RESEARCH ARTICLE

Phylogenetic analysis based on mitochondrial DNA sequences of wild rats, and the relationship with Seoul virus infection in Hubei, China

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Seoul virus (SEOV), which is predominantly carried by *Rattus norvegicus*, is one of the major causes of hemorrhagic fever with renal syndrome (HFRS) in China. Hubei province, located in the central south of China, has experienced some of the most severe epidemics of HFRS. To investigate the mitochondrial DNA (mtDNA)-based phylogenetics of wild rats in Hubei, and the relationship with SEOV infection, 664 wild rats were captured from five trapping sites in Hubei from 2000–2009 and 2014–2015. Using reverse-transcription (RT)-PCR, 41 (6.17%) rats were found to be positive for SEOV infection. The SEOV-positive percentage in Yichang was significantly lower than that in other areas. The mtDNA D-loop and cytochrome b (*cyt-b*) genes of 103 rats were sequenced. Among these animals, 37 were SEOV-positive. The reconstruction of the phylogenetic relationship (based on the complete D-loop and *cyt-b* sequences) allowed the rats to be categorized into two lineages, *R. norvegicus* and *Rattus nitidus*, with the former including the majority of the rats. For both the D-loop and *cyt-b* genes, 18 haplotypes were identified. The geographic distributions of the different haplotypes were significantly different. There were no significant differences in the SEOV-positive percentages between different haplotypes. There were three sub-lineages for the D-loop, and two for *cyt-b*. The SEOV-positive percentages for each of the sub-lineages did not significantly differ. This indicates that the SEOV-positive percentage is not related to the mtDNA D-loop or *cyt-b* haplotype or the sub-lineage of rats from Hubei.

KEYWORDS Seoul virus(SEOV); *Rattus norvegicus*; mitochondrial DNA; phylogenetic analysis

INTRODUCTION

Seoul virus (SEOV) (genus *Hantavirus*, family *Bunyaviridae*), which is mainly carried by *Rattus norvegicus* (Norway rat), is a major cause of hemorrhagic fever with renal

syndrome (HFRS) in China. Currently, the *Hantavirus* genus includes 23 distinct species and 30 provisional species (Plyusnin et al., 2012). Hantaviruses are hosted and transmitted by rodents, insectivores, and bats (Zhang, 2014). In most cases, there is a “one hantavirus, one host” relationship. Both co-speciation (Plyusnin and Sironen, 2014) and host-switching (Ramsden et al., 2009) have had an important impact on hantavirus evolution (Guo et al., 2013).

Two human diseases are caused by hantaviruses: HFRS in Eurasia, and hantavirus pulmonary syndrome (HPS) in

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the Americas (Watson et al., 2014). Studies on the ecological and phylogenetic relationships between hantaviruses and their hosts have suggested that the distribution and evolution of the reservoir hosts play important roles in the geographic distribution and epidemiology of hantavirus-related diseases (Schlegel et al., 2012; Bennett et al., 2014; Yanagihara et al., 2014; Schmidt et al., 2016).

HFRS is highly endemic in China. Hantaan virus (HTNV), which is mainly carried by *Apodemus agrarius* (Striped Field Mouse), and SEOV, which is mainly carried by *R. norvegicus*, are the major causes of HFRS (Chen et al., 1986a; Song, 1999; Zhang et al., 2014a; Cao et al., 2016). Hubei province, which is located in the central south of China, has experienced some of the most severe epidemics of HFRS (Zhang, 1990; Zhang et al., 2014b). SEOV and HTNV co-circulate in Hubei (Kang et al., 2012). Phylogenetic analysis (based on partial S- and M-segment sequences) revealed that there are several lineages of SEOV and a novel genetic lineage of HTNV in Jiangxia, Xinzhou, Qichun and Nanzhang of Hubei (Li et al., 2012; Liu et al., 2012). Analysis of epidemiological data from Hubei has indicated that the HFRS cases that occurred in the 1980s and 1990s were mainly caused by HTNV, whereas the proportion of HFRS cases caused by SEOV increased greatly in the 2000s (Zhang et al., 2014b).

