeng Cheng
Institute of Special Economic Animal and Plant Science, CAAS, No. 4899, Juye Street, Changchun, 130122, China

Corresponding Author: Shipeng Cheng, Institute of Special Economic Animal and Plant Science, CAAS, No. 4899, Juye Street, Changchun, 130122, China Tel: +86-431-81919840 Fax: +86-431-81919840

ABSTRACT

In January 2013, several clinical signs of Chinese raccoon dog with diarrhea, cough, nasal discharge and fever were reported in Jilin province, China. One virus named CDV-RD-JL was isolated and identified. Complete genome of the virus is 13590nt in length and contains 6 genes including 3' leader sequence,the nucleocapsid gene (N), the phosphoprotein gene (P), the matrix protein gene (M), the fusion protein gene (F), Hemagglutinin protein gene (H), the large protein gene (L), 5' trailer sequence. Phylogenetic analysis of N, P, H and F gene demonstrated the virus belonged to Asia-1 and genetically related to the CDV strains CYN07-hV and CDV SY from China in 2010. This was the first confirmed isolation of CDV circulating in Chinese raccoon dogs.

Key words: *Canine distemper* virus, genome analysis, China

INTRODUCTION

Canine distemper virus (CDV) belongs to Paramyxoviridae, Paramyxovirinae and measles virus (*Morbillivirus*) which is an acute and highly contagious disease. Canine Distemper (CD) caused by canine distemper (Stettler *et al*., 1997). The disease can cause morbidity of a large number of dog, fox, raccoon, mink and other susceptible animals. The mortality rate is as high as 30~80% with the mortality rate of ferrets as 100% (Sakai *et al*., 2013; Terio and Craft, 2013).

CDV particle has capsule and its interior genome is coated with helical nucleocapsid protein (N). The genome is single-strand and unsegmented non overlapping negative chain RNA with size of about 15690 nucleotides (Lempp *et al*., 2014; Cunningham *et al*., 2009). Its coding genes from the genome of 3' end to 5' end are as follows: The 3' end of the leader sequence (3' leader sequence), the nucleocapsid protein gene (N), phosphoprotein gene (P), matrix membrane protein gene (M), fusion protein gene (F), blood coagulation protein gene (H) and large protein gene (L) these 6 non overlapping structure genes and 5' end trailer sequence (5' Trailer sequence).

In recent years, Canine distemper also often occurs in fur animal farms in various placed of China along with the development and growth of fur animal breeding industry in the northern China especially the northeast area which causes huge economic losses in fur animal breeding industry including Chinese raccoon dogs (Zhao *et al*., 2010). At present, it lacks of extensive investigation and research of molecular epidemiology for Canine distemper infection and epidemic in Chinese raccoon dogs. The genetic variation was expounded and the source of the parental strain isolated from Chinese raccoon dogs was traced by the analysis and comparison with the published main sequences of the CDV wild strain and vaccine strain.

MATERIALS AND METHODS

Total RNA preparation: Total RNA was extracted from the virus-infected Vero cells using QIAamp viral RNA mini kit (Qiagen) according to the manufacturer's instruction.

Primer pair and PCR amplification: To understand the genetic characteristics of the newly isolated CDV strains, nine pairs of primers covering the whole genome were designed according to reference CDV sequences from GenBank (Fig. 1). RT-PCR was carried out using mRNA as template.

The PCR reactions contained 2 μL DNA Template, 2 μL primer pairs, 2 μL dNTPs (TaKaRa, Dalian, China), 2.5 μL 10×Ex *Taq* buffer and 0.5 U Ex *Taq* polymerase (TaKaRa, Dalian, China) in a total volume of 25 μL. The PCR amplification was initiated by a pre-denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at temperatures 55°C for 30 sec and an extension at 72°C for 30 or 90 sec (depending on the amplified fragment length). Subsequently, the target fragment of PCR products were purified and cloned into pMD18-T vector (TaKaRa, Japan) and then transfected into *E. coli* DH5α cells. The positive clones were selected for sequencing by Shanghai Invitrogen Biotechnology Co., Ltd. The sequence assembly was carried out using the SeqMan program of the DNASTAR Software (Madison, WI).

Sequences alignment analysis: To investigate the differences between the published main sequences of the CDV wild strain and vaccine strain. Multiple sequence alignment was carried out using the software package MegAlign (DNASTAR).

Amino acid analysis: The potential N-linked glycosylation sites of the H protein were determined using the software NetNGlyc1.0. Phylogenetic trees were constructed based on the H, F, N and whole genome by the neighbor-joining method and a bootstrap analysis with 1000 replicates was done to assess the confidence level of the branch pattern. Bootstrap values >70% were considered to be significant.

