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Tacheng Tick Virus 1 and Songling Virus Infection in Great Gerbils (*Rhombomys opimus*) in Northwestern China

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ABSTRACT: Tacheng tick virus 1 (TcTV-1) and Songling virus (SGLV) were originally found in human patients in China who had had tick bites. Tamdy virus (TAMV) was detected for the first time in a tick-infested person from Kyrgyzstan in 1973. In this study, 276 great gerbils (*Rhombomys opimus*) were collected in Xinjiang Uygur Autonomous Region in northwestern China. The total RNA of individual spleen samples was extracted, and the viral L segments of TcTV-1, SGLV, and TAMV were detected by nested reverse transcription PCR. Overall, 2.9% (8/276) and 2.2% (6/276) of spleen samples tested positive to the viral L segments for TcTV-1 and SGLV, respectively; TAMV was not detected in any samples. The SGLV from the great gerbils shared 93.7% (236/252 nucleotide [nt]) and 94.0% (78/83 amino acid [aa]) identities to SGLV detected in patients infected with SGLV in northeastern China. The TcTV-1 in great gerbils was closest to TcTV-1 from a patient in China, with 98.5% (797/809 nt) and 98.9% (265/268 aa) sequence identities. This is the first molecular evidence for the presence of TcTV-1 and SGLV in great gerbils. High genetic diversity in SGLV was observed among geographical locations. Multiregion surveillance of Tamdy orthonairoviruses in more wildlife species is necessary.

Key words: China, great gerbil, *Rhombomys opimus*, Songling virus, Tacheng tick virus 1.

Tickborne orthonairoviruses (Nairoviridae: Bunyavirales) have been characterized as a global health threat to humans, domestic animals, and wildlife (Garrison et al. 2020). Tamdy virus (TAMV) was first detected in a febrile patient from Kyrgyzstan in 1973 (L'vov et al. 2014) and subsequently isolated from *Hyalomma asiaticum* ticks from Bactrian camels (*Camelus bactrianus*) in Xinjiang Uygur Autonomous Region (XUAR) in 2018 (Zhou et al. 2019). Tacheng tick virus 1 (TcTV-1) was first isolated from a patient who had tick bites plus fever and rash and was then detected in cattle and sheep from XUAR in 2019 (Liu et al. 2020). Recently, Songling virus (SGLV) has been identified in 42 of 658 hospitalized patients who had tick bites in Heilongjiang Province and Inner Mongolia Autonomous Region (IMAR) in northern China (Ma et al. 2021). However, information is lacking regarding tickborne orthonairoviruses, such as SGLV, TcTV-1, and TAMV, in wildlife, especially in northwestern China.

The great gerbil (*Rhombomys opimus*) belongs to the order Rodentia, family Cricetidae, subfamily Gerbillinae (Liu et al. 2012). Its distribution encompasses arid and semiarid regions throughout central and south Asia (Kamranrashani et al. 2013). It is a dominant mammalian species in the Gurbantünggüt Desert, XUAR, in northwestern China (covering 48,800 km^2) and is a valuable sentinel animal for multiple vectorborne diseases (Wilschut et al. 2013). It is considered a natural reservoir for *Yersinia pestis*, *Leishmania donovani*, Crimean-Congo hemorrhagic fever virus (CCHFV), Phlebotomus fever Sicilian virus, lymphocytic choriomeningitis virus, and Chim virus (Darwish et al. 1983; Hardestam et al. 2007; Kamranrashani et al. 2013; Ishii et al. 2014; Zhang et al. 2018). It remains unclear whether great gerbils could be potential reservoirs for SGLV, TcTV-1, and TAMV.

FIGURE 1. Map showing sites (in red) at which great gerbils (*Rhombomys opimus*) were caught and sampled for viruses during 2019–2021, providing new confirmed locations for Tacheng tick virus 1 (TcTV-1) and Songling virus (SGLV). Areas in which SGLV and TcTV-1 have previously been detected in China are also shown on the map, in pale blue and dark blue, respectively.

