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BRIEF REPORT

Identification of a novel orthohepadnavirus in pomona roundleaf bats in China

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Abstract Bats in Myanmar, Gabon, and Panama have been found to harbor diverse hepadnaviruses. Here, we report a novel hepadnavirus in 4 of 20 pomona roundleaf bats from Yunnan province, China. This virus contains 3,278 nucleotides (nt) in the full circularized genome, with four predicted open frames (ORFs) reading in the same direction. Full genomic sequence comparisons and evolutionary analysis indicate that this virus is a member of a new species within the genus *Orthohepadnavirus*

Keywords Bat · Orthohepadnavirus · Genetic diversity

Introduction

Bats are important reservoir animals, harboring pathogenic zoonotic viruses including henipaviruses, lyssaviruses, and SARS-like coronaviruses [1]. The number of novel viruses identified in bats has been significantly increasing due to extensive studies by next-generation sequencing over the last few years [7]. Since the identification of a novel bat orthohepadnavirus (BtHV) from *Miniopterus spp.* in Myanmar [3], further similar viruses have been found in *Uroderma spp.* in Panama and *Rhinolophus spp.* and *Hipposideros spp.* in Gabon [2]. We report here another BtHV identified in bats in Yunnan province of China.

In 2011, 42 bats were collected in Pu'er city in Yunnan province from bats of three species: *Hipposideros pomona* (n = 20), *Rhinolophus affinis* (n = 20), and *Rhinolophus sinicus* (n = 2). 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 number JSY-DW-2010-02). All organs were pooled and subjected to viral metagenomic analysis as per our published method [4]. Results revealed 1,436 reads relating to orthohepadnaviruses, with an average length of 147 nucleotides (nt) and showing 68-74 % nt sequence identity to a *Hipposideros* BtHV from Gabon in its partial S and C genes. All liver samples were screened by PCR for orthohepadnavirus as described previously [3]. The results showed that 20 % (4/20) of pomona roundleaf bats (*Hipposideros pomona*) were positive, with all amplicons sharing >99 % nt sequence identity. All remaining bats were negative.

Three of the positives were randomly selected for full genomic amplification and sequencing. the results showed that their full genomic sequences (BtHV PEPR6, PEPR7 and PEPR13, GenBank accession numbers: KF939648-KF939650) were >99 % identical at the nucleotide level, with the same size of 3,278 nt; i.e., slightly larger than the genome of BtHV found in Myanmar (3230 nt) [3]. The genomic organizations were identical to those of other orthohepadnaviruses,

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containing four open reading frames (ORFs) encoding Pol, preS1/preS2/S, preC/C and X proteins in a circular and compact configuration. Orthohepadnaviral genomic characteristics, such as a highly conserved YMDD motif [6] and encapsidation signal ε [5], were also found in the PEPR genomes and were essentially identical to those of other orthohepadnaviruses. Predicted genomic structures and sequence comparisons are summarized in Table 1. The results showed that PEPR shared the highest nt and amino acid (aa) sequence identities in the *Pol*-, *preS1/preS2/S*-, and *X* genes with strain HBHBV identified in *Hipposideros spp.* from Gabon [2], while the *preC/C* gene of PEPR alone showed highest nt and aa sequence identity with isolate 776, a representative of Miniopterus BtHV in Myanmar [3].

