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Detection and phylogenetic analysis of torque teno virus (TTV) carried by murine rodents and house shrews in China

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ABSTRACT

Between May 2015 and May 2017, 496 animals (473 murine rodents and 23 house shrews) were captured in six regions of China. A total of 22.8% (113/496) of throat swabs, 29.1% (142/488) of fecal samples and 23.8% (54/227) of serum samples tested positive for rodent torque teno virus 3 (RoTTV3). The positive rate in *Rattus norvegicus* was higher than the rate in *Rattus tanezumi* and *Rattus losea*. Of 23 house shrews, one throat swab and one serum sample were positive for RoTTV3. Ten murine rodents were simultaneously positive for RoTTV3 in throat swab, fecal and serum samples. Phylogenetic analysis showed that the 12 near-full length genomes of RoTTVs sequences obtained in this study represented a novel RoTTV genotype (RoTTV3). In conclusion, high prevalence rates of RoTTV3 were found in three common murine rodents in China, and the RoTTV3 obtained in this study were classified as a novel genotype of RoTTV.

1. Introduction

Torque teno virus (TTV) belongs to the genus *Anelloviridae*, which has a nonenveloped single-stranded DNA genome (Hino and Miyata, 2007). TTV was first discovered in 1997 in a Japanese patient with post-transfusion non-A–G hepatitis (Nishizawa et al., 1997). TTV is widely distributed throughout the world (Hettmann et al., 2016; Bendinelli et al., 2001; Cancela et al., 2016), and it has been suggested that it is associated with various diseases, such as hepatitis, respiratory diseases, cancers, hematological disorders and autoimmune disorders (Spandole et al., 2015).

Abundant epidemiological surveys have shown that TTV is extensively dispersed among humans, and among a wide variety of wild and domesticated mammalian species (Hino and Miyata, 2007; Spandole et al., 2015; Leary et al., 1999; Okamoto et al., 2002; Martinez et al., 2006). However, there are only rare reports of TTV being found in rodents and shrews. Rodents are known to serve as the natural reservoirs of myriad zoonotic agents, including *Arenaviridae*, *Reoviridae*, *Togaviridae* and *Picornaviridae* (Phan et al., 2011; Himsworth et al., 2013). Shrews are small mole-like mammals that bear a close resemblance to long-nosed mice. Similar to rodents, shrews can act as reservoirs for a number of zoonotic disease pathogens (Sasaki et al., 2014; Yashina et al., 2010; Hsieh et al., 2010). Some species of rodents and shrews prefer to live on the ground and are commonly found near urban settings, which offer numerous opportunities for cross-species viral transmission.

In 2014, a study investigated TTV in wild rodents in the UK and identified novel anelloviruses, designated rodent TTV (RoTTV) (Nishiyama et al., 2014). The identified RoTTVs had a genomic organization consistent with other anelloviruses, but they formed two clear phylogenetic groups that were as distinct from each other as from previously defined genera (Nishiyama et al., 2014). Furthermore, the same research team investigated the prevalence of RoTTV infection in commonly used laboratory rats. They found that all ten laboratory rats (*Rattus norvegicus*) were RoTTV-positive (Nishiyama et al., 2015). No related research reports were identified except for the above two reports.

Research on TTV in rodents is important for better understanding of the origin and molecular relatedness of these viruses. In this study, we investigated the prevalence of RoTTV in murine rodents and house shrews in six regions of four provinces in China by detecting RoTTV DNA in throat swab, fecal and serum samples. The partial nucleic acid sequence of open reading frame 1 (ORF1) in RoTTV-positive specimens was amplified. Twelve near full-length genomes were obtained to elucidate the characteristics of RoTTV.

2. Methods

2.1. Samples

A total of 496 samples (473 murine rodents and 23 house shrews) were captured using cage traps between May 2015 and May 2017.

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Table 1
Prevalence of rodent torque teno virus (RoTTV) in murine rodents and house shrews (%, n).

		XM (2015.10)	SZ (2015.05)	GZ* (2016.02–2017.02)	YY (2017.03)	MM (2017.04)	MLP (2017.05)	Total
Throat swab samples	*Rattus norvegicus*	15.0 (3/20)	66.7 (2/3)	31.3 (46/147)	20.0 (15/75)	19.4 (18/93)	42.3 (22/52)	27.2 (106/390)
	Rattus tanezumi	0 (0/13)	0 (0/1)	16.7 (1/6)	13.3 (2/15)	—	—	8.6 (3/35)
	Rattus losea	6.3 (3/48)	—	—	—	—	—	6.3 (3/48)
	Suncus murinus	0 (0/8)	0 (0/4)	9.1(1/11)	—	—	—	4.3 (1/23)
	Subtotal	6.7 (6/89)	25.0 (2/8)	29.3 (48/164)	18.9 (17/90)	19.4 (18/93)	42.3 (22/52)	22.8 (113/496)
Fecal samples	*Rattus norvegicus*	50.0 (10/20)	—	42.2 (62/147)	20.0 (15/75)	16.1 (15/93)	55.8 (29/52)	33.9 (131/387)
	Rattus tanezumi	0 (0/13)	—	16.7 (1/6)	13.3 (2/15)	—	—	8.8 (3/34)
	Rattus losea	16.7 (8/48)	—	—	—	—	—	16.7 (8/48)
	Suncus murinus	0 (0/8)	—	0 (0/11)	—	—	—	0 (0/19)
	Subtotal	20.2 (18/89)	—	38.4 (63/164)	18.9 (17/90)	16.1 (15/93)	55.8 (29/52)	29.1 (142/488)
Serum samples	*Rattus norvegicus*	58.3 (7/12)	100.0 (2/2)	45.5 (35/77)	—	0 (0/58)	0 (0/24)	25.4 (44/173)
	Rattus tanezumi	22.2 (2/9)	0 (0/1)	0 (0/2)	—	—	—	16.7 (2/12)
	Rattus losea	21.2 (7/33)	—	—	—	—	—	21.2 (7/33)
	Suncus murinus	0 (0/2)	100.0 (1/1)	0 (0/6)	—	—	—	11.1 (1/9)
	Subtotal	28.6 (16/56)	75.0 (3/4)	41.2 (35/85)	—	0 (0/58)	0 (0/24)	23.8 (54/227)