Mitochondria are involved in virus-host interaction processes, such as apoptosis (Neumann et al., 2015; Zan et al., 2016) and auto/mitophagy (Ruggieri et al., 2014). Mitochondrial DNA (mtDNA) activates several innate immune pathways involving Toll-like receptor 9 (TLR9), the Nod-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome, and stimulator of interferon genes (STING) signaling. In addition to regulating antiviral signaling, mtDNA contributes to inflammatory diseases after cellular damage and stress (Fang et al., 2016).

Genetic analysis based on mtDNA sequences has been carried out to identify the animal species that act as hantavirus reservoirs and to clarify the evolutionary relationships among the reservoir hosts (Morzunov et al., 1998; Torres-Perez et al., 2010; Lin et al., 2012; Liu et al., 2012; Hugot et al., 2014; Schmidt et al., 2016). However, the association between SEOV infection and the mtDNA characteristics of the reservoir hosts in Hubei, China, has not been fully demonstrated. This information would provide a better understanding of the mtDNA genetic background of wild rats in Hubei, shed light on the relationship between SEOV infection and mtDNA genetic diversity, and could be valuable for the control of HFRS. To fill this gap, wild rats were captured in Hubei during 2000–2009 and 2014–2015. The lungs of the rats were screened for SEOV using specific reverse-transcription (RT)-PCR. The mitochondrial D-loop and cytochrome b (*cyt-b*) gene sequences were then identified from the lung tissues and subjected to phylogenetic analysis.

MATERIALS AND METHODS

Rodent samples

During 2000–2009 and 2014–2015, wild rats were captured with snap-traps, which were generally set at 5 m apart and baited with peanuts in both residential areas and fields. Trapping was conducted at five locations that are known to be HFRS epidemic areas in Hubei: Nanzhang (NZ), Yichang (YC), Xinzhou (XZ), Jiangxia (JX), and Qichun (QC) (Figure 1). All the animal research was conducted in accordance with internationally accepted principles and the Wuhan University Guidelines on the Care and Use of Laboratory Animals. The trapped animals were identified as described previously (Chen et al., 1986b). Lung tissues were collected and stored at -80°C until use.

RT-PCR of the SEOV partial S-segment sequence

Total RNA was extracted from the rodent lung tissues using an RNAPrep Tissue Kit (Tiangen, Beijing, China). First-strand cDNA was generated from the total RNA using random primers and Moloney murine leukemia virus (MMLV) reverse transcriptase (Promega, Beijing, China). The SEOV partial S-segment sequences were amplified with primers S927F (5'-GATTGAAGATAT TGAGTCACC-3') and S1168R (5'-GTTGTATCCCCATT GATTGTG-3'). The total volume of each PCR reaction was 50 μL , comprising 5 μL cDNA, 0.2 $\mu\text{mol/L}$ of the forward and reverse primers, 200 $\mu\text{mol/L}$ deoxynucleotide triphosphates (dNTPs), 1 unit *Taq* DNA polymerase (Tiangen, Beijing, China), 10 mmol/L Tris/HCl, (pH 8.3), 50 mmol/L KCl, and 1.5 mmol/L MgCl_2 . The PCR cycling conditions were as follows: 5 min at 94°C ; 30 cycles consisting of 45 s at 94°C , 45 s at 55°C , and 20 s at 72°C ; and a final extension step of 5 min at 72°C . The PCR products were detected by running them on an agarose gel and staining them with ethidium bromide, and they were then view under ultraviolet light.

PCR and sequencing of the mtDNA D-loop and *cyt-b* genes

To verify the species carried by the hantavirus-infected rodents and to study their phylogenetic relationships, genomic DNA was extracted from the lung tissues using a DNAPrep Tissue Kit (Tiangen, Beijing, China). The entire 899-nucleotide D-loop region of the mtDNA was amplified by PCR, using primers DF (5'-GTCAACT CCCAAAGCTGAAATTC-3') and DR (5'-TCTCGAG ATTTTCAGTGTCTTGCTTT-3'). The entire 1143-nucleotide *cyt-b* region of the mtDNA was amplified by PCR, using primers BF (5'-CGAAGCTTGATATGAAA AACCATCGTTG-3') and BR (5'-AACTGCAGTC

Figure 1. Map showing the trapping sites (▲) for wild rats in Hubei province, China.

