LETTER TO THE EDITOR

Segment 2 sequences analysis of genogroup II picobirnavirus in pig stool in China

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Picobirnaviruses (PBVs) were initially discovered in the fecal specimens in humans during investigation of children gastroenteritis causative agent in Brazil in 1988 (1). PBVs are found in the feces from rats, rabbits, simian and others, thus, PBVs appear to infect a very extensive range of hosts (2, 3, 4).

PBVs belong to the family Picobirnaviridae, which are a group of small, non-enveloped viruses measuring 35–40 nm in diameter with bi-segmented dsRNA genome. The larger genomic segment, or segment 1 ranges from 2.2 to 2.7 kbp, encoding for the capsid protein, and the smaller one, or segment 2 ranging from 1.2 to 1.9 kbp, encoding for the RNA-dependent RNA polymerase (RdRp) (5). PBVs are classified into genogroup I (prototype 1-CHN-97) and II (prototype 4-GA-91) according to the segment 2 genome (6, 7, 8).

Here, we amplified nearly complete segment 2 nucleotide sequences of a genogroup II picobirnavirus strain from swine fecal specimen using 4 sets of primers (Tab.1) designed according to picobirnavirus 4-GA-91 strain (prototype) available in GenBank (AF246940).

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Fecal specimens were resuspended in PBS (0.01 M phosphate, pH 7.2–7.4) to make 20% (w/v) solution for RNA extraction and centrifuged at 12,000 × g for 5 min at room temperature. Total RNA was extracted from 300 µl supernatant by using TRIZOL reagent (Invitrogen), according to the manufacturer's instructions. The final precipitate was resuspended in 25 µl RNase-free water and stored in -20°C.

cDNA synthesis was performed using PrimeScript Reverse transcriptase kit (TaKaRa) according to the protocol established by the manufacturer. The amplicons were analyzed by 1% agarose gel electrophoresis in TAE buffer, followed by staining with ethidium bromide (0.5l g/ml) and visualised under UV light.

The amplicons were purified by AxyPrep DNA gel extraction kit (Axygen), cloned into pMD-18T vector (TaKaRa), and sequenced (Invitrogen). The sequences obtained in this study were analyzed with the MegAlign software (DNAStar), and sequence splicing was performed using SeqMan (DNAStar). The segment 2 nucleotide sequences of a genogroup II porcine picobirnavirus CYZ-II-1 strain was deposited in GenBank Acc. No. KP984805.

The phylogenetic relationships of the PBV CYZ-II-1 strain in this study and the reference strains were assessed by employing the neighbor-joining distance algorithm in MEGA5.1. Bootstrap values of >50% were indicated for the corresponding nodes based on a bootstrapping with 1,000 replicates.

Based on the published sequences of picobirnavirus 4-GA-91 (AF246940), nearly complete segment 2 nucleotide sequences of genogroup II picobirnavirus were obtained by using specific primers from pig stool and named as CYZ-II-1. The nucleotide sequence of CYZ-II-1 strain was 1591nt long and contained one ORF (from 19 nt to 1572 nt) encoding a putative RNA-dependent RNA polymerase (RdRp) of 518 amino acids (aa). The base composition of
nearly complete segment 2 nucleotide sequence of CYZ-II-1 was found to be 28.72% A, 24.45% G, 26.27% U and 20.25% C. Segment 2 sequences of CYZ-II-1 strain obtained in this study and the other known reference strains, were found to share the highest nucleotide sequence identities (97.7%) with the genogroup II prototype 4-GA-91, while the RdRp shared 97.9% amino acid identities. To determine genetic relationships with reported PBVs, the CYZ-II-1 strain was used to construct the phylogenetic tree (Fig. 1) on the basis of RdRp coding region. Results confirmed that porcine picobirnavirus CYZ-II-1 strain belonged to genogroup II and closely clustered with human strain of picobirnavirus 4-GA-91 (AF246940). Genetic relationships of genogroup II picobirnaviruses between pigs and humans suggests crosspieces transmission of PBVs.

Since the discovery of picobirnavirus in 1988, PBVs are a group of worldwide distributed viruses that infect a broad host range and wide genetic diversity (9, 10, 11). Picobirnaviruses have been found in humans and animals that are healthy or with diarrhea, and in samples with or without the presence of other known enteric pathogens, so it is difficult to establish an etiological relationship between PBV and diarrheic syndrome. Picobirnaviruses can be also detected in fecal specimens during the course of different pathological situations in the host.

To our best knowledge, the high genetic diversity of genogroup I PBVs is caused by high variety of host species. However, in this study, the CYZ-II-1 strain was found to share 97.7% nucleotide homology with the genogroup II prototype 4-GA-91. Genogroup II PBVs were conservative between humans and pigs (12). Analysis of genetic relationship suggests crosspieces transmission of genogroup II PBVs between pigs and humans. These findings suggest the potential danger of locally acquired infection. Therefore, the prevalence and spread of picobirnaviruses should be monitored diligently.

References