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Short communication

Isolation of serotype 2 porcine teschovirus in China: Evidence of natural recombination

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ABSTRACT

Porcine teschovirus (PTV), the pathogen of porcine polioencephalomyelitis, is a member of the family Picornaviridae. In this study, a new PTV strain (designated as JF613) was isolated from pigs in China. It was confirmed by the specific CPE on susceptible cells, RT-PCR and nucleotide sequencing. Analysis of its amino acids sequence of complete polyprotein indicated that the isolate belongs to serotype 2. Genetic recombination is a well-known phenomenon for picornavirus which has been demonstrated in many other members of the family, but it remains so far unclear whether recombination occurs in PTV. To detect possible recombination events, 30 sequences of complete coding regions of PTV strains accessible in GenBank were examined. Putative recombinant sequence was identified with the use of SimPlot program. The result showed that the genomic sequence of our isolate exhibited highest similarities with strains of serotypes 2 and 5, respectively, in two crossover regions, suggesting the recombination event in PTV. Then the mosaic structure of viral genome was confirmed by bootscanning and genetic algorithm for recombination detection (GARD). This represents the first PTV-2 isolate in China. Furthermore, our study provided the first evidence of natural recombination in PTV and indicated that homologous recombination may be a driving force in PTV evolution.

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1. Introduction

Teschovirus encephalomyelitis (previously known as Teschen/Talfan diseases) is an acute disease of pigs characterized by central nervous system (CNS) disorders. This disease, caused by some serotypes of porcine teschovirus (PTV), was initially observed in Czechoslovakia in 1929 (Trefny, 1930). Although infections with PTV are most often asymptomatic, virulent strains can give rise to a wide range of clinical symptoms. During the 1940s and 1950s, the disease spread throughout Europe, followed by the United States, Canada, Australia and finally into Uganda and Madagascar, caused extensive losses to the swine industry.

PTV belongs to the genus teschovirus of family Picornaviridae. Similar to all Picornaviridae members, PTV contains a single-stranded, polyadenylated and positive-sense genomic RNA, with a VPg protein covalently linked to the 5′ end (Honda et al., 1990). Molecular mechanisms of picornavirus variation and evolution result from point mutations and genomic rearrangements, in particular recombination (Agol, 1997; Agol et al., 1999),

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which plays a significant role in the evolution by creating genetic variation, by reducing mutational load, and by creating viruses with new properties. For example, the frequent recovery of recombinant isolates of poliovirus that result from recombination involving vaccine strains shows that recombination has the potential to produce "escape mutants" in nature as well as in experiments (Georgescu et al., 1994). Recombination is also a major determinant of viral virulence, being implicated in the emergence of new viral strains (Worobey and Holmes, 1999). For members of Picornaviridae, besides poliovirus, recombination has been demonstrated in foot-and-mouth disease virus (FMDV) (Carrillo et al., 2005; Mohapatra et al., 2008) and human enteroviruses (HEV) (Huang et al., 2008). Recent reports described the recombination within human parechoviruses (HPeV) (Benschop et al., 2008; Zoll et al., 2009). To date, there was no evidence for recombination in PTV, although the possibility had been considered (Zell et al., 2001).

Comparison of nucleotide sequences of variants recovered from different individuals and geographical regions has revealed the existence of at least eleven distinct serotypes (Fauquet et al., 2005; Zell et al., 2001). Currently, there is only one Chinese PTV strain has been documented, which belongs to serotype 1 (Feng et al., 2007). In this paper, we report the isolation of a PTV-2 strain in China and, furthermore, provide the evidence of natural recombination in PTV.

2. Materials and methods

2.1. Virus isolation

In Jul 2009, some pigs in a commercial pig farm in Heilongjiang Province showed symptoms of pyrexia, diarrhea, respiratory distress and nervous disorders (difficulty in standing, recumbent and hindlimb paralysis), suggesting the possible infection of PTV. To isolate the pathogen, brain samples of the diseased piglets were collected and homogenized in phosphate-buffered saline (PBS) containing antibiotics (0.1 mg/ml of streptomycin and 100 IU/ml of penicillin). Tissue suspension was harvested by refrigerated centrifugation at 12,000 × *g* for 20 min and supernatants were then passed through 0.45 μm filters (Millex, Millipore). Subsequently 300 μl of filtrates was inoculated onto IBRS-2 cell monolayers cultured in Dulbecco's modified Eagle's medium (PAA, Somerst, UK) plus 8% fetal bovine serum (PAA). Cells were kept at 37 °C for 3 h to allow for virus adsorption, after that serum-containing medium was replaced by fresh medium. The cells were observed daily for cytopathic effect (CPE). After 3 passages, when CPE reached 80%, the culture media were collected as viral stocks and kept at −80 °C until further manipulation.

2.2. RNA extraction, RT-PCR and nucleotide sequencing

Briefly, viral RNA was directly extracted from both brain samples and CPE-inducing cells using commercially available RNA extraction kit (Qiagen, Chatsworth, CA) according to the manufacturer's instructions. Reverse transcription (RT) was carried out under standard conditions using random 9-mers. PCR was performed in a total volume of 25 μl with previously published primers to confirm the presence of PTV in tissue and CPE-inducing cells (Kaku et al., 2001; Palmquist et al., 2002). Once the PCR results were found positive by electrophoresis analysis, four synthetic oligonucleotide primer pairs (A, C, D and E) were designed to amplify partial sequences of the viral genome. The sequences of the primers are listed in Table 1. Amplification products were purified using Gel Extraction Kits (Qiagen, Inc.) and cloned into pMD18-T vector (TaKaRa, Dalian). The positive clones were sequenced in both directions by a commercial service (Invitrogen). Three further sets of primers (F, G and H) were designed based on the sequences that had been obtained to close gaps between the original PCR products (Table 1).

2.3. Sequence comparison and phylogenetic tree construction

The nucleotide sequences were compiled from the sequence contigs employing the SeqMan II program of

Table 1
List of primers utilized in this study.

Primer name	Nucleotide sequence (5′–3′)	Purpose	Reference
P1-F	GTGGCGACAGGGTACAGAAGAG	PTV genome detection	Palmquist et al. (2002)
P1-R	GGCCAGCCGCGACCCTGTCAG		
P2-F	ATGCTGCTGAGAATGCTG	PTV genome detection	Kaku et al. (2001)
P2-R	ACATCAGTGCCTTGAATG		
A-F	TGGAAGCTAGGTACTTGTTACGC	Sequencing	This study
A-R	TTCTGGCTTGTACCAGTGCTTRT		
C-F	ACACTGAAYCTGCTDTATGTTCC	Sequencing	This study
C-R	GTGTCTTGCCCAAGATCATCAAT		
D-F	CACCYGGTCAAGGCAAGAGTGT	Sequencing	This study
D-R	GACACAGCCTTCATAAGTGTTC		
E-F	GTTCAGCAAGTTCAGGCCATATT	Sequencing	This study
E-R	ACTACTTAGGCAACAGGCAG		
F-F	GACAGATAAGCACTGGT	Sequencing	This study
F-R	ACAGGTGTCTGATTTGGYTTCC		
G-F	CAGTTGAGAGCATTGG	Sequencing	This study
G-R	ACTCTTGCCTTGACCAG		
H-F	ACTCACTGCAGCTACC	Sequencing	This study
H-R	TTAGGCAACAGGCAG		

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