



Short communication

Molecular detection and sequence characterization of diverse rhabdoviruses in bats, China

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ABSTRACT

The *Rhabdoviridae* is among the most diverse families of RNA viruses and currently classified into 18 genera with some rhabdoviruses lethal to humans and other animals. Herein, we describe genetic characterization of three novel rhabdoviruses from bats in China. Of these, two viruses (Jinghong bat virus and Benxi bat virus) found in *Rhinolophus* bats showed a phylogenetic relationship with vesiculoviruses, and sequence analyses indicate that they represent two new species within the genus *Vesiculovirus*. The remaining Yangjiang bat virus found in *Hipposideros larvatus* bats were only distantly related to currently known rhabdoviruses.

The family *Rhabdoviridae* within the order *Mononegavirales* is a large group of bullet- or rod-shaped viruses with great genetic diversity and complexity (Walker et al., 2015). The virions contain a single or segmented molecule of linear, negative sense single-stranded RNA of size 11–15 kb (Dietzgen et al., 2012; Lyles et al., 2013). Rhabdoviruses have been found replicating in plants, invertebrates and vertebrates, and many members are significant medical, veterinary, or agricultural pathogens, such as lyssaviruses, vesiculoviruses and ephemeroviruses (Lyles et al., 2013). The genus *Lyssavirus* is represented by the well-known rabies virus (RABV), one of the most wide spread and lethal infectious agents worldwide (Giesen et al., 2015), and also includes other members causing rabies-related human diseases with mortalities approaching 100%, such as Australian bat lyssavirus (ABLV) (Francis et al., 2014), European bat lyssavirus 2 (EBLV-2) (Nathwani et al., 2003) and Irkut virus (IRKV) (Leonova et al., 2009). The genus *Vesiculovirus* includes Vesicular stomatitis Indiana virus (VSIV), the most intensively studied prototype species for nonsegmented negative-stranded RNA viruses, which infects cattle, horses and pigs causing fever, vesicles in the mucosa of the oral cavity and in the skin of the coronary band and teat, and acute febrile disease in humans (Lyles et al., 2013). Bovine ephemeral fever virus (BEFV) of the genus *Ephemerovirus* infects cattle and water buffaloes and gives rise to an acute febrile illness, bovine ephemeral fever, which causes severe economic

losses by malaise and decreased milk production in cattle (Lyles et al., 2013; Walker and Klement, 2015).

As the second diverse order of mammals (after rodents), bats are important natural reservoirs of viruses of which more than 200 have been discovered, including several agents that are highly pathogenic for humans such as Hendra and Nipah viruses (Halpin et al., 2000; Yob et al., 2001), SARS-related coronavirus (Li et al., 2005), and Ebola virus (Johnson et al., 2010). Bats also harbor and transmit diverse rhabdoviruses such as lyssaviruses, ledanteviruses and vesiculoviruses. Rabies virus of the genus *Lyssavirus* was the first lethal pathogen recognized to be harbored and spread by bats. Currently, 14 approved species are included within the genus, of which 12 originate from bats, exclusively or partially. Ledanteviruses hold strong ecological associations with bats, with viruses assigned to six of the 14 species discovered in bats, such as Mount Elgon bat virus (MEBV) and Oita virus (OITAV) (Iwasaki et al., 2004; Metselaar et al., 1969). Le Dantec virus (LDV), which is assigned to the type species (*Le Dantec ledantevirus*) of the genus *Ledantevirus*, was isolated from the serum of a Senegalese girl with acute febrile illness and signs of hepatosplenomegaly (Cropp et al., 1985), and its RNA was detected in a serotine bat, *Eptesicus isabellinus* (Vazquez-Moron et al., 2008). Within the genus *Vesiculovirus*, only one bat-associated member has been reported: American bat vesiculovirus (ABVV), detected in big brown bats (*Eptesicus fuscus*) in the USA and

