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Hantaviruses, responsible for hantavirus cardiopulmonary syndrome (HCPS) in the Americas and hemorrhagic fever with renal syndrome (HFRS) in Asia and Europe, are major life-threatening pathogens in human (Schmaljohn and Nichol, 2007). About 60,000–150,000 cases of HFRS are reported yearly worldwide with more than 90% in Asia, mostly in China with 29 of its 31 provinces being affected (Jonsson et al., 2010; Zhang et al., 2010; Kariwa et al., 2007).

Rodents are natural reservoirs of hantaviruses (Schmaljohn and Nichol, 2007; Jonsson et al., 2010; Zhang et al., 2010); however, recent studies have demonstrated that the natural hosts also include insectivores and bats (Zhang, 2014; Guo et al., 2013; Weiss et al., 2012). Bats are important reservoirs of many emerging deadly viral pathogens, such as filoviruses, lyssaviruses, and SARS-related coronaviruses (Calisher et al., 2006). Genetically divergent bat-borne hantaviruses have recently been identified in Africa (Weiss et al., 2012; Sumibcay et al., 2012) and Asia (Guo et al., 2013; Zhang, 2014; Gu et al., 2014), but very limited sequences are available, with none having complete sequences of all three gene segments (Table 1). Lack of complete genomic sequences impairs the phylogenetic and evolutionary comparison of bat-borne hantaviruses with those of human, rodents and insectivores.

Here, we report the complete genomic sequence of a novel hantavirus identified from a South China bat.

As part of a survey of bat-borne pathogens, thirty-two insectivorous black-bearded tomb bats (Taphozous melanopogon) were captured in July 2012 in Laibin city, Guangxi province, China. The bat species was morphologically identified by an experienced zoologist and further confirmed by PCR of its mitochondrial cytochrome b gene sequence (Wang et al., 2003). Intestines with contents, and lungs were collected and the samples of each tissue were pooled for viral metagenomic analysis as described elsewhere (He et al., 2013b). Among sequences annotated to mammal viruses, 250 identical reads with an average length of 135 nucleotides (nt) were found to exhibit 70% nt identity with the S gene of Dobrav-Belgrade hantavirus (DOBV) (Nemirov et al., 1999). To further screen for hantaviruses, total RNA was extracted from each sample using a QIAamp RNeasy Mini Kit (Qiagen), then subjected to hantavirus-specific RT-PCR using published primers (Klempl et al., 2006). Hantavirus was identified in one bat lung tissue, and designated Laibin virus (LBV). To obtain the full genome of LBV, primers were designed according to bat-borne hantavirus sequences retrieved from GenBank (Table S). The end sequences of each segment were obtained by using conserved terminal nt sequences as primers. Overlapping amplicons were obtained by using a Fast HiFidelity PCR Kit (Tian-gen), then assembled into three full gene segments. The complete sequences of S, M and L gene segments were 6,531 nt in length, encoding 427, 1,127 and 2,145 aa proteins respectively (GenBank accession numbers: KM102247 and KM102249). Notably, LBV exhibited the longest M segment,
107 nt longer in the 3' non-coding region than the largest M segment previously reported (3,801 nt; El Moro Canyon hantavirus RM-97, accession number U26828). The three segments were aligned with reported sequences using Clustal W (Version 2.0). Since most of the bat-borne hantavirus sequences were incomplete (Table 1), phylogenetic analyses were conducted based on partial sequences of the three gene segments (Fig. 1). The resulting topologies were similar to that described previously (Guo et al., 2013), which divided all sequences into four clades, and all bat-borne hantaviruses were clustered together. Further sequence comparison showed that the three gene segments of LBV had 46.4–75.3% nucleotide identity with other bat-borne hantaviruses (Table 1) and 44.6–67.4% with hantaviruses from humans, rats, mice, shrews and moles (Table 2). These results characterize LBV as being a novel bat-borne hantavirus exhibiting highest nucleotide identities with the three gene segments of Vietnamese XSV (60.8–75.3%) (Table 1).

Five blind passages in Vero-E6 cells failed to isolate the virus from the positive lung sample. However, a single viral particle of about 100 nm in diameter was observed in lung tissue by electron microscopy (EM) using previously described methods (He et al., 2013a) although no grid-like pattern typical of hantaviruses (Schmaljohn and Nichol, 2007) was seen (Fig. S). In support of the conclusion that the EM sample contained LBV, specific RT-PCR above was performed and revealed specific amplification of an LBV gene fragment.

