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Seoul virus in the Brown Rat (*Rattus norvegicus*) from Ürümqi, Xinjiang, Northwest of China

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ABSTRACT: *Hantavirus* infections among human populations are linked to the geographic distribution of the host rodents that carry the viruses. To determine the presence and distribution of hantaviruses in the northern region of Xinjiang Uygur Autonomous Region (XUAR), northwestern China, 844 rodents were captured from five locations in four dissimilar habitats during 2010–14 and examined for *Hantavirus* infection. *Hantavirus* nucleic acids were firstly detected in the brown rat (*Rattus norvegicus*) from Ürümqi, China, indicating that the *Hantavirus* was transmitted into Ürümqi in XUAR and circulated by the brown rat. Our results suggest that the brown rat may act as a natural reservoir for the virus in XUAR.

Key words: Brown rat, hantavirus, *Rattus norvegicus*, rodent, Seoul virus, Xinjiang Uygur Autonomous Region.

In China, hemorrhagic fever with renal syndrome (HFRS) remains a severe public health problem. There may be as many as 150,000 HFRS cases each year, with more than half occurring in China (Song et al. 1999). The case fatality rate for HFRS is as high as 12% (Vapalahti et al. 2003). *Hantaan virus* and *Seoul virus* (SEOV) have mainly caused HFRS in China (Zhang et al. 2010). *Seoul virus* is carried by the brown rat (*Rattus norvegicus*) in the urban districts of China (Lin et al. 2012). In 1982, SEOV was isolated from the brown rat in Henan Province, China, where the virus is endemic. *Seoul virus* is found in all areas of China endemic to HFRS, except for Qinghai Province and the Xinjiang Uygur Autonomous Region (XUAR) (Zhang et al. 2010). Interestingly, HFRS caused by SEOV occurs in areas in which HFRS has not been previously reported, suggesting that both the virus and the disease are spreading into other regions of China (Huang et al. 2012).

Xinjiang Uygur Autonomous Region is adjacent to eight countries. Ürümqi, the provincial capital of XUAR, and has a human population of more than 3.5 million. Only an indirect immunofluorescence assay was used to detect HFRS antigens in captured brown rats in Turpan city (east XUAR) during the 1980s (Xinjiang Center for Disease Control and Prevention unpubl. data). Further investigation is warranted because reports regarding *Hantavirus* in XUAR are limited, and the virus is emerging further into China.

To investigate the presence of hantaviruses in northern XUAR, we collected rodent samples from representative rodent habitats, which consisted of woodlands (around Shihezi City, XUAR), semideserts (surrounding the port of Alashankou, XUAR), urban areas (Ürümqi and Yining City, XUAR), and high mountain meadows (around Jinghe County, XUAR). Each habitat selection consisted of two to four trapping sites in areas in which there was a high density of the dominant species. The rodents were captured with rodent traps during 2010–14 (Table 1). The great gerbil (*Rhombomys opimus*) and the long-tailed ground squirrel (*Spermophilus undulatus*) were captured in Sherman traps (H. B. Sherman Traps, Tallahassee, Florida, USA), which were placed at the entrances of occupied burrows. Each survey site included >150 traps that were checked twice a day for a 1–2 d period. Each trap was removed before nightfall and replaced on the survey site the following day. The brown rat, gray dwarf hamster (*Cricetulus migratorius*), and other less-abundant rodents were captured using snap traps (Victor® Easy Set® Rat Trap, Woodstream Corporation, Lititz, Pennsylvania, USA), which

TABLE 1. Prevalence of *Hantavirus* nucleic acids in rodents located in North Xinjiang, China, during 2010–14.

Species	No. positive/ no. captured	Place of capture	Habitat	Coordinates	Elevation (m)	Year of capture
Rhombomys opimus	0/255	Ashankou Port	Semidesert	45°6′N, 82°42′E	273	2012
Cricetulus migratorius	0/31	Shihezi City	Woodland	44°12′N, 86°0′E	562	2010
Rattus norvegicus	11/72	Urumuqi City	Urban district	43°48′N, 87°36′E	847	2010
	0/206	Yining City	Urban district	43°54′N, 81°18′E	646	2013
Citellus undulatus	0/252	Jinghe County	High mountain meadow	44°12′N, 83°0′E	2400	2014
Other[a]	0/28	Ashankou Port	Semidesert	45°6′N, 82°42′E	273	2012

[a] Includes 9 *Allactaga sibirica,* 5 *Meriones tamariscinus,* 5 *Meriones meridianus,* 7 *Meriones libycus,* and 2 *Suncus murinus.*

were set up at approximately 5-m intervals in the evening and collected the next morning, as described by Aplin et al. (2003). In addition, 200–250 mousetraps were used in each trapping site during each survey. This study was approved by the Animal Ethics Committee of Xinjiang Medical University, Ürümqi, China (approval AECXMU2010-11).