RESULTS AND DISCUSSION

In the present study, 9 cDNA fragments covering genome and whole length genome sequence were obtained by using RT-PCR. The full-length genome sequence was obtained by cloning, sequencing and splicing of the 9 overlapping gene fragments. The sequence named CDV-RD-JL strains was submitted to GenBank and the accession number is KJ848781.

Sequence analysis results showed that the complete genome of CDV-RD-JL was 15690 nt and the 3' end leader sequence and the 5' end tailer sequence were composed of 52 and 38 nucleotides, respectively. The N, P, M, F, H and L gene sizes were 1683, 1655, 1447, 2209, 1946, 6642 nt. M

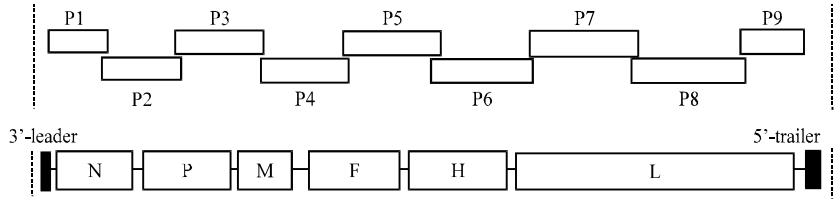

Fig. 1: Schema graph of nine pairs of primers covering the whole genome

and F genes were spaced by a length of about 400 bp non coding region. The recently research demonstrated that the spacer region played an important role in the regulation of F protein expression and control of virus virulence (Avila *et al*., 2014; Imhoff *et al*., 2007).

The sequence alignment analysis of the 15 different sources of CDV whole genomes deposited in the GenBank showed that the sequence homology of the genome sequences of the CDV-RD-JL strain and Asia-1 type CDV strains was as high as 98.1-98.8%. The genome homology of the CDV genomes in the present study with the other Genotype CDV strains was 92.9-97%. The genome homology of the CDV with Onderstepoort isolate was the lowest and genome homology of the CDV with HLJ1-06 and CDV SY in China were the highest. The identity values with CDV other reference sequences ranged from 93.2-98.9% for N gene, 93.8-99.0% for P gene, 90.6-98.4% for H gene and 90.6-98.1% for F gene, respectively (Table 1).

The CDV H protein N-linked glycosylation site prediction showed that there were 9 potential N-linked glycosylation sites in the CDV-RD-JL strain located in the amino acids 19-21, 149-151, 309-311, 391-393, 422-424, 456-458, 584-586, 587-589 and 603-605 (Table 2). Among them, six glycosylation sites (positions 19-21, 149-151, 391-393, 422-424, 456-458 and 587-590) were conserved for all of the CDV strains. The potential N-linked glycosylation sites number is the same as CDV Asian-1 strains in China such as strain HLJ1-06 and strain Hebei.

Phylogenetic trees were constructed based on the H, F, N and whole genome (Fig. 2). Results showed that the CDV strain CDV-RD-JL having highest identity with the strain HeBei (99.0%) originated from China in 2008, belonging to CDV Asia-1 type. The phylogenetic tree for the H, F and N regions had a topology similar to that of whole genome which clustered with CDV Asia-1 type.

With the rapid development of Fur-animal farm, the number of Chinese raccoon dog reached 8-15 million. Emergence of the CDV infection was more complex and need more attention to

Table 1: Nucleotide identity of CDV-RD-JL with reference sequences

				Nucleotide identity (%)				
Strains	Sequence accession	Genotype	Place	Genome	N	P	H	F
M25CR	AB475097	Asia-2	Japan	95.1	95.6	95.2	92.8	92.2
A75/17	AF164967	America-2	Switzerland	97.0	97.5	96.5	95.3	94.3
Onderstepoort	AF305419	America-1	USA	92.9	93.5	93.8	90.6	90.6
98-2654	AY466011	America-1	USA	95.4	96.8	95.5	91.3	92.8
CDV3	EU726268	America-1	China	93.2	93.2	93.9	91.1	90.9
Phoca/Caspian/2007	HM046486	Arctic-like	Kazakhstan	93.2	93.2	94.0	91.0	90.9
Shuskiy	HM063009	Arctic-like	Kazakhstan	93.3	93.3	94.0	91.0	91.0
HLJ1-06	HQ540293	Asia-1	China	98.8	98.7	99.0	98.2	97.9
Snyder Hill	JN896987	America-1	Canada	93.3	93.3	94.0	91.0	90.6
Hebei	KC427278	Asia-1	China	99.0	98.9	98.3	98.4	98.1
CDV2784/2013	KF914669	Arctic-like	Italy	94.7	95.7	95.2	92.6	92.4
171391-513	KJ123771	America-2	USA	96.2	96.9	96.0	94.5	93.6
CDV SY	KJ466106	Asia-1	China	98.8	98.7	98.6	98.2	97.4
CYN07-Hv	AB687721	Asia-1	Japan	98.1	97.7	97.7	97.5	97.1
007Lm-1vp	AB462810	Asia-2	Japan	95.2	96.0	ND	92.6	92.3