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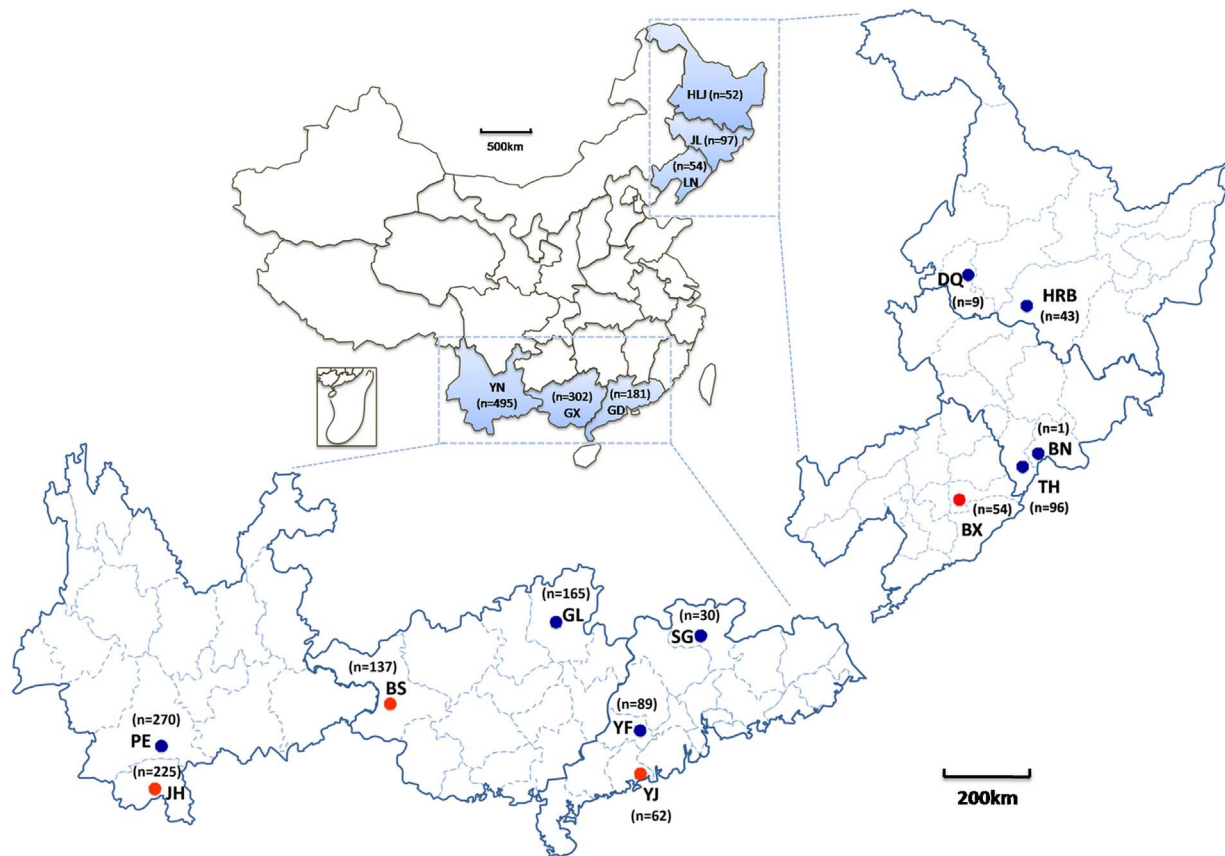


Fig. 1. Details of bat collection in Yunnan Province, Guangdong Province, Liaoning Province, Jilin Province, Heilongjiang Province and the Guangxi Zhuang Autonomous Region. Circles: bat-sampling locations. Samples positive for rhabdoviruses are identified in red, with negatives in blue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Bat rhabdoviruses detected by PCR.

