Virologica Sinica (2019) 34:471–473
https://doi.org/10.1007/s12250-019-00108-1

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www.springer.com/12250

LETTER

Genomic Characterization of the First Parechovirus in Bats

Chang Zhang[1,2] · Jinliang Sheng[3] · Zihan Zhao[2] · Chao Yan[2] · Changchun Tu[1,2,4] · Biao He[2,4]

Received: 15 January 2019 / Accepted: 5 March 2019 / Published online: 5 April 2019

Dear Editor,

Parechoviruses (PeVs) are non-enveloped, spherical viruses of genus *Parechovirus* and family *Picornaviridae*. Within the capsid is a naked monopartite, linear, single-stranded positive-sense RNA genome of 7.3 kb, comprising a single long open reading frame (ORF) encoding a polyprotein containing regions P1, P2, and P3. The P1 region encodes three structural proteins (VP0, VP3, and VP1); P2 and P3, non-structural proteins (P2 encoding proteins 2A, 2B, and 2C, and P3 encoding proteins 3A, 3B, 3C, and 3D) (ICTV 2018). PeVs are classified into four species: *Parechovirus A*, is composed of human parechoviruses (HPeVs) identified worldwide, causing gastrointestinal or respiratory diseases and being implicated in myocarditis and encephalitis (Harvala and Simmonds 2009); *Parechovirus B* and *C* have been reported in rodents, including Ljungan viruses (LVs) in bank voles and Sebokele virus (SEBV) in African wood mice (Niklasson *et al.* 1999; Joffret *et al.* 2013); *Parechovirus D* comprises a single virus, ferret parechovirus (FPeV), reported through metagenomics studies on healthy household ferrets in the Netherlands (Smits *et al.* 2013).

As important hosts for several pathogenic viruses, bats harbor viruses from almost all families of vertebrate viruses, including several genera of family *Picornaviridae*, including *Hepatovirus*, *Kobuvirus*, *Crohivirus*, and *Sapelovirus* (Drexler *et al.* 2015; Wu *et al.* 2016; Yinda *et al.* 2017). However, PeVs were not reported in bats until our recent viral metagenomic analysis of 122 adult healthy bats (*Pipistrellus pipistrellus*) obtained from two locations in Xinjiang (Xinyuan, n = 46; Qapqal, n = 76) in 2016, revealing thousands of reads related to PeV (Zhang *et al.* 2018). PCR-based screening revealed that 6.5% and 10.5% bats from Xinyuan and Qapqal, respectively, harbored this virus, and preliminary phylogenetic analysis of 396-nt-long amplicons targeting the VP1 region (GenBank accession numbers: MH921430–MH921443) revealed > 91.5% identities among each other and 63.7%–64.2% identity with their closest phylogenetic neighbor, FPeV (Smits *et al.* 2013; Zhang *et al.* 2018). This study reports the complete genomic characterization of the first bat PeV to better understand its evolutionary history.

One of the bats harboring the PeV from Qapqal was selected for whole-genome amplification and sequencing, wherein the gut sample contained a single, not multiple PeVs, and this viral isolate was named bat PeV QAPp32 (BtPeV QAPp32). PCR primer pairs were designed using Primer Premier 5.0 from the consensus sequences of contigs and FPeV (Supplementary Table S1). The gut was homogenized with SM buffer (50 mmol/L Tris, 10 mmol/L $MgSO_4$, and 0.1 mol/L NaCl; pH 7.5). After centrifugation at 8000 $\times g$ for 10 min at 4 °C, 200 μL of the supernatant was subjected to RNA extraction using the QIAamp RNA Mini Kit (Qiagen, Hilden, Germany) and reverse-transcribed using Reverse Transcription Kit (TaKaRa, Dalian, China) in accordance with the manufacturer's instructions. cDNA thus obtained was amplified using the LA PCR kit (TaKaRa) under the following cycling conditions: 35 cycles (outer PCR) or 40 cycles (inner PCR) of denaturation at 94 °C for 30 s, annealing at 56 °C (or adjusted in accordance with primer pairs) for 30 s, and extension at

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12250-019-00108-1) contains supplementary material, which is available to authorized users.

Chang Zhang and Jinliang Sheng have contributed equally to this work.