### Table 1

| Virus | Country (Province) | Bat species | Gene | Position at LBV genome | Length (nt) | nt Identity with LBV |
|-------|--------------------|-------------|------|------------------------|-------------|----------------------|
| LQUV  | China (Zhejiang)   | Rhinolophus sinicus | S Complete | 1,563 | 50.6 |
|       |                    |             | M Complete | 3,618 | 52.6 |
|       |                    |             | L 3,005–3,328 | 324 | 69.1 |
| HUPV  | China (Hubei)      | Pipistrellus abramus | S | 522–1,935 | 1,115 | 46.4 |
|       |                    |             | L 2,945–3,288 | 343 | 69.8 |
| XSV   | Vietnam (Tuyên Quang) | Hipposideros pomona | S | 1,956–2,618 | 663 | 60.8 |
|       |                    |             | L | 2,540–3,699 | 1,160 | 71.2 |
| MOUV  | Côte d'ivoire      | Neotycteris hipposiderus | L | 1,892–3,582 | 414 | 71.5 |
| MGBV  | Sierra Leone       |             | L | 2,933–3,370 | 414 | 71.5 |

*Abbreviation and accession No.: LQUV, Longquan virus Ra-25 (JX465415, JX465397, JX465381); HUPV, Huangpi virus Pa-1 (JX473273, JX465369); XSV, Xuan son virus F14292 (KF704705, KJ000533, KF704714); MOUV, Mouyassue virus KB576 (JQ287716); MGBV, Magboi virus 1209 (JQ287715).*

### Table 2

| Hantavirus | S (nt) | M (nt) | L (nt) |
|------------|--------|--------|--------|
| HTNV/CHN/Human | 48.4 | 52.5 | 49.7 |
| DOBV/RUS/Human | 49.9 | 53.0 | 49.4 |
| SEOV/CHN/Rodent | 50.2 | 52.9 | 52.3 |
| SNV/USA/Rodent | 50.2 | 52.9 | 48.4 |
| PUUV/USA/Rodent | 51.9 | 53.0 | 52.6 |
| PHV/USA/Rodent | 48.5 | 52.1 | 52.8 |
| THAV/MDC/Rodent | 53.0 | 52.8 | 48.9 |
| ASAV/PJN/Mole | 51.4 | 51.3 | 50.5 |
| NVAV/HUN/Mole | 58.3 | 59.6 | NA |
| CBNV/VIE/Shrew | 56.1 | 53.0 | 50.4 |
| MJNV/KOR/Shrew | 45.5 | 46.4 | 47.6 |
| TPMV/JNK/Shrew | 44.6 | 44.4 | 47.9 |

* Abbreviation and accession No.: HTNV, Hantaan virus CHHu2 (EU363813, EU363819); DOBV, Dobrava-Belgrade virusAp/Sochi/hu (JF920150, JF920149, JF920148); SEOV, Seoul virus CHN (AF288299, AF288298, AF288297); SNV, Sin Nombre virus NMH10 (NC_005216, NC_005219, NC_005222); PUUV, Puumala virus Sotkamo (NC_005224, NC_005223, NC_005225); PHV, Prospect Hill virus PH-1 (X55128, X55129, EF646763); THAV, Anjouzorobe virus Em/MDG/2009/ATD49 (KC490918, KC490919, KC490922); ASAV, Asa virus N10 (EU290989, EU290987); NVAV, Nova virus MSB9703 (FJ539168, FJ539498); CBNV, Cao Bang virus 3 (EF543524, EF543526, EF543525); MJNV, Imjin virus CD4-55 (EF641805, EF641806, EF641807); TPMV, Thottapalayam virus VRC66412 (NC_010704, NC_010708, NC_010707); NA, not available.*
Apart from the present report, five hantaviruses have been identified in bats worldwide; however, their complete genomic sequences have not been reported (Table 1). In present study a novel bat-borne hantavirus has been identified and its full genomic sequence has been determined. Comparison with available sequences shows that LBV is distantly related to all known bat-borne hantaviruses (Table 1), but that it is closer to Vietnamese XSV (60.8–75.3% nt identity) than to the two other Chinese strains LQUV and HUPV. Laibin city, however, is closer to Vietnamese Tuyên Quang province (the isolation site of XSV near the China-Vietnam border) than to Longquan city in Zhejiang province (LQUV) and Huangpi city in Hubei province (HUPV). Insufficient sequence data prevents a comprehensive analysis of the genetic diversities of the bat-borne hantaviruses from Côte d’Ivoire and Sierra Leone, but partial sequence analysis suggests that bat-borne hantaviruses have evolved worldwide within an independent and diverse phylogroup (Fig. 1). The successful determination of the complete sequences of the LBV gene segments may provide reference data for determining the complete genomic sequences of other bat-borne hantaviruses.

Divergent bat-borne hantaviruses have been found in a broad spectrum of bat species (Table 1) which emphasizes the importance of bats as natural worldwide reservoirs of these viruses and the need to investigate further the potential role of bats in transmission of hantaviruses to humans and other animals.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.meegid.2015.01.018.

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