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Original Research

Molecular characterization of feline panleukopenia virus isolated from mink and its pathogenesis in mink

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ABSTRACT

Six feline panleukopenia viruses (FPV) were detected in the intestinal samples from the 176 mink collected in China during 2015 to 2016, named MEV-SD1, MEV-SD2, MEV-SD3, MEV-SD4, MEV-SD5 and MEV-SD6. The VP2 genes of the isolates shared 98.9%–100% identity with the reference sequences. The substitution of residue V300A in VP2 protein differentiates the isolates from the reference MEVs, and A300 is a characteristic of FPV. Furthermore, phylogenetic analysis of VP2 genes indicated that the six isolates were clustered into the same branch of all the reference FPVs. The NS1 genes of the isolates shared 98.2%–100% identity with the reference sequences. The NS1 genes of the six isolates and the three reference FPVs formed one unique evolutionary branch. To clarify the pathogenicity of the isolates, animal experiments were performed on healthy mink, using MEV-SD1. As a result, the morbidity of the inoculated animals was 100% and the mortality was as high as 38.9%. It was implied that the FPV infection caused a high morbidity and mortality in mink and the inoculation dose had an effect on pathogenicity of MEV-SD1 in mink.

1. Introduction

Feline panleukopenia virus (FPV), mink enteritis virus (MEV) and canine parvovirus (CPV) are very closely related viruses, showing a genome identity of more than 98% (Mcmaster et al., 1981), and belong to the Protoparvovirus genus within the Parvovirinae subfamily of the Parvoviridae family of single-stranded DNA viruses. FPV-induced disease in cats has been known since the 1920s (Steinel et al., 2000) and can infect many species within the order Carnivora, including large and small cats, mink, raccoons and foxes (Steinel et al., 2001). MEV was first reported in southern Canada (Wills, 1952), and causes intestinal enteritis, myocarditis and lymphopenia in mink, especially in neonates and young mink (Uttenthal et al., 1990; Yuan et al., 2014). Whereas CPV-2 and its associated disease in dogs were recognized in the late 1970s and spread globally within a few months (Appel et al., 1979). CPV has undergone a series of evolutionary selections in nature which have resulted in the global distribution of new virus variants, including CPV-2a, CPV-2b and CPV-2c (Parrish et al., 1999; Buonavoglia et al., 2001 Buonavoglia et al., 2001). The viruses show different biological characteristics such as the pH dependence of haemagglutination (HA) and host cell specificity in vitro and in vivo. CPV can replicate in canine and feline cell lines, however, FPV and MEV do not replicate or replicate only poorly in canine cell lines such as A72 canine fibroma, MDCK and Cf2Th (Horiuchi et al., 1994).

FPV, CPV and MEV are linear, single-stranded DNA viruses with an average genome size of approximately 5000 bp, containing two large open reading frames (ORFs), one in the 5′ half and the other in the 3′ half of the genome, with encoding two nonstructural proteins (NS1 and NS2) and two capsid proteins (VP1 and VP2), respectively (Kariatsumari et al., 1991; Langeveld et al., 1995; Christensen and Tattersall, 2002). Several amino acid residues in VP2 protein influence the antigenicity and host range of both CPV-2 and FPV and have subsequent effects on the viral surface structure (Chang et al., 1992; Truyen et al., 1995, 1996; Langeveld et al., 1995; Parrish, 1999; Buonavoglia et al., 2001; Govindasamy et al., 2003). The amino acids N93, A103, and N323 are critical for CPV-2 replication in dogs, whereas K80, N564, and A568 are critical for FPV replication in cats. Amino acid differences at positions 87, 300, and 305 of VP2 protein distinguish CPV-2a and CPV-2b from CPV-2. The feline host range of the new antigenic types CPV-2a and CPV-2b is most likely determined by the

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amino acid changes M87L, A300G, and D305Y. The substitution of N426D differentiates CPV-2b from CPV-2a. In 2000, a new mutant with VP2 D426E, a strategic residue for the antigenicity of CPV, was reported in Italy and was designated CPV-2c (Buonavoglia et al., 2001).

MEV vaccines have been used with some success to prevent further spread of the viral disease with significant decreases in morbidity and mortality (Sun et al., 2013). However, due to the genetic variability of MEV, these vaccines are becoming increasingly inadequate (Sun et al., 2013). The objectives of the study were to clarify molecular characterization of the parvoviruses isolated from the mink in China, and whether experimental oral gavage infection of the mink results in clinical signs and leads to virus shedding.

2. Material and methods

2.1. Samples

During 2015–2016, the intestinal samples from 176 mink affected by enteritis at the age of 90–110 days were collected from seven mink farms throughout an east coast of China. The 176 mink were all vaccinated at the age of 50–60 days, using licensed inactivated cell-derived MEV vaccine in China. The samples were transported on ice to the laboratory and stored frozen at −20 °C until further use. 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 176 intestinal samples by using the EasyPure Genomic DNA Kit (TransGen Biotech, China). The samples were tested by PCR using the primers for FPV-like parvovirus, VP2-P1: 5′- CTTTGCCTCAATCTGAAGGAG-3′, VP2-P2: 5′- GAATTGGATTC-CAAGTATGAG-3′. The PCR conditions were available upon request. The 6 of the 176 intestinal samples were positive for VP2 gene by PCR and 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 feline kidney (CRFK) cells. The cells were cultured at 37 °C in a humidified 5% CO2 incubator in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal calf serum. A typical cytopathic effect of parvovirus infection was observed in the cell monolayer on day 4 postinoculation.

