Detection of bat hepatitis E virus RNA in microbats in Japan

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Abstract
Several recent studies have reported that various bat species harbor bat hepatitis E viruses (BatHEV) belonging to the family Hepeviridae, which also contains human hepatitis E virus (HEV). The distribution and ecology of BatHEV are not well known. Here, we collected and screened 81 bat fecal samples from nine bat species in Japan to detect BatHEV RNA by RT-PCR using HEV-specific primers, and detected three positive samples. Sequence and phylogenetic analyses indicated that these three viruses were BatHEVs belonging to genus Orthohepevirus D like other BatHEV strains reported earlier in various countries. These data support the first detection of BatHEVs in Japanese microbats, indicating their wide geographical distribution among multiple bat species.

Keywords Bat · Hepevirus · Epidemiology
Aomori
Asian parti-colored bat (Vespertilio inensis): 16
Blackish whiskered bat (Myotis pruinosus): 2
● Brown long-eared bat (Plecotus sacrimontis): 3
Greater horseshoe bat (Rhinolophus ferrumequinum): 9
Ikonnikov’s whiskered bat (Myotis ikonnikovi): 1
Ussuri tube-nose bat (Murina ussuriensis): 1

Iwate
Ikonnikov’s whiskered bat (Myotis ikonnikovi): 2

Tochigi
Brown long-eared bat (Plecotus sacrimontis): 1
Japanese large-footed bat (Myotis macrodactylus): 1

Tokyo
Asian parti-colored bat (Vespertilio inensis): 2
Japanese large-footed bat (Myotis macrodactylus): 18
Tube-nosed bat (Murina hilgendorfi): 2

Nagano
Greater horseshoe bat (Rhinolophus ferrumequinum): 2
Ikonnikov’s whiskered bat (Myotis ikonnikovi): 2
● Japanese short-tailed bat (Eptesicus japonensis): 19

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B

Rabbit-HEV
HEV genotype 3
HEV genotype 1
HEV genotype 2

Wild boar-HEV
HEV genotype 4

Avian-HEV
HEV genotype C1

BIHEV/NMS09125R (Myotis daubentonii; Germany)
BIHEV/NMS098B (Myotis daubentonii; Germany)
BIHEV/NMS98AC156 (Myotis bechsteinii; Germany)
BIHEV/Ps1 (Plecotus sacrimontis; Japan)
BIHEV/Md2350 (Myotis davidii; China)
RI-HEV (Rhinolophus ferrumequinum; China)
BIHEV/Ej1 (Eptesicus japonensis; Japan)
BIHEV/Ej2 (Eptesicus japonensis; Japan)
BIHEV/BS7 (Eptesicus serotinus; Germany)
BIHEV/G19E36 (Hipposideros abae; Ghana)
BIHEV/G19E38 (Hipposideros abae; Ghana)
Cutthroat trout virus

C

Rabbit-HEV
HEV genotype 3
HEV genotype 4
HEV genotype 1
HEV genotype 2

Wild boar-HEV

Avian-HEV
HEV genotype C1

RI-HEV (Rhinolophus ferrumequinum; China)
BIHEV/Ps1 (Plecotus sacrimontis; Japan)
BIHEV/Md2350 (Myotis davidii; China)
BaHEV/BS7 (Eptesicus serotinus; Germany)
BIHEV/Ej1 (Eptesicus japonensis; Japan)
BIHEV/Ej2 (Eptesicus japonensis; Japan)
Avian-HEV
Cutthroat trout virus

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Orthohepevirus A
Orthohepevirus C
Orthohepevirus D
Orthohepevirus B
Virus Genes (2018) 54:599–602