To determine the phylogenetic relationship of PEPR to other orthohepadnaviruses, *Pol* genes from all known members of the family *Hepadnaviridae* [8] and including previously reported BtHVs [2, 3] were aligned using ClustalW, available in MEGA5.1 [9]. The phylogenetic tree showed that all sequences clustered into seven distinct groups sharing <74 % nt identity. Three consisted of avian, primate, and rodent hepatitis viruses and corresponded to the approved species listed in the Ninth Report of the International Committee on Taxonomy of Viruses (ICTV) from 2011 [8] (Fig. 1). The bat-associated orthohepadnaviruses identified in Myanmar, Panama, and Gabon [2, 3] and in this study were genetically diverse and classified into four distinct groups (Fig. 1). Comparisons of the full genome sequence of PEPR with those of BtHVs showed that sequence identity ranged from 60 % (with TBHBV) to 73 % (with HBHBV), and around 63 % with primate and rodent hepatitis viruses. Notably, PEPR was only 70 % identical to BtHVs in Myanmar, showing wide genetic heterogeneity. Although PEPR in Yunnan and HBHBV from Gabon both originated from *Hipposideros spp.*, they shared only 73 % nt sequence identity; i.e., less than within both the primate hepatitis virus group (e.g., HBV and WMHBV; ~80 %) and the rodent hepatitis virus group (e.g., WHV and GSHV; ~85 %) [8]. These results show that BtHVs of wide genetic diversity exist within the genus *Orthohepadnavirus*, differing according to the bat genus or species and therefore requiring classification into different species.

Pu'er is about 430 km southeast of Sedon and Wutao in Myanmar, where BtHV 776 was found in *Miniopterus spp.* but not in *Hipposideros* and other bat species [3]. BtHVs were also absent from *Rhinolophus spp*. in Pu'er. This is in contrast to HBHBV and RBHBV, both detected in Gabon in *Hipposideros* and *Rhinolophus spp*., respectively, and sharing <82 % identity [2]. These results suggest the existence of diverse orthohepadnaviruses naturally circulating in various bat species worldwide, but further investigation with expanded bat sampling will be needed to determine if BtHVs have restricted host ranges and geographical distributions.

HBV causes hepatitis B, cirrhosis and hepatocellular carcinoma in humans, resulting in >240 million chronic liver infections worldwide and about 600,000 deaths every year [10]. A previous study has shown that a BtHV cross-reacts with sera against HBV core protein, and reverse genetics has indicated that TBHBV from Panama has the ability to infect primary human hepatocytes [2], suggesting a potential zoonotic threat of BtHVs to humans.

Table 1 Gene lengths and percent identity between BtHV and other hepadnaviruses

Virus	Full genome		*Pol* gene				*preS1/preS2/S* gene				*preC/C* gene				*X* gene			
	nt	%ID	nt	%ID	aa	%ID	nt	%ID	aa	%ID	nt	%ID	aa	%ID	nt	%ID	aa	%ID
PEPR6	3,278		2,607		868		1,248		415		654		217		429		142	
776	3,230	70.3	2,562	71.7	853	64.1	1,200	74.3	399	62.9	654	***81.0***	217	***80.1***	435	73.7	144	55.8
TBHBV	3,149	59.8	2,484	59.6	827	55.2	1,149	67.6	382	57.3	663	65.1	220	58.0	408	57.0	135	43.3
HBHBV	3,377	***73.0***	2,709	***73.8***	902	***70.0***	1,347	***76.9***	448	***69.2***	654	74.7	217	75.6	426	***78.6***	141	***64.8***
RBHBV	3,368	72.5	2,700	73.4	899	69.7	1,338	76.3	445	66.4	654	76.5	217	76.9	426	77.7	141	62.0
HBV	3,215	63.1	2,532	63.0	843	57.0	1,203	64.1	400	57.0	639	76.3	212	75.9	465	56.5	154	43.4
WMHBV	3,179	63.2	2,508	63.3	835	55.8	1,176	64.7	391	59.0	636	76.2	211	73.7	459	60.4	152	43.8
WHV	3,308	62.8	2,640	60.6	879	55.0	1,281	64.8	426	47.5	678	78.9	225	78.0	426	67.8	141	51.0
ASHV	3,302	63.4	2,634	60.5	877	54.5	1,284	64.5	427	48.5	654	79.3	217	77.6	417	68.0	138	55.0
DHBV	3,006	37.5	2,376	36.3	791	31.9	1,104	37.7	367	18.1	888	30.9	295	10.8	NA	/	NA	/

nt, the length of the gene in nucleotides; %ID, the percent identity of nucleotide sequence and amino acid sequence between BtHV PEPR6 and the others; aa, the length of the gene in amino acids; NA, X gene not available; the accession numbers of 776 (BtHV), TBHBV, HBHBV, RBHBV, HBV, WMHBV, WHV, ASHV and DHBV are JX941466, KC790381, KC790377, KC790376, D00329, AF046996, AY344076, U29144 and EU429324, respectively. The highest identities are in bold italic