We captured 844 rodents from the four habitats that were examined. We trapped 283 rodents (255 great gerbils [*Rhombomys opimus*], 9 Mongolian five-toed jerboas [*Allactaga sibirica*], 5 tamarisk jirds [*Meriones tamariscinus*], 5 midday jirds [*Meriones meridianus*], 7 Libyan jirds [*Meriones libycus*], and 2 Asian house shrews [*Suncus murinus*]) in the semidesert region located near Alashankou Port, 31 gray dwarf hamsters (*Cricetulus migratorius*) in the forest region near Shihezi City, 278 brown rats in the urban areas (72 in Ürümqi and 206 in Yining City), and 252 long-tailed ground squirrels (*Citellus undulatus*) in the high mountain meadow near Jinghe County.

After each captured animal was identified and euthanized, approximately 150 mg of lung tissue was collected, cut aseptically into slices <0.5 cm thick, and placed into tubes containing 5 mL RNA stabilization reagent (Ambion RNAlater®, Thermo Fisher Scientific Life Sciences, Carlsbad, California, USA). Each tube was assigned a classification number, and the samples were then placed at 5 C overnight. On the following day, the samples were transferred to –80 C for long-term cryopreservation.

Total RNA was extracted from lung tissue using the TRIzol reagent (Thermo Fisher) according to the manufacturer's instructions. The detailed cycling conditions for complementary DNA synthesis and coding DNA targeted amplification are shown in the Supplementary Material (primer nucleotide sequences; Table S1). The amplified products were analyzed on a 2% agarose gel. Positive samples were purified and ligated according to the instructions provided in the TIANgel Midi Purification Kit (TIANGEN Biotech Co., Ltd., Beijing, China) and pGEM®T Easy Vector System I (Promega, Madison, Wisconsin, USA). All of the *Hantavirus*-positive clones were selected for sequencing and analyzed using Clustal X 2.0 software (Larkin et al. 2007). The M gene fragment of SEOV was first amplified in 15% (11 or 72) of the brown rat samples from Ürümqi only. The captured *Hantavirus*-positive rodents were identified by PCR and the complete cytochrome b gene was sequenced as a phylogenetic marker (Schlegel 2012). All clones from the positive samples were classified as two representative strains, namely Ürümqi55 and Ürümqi59 (deposited in GenBank as Ürümqi55: KT188829 and Ürümqi59: KT188830 accessions). The two clones had 99.5% nucleotide identity. The silent mutations (A to G) were detected in two nucleotide sites of the M gene (2230 and 2258) in both clones.

Thirty-four *Hantavirus* sequences that were retrieved from GenBank are available in Table S2. The phylogenetic tree based on the M gene fragments revealed that the Ürümqi55 and Ürümqi59 branches were related to SEOV and