Fig. 1 Bat capture sites and phylogenetic trees of the detected viruses and bats. a Prefectures where bats were captured are indicated in red. Bat species and the number of samples are noted in each prefecture. To obtain fresh feces, bats were kept in a pouch for an hour, and the feces were then collected by a sterilized cotton bud and transferred to 1 mL of Dulbecco’s modified medium Eagle’s minimum essential medium (DMEM) supplemented with 100 U/mL of penicillin, 1 mg/mL of streptomycin, 100 µg/mL of gentamycin, and 2 µg/mL of amphotericin. The feces were suspended well and then centrifuged at 10,000×g for 15 min at 4 °C. The supernatants were used for RNA extraction with ISOGEN LS reagent (Nippon Gene). cDNA was synthesized using Prime Script RT reagent kit (Takara Bio) with a mixture of random hexamer and oligo dT primers. PCR amplifications were performed using the KOD FX Neo (Toyobo) with consensus HEV primer sets (PanHEV F and R), which were designed in this study to amplify a 191-bp fragment of the RNA-dependent RNA polymerase gene (corresponding to nt 4084–4274 of BatHEV/BS7) from the BatHEV genome. Black dots indicate Pan-HEV RT-PCR-positive bats. b and c Maximum likelihood phylogenetic trees of the partial RdRp and capsid genes (corresponding to nt 3970–4296 and nt 4777–6690 of BatHEV/BS7, respectively) of BtHEVs including the novel Japanese viruses (bold underline) at the amino acid level with Jones–Taylor–Thornton model. All phylogenetic trees were generated using ClustalW and MEGA software version 7.0 [9]. Bootstrap values are shown above and to the left of the major nodes. Scale bars indicate the number of substitutions per site. The accession numbers of sequences used in these trees are as follows: BtHEV-Ej1 (LC340968), BtHEV-Ps1 (LC340969), BtHEV-Ej2 (LC340970), HEV genotype 1 (D11092), HEV genotype 2 (M74506), HEV genotype 3 (EU723512), HEV genotype 4 (AB220974), Rabbit-HEV (FJ906895), Wild boar-HEV (AB573435), Avian-HEV (AM943647), HEV genotype C1 (GU345042), RF-HEV (KJ562187), Bat-HEV/BS7 (JQ001749), BtHEV-Md2350 (KX513953), BtHEV/G19E36 (JQ001744), BtHEV/G19E38 (JQ001746), and Cutthroat trout virus (NC015521).

We then phylogenetically analyzed the sequences by maximum-likelihood analysis using ClustalW and MEGA version 7.0 [9]. A phylogenetic tree constructed using the partial amino acid sequences of RdRp indicated that the Japanese viruses were included in Orthohepevirus D (Fig. 1b), demonstrating that all BatHEVs are classified in this species. We also amplified the full-length capsid (ORF2) sequences by RT-PCR and analyzed them phylogenetically. The resulting tree confirmed that the novel Japanese viruses were included in Orthohepevirus D (Fig. 1c). The sequences of BtHEV-Ej1/-Ej2 were placed in a position neighboring the German BatHEV/BS7 strain, confirming the phylogenetic similarity between these strains.

All bats captured in this study were insectivores and hibernate in winter. Although there is no information about migration of E. japonensis and P. sacrimontis, bat species closely related to them were reported to migrate only a few kilometers from their colonies per night [10, 11]. They had different ecology in the terms of the habitat. BtHEV-Ej1 and -Ej2 were detected on different days from two E. japonensis bats, both of which used eaves of the same house as night roost in Nagano. Although the E. japonensis bats formed mixed colony at the roost with Myotis ikikonikovi and Rhinolophus ferrumequinum bats, BatHEVs were only detected in E. japonensis, implying BtHEV-Ej1/-Ej2 might have a narrow host range. P. sacrimontis bats usually form a small colony without other species of bats. Indeed, P. sacrimontis bats, from which BtHEV-Ps1 was detected in this study, were captured near such small colony in a ruin in Aomori. Thus, BtHEV-Ps1 is likely circulating in the P. sacrimontis since the bats have low opportunity to come in contact with other species of bats.

The closely related BatHEVs (BtHEV-Ej1/-Ej2 and Bat HEV/BS7) have been detected in different species of Eptesicus bats (E. serotinus and E. japonensis). Since the distribution areas of these bats are not overlapping, viruses ancestral to BtHEV-Ej1/-Ej2 and BatHEV/BS7 might have infected ancestral Eptesicus and might have branched into different species in the process of evolution.