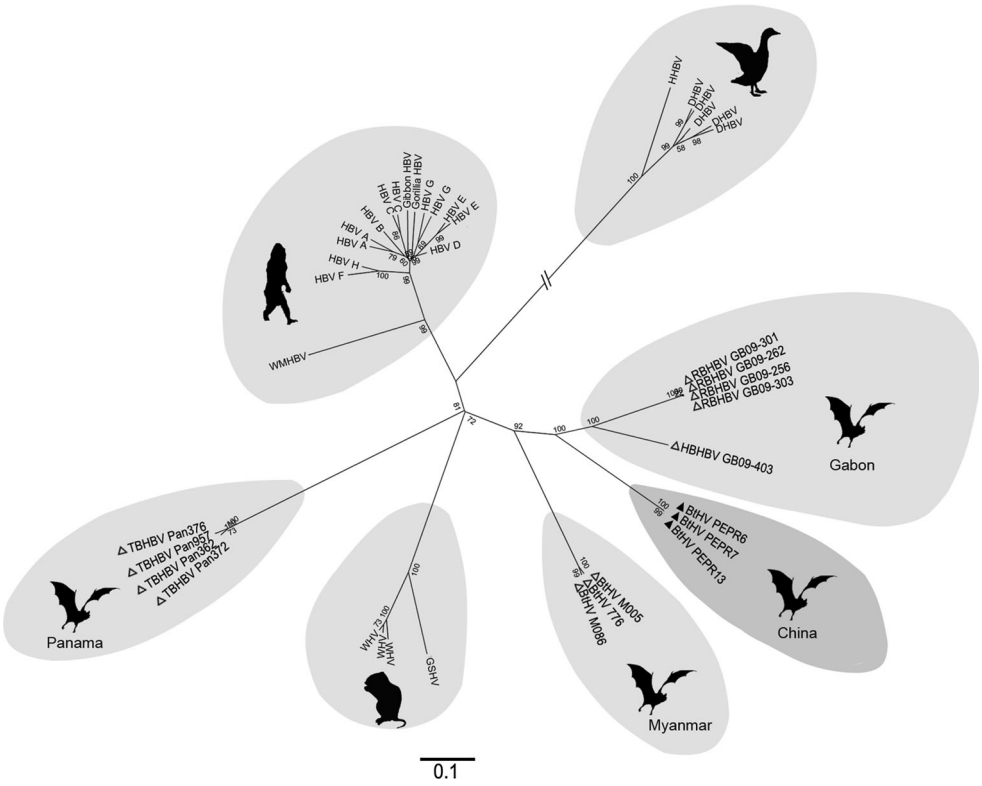

Fig. 1 Evolutionary analysis of BtHV and representatives of other orthohepadnaviruses based on their complete *Pol* nucleotide sequences, with avian hepatitis viruses used as an outgroup. The phylogenetic tree was constructed by the maximum-likelihood method using MEGA 5.1. Viruses identified in this study are indicted by filled triangles; previous BtHVs, with open triangles. The hosts of RBHBV and HBHBV in Gabon, TBHBV in Panama, BtHV in Myanmar and BtHV in China are *Rhinolophus alcyone*, *Hipposideros cf. ruber*, *Uroderma bilobatum*, *Miniopterus fuliginosus* and *Hipposideros pomona.* The scale bar indicates nucleotide substitutions per site

In conclusion, BtHVs are a novel group of orthohepadnaviruses of wide genetic diversity that are widely distributed among different bat species. They may provide new insights into HBV transmission, infection, and prevention, and their impact on public health merits further investigation.

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

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