ATCTCCGGTTTACAAGAC-3'). The total volume of each PCR reaction was 50 μ L, comprising 0.5 μ g of DNA template, 0.1 μ mol/L of each primer, 200 μ mol/L of dNTPs, and 2 units of *Taq* DNA polymerase (Tiangen, Beijing, China). The cycling conditions consisted of an initial denaturation of 3 min at 94 $^{\circ}$ C; 35 cycles consisting of 1 min at 94 $^{\circ}$ C, 1 min at 50 $^{\circ}$ C, and 1 min at 72 $^{\circ}$ C; and a final extension step at 72 $^{\circ}$ C for 10 min. The PCR products were gel-purified, and sequenced using an ABI 3730 automatic sequencer (Torrance, CA, USA).

Molecular diversity analysis

For the mtDNA D-loop and *cyt-b* sequences, several population parameters, i.e., haplotype number (*h*), haplotype diversity (*h.d.*), and nucleotide diversity (π), were calculated using DnaSP v5.0 (Librado and Rozas, 2009). All positions containing gaps and missing data were eliminated from the dataset.

Phylogenetic analyses

Multiple sequence alignment was carried out using ClustalW (Larkin *et al.*, 2007) with default parameters, and the analysis was revised using BioEdit 7.2 (Hall, 1999). D-loop or *cyt-b* sequences of rats from other parts of China and other countries were also included in the phylogenetic analysis. For each haplotype, one sequence was included in the phylogenetic analysis. The phylogenetic relationships for first the D-loop and then the *cyt-b* sequences were reconstructed using Bayesian Markov Chain Monte Carlo (MCMC) runs over 500,000 generations, as implemented in MrBayes 3.1 (Ronquist and Huelsenbeck, 2003). For both analyses, a majority rule (50%) consensus tree was constructed after burn-in of an

initial 1250 trees. For both the mtDNA D-loop and *cyt-b* sequences, *Microtus kikuchii* (a species of vole that belongs to the family *Crictidae*), was used as the out-group to root the phylogenetic tree.

Statistical analyses

Using the likelihood ratio chi-square test, the SEOV-positive percentage among different geographic locations, different mtDNA haplotypes, and different mtDNA phylogenetic sub-lineages were evaluated, along with the geographic distribution of the mtDNA haplotypes.

Nucleotide sequence accession numbers

The new mtDNA D-loop and *cyt-b* sequences of the rodents described in this study have been deposited in GenBank under accession numbers KY356101-KY356194, and MF062462-MF062465 (Supplementary Table S1). Other previously published sequences used in the study were obtained from GenBank (Supplementary Table S2).

RESULTS

Hantavirus infection in rodents

During 2000–2009 and 2014–2015, 664 wild rats were captured from five trapping sites in Hubei. Using RT-PCR, 41 (6.17%) wild rats were found to be positive of SEOV infection (Table 1). The SEOV-positive percentage in Yichang was significantly lower than that in other areas ($P < 0.01$).

mtDNA sequencing, phylogeny, and relationship with SEOV infection

In this study, the D-loop and *cyt-b* genes of 103 rats were

sequenced. Among these animals, 37 were SEOV positive. For both the D-loop and *cyt-b* genes, 18 haplotypes were identified (Tables 2, 3).

Using Bayesian methods to reconstruct the phylogenetic relationships based on complete D-loop or *cyt-b* sequences allowed the rats to be categorized into two lineages, *R. norvegicus* and *Rattus nitidus*, both with high support values (Figure 2). Lineage *R. norvegicus* contained the majority of the rats, i.e., 101 rats, with 37 (36.63%) being SEOV positive. Lineage *R. nitidus* included two rats (both from Qichun in Hubei), both of which were SEOV negative.

mtDNA D-loop

The lineage *R. norvegicus* consisted of 17 haplotypes, with a haplotype diversity of 1.00 ± 0.02 and a nucleotide diversity of $0.74 \pm 0.08\%$ (Table 4). Some of the rats from Hubei formed three sub-lineages, D-I to D-III, while the others could not be classified into clusters (Figure 2A). Sub-lineage D-I included 40 rats, with six haplotypes (D2, D5–D7, D11, and D17). Sub-lineage D-II included six rats, with two haplotypes. Sub-lineage D-

