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Identification and characterization of Jingmen tick virus in rodents from Xinjiang, China

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ABSTRACT

Jingmen tick virus (JMTV) is a recently identified virus which provides an unexpected connection between segmented and unsegmented RNA viruses. Recent investigations reveal that JMTV including JMTV-like virus (Alongshan virus) could be associated with human disease, suggesting the significance of JMTV in public health. To better understand the genetic diversity and host range of JMTV, a total of 164 rodents representing 8 species were collected in Qapqal Xibe county of Xinjiang Uygur Autonomous Region, China, and were screened for JMTVs using RT-PCR. Consequently, JMTV was identified in 42 rodents including 23 Microtus arvalis voles (24.5%), 9 Apodemus uralensis mice (29.0%), 5 Mus musculus mice, 1 Rhombomys opimus gerbil, 1 Meriones amariiscinus gerbil, 1 Meriones libycus gerbil, 1 Orientalus migratorius hamster and 1 Microtus gregalis vole. Interestingly, nearly complete genome sequences were successfully recovered from 7 JMTV positive samples. Although the newly identified rodent JMTVs were closely related to those previously identified in ticks from China, based on the phylogenetic analysis of the S1, S2 and S3 segments, the newly identified rodent viruses clustered into two genetic groups. One group comprised of viruses only found in China, based on the phylogenetic analysis of the S1, S2 and S3 segments, the newly identified rodent viruses clustered into two genetic groups. One group comprised of viruses only found in China, while another group included viruses from A. uralensis, C. migratorius and M. gregalis. However, all rodent viruses clustered together in the S4 tree. Considering rodents live in close proximity to humans, more efforts are needed to investigate the role of rodents in the evolution and transmission of JMTV in nature.

1. Introduction

Jingmen tick virus (JMTV) was first identified in ticks sampled from Jingmen city of Hubei province and Wenzhou city of Zhejiang province of China in 2010 (Qin et al., 2014). The JMTV genome comprises four separate segments of linear, positive-sense single-stranded RNA, which are referred to as the segments S1, S2, S3 and S4, respectively. The segments S1 and S3 encode nonstructural proteins (NSP1 and NSP2), while the segments S2 and S4 encode structural proteins (VP1, VP2 and VP3). The segments S1 and S3 are related to the nonstructural protein genes (NS3 and NS5) of classic flaviviruses of the family Flaviviridae, the remaining two segments (S2 and S4) share no homology with viral sequences of any known viruses (Qin et al., 2014). JMTV is as such one of the most recent discovered viruses to provide an unexpected link between segmented and unsegmented RNA viruses. Since the discovery of JMTV in 2010, a group of JMTVs and JMTV-like viruses, which are named Jingmenivirus (Shi et al., 2016), have been identified in arthropods and mammals including cattle and monkey sampled from Asia, Africa, Europe and America (Qin et al., 2014; Ladner et al., 2016; Shi et al., 2016; Villa et al., 2017; de Souza et al., 2018; Emmerich et al., 2018; Samero et al., 2019; Temmam et al., 2019), showing a remarkable diversity and a global geographic distribution. In addition, Jingmenivirus also exhibit diverse genome organization strategies such as variation in segment numbers, monopartite and multipartite forms (Ladner et al., 2016; Shi et al., 2016; Villa et al., 2017; de Souza et al., 2018; Emmerich et al., 2018; Samero et al., 2019; Temmam et al., 2019), showing a remarkable diversity and a global geographic distribution. In addition, Jingmenivirus also exhibit diverse genome organization strategies such as variation in segment numbers, monopartite and multipartite forms (Ladner et al., 2016; Shi et al., 2016; Villa et al., 2017). More importantly, JMTV has been identified in humans suffering with hemorrhagic fever in Kosovo (Emmerich et al., 2018). Recently, human febrile illness associated with JMTV and JMTV-like virus [named Alongshan virus (ALSV)] was also described in China (Jia et al., 2019; Wang et al., 2019). All these data reveal the importance of
Jingmenviruses in both virus evolution and public health, therefore calling for further efforts to better understand the diversity, prevalence, and transmission of JMTV in nature to prevent its emergence.