XM, Xiamen city in Fujian province; SZ, Shenzhen city in Guangdong province, GZ, Guangzhou city in Guangdong province; YY, Yiyang city in Hunan province; MM, Maoming city in Guangdong province; MLP, Malipo county in Yunnan province.

* There were two sample collection regions in Guangzhou city: Baiyun district (BY) and Huadu district (HD).

These samples were captured in six regions of four provinces in China: Xiamen city in Fujian province (XM, n = 89); Shenzhen city (SZ, n = 8), Maoming city (MM, n = 93) and Guangzhou city (GZ, n = 164) in Guangdong province; Yiyang city in Hunan province (YY, n = 90); and Malipo county in Yunnan province (MLP, n = 52) (Table 1). There were two collection regions in Guangzhou city: Baiyun district (BY) and Huadu district (HD). All the rodents and shrews were captured in or close to human residences.

The rodents and shrews were anesthetized with diethyl ether to draw cardiac blood, and serum samples were subsequently obtained using centrifugation. Throat swab samples were obtained by swabbing the posterior oropharynx and then the swabs were soaked in phosphate-buffered saline (PBS). No serum samples were collected from Yiyang city (YY) and no fecal samples were collected from Shenzhen city (SZ). All the throat swab, fecal and serum samples were frozen immediately at −80°C for storage and thawed at 4°C prior to processing.

2.2. Nucleic acid extraction and detection of TTV

Using a MiniBEST Viral RNA/DNA Extraction Kit (TaKaRa, Japan), a 200 µl aliquot of each serum, fecal and throat swab sample was extracted according to the manufacturer's instructions, and it was eluted to produce a final volume of 50 µl. When the serum samples contained < 200 µl, they were excluded from the nucleic acid extraction process. Finally, nucleic acid was extracted from 496 throat swab samples, 488 fecal samples and 227 serum samples.

Polymerase chain reaction (PCR) was conducted to detect TTV DNA. Based on a TTV reference sequence (GenBank accession number KM609325), we designed a pair of primers, TTV-F-1171 (forward, 5′-CGGGCTTCGGATACAACCAAGAA-3′) and TTV-R-1917 (reverse, 5′-CTACCGTCAAAGGTCGTCGGATT-3′), to amplify a 747-bp fragment containing a partial nucleic acid sequence of ORF1. Two microliters of DNA were added to a 25 µl system containing Premix Taq™ according to the manufacturer's protocol (TaKaRa, Japan). Amplification was carried out under the following conditions: 94 °C, 5 min (1 cycle); 94 °C, 30 s; 60 °C, 35 s; 72 °C, 45 s (40 cycles); 72 °C, 10 min (1 cycle); 4 °C, infinity. The amplified products were separated on a 1% agarose gel, and virus-positive samples were sent to TaKaRa Biotechnology for sequencing.

2.3. Genome sequencing

Based on the RoTTV reference sequence (GenBank accession number KM609325.1), six pairs of primers were designed to amplify the viral genome. Information regarding these primers is shown in Table S1. After all the fragments had been sequenced, Lasergene SeqMan software (DNASTAR, Madison, Inc.) was used to assemble the sequences. Finally, 12 near full-length genomes of RoTTV3 were obtained: XM.73, HD.69, HD.78, MM.51, MM.59, MM.86, MLP.31, MLP.44, BY.483, BY.500, BY.533 and SZ.1 (the GenBank accession numbers are MF926271 to MF926282, respectively).

2.4. Rodent and shrew species identification

The species of murine rodents and house shrews were confirmed by preliminary morphological identification and sequencing of the cytochrome b gene (cyt b) fragment in the mitochondrial genome, which is recognized as an accurate technique that can be used for almost all kinds of biological samples. The primers and PCR amplification conditions were as previously described (Arai et al., 2008).

2.5. Phylogenetic analysis and statistical analysis

All nucleic acid sequences revealed in this study have been deposited in the GenBank database. Multiple alignments were performed using a ClustalW multiple sequence alignment program, which was implemented in MEGA version 6.0 (Oxford Molecular Ltd., UK). The percent identity was selected to display the similarity of each sequence pair after multiple alignment by using Lasergene MegAlign software (DNASTAR, Madison, Inc.). Phylogenetic trees were constructed using the neighbor-joining (NJ) and maximum likelihood (ML) methods based on the results of alignment with MEGA version 6.0. The robustness of the tree topology was assessed using 1000 bootstrap replicates, as implemented in the program. The data were analyzed using SPSS software (version 13.0, Chicago, IL) and the chi-square test was used to compare the positive rates among different species and sample collection regions. A P value of 0.05 was considered to be statistically significant.

2.6. Compliance with ethics guidelines

The study protocol was approved by the ethics committee of the Institutional Animal Care and Use Committee of Southern Medical University.

3. Results

3.1. Prevalence of RoTTV in rodents and shrews

Between May 2015 and May 2017, a total of 496 samples were collected, including 390 *Rattus norvegicus*, 35 *Rattus tanezumi*, 48 *Rattus*