III included 19 rats, with two haplotypes. There were 36 rats, with seven haplotypes (D-others), that were not classified into sub-lineages (Table 4). The SEOV-positive percentages for the sub-lineages were 35% (D-I), 16.67% (D-II), 36.84% (D-III), and 41.67% (D-others). There were no significant differences in the SEOV-positive percentages between the sub-lineages

Table 1. Detection of SEOV (using RT-PCR) in wild rats from Hubei, China

Site	RT-PCR positive	Total	Percentage
Nanzhang	16	160	10.00
Yichang	1	126	0.79**
Xinzhou	10	178	5.62
Jiangxia	7	125	5.60
Qichun	8	75	10.67
Total	41	664	6.17

Note: ** $P < 0.01$ (chi-square test)

Table 2. Number of rats with each D-loop haplotype (D1–18) by geographic location

Haplotype	Nanzhang	Yichang	Xinzhou	Jiangxia	Qichun	All
D1	4/14 (28.57)	1/7 (14.29)	–	–	–	5/21 (23.81)
D2	–	–	4/19 (21.05)	–	–	4/19 (21.05)
D3	6/9 (66.67)	0/9 (0)	–	–	–	6/18 (33.33)
D4	5/7 (71.43)	–	–	–	–	5/7 (71.43)
D5	–	–	–	4/6 (66.67)	1/1 (100)	5/7 (71.43)
D6	–	–	–	–	3/6 (50.00)	3/6 (50.00)
D7	–	–	2/5 (40.00)	–	–	2/5 (40.00)
D8	–	–	–	–	0/4 (0)	0/4 (0)
D9	–	–	2/3 (66.67)	–	–	2/3 (66.67)
D10	–	–	–	–	1/2 (50.00)	1/2 (50.00)
D11	–	–	0/2 (0)	–	–	0/2 (0)
D12	–	–	1/2 (50.00)	–	–	1/2 (50.00)
D13	–	–	–	1/1 (100.00)	–	1/1 (100.00)
D14	0/1 (0)	–	–	–	–	0/1 (0)
D15	–	–	–	–	1/1 (100.00)	1/1 (100.00)
D16	–	–	–	–	1/1 (100)	1/1 (100.00)
D17	–	–	0/1 (0)	–	–	0/1 (0)
D18	–	–	–	–	0/2 (0)	0/2 (0)
All	15/31 (48.39)	1/16 (6.25)	9/32 (28.13)	5/7 (71.43)	7/17 (41.18)	37/103 (35.92)

Note: For each cell, the figures are the no. of SEOV-positive rats/no. of sequenced rats (%). The chi-square test of the difference in the SEOV-positive percentage among the different haplotypes indicated no significant differences ($P > 0.05$). The chi-square test of the difference in the geographic distribution among the different haplotypes indicated a significant difference ($P < 0.01$).

Table 3. Number of rats with each *cyt-b* haplotype (B1–18) by geographic location

Haplotype	Nanzhang	Yichang	Xinzhou	Jiangxia	Qichun	All
B1	5/14 (35.71)	1/7 (14.29)	1/2 (50.00)	—	—	7/23 (30.43)
B2	6/9 (66.67)	0/9 (0)	—	1/1 (100.00)	—	7/19 (36.84)
B3	—	—	5/19 (26.32)	—	—	5/19 (26.32)
B4	—	—	—	—	1/6 (14.28)	1/6 (16.67)
B5	—	—	—	4/6 (66.66)	—	4/6 (66.66)
B6	4/6 (66.66)	—	—	—	—	4/6 (66.66)
B7	—	—	—	—	3/6 (50.00)	3/6 (50.00)
B8	—	—	2/4 (50.00)	—	—	2/4 (50.00)
B9	—	—	0/3 (0)	—	—	0/3 (0)
B10	—	—	0/2 (0)	—	—	0/2 (0)
B11	0/1 (0)	—	—	—	—	0/1 (0)
B12	1/1 (100.00)	—	—	—	—	1/1 (100.00)
B13	—	—	—	—	1/1 (100.00)	1/1 (100.00)
B14	—	—	—	—	1/1 (100.00)	1/1 (100.00)
B15	—	—	—	—	1/1 (100.00)	1/1 (100.00)
B16	—	—	0/1 (0)	—	—	0/1 (0)
B17	—	—	0/1 (0)	—	—	0/1 (0)
B18	—	—	—	—	0/2 (0)	0/2 (0)
All	16/31 (51.61)	1/16 (6.25)	8/32 (25.00)	5/7 (71.43)	7/17 (38.89)	37/103 (35.92)

