Veterinary Microbiology 216 (2018) 7–12

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Short communication

Emergence of novel canine parvovirus type 2 and its pathogenesis in raccoon dogs

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ARTICLE INFO

Keywords:
Canine parvovirus
Raccoon dog
Animal experiment

ABSTRACT

Three parvoviruses were isolated from the raccoon dogs experiencing severe enteritis, named RDPV-DP1, RDPV-DP2 and RDPV-DP3, respectively. The VP2 genes of the 3 isolates showed 99.9% identity at the nucleotide level, and shared 99.1%-99.5% identity with the reference CPVs. The RDPVs resembled original CPV-2, but with four mutations. The RDPVs displayed S297A of VP2 protein as CPV-2a or CPV-2b prevalent throughout most of the world. Residue N375D was found in the 3 isolates, resembling CPV-2a/2b/2c. And the 3 isolates had a natural mutation of VP2 residue V562L, which is adjacent to residue 564 and 568 and might be involved in host range. Interestingly, VP2 S27T was firstly found in the isolates. Phylogenetic analysis of VP2 genes revealed that the RDPVs were clustered into one small evolutionary branch and shared the identical branch with 7 CPV-2 isolates from raccoon dogs and one CPV-2 isolate from fox, not with CPV vaccine viruses. Phylogenetic analysis of NS1 genes demonstrated that the RDPVs shared the identical branch with the reference CPV-2a/2b/2c. Experimental infection showed that RDPV infection caused a high morbidity in raccoon dogs. It implied that the RDPV was virulent to raccoon dogs and continued to evolve in China.

1. Introduction

Canine parvovirus (CPV) is one of the most important enteric pathogens of dogs and very closely related to Feline panleukopenia virus (FPV), belongs to the genus Protoparvovirus, a member of the Parvoviridae family, which has been included within the species Carnivore protoparvovirus 1, according to the International Committee on Taxonomy of Viruses (Miranda and Thompson, 2016). In the 1970s, CPV emerged as a new pathogen of dogs, and spread globally in 1978 (Parrish et al., 1985), and was identified as CPV-2 to distinguish it from the distantly related minute virus of canine (MVC). CPV and FPV are over 98% identity at the nucleotide level, but show different biological characteristics such as the pH dependence of haemagglutination (HA) and host cell specificity *in vitro* and *in vivo* (Chang et al., 1992; Truyen et al., 1995). Several residues in VP2 protein have subsequent effects on the viral surface structure and can influence the antigenicity and host ranges of both CPV-2 and FPV (Chang et al., 1992; Truyen et al., 1995; Truyen et al., 1996; Parrish, 1999; Buonavoglia et al., 2001). Three substitutions of K93N, V103A and D323N between FPV and CPV-2 could introduce the canine host range (Chang et al., 1992; Truyen et al., 1995). The substitutions of K80R, N564S and A568G were associated with the loss of ability to replicate in cats (Truyen et al., 1994).

Since its emergence, CPV-2 has undergone a series of evolutionary selections in nature and the new variants are now circulating worldwide. CPV-2a had regained the ability to infect cats and became the most common virus in many other carnivores, which emerged in 1979 and differed in only five or six amino acids from CPV-2 (Parrish et al., 1991; Truyen et al., 1996). The feline host range of CPV-2a is most likely determined by VP2 M87L, A300G and D305Y. Furthermore, VP2 I101T, S297A and V555I occurred between original CPV-2 and CPV-2a (Tsao et al., 1991; Truyen et al., 1996; Miranda and Thompson, 2016). CPV-2b was first identified in 1984, and the substitution of VP2 N426D differentiates CPV-2b from CPV-2a (Parrish et al., 1991). In 2000, CPV-2c with VP2 D426E was reported in Italy (Buonavoglia et al., 2001). But the oldest CPV-2c strain was isolated in 1996 and had been circulating in Germany 4 years before its first detection in Italy (Decaro et al., 2007).

CPV-2a, CPV-2b and CPV-2c co-circulated in China (Zhang et al.,

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https://doi.org/10.1016/j.vetmic.2018.01.016
Received 29 September 2017; Received in revised form 22 January 2018; Accepted 22 January 2018

Table 1
Amino acid sequence variations in the VP2 proteins of the isolates and the reference viruses.

Isolates	Host	Country	Amino acid residues					Accession numbers
			27	297	375	426	562	
FPV	Jaguar	China	S	S	D	N	V	KX900570
CPV-2	Dog	USA	S	S	N	N	V	EU659116
CPV-2	Dog	China	S	S	D	N	L	FJ435344
CPV-2	Dog	China	S	S	D	N	L	FJ435342
CPV-2	Dog	China	S	S	D	N	L	FJ435348
CPV-2	Fox	China	S	S	D	N	L	GU392237
CPV-2	Raccoon dog	Finland	S	S	N	N	V	U22192
CPV-2	Raccoon dog	Finland	S	S	N	N	V	U22193
CPV-2	Raccoon dog	China	S	S	D	N	L	KJ194463
CPV-2	Raccoon dog	China	S	S	D	N	L	GU392241
CPV-2	Raccoon dog	China	S	S	D	N	L	GU392240
CPV-2	Raccoon dog	China	S	S	D	N	L	GU392244
CPV-2	Raccoon dog	China	S	S	D	N	L	GU392242
CPV-2	Raccoon dog	China	S	S	D	N	L	KJ170679
CPV-2	Raccoon dog	China	S	S	D	N	L	GU392239
RDPV-DP1	Raccoon dog	China	T	A	D	N	L	MF996332
RDPV-DP2	Raccoon dog	China	T	A	D	N	L	MF996333
RDPV-DP3	Raccoon dog	China	T	A	D	N	L	MF996334
CPV-2a	Dog	China	S	A	D	N	V	KF803615
CPV-2b	Dog	Italy	S	A	D	D	V	FJ005263
CPV-2c	Dog	Australia	S	A	D	E	V	KU508692