ND: Not determined

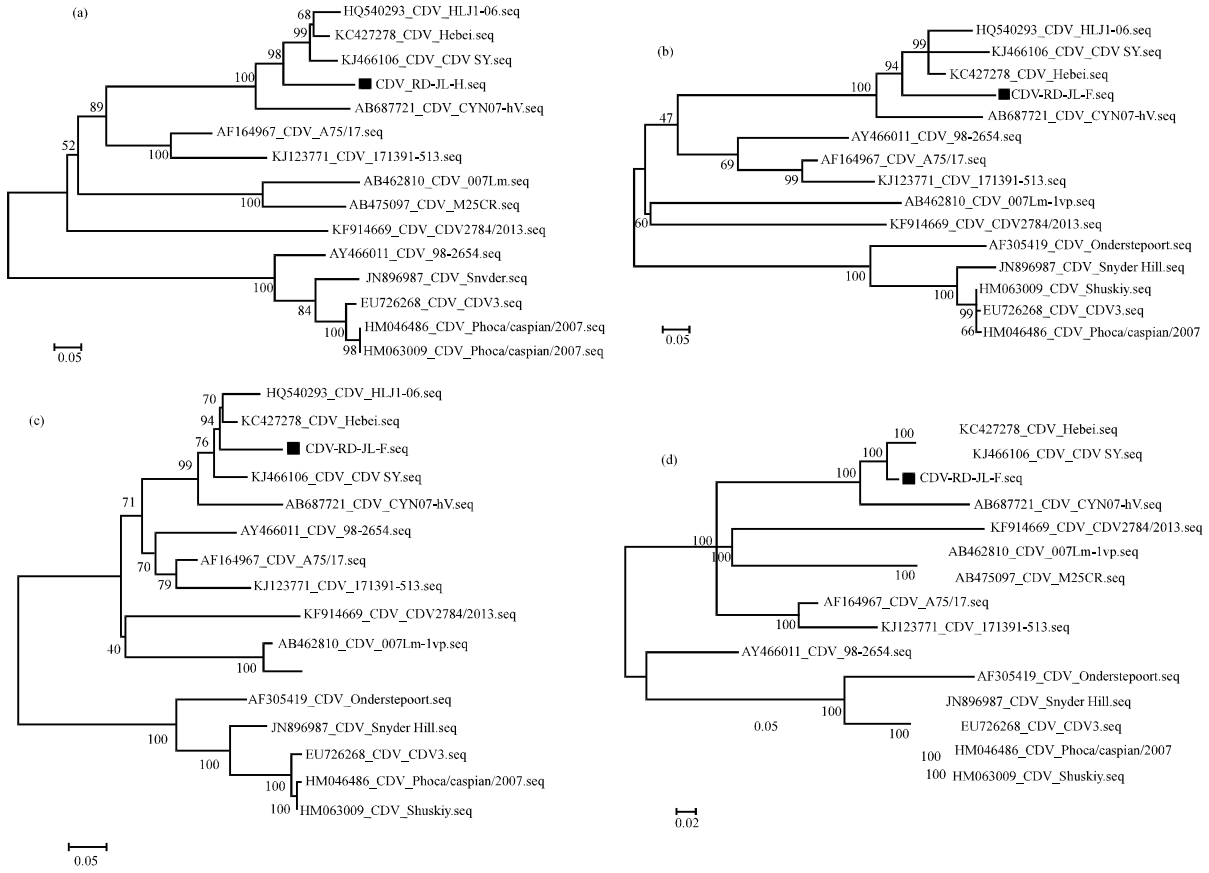

Fig. 2(a-d): Phylogenetic trees of (a) H, (b) F, (c) N and (d) Whole genome

Table 2: CDV H protein N- linked glycosylation site prediction

	Potential N-link glycosylation sites (N-X-S/T) in the amino acids of H protein								
Strains	No. of sites	19-21	149-151	309-311	391-393	422-424	456-458	584-586	587-589	603-605
CDV-RD-JL	9	N-S-S*	N-F-T*	N-G-S*	N-Q-T*	N-I-S*	N-G-T*	N-I-T*	N-S-T*	N-R-S*
M25CR	8	N-S-S*	N-F-T*	N-G-S*	N-Q-T*	N-I-S*	N-G-T*	D-I-T	N-S-T*	N-R-S*
A75/17	7	N-S-S*	N-F-T*	D-G-S	N-Q-T*	N-I-S*	N-G-T*	D-S-T	N-S-T*	N-R-S*
Onderstepoort	5	N-S-S*	N-F-T*	S-G-S	N-Q-A	N-I-S*	D-G-T	D-I-A	N-S-T*	N-R-S*
98-2654	7	N-P-S*	N-F-T*	R-G-S	N-Q-T*	N-I-S*	N-G-T*	N-I-A	N-S-T*	N-R-S*
CDV3	7	N-S-S*	N-F-T*	R-G-S	N-Q-T*	N-I-S*	N-G-T*	N-I-A	N-S-T*	N-R-S*
Phoca/Caspian/2007	7	N-S-S*	N-F-T*	R-G-S	N-Q-T*	N-I-S*	N-G-T*	N-I-A	N-S-T*	N-R-S*
Shuskiy	7	N-S-S*	N-F-T*	R-G-S	N-Q-T*	N-I-S*	N-G-T*	N-I-A	N-S-T*	N-R-S*
HLJ1-06	9	N-S-S*	N-F-T*	N-G-S*	N-Q-T*	N-I-S*	N-G-T*	N-I-T*	N-S-T*	N-R-S*
Snyder Hill	7	N-P-S*	N-F-T*	R-G-S	N-Q-T*	N-I-S*	N-G-T*	N-I-A	N-S-T*	N-R-S*
Hebei	9	N-S-S*	N-F-T*	N-G-S*	N-Q-T*	N-I-S*	N-G-T*	N-I-T*	N-S-T*	N-R-S*
CDV2784/2013	8	N-L-S*	N-F-T*	N-D-S*	N-Q-T*	N-I-S*	N-G-T*	D-I-T	N-S-T*	N-R-S*
171391-513	8	N-S-S*	N-F-T*	N-G-S*	N-Q-T*	N-I-S*	N-G-T*	D-S-T	N-S-T*	N-R-S*
CDV SY	9	N-S-S*	N-F-T*	N-G-S*	N-Q-T*	N-I-S*	N-G-T*	N-I-T*	N-S-T*	N-R-S*
CYN07-Hv	9	N-S-S*	N-F-T*	N-G-S*	N-R-T*	N-I-S*	N-G-T*	N-I-T*	N-S-T*	N-R-S*
007Lm-1vp	9	N-S-S*	N-F-T*	N-G-S*	N-Q-T*	N-I-S*	N-G-T*	D-I-T	N-S-T*	N-R-S*

*Potential N-link glycosylation sites (N-X-S/T), Among them, the 309-311 N-glycosylation site, which is specific for the wild-type strain, is boxed in red and the 584-586 N-glycosylation site, acquired in the Asian-1 strains