Note: For each cell, the figures are the no. of SEOV-positive rats/no. of sequenced rats (%). The chi-square test of the difference in the SEOV-positive percentage among the different haplotypes indicated no significant differences ($P > 0.05$). The chi-square test of the difference in the geographic distribution among the different haplotypes indicated a significant difference ($P < 0.01$).

when evaluated with a chi-square test ($P > 0.05$).

Haplotype D12 clustered with a rat from Hainan, China. Haplotype D15 clustered with a rat from Denmark. Other rats from Japan, France, and Germany did not cluster with any of the rats from Hubei in our study.

For each D-loop haplotype, the SEOV-positive percentage varied, but there were no significant differences when evaluated with a chi-square test ($P > 0.05$) (Table 2). Most of the rats in this study were in haplotypes D1–D3, and they were distributed in Nanzhang, Yichang, and Xinzhou. The numbers of D-loop haplotypes in Qichun (seven haplotypes) and Xinzhou (six haplotypes) were larger than that in any other area (Table 2, Figure 3A). The geographic distributions of the different D-loop haplotypes were significantly different when evaluated with a chi-square test ($P < 0.01$).

cyt-b gene

The lineage *R. norvegicus* comprised 17 haplotypes, with a haplotype diversity of 1.00 ± 0.02 and a nucleotide diversity of $0.50 \pm 0.10\%$ (Table 4). Two sub-lineages, B-I and B-II, were formed. Sub-lineage B-I included six

Figure 2. Bayesian reconstruction of (A) D-loop and (B) *cyt-b* phylogenetic trees. The haplotypes of mtDNA D-loop and *cyt-b* identified in this study are presented in boldface. The number of SEOV-positive rats/number of sequenced rats (percentage) and geographic locations are noted after each haplotype. The haplotype numbers of reference sequences (obtained from GenBank) are marked according to previous descriptions (Song et al., 2014). The branches are labeled with the Bayesian posterior possibilities (cut-off >50%). The scale bar shows number of substitutions per nucleotide.

rats, with two haplotypes. Sub-lineage B-II involved four rats, with two haplotypes. There were 90 rats, with 13 haplotypes (B-others), that were not classified into sub-lineages (Figure 2B, Table 4). The SEOV-positive percentage for the sub-lineages were 71.42% (B-I), 0% (B-II), and 32.56% (B-others). There were no significant differences in the SEOV-positive percentages between the sub-lineages when evaluated with a chi-square test ($P > 0.05$).

Lineage B-I was closely related to a rat from Guangdong, China. Haplotype B13 clustered with a rat from Wuhan, Hubei, China (Lin et al., 2012), and two from Europe. Haplotype B11 clustered with a rat from Fujian, China (Lin et al., 2012). Other previously described rat *cyt-b* sequences did not cluster with any of the sequences in this study.

For each *cyt-b* haplotype, the SEOV-positive percentage varied, but there were no significant differences when evaluated with a chi-square test ($P > 0.05$) (Table 3).

The haplotypes B1-B3 included most of the rats in this study, and they were distributed in Nanzhang, Yichang, Xinzhou, and Jiangxia. The numbers of *cyt-b* haplotypes in Xinzhou (seven haplotypes) and Qichun (six haplotypes) were larger than that in any of the other areas (Table 3, Figure 3B). The geographic distributions of different *cyt-b* haplotypes were significantly different when evaluated with a chi-square test ($P < 0.01$).