✉ Biao He
heb-001001@163.com

1 College of Animal Science, Fujian A&F University, Fuzhou 350002, China

2 Key Laboratory of Jilin Province for Zoonosis Prevention and Control, Military Veterinary Institute, Academy of Military Medical Sciences, Changchun 130122, China

3 College of Animal Science and Technology, Shihezi University, Shihezi 832003, China

4 Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonosis, Yangzhou 225009, China

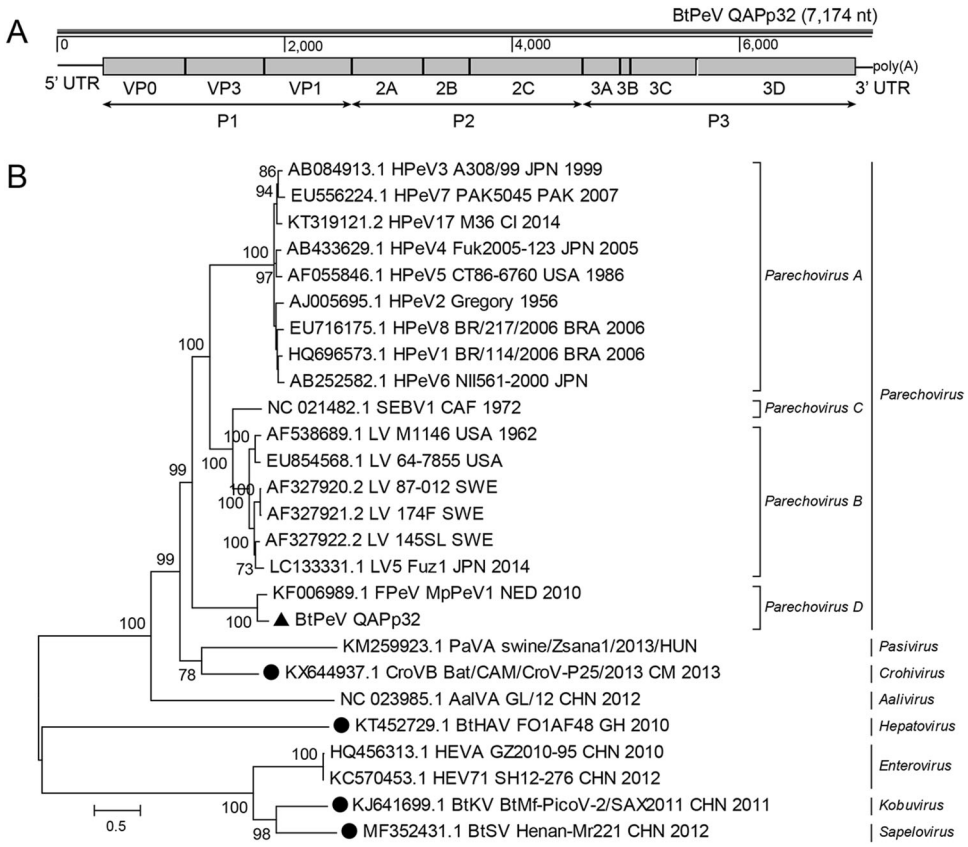

Fig. 1 **A** A schematic representation of the genome structure of QAPp32. Boxes represent the open reading frames encoding structural proteins (P1) and nonstructural proteins (P2, P3). **B** The maximum-likelihood phylogenetic tree for QAPp32 (filled black triangle) with other prototypical members of family *Picornaviridae*, based on entire polyprotein sequences, wherein other bat viruses are indicated by filled black circles.

72 °C for 1 min, with double-distilled water replacing cDNA as the negative control. Expected products of ~ 1500 nt were directly sequenced using an ABI 3730 Sanger sequencer (Comate, Changchun, China), and their 5′ and 3′ terminal sequences were determined using Rapid Amplification of cDNA Ends (RACE) Kits (TaKaRa) in accordance with the manufacturer's instructions.

The genome of BtPeV QAPp32 (GenBank accession number: MK348056) was determined to be 7174-nt-long, organized as a typical PeV genome (Fig. 1A). It contains a 343-nt-long 5′ untranslated region (UTR), followed by a 6624-nt-long ORF and a 178-nt-long 3′ UTR (Fig. 1A). This ORF encodes a 2207-aa polyprotein further divided into a 730-aa-long P1, 676-aa-long P2, and 801-aa-long P3 regions. Genomic differences between BtPeV QAPp32 and other PeV prototypes are summarized in Table 1. The BtPeV QAPp32 ORF has the same size as that of FPeV; however, it is larger than that of HPeV and smaller than that of LV and SEBV (Table 1), displaying the highest identity with FPeV (72.4% in nt and 79.3% in aa) and ≤ 51.3% with other PeVs (Table 1). To determine its phylogenetic associations, the polyprotein aa sequence of BtPeV QAPp32 was aligned with that of its other counterparts, using ClustalW, available in MEGA6, and a phylogenetic tree was constructed using the maximum-likelihood method with 1000 bootstrap replicates with the best substitution model. The phylogenetic tree showed that those PeVs constituted an independent branch from other picornaviruses such as pasivirus and hepatovirus and further clustered into 4 clades, corresponding to 4 species, and BtPeV QAPp32 clustered nearly together with FPeV within the clade of *Parechovirus D* (Fig. 1B).