During 2019–21, 276 great gerbils were collected at 16 sampling sites in Alataw City (45°19′N, 82°56′E) and Manas County (43°28′N, 85°34′E), Gurbantünggüt Desert, XUAR (Fig. 1). The gerbils were captured using 390 Sherman live traps (30 cm × 15 cm × 15-cm wire mesh; Alataw City) baited with walnut, cucumber, or tomato. Traps were checked twice daily and rebaited whenever necessary; trap success rate was from 0.5% to approximately 3% (Kamranrashani et al. 2013; Zhao et al. 2020; Ji et al. 2021). To prevent the spread of highly pathogenic infections, the rodents were trapped and handled following biosafety guidelines (Mills et al. 1995). All wild rodents were morphologically identified by an experienced zoologist. The rodents in Manas County were kept in a cool, shaded place with sufficient food and then transported to our laboratory, whereas the wild animals captured in Alataw City were transported to the Vectorborne Laboratory at Alataw Customs. Tiletamine-zolazepam (Zoletil 50, Virbac, Paris, France) was given by intramuscular injection to anesthetize the gerbils (Yan et al. 2020). The rodents were killed by cervical dislocation while anesthetized (Feldman and Hillman 1969), and necropsy was performed. The spleen was aseptically removed from each individual, placed in an individual sterile polypropylene tube, and stored frozen at –80 C (Laakkonen et al. 2001). This study was reviewed and approved by the ethical stan-

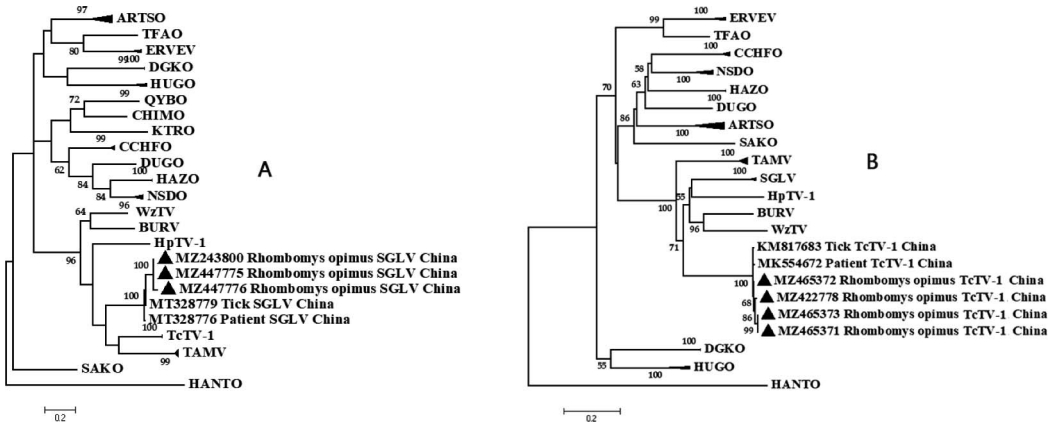

FIGURE 2. Phylogenic tree based on partial sequences of the L segment gene (820 base pairs) of spleens from great gerbils (*Rhombomys opimus*) in border regions of northwestern China. The evolutionary history was inferred using the maximum likelihood method (bootstrap replicates: 1,000) with MEGA7 (Kumar et al. 2016). Sequences obtained in this study are indicated by black triangles. (A) Songling virus. (B) Tacheng tick virus 1.

dards of Animal Ethics Committee of Shihezi University (approval A2018-143-01).