DISCUSSION

Norway rats are reservoir hosts for several zoonotic pathogens that infect humans, such as hantaviruses (particularly SEOV), hepatitis E virus, *Leptospira interrogans*, and *Toxoplasma gondii* (Meerburg et al., 2009). As the main reservoir of SEOV, the distribution of Norway rats determines the spread of SEOV (Lin et al., 2012). SEOV has been found across the world, from

Table 4. Descriptive statistics of genetic variation of D-loop and *cyt-b* sequences of *R. norvegicus* and *R. nitidus* in Hubei, China

Gene	Lineage	N ^a	Nh ^b	S ^c	Hd ^d (mean ± SD)	π ^e (%) (mean ± SD)
D-loop	D-I	40	6	12	1.00 ± 0.10	0.47 ± 0.09
D-loop	D-II	6	2	1	1.00 ± 0.50	0.11 ± 0.06
D-loop	D-III	19	2	2	1.00 ± 0.50	0.22 ± 0.11
D-loop	D-others	36	7	18	1.00 ± 0.76	0.65 ± 0.16
D-loop	<i>R. norvegicus</i>	101	17	35	1.00 ± 0.02	0.74 ± 0.08
D-loop	<i>R. nitidus</i>	2	1	0	0	NA
D-loop	All	103	18	88	1.00 ± 0.02	1.48 ± 0.64
<i>cyt-b</i>	B-I	7	2	1	1.00 ± 0.50	0.09 ± 0.04
<i>cyt-b</i>	B-II	4	2	1	1.00 ± 0.50	0.09 ± 0.04
<i>cyt-b</i>	B-others	90	13	19	1.00 ± 0.03	0.33 ± 0.04
<i>cyt-b</i>	<i>R. norvegicus</i>	101	17	31	1.00 ± 0.02	0.50 ± 0.10
<i>cyt-b</i>	<i>R. nitidus</i>	2	1	0	0	NA
<i>cyt-b</i>	All	103	18	84	1.00 ± 0.02	1.04 ± 0.48

Note: ^aN, number of rats; ^bNh, number of haplotypes; ^cS, number of polymorphic (segregating) sites; ^dHd, haplotype diversity; ^eπ, nucleotide diversity; NA, non-applicable, because the number of samples was less than seven.

Figure 3. Geographic distribution of haplotypes of mtDNA (A) D-loop or (B) *cyt-b*. The geographic distributions among the different D-loop and *cyt-b* haplotypes were evaluated with chi-square tests, and there was a significant difference for both ($P < 0.01$).

Asia (Kariwa *et al.*, 1994; Ibrahim *et al.*, 1996; Guo *et al.*, 2016) to Africa (Avsic-Zupanc *et al.*, 2015), Europe (Heyman *et al.*, 2004; Heyman *et al.*, 2009; Plyusnina *et al.*, 2012; Dupinay *et al.*, 2014), and the Americas (Childs *et al.*, 1987; Cueto *et al.*, 2008; Costa *et al.*, 2014).

In the past, it was thought that Norway rats originated in the area bordering northern China and Mongolia (Hedrich, 2000; Lin *et al.*, 2012). However, recent fossil analyses indicate that they may have originated in South-western China around 1.2–1.6 million years ago (Mya) (Jin *et al.*, 2008; Wu and Wang, 2012). In addition, a phylogeographic analysis based on mtDNA *cyt-b* and D-loop sequences indicated that the Norway rat originated

in southern China about 1.3 Mya (Song *et al.*, 2014). The analysis of rat fossils collected in the Choukoutien Cave in northern China further indicated that the species arrived there about 0.14 Mya and was widely distributed across most of China and adjacent Asian countries about 0.01–0.13 Mya (Wu and Wang, 2012).

During the 15th century, Norway rats began their spread across the globe (Aplin *et al.*, 2003). The Norway rats migrated to Europe in the 18th century (Barnett, 2002). They reached North America on the ships of the new settlers by the middle of the 18th century (Grzimek, 1968). The worldwide spread of Norway rats can be directly attributed to their relationship with humans (Robin-

son, 1965). The current worldwide distribution of the Norway rats followed the distribution of human activities, and it might have taken place within the last few centuries (Lin et al., 2012).