Rodents are mammals of the order Rodentia, which includes approximately 2277 species worldwide (Wilson and Reeder, 2005). They represent more than 40% of the world’s mammalian biodiversity and are found in all continents with the exception of Antarctica. Due to their high diversity, global distribution and frequent contact with humans, rodents are a major reservoir for a broad range of human pathogens such as arenaviruses, hantaviruses, and rodents are a major reservoir for a broad range of human pathogens such as arenaviruses, hantaviruses, and rodents are a major reservoir for a broad range of human pathogens such as arenaviruses, hantaviruses, and Yersinia pestis bacteria (Li et al., 2015; Milholland et al., 2018; Meerburg et al., 2009). Considering that JMTV have been shown to infect mammals (Qin et al., 2014; Ladner et al., 2016; de Souza et al., 2018), and that rodents are natural hosts of a broad range of ticks, we hypothesized that rodents may harbor JMTV and play an important role in its transmission and evolution.

In this study, we performed a molecular epidemiology survey of JMTV in rodents sampled from Qapqal Xibe Autonomous County, Yili Autonomous Prefecture, Xinjiang Uygur Autonomous Region of China, in order to investigate the presence and diversity of JMTV in rodents.

2. Materials and methods

2.1. Collection of animals

During 2016, a total of 164 rodents were captured trapped in Qapqal Xibe Autonomous county of Autonomous Yili prefecture of Xinjiang Uygur Autonomous Region, China. All captured rodents were first identified to species by morphological examination. Rodent species were then confirmed by analyzing sequence of mitochondrial cytochrome b (mt-cyt b) gene (Guo et al., 2013; Chen et al., 2019). Tissue samples of heart, liver, spleen, lung, and kidney were collected from rodents to detect JMTV.

The study was reviewed and approved by the ethics committee of the National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention (CDC). All animals were handled following the protocols approved by the Laboratory Animal Use and Care Committee, CDC. Particularly, rodents were anesthetized with ether before surgery as previously described (Guo et al., 2013), and then liver, lung and other tissue samples were collected and stored at −80 °C.

2.2. Extraction of DNA and RNA, RT-PCR and sequencing

DNA and RNA were extracted from liver, lung and other tissue samples using a DNA/RNA isolation kit (Omega biotek, USA) according to the manufacturer’s instruction. A one step RT-PCR kit (TaKaRa, Dalian, China) was used to reverse transcribe total RNA. JMTV and JMTV-like viruses were detected by a nested RT-PCR as described previously (Qin et al., 2014; Shi et al., 2016). We also attempted to recover the complete genome of JMTV using primers designed previously by our laboratory (Qin et al., 2014; Shi et al., 2016). The primers used in this study are described in Table S1. The 5’ and 3’ ends of the four segments were amplified by using a RACE kit (TaKaRa, Dalian, China). Simple PCR was used to amplify mt-cyt b gene as described previously (Guo et al., 2013). Finally, as cross contamination is an often issue for PCR amplification, strict protocols for sample collection, animal dissection, tissue homogenization, RNA or DNA isolation, negative and positive controls were performed to prevent from false positive. Our criteria for positive sample was based on next PCR positive for 2–3 segments of JMTV, apart from negative and positive controls.

The PCR products were purified using QIAquick Gel Extraction kit (Qiagen, Valencia, USA) for sequencing. Purified DNA with < 700 bp was sequenced directly using the standard Sanger sequencing method by Shanghai Sangon Biotechnology Company (Shanghai, China). However, those with > 700 bp was firstly cloned into pMD18-T vector (TaKaRa, Dalian, China), and then transformed into JM109-143 competent cells. For each sample, at least three clones were selected for sequencing. All the sequencing data were assembled by Seqman.

All viral genome sequences obtained in this study have been deposited in GenBank and assigned accession numbers (virus: MK174230 to MK174257 and MN369292-MN369308; mt-cyt b: MN454323-MN454334 and MT024684-MT024691) (Table S2).