highlight the infection of CDV. This new finding give us a support to pay more attention to the CDV in China. It should take appropriate measures to control the disease and take a surveillance program in a larger Chinese raccoon dog population in vast geographical areas.

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REFERENCES

Avila, M., L. Alves, M. Khosravi, N. Ader-Ebert and F. Origgi *et al*., 2014. Molecular determinants defining the triggering range of prefusion f complexes of canine distemper virus. J. Virol., 88: 2951-2966.

Cunningham, M.W., D.B. Shindle, A.B. Allison, S.P. Terrell, D.G. Mead and M. Owen, 2009. Canine distemper epizootic in Everglades mink. J. Wildlife Dis., 45: 1150-1157.

Imhoff, H., V. von Messling, G. Herrler and L. Haas, 2007. Canine distemper virus infection requires cholesterol in the viral envelope. J. Virol., 81: 4158-4165.

Lempp, C., I. Spitzbarth, C. Puff, A. Cana and K. Kegler *et al*., 2014. New aspects of the pathogenesis of canine distemper leukoencephalitis. Viruses, 6: 2571-2601.

Sakai, K., T. Yoshikawa, F. Seki, S. Fukushi and M. Tahara *et al*., 2013. Canine distemper virus associated with a lethal outbreak in monkeys can readily adapt to use human receptors. J. Virol., 87: 7170-7175.

Stettler, M., K. Beck, A. Wagner, M. Vandevelde and A. Zurbriggen, 1997. Determinants of persistence in canine distemper viruses. Vet. Microbiol., 57: 83-93.

Terio, K.A. and M.E. Craft, 2013. Canine Distemper Virus (CDV) in another big cat: Should CDV be renamed carnivore distemper virus? mBio, Vol. 4, No. 5. 10.1128/mBio.00702-13

Zhao, J.J., X.J. Yan, X.L. Chai, V. Martella and G.L. Luo *et al*., 2010. Phylogenetic analysis of the haemagglutinin gene of canine distemper virus strains detected from breeding foxes, raccoon dogs and minks in China. Vet. Microbiol., 140: 34-42.