2010; Wang et al., 2016; Zhao et al., 2016; Zhou et al., 2016). Although CPV-2 viruses are no longer considered to be prevalent worldwide, CPV-2 viruses were detected in New Zealand, Japan, USA and China (Kapil et al., 2007; Zhang et al., 2010; Soma et al., 2013; Ohneiser et al., 2015). It was likely that the viruses detected in the puppies were CPV-2 vaccine viruses (Parrish et al., 1991). But here, we described the successful isolation of novel CPV-2 viruses from the raccoon dogs affected by enteritis in the Midwest Shandong province, China, in 2016. The objectives of the study were to clarify molecular characterization of the novel CPV-2 isolates, and whether experimental oral gavage infection of the raccoon dogs resulted in clinical signs and led to virus shedding.

2. Material and methods

2.1. Samples

In August 2016, the raccoon dogs inhabiting three raccoon dog farms in the Midwest Shandong province, China, experienced severe enteritis, including vomiting, haemorrhagic diarrhoea, depression, loss of appetite and dehydration. The raccoon dogs in the farms were all unvaccinated with CPV vaccine. Three intestinal samples from the raccoon dogs died of enteritis were collected from the three farms. The samples were transported on ice to the laboratory and stored frozen at −20 °C until further used. The study was approved by the Shandong Agricultural University's Animal Care and Use Committee and also complied with the European Union (EU) Animal Welfare legislation.

2.2. Virus isolation

DNA was extracted from the 3 intestinal samples by using the EasyPure Genomic DNA Kit (TransGen Biotech, China), respectively. The samples were detected by PCR using the primers VP2-P1 5′- GGA TGG GTG GAA ATC ACA GC-3′ and VP2-P2 5′- ATA ACC AAC CTC AGC TGG TC-3′. The PCR conditions were available upon request. The intestinal samples were all positive for VP2 gene by PCR. Virus isolation was performed. In brief, the intestinal samples were homogenized in phosphate-buffered saline solution supplemented with 2000 unit/ml penicillin and 2000 mg/ml streptomycin, immediately centrifuged at 5000 × *g* for 5 min to precipitate debris. Subsequently, the intestinal suspensions were filtered through a 0.22-μm Millipore filter (Millipore, Bedford, MA, U.S.A.) for virus isolation in Crandell-Rees feline kidney (CRFK) cells (Shandong center for animal disease control and prevention), respectively.

2.3. Nucleotide sequencing and phylogenetic analysis

DNA was extracted from the 3 intestinal samples by using the EasyPure Genomic DNA Kit (TransGen Biotech, China). PCR was used to amplify a 1755 bp segment of entire VP2 gene using the primers VP2-F 5′-ATGAGTGATGGAGCAGTTCAACCAG-3′ and VP2-R 5′-TTAATATA ATTTTCTAGGTGCTAG-3′, and a 2007 bp segment of entire NS1 gene using the primers NS1-F 5′-ATGTCTGGCAACCAGTATACTGAG-3′ and NS1-R 5′-TTAATCCAAGTCGTCTCGAAAATC-3′, as described previously (Fei-Fei et al., 2017). The PCR conditions were available upon request.

The PCR products were extracted from agarose gels, using a GenScript QuickClean gel extraction kit (GenScript, Piscataway, NJ, USA), and sequencing was performed in Sangon Biological (Shanghai) Co., Ltd (Shanghai, China). The DNA sequences were compiled and edited using the Lasergene sequence analysis software package (DNAStar, Madison, WI, USA). The sequences of VP2 and NS1 genes of the 3 isolates were submitted to GenBank, and were assigned GenBank accession number individually, accession numbers MF996332 to MF996337.

To more precisely investigate the genotype and genetic origin of the isolates, BLAST analyses (http://blast.ncbi.nlm.nih.gov/Blast.cgi) were used on each sequence to identify the related reference viruses and the nucleotide sequences of the reference viruses were retrieved from the GenBank database. The deduced amino acid sequences were also compared using DNASTAR software. Multiple sequence alignment was carried out by using CLUSTAL W. Phylogenetic trees were constructed using MEGA6.0 software by the neighbor-joining method and the maximum composite likelihood model was used to calculate distances between sequences. Bootstrap values were calculated on 1000 replicates of the alignments.

2.4. Raccoon dog pathogenesis experiments

To determine the pathogenicity of the RDPV, animal experiments were performed on 10 healthy raccoon dogs (2–3 months of age) that were negative for parvovirus antigen and anti-parvovirus antibody. The ten raccoon dogs were divided into 2 groups on average. RDPV-DP1 was titrated by 50% tissue culture infectious dose ($TCID_{50}$) assay in