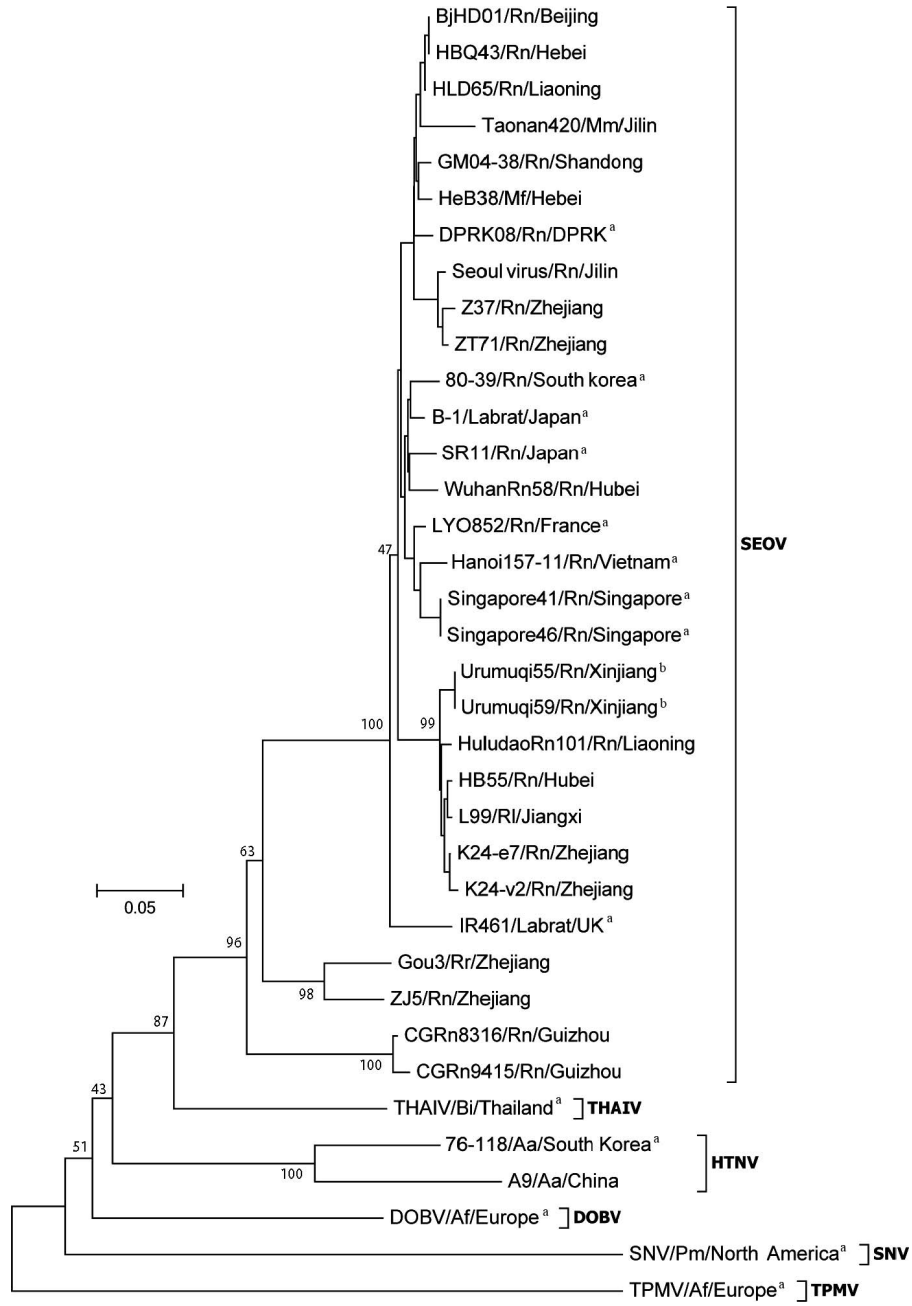

FIGURE 1. Phylogenetic relationship between *Seoul virus* (SEOV) detected or isolated within and external to China. Neighbor-joining tree was based on the partial M (418 base pair) of SEOV, including a reference for the sequencing of the *Thailand virus*, *Sin Nombre virus*, *Thottapalayam virus*, *Dobrava-Belgrade virus*, and *Hantaan virus* (76–118, A9) as outgroups. The nucleotide position of the partial M fragment sequence was from nucleotide position 1936 to 2353. The percentage (>70%) of replicate trees in which the associated viruses clustered together in the bootstrap test (1,000 replicates) are shown next to the branches. Rr=*Rattus rattus*; Rn=*Rattus norvegicus*; Rl=*Rattus losea*; Mm=*Mus musculus*; Mf=*Microtus fortis*; Pm=*Peromyscus*; Sm=*Suncus murinus*; Af=*Apodemus flavicollis*; Aa=*Apodemus agrarius*; THAIV=Thailand virus; SNV=Sin Nombre virus; TPMV=Thottapalayam virus; DOBV=Dobrava-Belgrade virus; HNTV (76-118, A9)=Hantaan virus; [a]=virus strain that originated from outside of China; [b]=virus strain reported in this article.

more closely related to HuludaoRn101, HB55, L99, k24-e7, and k24-v2, which were isolated in Liaoning, China; Hubei, China; Jiangxi, China; and Zhejiang Province, China, respectively (Fig. 1). However, we found no association between the homology of the SEOV and the geographic distribution of host rodents. Our attempts to obtain the whole nucleotide sequence of L, M, S segments were unsuccessful because of nucleic acid degradation.

Many data indicate that the translocation or trafficking of domestic and wild animals has an important role in the rapid dispersal of many zoonotic pathogens (Cutler et al. 2010). More specifically, the migration of the brown rat resulted in the worldwide distribution of the *Seoul virus* (Lin et al. 2012). Kang et al. (2012) showed that the brown rat entered XUAR by way of the Lanzhou-Xinjiang Railway line and formed a stable population in the mid-1980s, which gave rise to the gradual spread of this species throughout XUAR, resulting in the dissemination of parasites and pathogens, although local human *Hantavirus* infections that result in HFRS have not been reported. Our results indicated that 15% of brown rats carry SEOV, suggesting a high risk for HFRS in Ürümqi City. *Seoul virus* has not yet been detected in the brown rats of Yining City. It is likely virus distribution area in XUAR is not restricted to Ürümqi City and may spread to other regions in Xinjiang and even to neighboring countries, including Kazakhstan, through transportation. *Seoul virus* circulates in Ürümqi City, where the brown rat may act as a natural reservoir, as is indicated by its high prevalence in that species.

To our knowledge, there have been no reports demonstrating that hantaviruses are naturally carried by the great gerbil, long-tailed ground squirrel, or gray dwarf hamster. Our negative results indicate the possibility that these species are not susceptible to *Hantavirus* infections or that they lack exposure the viruses. Based on our studies, long-term surveillance and examination of hantaviruses in the human and rodent populations located in XUAR, and especially in Ürümqi City, are necessary to identify novel *Hantavirus* strains and ultimately to reduce the rate of human exposure.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/2015-08-210.

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