A minced spleen sample (~0.2 g) was used to extract genomic RNA by using the RNAprep Pure Tissue Kit (Tiangen, Beijing, China). The cDNAs were obtained by using a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Shanghai, China), following the manufacturer's instructions. The quantity and purity of RNA were assessed with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific) using a representative number of randomly selected samples. We used a concentration of RNA >50 ng/μL (50–100 μL); we have found this concentration to be sufficient to detect pathogens, although Gilbert et al. (2004) suggested 100 ng/μL. We carried out specific nested reverse transcription (nRT)-PCR for partial L segments of TcTV-1 (809 base pairs [bp]), SGLV (229 bp), and TAMV (228 bp) on the 276 spleen samples. Based on genome sequences available in GenBank (TcTV-1: KM817683, MK554672; SGLV: MT328776, MT328779; and TAMV: MK757580, MN792651), primer design was carried out using DNAMAN software (Lynnon Biosoft, San Ramon, California, USA). For the primers and nRT-PCR cycling conditions, see the Supplementary Material. Positive and negative controls were included in each amplification (Liu et al. 2020; Ma et al. 2021). The amplified products were cloned into the pGEM-T Easy vector (TransGen Biotech, Beijing, China) and then sequenced (Song et al. 2018). In addition, we used a BLASTn (NCBI 2021) search to identify closely related sequences. Molecular phylogenetic analyses were conducted using MEGA7 software (Kumar et al. 2016).

The viral L segments of TcTV-1 and SGLV were detected in 2.9% (8/276) and 2.2% (6/276) of spleen samples, respectively; TAMV was not detected in any samples. The BLASTn alignments indicated that 1) SGLV in great gerbil shared 93.7% (236/252 nucleotide [nt]) and 94.0% (78/83 amino acid [aa]) sequence identities to SGLV (GenBank no. MT328776) detected in human patients who had had tick bites in northeastern China; and 2) the viral segment in great gerbil was the closest to that of TcTV-1 from a human patient in China (GenBank no. MK554672), having 98.5% (797/809 nt) and 98.9% (265/268 aa) sequence identities. Phylogenetic analysis showed that the segments of SGLV in great gerbil from XUAR were different from viral sequences from patients (GenBank no. MT328776; Fig. 2). The viral segment of TcTV-1 in our study was most closely related to that from a human patient sampled in 2018 in XUAR (GenBank no. MK554672), although four sequences from the spleen samples of

great gerbils showed remarkable genetic diversity (98.8–99.0%; Fig. 2).

Tamdy orthonairoviruses (Nairoviridae: *Orthonairovirus*) include at least six valid viruses (Zhou et al. 2019; Liu et al. 2020; Ma et al. 2021). To date, TcTV-1, SGLV, and TAMV have been considered human pathogens with public health significance. However, potential reservoirs for TcTV-1, SGLV, and TAMV are unknown. Our detections of TcTV-1 and SGLV in spleen samples of great gerbils suggest that these rodents may be reservoirs in the epidemiologic cycles of TcTV-1 and SGLV, although the presence of other members of Tamdy orthonairoviruses should be investigated in more wild mammal species in follow-up studies.

Previous phylogenetic analyses indicated that CCHFV isolates collected in a worldwide context belonged to seven distinct clades according to geographical location (Deyde et al. 2006). Our finding that the viral L segment of SGLV in great gerbil shared 93.7% (236/252 nt) and 94.0% (78/83 aa) identities with those of SGLV (accession no. MT328776) in a human patient from northeastern China who had had tick bites indicates that SGLVs show high genetic diversity between geographic regions, similar to the polymorphism of CCHFV among different countries, and even within a country (Drosten et al. 2002; Deyde et al. 2006). In addition, TcTV-1 presented genetic diversity (98.8–99.0%) even among great gerbils captured in the same region. Future studies examining whether links exist between viral genetic diversity, geographic locations, and hosts of TcTV-1 may provide additional insights.

Previously, TcTV-1 had been found in seven counties or cities of northwestern China (Liu et al. 2020) and SGLV had been detected from Heilongjiang Province and IMAR (Ma et al. 2021). Our study expands knowledge of the geographical distribution of TcTV-1 and SGLV.

To gain further understanding of Tamdy orthonairovirus infections in great gerbils, future studies should examine whether pathologic changes occur after natural infection with SGLV or TcTV-1 and evaluate whether other species of Tamdy orthonairoviruses infect great gerbils.

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SUPPLEMENTARY MATERIAL

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