LETTER TO THE EDITOR

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

M. CHEN, H. SUN, X. HUA*

Shanghai Key Laboratory of Veterinary Biotechnology, School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai 200240, P. R. China

Received October, 16, 2018; accepted January 18, 2019

Keywords: picobirnavirus; RdRp; pig; China

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).

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

*Corresponding author. E-mail: hxg@sjtu.edu.cn; phone:+86-21-34206149.

Abbreviations: PBVs = picobirnaviruses; RdRp= RNA-dependent RNA polymerase

Table1. Primers used to amplify segment 2 nucleotide sequences of picobirnavirus CYZ-II-1

Primer	Nucleotide sequence	Position	Length (nt)
AFsense70F	5'TTCGAAAGGAGGTTTACTATG3'	70	698
AF767R	5'GTTATGGAGGATTGCCATTC3'	767	
AFsense683F	5'CGG TAT GGA TGT TTC3'	683	369
AFantisense1051R	5'AAG CGA GCC CAT GTA3'	1051	
AF sense953F	5'AGTATTGGCCTAAACTCTATCA3'	953	361
AF antisense1313R	5'GCTCCGAAAACCCCTCCGGC3'	1313	
AF sense1201F	5'GGACTGCCTGCTAATCCGACAA3'	1201	460
AF antisense1660R	5'AACCCCACAACCCGCCTGATT3'	1660	

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 cross-pieces transmission of PBVs.

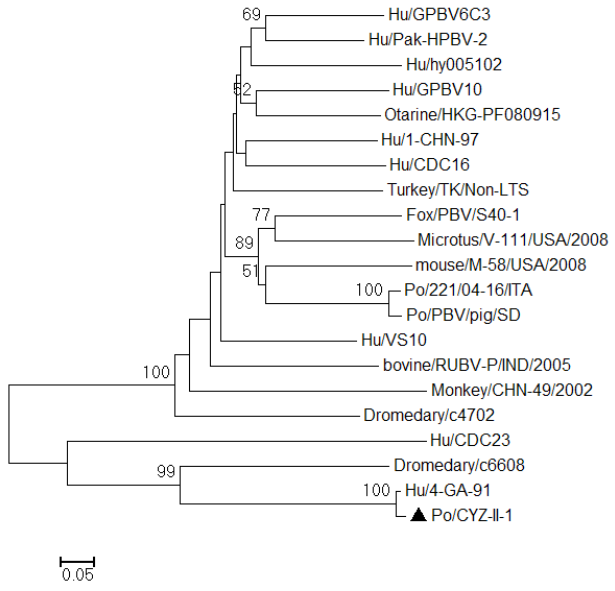

Fig.1

Phylogenetic analysis of PBV CYZ-II-1 based on the RdRp coding region

The "Po" or "Hu" prefix of strain indicates porcine or human origin, respectively. ▲: picobirnavirus obtained in our study.

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 suggestes 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

1. Pereira HG, Fialho AM, Flewett TH, Teixeira JMS, Andrade ZP, Lancet 103–104, 1988. https://doi.org/10.1016/S0140-6736(88)90032-3
2. Pereira HG, Flewett TH, Candeias JAN, Barth OM, J. Gen. Virol. 2749–2754, 1998.
3. Ludert JE, Abdul-Latiff L, Liprandi A, Liprandi F, Res. Vet. Sci. 222–225, 1995. https://doi.org/10.1016/0034-5288(95)90006-3
4. Wang Y, Tu X, Humphrey C, McClure H, Jiang X, Qin C, Glass RI, Jiang B, J. Med. Primatol. 101–107, 2007. https://doi.org/10.1111/j.1600-0684.2006.00167.x
5. Gatti MS, de Castro AF, Ferraz MM, et al., Res. Vet. Sci. 397–398, 1989. https://doi.org/10.1016/S0034-5288(18)31269-4

6. Rosen BI, Fang ZY, Glass R I, Monroe SS, Virology 316–329, 2000. https://doi.org/10.1006/viro.2000.0594

7. Banyai K, Jakab F, Reuter G, Bene J, Uj M, Melegh B, Szucs G, Arch. Virol. 2281–2291, 2003. https://doi.org/10.1007/s00705-003-0200-z

8. Gallimore CI, Appleton H, Lewis D, Green J, Brown DW, J. Med. Virol. 135–140, 1995. https://doi.org/10.1002/jmv.1890450204

9. Woo PC, Teng JL, Bai R, Wong AY, Martelli P, Hui SW., et al., Front. Microbiol. 1886, 2016.

10. Gallagher CA, Navarro R, Cruz K, Aung MS, Ng A, Bajak E., et al., Virus. Res. 13–18, 2017. https://doi.org/10.1016/j.virusres.2016.12.021

11. Navarro R, Yibin C, Nair R, Peda A, Aung MS, Ketzis J. et al., Infect. Genet. Evol. 200–204, 2017. https://doi.org/10.1016/j.meegid.2017.07.006

12. Chen ML, Sun H, Lan DL, Hua XG, Cui L, Yuan CL, et al., Virus Genes 553–556, 2014. https://doi.org/10.1007/s11262-014-1058-8