For virus isolation, we inoculated the RT-PCR-positive fecal samples not only into several bat cells (BKT, FBKT, and DemKTI cells) but also into other mammalian cell lines (Madin-Darby canine kidney (MDCK), African green monkey VeroE6, human A549, Madin-Darby bovine kidney (MDBK), and swine PK15 cells) since we suspected that the bat fecal samples may contain several pathogens other than BatHEVs. All inoculated cells were incubated for 12–15 days with media changes at 2–3 days interval. After the incubation, cells were blindly passaged three times. However, we could neither recover any infectious viruses nor detect BatHEV RNA in the inoculated cells by RT-PCR.

In conclusion, the present study showed the presence of several BatHEV strains, which were independently classified.
into Orthohepevirus D, in Japanese bats, suggesting wide geographical distribution of BatHEV among multiple bat species. Although these data suggest limited transmissibility of BatHEV to other animals, further studies are needed to determine its zoonotic potential.

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Author contributions TK, SM, and TH conceived and designed the experiments. TK, TY, KMi, MS, RS, and KMa performed the experiments. TK and SM analyzed the data. TK, SM, and TH wrote the paper and designed the figure. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest There is no conflict of interest associated with this article.

Research involving human participants and/or animals This article does not contain any studies with human participants performed by any of the authors. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Informed consent Informed consent is not required because no human participants were involved in this article.

References

1. W.J. Pape, T.D. Fitzsimmons, R.E. Hoffman, Emerg. Infect. Dis. 5, 433–437 (1999)
2. J.M. Yob, H. Field, A.M. Rashdi, C. Morrissy, B. Van der Heide, P. Rota, A. bin Adzhar, J. White, P. Daniels, A. Jamaluddin, T. Ksiazek, Emerg. Infect. Dis. 7, 439–441 (2001)
3. X.Y. Ge, J.L. Li, X.L. Yang, A.A. Chmura, G. Zhu, J.H. Epstein, J.K. Mazet, B. Hu, W. Zhang, C. Peng, Y.J. Zhang, C.M. Luo, B. Tan, N. Wang, Y. Zhu, G. Cramer, S.Y. Zhang, L.F. Wang, P. Daszak, Z.L. Shi, Nature 503, 535–538 (2013)
4. J.F. Drexler, A. Seelen, V.M. Corman, A.F. Tateno, V. Cottontail, R.M. Zerbinati, F. Gloza-Rausch, S.M. Klose, Y. Adu-Sarkodie, S.K. Oppong, E.K. Kalko, A. Osterman, A. Rasche, A. Adam, M.A. Müller, R.G. Ulrich, E.M. Leroy, A.N. Lukashev, C. Drosten, J. Virol. 86, 9134–9147 (2012)
5. B. Wang, X.L. Yang, W. Li, Y. Zhu, X.Y. Ge, L.B. Zhang, Y.Z. Zhang, C.T. Bock, Z.L. Shi, Virol. J. 14, 40 (2017)
6. S.U. Emerson, R.H. Purcell, in Fields Virology, 5th edn, by D.M. Knipe, P.M. Howley (Lippincott-Raven, Philadelphia, 1996), pp. 3047–3058
7. D.B. Smith, P. Simmonds, members of the International Committee on the Taxonomy of Viruses Hepeviridae Study Group, S. Jameel, S.U. Emerson, T.J. Harrison, X.J. Meng., H. Okamoto, W.H. Van der Poel, M.A. Purdy, J. Gen. Virol. 96, 1191–1192 (2015)
8. R. John, P. Dremsek, J. Reetz, G. Heckel, M. Hess, R.G. Ulrich, Infect. Genet. Evol. 27, 212–229 (2014)
9. S. Kumar, G. Stecher, K. Tamura, Mol. Biol. Evol. 33, 1870–1874 (2016)
10. C.M.C. Catto, A.M. Hutson, P.A. Racey, P.J. Stephenson, J. Zool. 238, 623–633 (1996)
11. A.C. Entwistle, P.A. Racey, J.R. Speakman, Philos. Trans. R. Soc. Lond. B. 351, 921–931 (1996)