Homogenates of 4 PeV-positive gut tissues were inoculated onto Vero E6, MDCK, and PK-WRL cells in an attempt to culture the viruses; however, after five passages, all cells were normal and RT-PCR analysis did not detect the virus.

BtPeV QAPp32 is a variant and a new member of species *Parechovirus D*, since its aa identity with FPeV was significantly higher than the species criterion (70%) proposed by International Committee on Taxonomy of Viruses (ICTV 2018). We previously reported that this virus is prevalent in bats sampled from Xinyuan and Qapqal, which are 170 km apart, and this virus was detected in the lung and gut tissues, suggesting that this virus replicates in these organs in *P. pipistrellus* and is hence unlikely to be transmitted from other animals to bats (Zhang *et al.* 2018).

Table 1 Comparison of amino acid and nucleotide sequence identity between QAPp32 and other representative parechoviruses.

ORF/region	QAPp32		HPeVNII561-2000 (AB252582)				LV 87-012G (EF202833)				SEBV (NC021482)				FPeVNED 2010 (KF006989)			
	nt	aa	nt	%	aa	%	nt	%	aa	%	nt	%	aa	%	nt	%	aa	%
VP0	726	242	867	42.2	289	34.3	777	48.7	259	39.9	777	49.9	259	39.0	726	**70.0**	242	**77.3**
VP3	693	231	756	46.4	252	38.3	732	51.2	244	44.3	729	48.5	243	41.0	693	**68.9**	231	**81.0**
VP1	771	257	702	44.0	234	34.6	951	41.3	317	28.7	930	41.8	310	31.5	771	**70.8**	257	**74.0**
P1	2190	730	2325	45.6	775	35.3	2460	46.6	820	36.9	2436	46.3	812	36.5	2190	**69.9**	730	**77.3**
2A	627	209	450	37.4	150	28.0	405	35.6	135	31.1	402	34.0	134	30.6	627	**75.6**	209	**68.6**
2B	408	136	366	47.9	122	36.0	414	54.5	138	41.8	411	48.1	137	40.1	408	**72.5**	136	**80.3**
2C	993	331	987	53.2	329	41.7	999	55.1	333	44.7	999	52.7	333	43.7	993	**74.0**	331	**83.7**
P2	2028	676	1803	47.7	601	36.8	1818	49.7	606	40.3	1812	46.3	604	39.4	2028	**74.5**	676	**78.7**
3A	330	110	351	40.8	117	22.0	390	39.5	130	15.4	372	43.0	124	21.0	330	**66.4**	110	**71.8**
3B	90	30	60	34.4	20	37.5	87	49.5	29	34.4	87	53.3	29	43.3	90	**74.4**	30	**90.0**
3C	582	194	600	48.8	200	36.3	594	50.4	198	38.9	582	50.9	194	36.6	582	**75.4**	194	**86.6**
3D	1401	467	1407	51.5	469	41.7	1410	52.9	470	44.4	1410	52.3	470	43.7	1401	**72.9**	467	**80.7**
P3	2403	801	2418	49.4	806	37.0	2481	50.1	827	38.6	2451	50.5	817	38.8	2403	**72.6**	801	**81.3**
Polyprotein	6624	2207	6549	47.8	2182	36.5	6762	51.3	2253	39.6	6702	50.3	2233	39.4	6624	**72.4**	2207	**79.3**

The highest identities are indicated in bold.

nt, nt length; %, identity; aa, aa length.

Acknowledgements This work was supported by the NSFC-Xinjiang joint fund (U1503283).

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Animal and Human Rights Statement The sampling of bats was approved by the Administrative Committee on Animal Welfare of the Military Veterinary Institute, Academy of Military Medical Sciences, China (Laboratory Animal Care and Use Committee Authorization, Permit No. JSY-DW-2015-01).

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