In China, HFRS is still serious public health problem even though comprehensive prevention measures have been implemented. HTNV and SEOV are the main causes of HFRS in China. SEOV-positive rats have been found in all the provinces of China except for Qinghai (Zhang et al., 2010; Hu et al., 2015). The proportion of SEOV-related HFRS cases increased greatly in the 2000s in Hubei (Zhang et al., 2014b).

In this study, using RT-PCR, SEOV was detected in rats captured in five areas in Hubei. The SEOV-positive percentage was 6.17%. The SEOV-positive percentage in Yichang was significantly lower than that in all the other areas ($P < 0.01$). It has previously been reported that rodent density and incidences of HFRS decreased and were maintained at low levels in the Three Gorges reservoir region, which includes Yichang (Chang et al., 2016). Further continuous surveillance will be needed to monitor hantavirus infections in rodent populations.

Mitochondrial genes are associated with the course of disease caused by several viruses. Hepatitis C virus persistence is strongly associated with mitochondrial dysfunction, with liver mtDNA genetic diversity being linked to disease progression (Campo et al., 2016). The Amerindian mtDNA haplogroup B2 enhances risk of cervical cancer caused by human papillomavirus, and deregulation of mitochondrial genes may be involved (Guardado-Estrada et al., 2012). The frequency of mtDNA D-loop mutations in non-neoplastic tissue was found to be higher in HBV-infected patients with hepatocellular carcinoma than in HBV non-infected patients with hepatocellular carcinoma (Gwak et al., 2011). Mitochondrial *cyt-b* is a mediator of FAS-induced apoptosis (Komarov et al., 2008). Forced expression of *cyt-b* mutations induces mitochondrial proliferation and prevents apoptosis in human uroepithelial SV-HUC-1 cells (Dasgupta et al., 2009). It has been reported that, according to phylogenetic trees based on mtDNA D-loop or *cyt-b* gene sequences of *Apodemus agrarius*, hantavirus-positive and hantavirus-negative mice belonged to the same cluster (Yang et al., 2016).

In this study, the reconstruction of the phylogenetic relationship between rats based on complete D-loop or *cyt-b* sequences allowed the rats to be categorized into two lineages, *R. norvegicus* and *R. nitidus*, with the former group containing the majority of the rats. These two species are sister species in the genus *Rattus*, and cannot be easily distinguished from one another. *R. norvegicus* lives with humans in and around residential areas, while *R. nitidus* lives only on farmland in hilly areas. In the mountainous areas of Guizhou, Yunnan, Zhejiang, and

Hunan provinces of China, hantaviruses were isolated from *R. nitidus* (Zou et al., 2008; Zhou et al., 2009; Lin et al., 2012). In this study, both of the rats that were categorized as *R. nitidus* were SEOV negative. More samples are therefore needed to clarify the level of hantavirus infection in *R. nitidus* in Hubei province.

The geographic distribution of the different mtDNA D-loop or *cyt-b* haplotypes were significantly different. This indicates that the mtDNA genes of rats vary in different areas of Hubei. For *R. norvegicus*, 17 haplotypes of mtDNA D-loop or *cyt-b* genes were identified in Hubei. There were no significant differences among the SEOV-positive percentages for the different haplotypes. There were three sub-lineages based on the D-loop sequences, and two based on the *cyt-b* sequences. The SEOV-positive percentages for the sub-lineages did not significantly differ. Further studies with immunity-related genes, such as major histocompatibility complex (MHC) class I, MHC class II, C4A component of the complement system, tumor necrosis factor (TNF), and interleukin-1 receptor antagonist (IL-1RA) (Charbonnel et al., 2014) may shed more light on the relationship between hantavirus infections and host genetic background.

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COMPLIANCE WITH ETHICS GUIDELINES

The authors declared that they have no conflict of interests. The study was approved by the Ethics Committees of School of Basic Medical Sciences, Wuhan University. All institutional and national guidelines for the care and use of laboratory animals were followed.

AUTHOR CONTRIBUTIONS

ZQY and DYL designed the experiments. JL, BYL, YYL, HRX and WH carried out the experiments. DYL analyzed the data. DYL and ZQY wrote the manuscript. All authors read and approved the final manuscript.

Supplementary tables are available on the websites of *Virologica Sinica*: www.virosin.org; link.springer.com/journal/12250.

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