2.3. Nucleotide sequencing and phylogenetic analysis

DNA was extracted from the 6 intestinal samples by using the EasyPure Genomic DNA Kit (TransGen Biotech, China). PCR was used to amplify a 1755 bp segment of VP2 gene using primer pair VP2-F 5′-ATGAGTGATGGAGCAGTTCAACCAG-3′ and VP2-R 5′-TTAATATAAT-TTTCTAGGTGCTAG-3′, and a 2007 bp segment of NS1 gene using primer pair NSI-F 5′-ATGTCTGGCAACCAGTATACTGAG-3′ and NS1-R 5′-TTAATCCAAGTCGTCTCGAAAATC-3′. PCR conditions used 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 sequences of the VP2 and NS1 genes of the 6 isolates were submitted to GenBank, and were assigned GenBank accession number individually, accession numbers KY094112 to KY094117 and KY094120 to KY094125.

BLAST analyses (http://blast.ncbi.nlm.nih.gov/Blast.cgi) were used on each sequence to identify related reference viruses. To investigate more precisely the genotype and genetic origin, the DNA sequences were compiled and edited using the Lasergene sequence analysis software package (DNASTAR, Madison, WI, USA). 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. Mink pathogenesis experiments

To determine the pathogenicity of the isolates, animal experiments were performed on 42 healthy mink (2 months of age) which were negative for parvovirus antigen and anti-parvovirus antibody. Forty-two mink were divided into 7 groups on average. MEV-SD1 was titrated by 50% tissue culture infectious dose ($TCID_{50}$) assay (Yuan and Parrish, 2000) in CRFK cells. The mink in group 1 to 6 were lightly anesthetized with ketamine chloride (Ketalar, Parke-Davis) and were inoculated via oral gavage with $10^{6.0}$ $TCID_{50}$, $10^{5.0}TCID_{50}$, $10^{4.0}$ $TCID_{50}$, $10^{3.0}$ $TCID_{50}$, $10^{2.0}$ $TCID_{50}$ and $10^{1.0}$ $TCID_{50,}$ using MEV-SD1. Six mink in group 7 were inoculated via oral gavage with 0.9% NaCl solution, serving as the unchallenged group. The animals were housed individually and fed twice daily on a commercial meat-based diet, and water was freely available at all times. The animal experiments were performed in accordance with regulatory standards and guidelines approved by the Shandong Agricultural University's Animal Care and Use Committee, and the approved NO. is SDAUA-2015-002.

From postinfection (p.i.) onwards, clinical signs of the mink were monitored and scored daily for 15 days or until the inoculated mink died from MEV-SD1 infection. To determine virus shedding, rectal swabs were collected from the animals for 15 days and were confirmed by PCR as above. The tissue samples were collected from the mink either killed by MEV-SD1 infection or euthanized on days 15 after MEV-SD1 inoculation, including cerebrum, cerebellum, tonsil, retropharyngeal lymph node, thymus, lung, myocardium, bone marrow, liver, spleen, kidney, bladder, mesenteric lymph node, ileum, jejunum, colon and rectum. The samples were rapidly immersed in 10% neutral formalin buffer to prevent autolysis, and then processed into paraffin, sectioned at 4 um using the microtome Leica RM2235 (Leica Microsystems Ltd.), and stained with hematoxylin and eosin (HE) for the detection of histological lesions by light microscopy. Serum samples were collected on day 15 p.i. for serological testing using HI (Yuan and Parrish, 2000). The LD_{50} of MEV-SD1 in mink was titrated using Reed and Muench.

3. Results

3.1. Six parvoviruses isolated from the mink

In the study, six parvoviruses were isolated from the mink exhibiting diarrhea disease, named MEV-SD1, MEV-SD2, MEV-SD3, MEV-SD4, MEV-SD5 and MEV-SD6, respectively.

3.2. Molecular characterization of the six parvoviruses

The VP2 genes of the 6 isolates showed 99.8%–100% identity at the nucleotide level, which shared 98.9%–100% identity with the reference strains. Phylogenetic analysis of VP2 genes revealed that the isolates and the reference were clustered into 4 branches (Fig. 1). The isolates were clustered into one evolutionary branch and shared the identical branch with the reference FPVs. To identify the molecular characteristics of the isolates in detail, the deduced amino acid sequences of the VP2 proteins of the isolates were analyzed and aligned using DNASTAR software, and had 99.3%–100% similarity, which shared 98.1%-100% similarity with the reference strains. The mutations were shown in Table 1. The NS1 genes of the six isolates shared 99.9%–100% at the