Sampling location	Host ^a	Tissue	Virus	Positive rate (positive No./total No.)
Jinghong, Yunnan	<i>Rh. affinis</i>	intestines	JHBV	4.2% (1/24)
Benxi, Liaoning	<i>Rh. ferrumequinum</i>	intestines & lungs	BXBV	18.8% (3/16)
Yangjiang, Guangdong	<i>Hi. larvatus</i>	intestines	YJBV	16.7% (2/12)
Baise, Guangxi	<i>Hi. larvatus</i>	intestines	YJBV	4.1% (2/49)

^a *Rh.*: *Rhinolophus*; *Hi.*: *Hipposideros*.

forming a distinct lineage of vesiculovirus (Ng et al., 2013).

To investigate bat virus ecology in China, 1181 bats were collected at 13 locations in the south (Yunnan Province, Guangdong Province and Guangxi Zhuang Autonomous Region) and northeast (Liaoning Province, Jilin Province and Heilongjiang Province) between 2005 and 2014 (Fig. 1 and Table S1). The collection was comprised of 25 species within 12 genera of 5 families: *Rhinolophidae* (n = 302), *Hipposideridae* (n = 483), *Vespertilionidae* (n = 233), *Emballonuridae* (n = 63), and *Pteropodidae* (n = 100). Intestines (with contents) and lungs of bats from each location were pooled and subjected to viral metagenomic analysis as per our published method (He et al., 2013). The procedures for sampling of bats in this study were reviewed and approved by the Administrative Committee on Animal Welfare of the Institute of Military Veterinary, Academy of Military Medical Sciences, China (Laboratory Animal Care and Use Committee Authorization, permit number: JSY-DW-2015-01). All live bats were maintained and handled

according to the Principles and Guidelines for Laboratory Animal Medicine (2006), Ministry of Science and Technology, China.

A total of 210 reads with lengths of ~140 nucleotides (nt) were annotated to rhabdovirus, corresponding to the RNA-dependent RNA polymerase domain of the L gene. These reads could be classified into two groups, showing identities of 84% (nt) and 91% (amino acid, aa) with vesiculoviruses and 68% (nt) and 52% (aa) with lyssaviruses. The reads and their phylogenetic neighbors from GenBank were used as a template to design degenerate nested or semi-nested PCR primers targeting the conserved L gene (252–715 nt, shown in Table S2). Viral RNA of each sample was extracted and submitted to RT-PCR screening. Results showed that eight bats were positive for rhabdoviruses: an intermediate horseshoe bat (*Rhinolophus affinis*) (4.2%, 1/24) in Jinghong City, Yunnan; three greater horseshoe bats (*Rhinolophus ferrumequinum*) (18.8%, 3/16) in Benxi City, Liaoning; two intermediate roundleaf bats (*Hipposideros larvatus*) in Baise City, Guangxi (4.1%, 2/49) and two in Yangjiang City, Guangdong (16.7%, 2/12) (Table 1).

We tried several times to isolate the rhabdoviruses by BHK-21 and Vero E6 cells as well as 3-day-old Kunming mice, however, results of RT-PCR detection of the cell culture and mouse internal organs were negative, resulting in a failure to isolate any virus.

The amplicons obtained (252 bp) were aligned by ClustalW with representatives of all rhabdoviruses except five genera of plant and fish rhabdoviruses (*Cytorhabdovirus*, *Dichorhavivirus*, *Nucleorhabdovirus*, *Varicosavirus* and *Novirhabdovirus*) as well as some currently unclassified rhabdoviruses, such as Bat rhabdovirus (BtMp-RhaV/SX2013) (Wu et al., 2016), Muir Springs virus (MSV) (Walker et al., 2015) and Huangpi tick virus 3 (HTV3) (Li et al., 2015). Details of reference sequences were shown in Table S3. Identities were calculated using MegAlign (Table S4) and the phylogenetic tree was constructed by MEGA7 using the Maximum Likelihood method with 1000 bootstrap

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