2.3. Phylogenetic analysis

Viral sequences were aligned using the Clustal W method implemented in the MEGA software, version 6.0 (Tamura et al., 2013). Identities of viral sequences were calculated by DNAStar (version 5.01). Viral sequences used for phylogenetic analysis in this study are described in Table S2.

Phylogenetic trees were constructed by using the maximum likelihood (ML) method available within the PhyML version 3.0 program (Guindon et al., 2010). jModelTest was used to determine the best-fit model of nucleotide substitution of all aligned sequences (Posada, 2008). Subtree Pruning and Regrafting (SPR) branch-swapping algorithm was chosen, then Shimodaira-Hasegawa-like (SH-like) procedure was used in the approximate likelihood ratio test (aLRT). Bootstrap analysis was performed with 1000 replicates with bootstrap values > 70% considered significant.

3. Results

3.1. Detection and organ distribution of JMTV in rodents

During 2016, a total of 164 rodents, which included 94 Microtus arvalis (common vole), 31 Apodemus uralensis (Ural field mouse), 13 Rhombomys opimus (great gerbil), 12 Mus musculus (house mouse), 6 Meriones tamariscinus (Kalmkyk gerbil), 5 Meriones libycus (Lybian jird), 2 Cricetulus migratorius (grey hamster) and 1 Microtus gregalis (narrow-headed vole), were captured in Qapqal Xibe Autonomous county of Yili Autonomous prefecture of Xinjiang Uygur Autonomous Region, China. The RT-PCR, which targets the conserved region of the S1 to S4 segments of JMTV, as described previously (Qin et al., 2014; Shi et al., 2016), was used to screen for JMTV and JMTV-like viruses in liver tissues from these rodents. Notably, 42 liver samples were found positive by RT-PCR, including 23 M. arvalis (24.5%), 9 A. uralensis (29.0%) and 5 M. musculus, 1 R. opimus, 1 M. tamariscinus, 1 M. libycus, 1 C. migratorius, 1 M. gregalis, with a total detection rate of 25.6% (Table 1). Genetic analysis of the recovered viral sequences revealed that they were closely related to each other with up to 90.7% nucleotide identity and shared the high similarity (up to 87.2%) with known JMTVs discovered in ticks sampled from China (Qin et al., 2014; Jia et al., 2019), indicating the circulation of JMTV in rodents sampled from Xinjiang of China. However, JMTV-like viruses had not yet been found from these rodents.

To determine the tissue distribution of JMTV in rodents, organ

| Table | Prevalence of Jingmen tick virus in liver of rodents in Qapqal of Xinjiang, China. |
|-------|----------------------------------------------------------------------------------|
| Species                  | PCR positive/Samples collected | Total          |
|                        | Male     | Female    |                  |
| Microtus arvalis        | 7/28     | 16/66     | 23/94           |
| Apodemus uralensis      | 5/17     | 4/14      | 9/31            |
| Rhombomys opimus        | 0/10     | 1/3       | 1/13            |
| Mus musculus            | 3/7      | 2/5       | 5/12            |
| Meriones tamariscinus    | 1/3      | 0/3       | 1/6             |
| Meriones libycus        | 1/5      | 0/0       | 1/5             |
| Cricetulus migratorius  | 1/2      | 0/0       | 1/2             |
| Microtus gregalis       | 1/1      | 0/0       | 1/1             |
| Total (%)               | 19/73    | 23/91(25.3)| 42/164 (25.6)   |
samples from individual rodents were screened by PCR for detecting JMTV. Consequently, JMTV was found in the heart, liver, spleen, lung, and kidney sampled from rodents collected in Xinjiang (Table 2). However, the detection rate in tissue samples was different, with the highest in liver (25.6%).

3.2. Genetic analysis of the newly identified rodent-borne JMTVs

To better characterize the JMTVs harbored by rodents, 5 partial and 7 nearly complete or complete coding genome sequences were recovered from viral RNA-positive samples (XJ9, XJ26, XJ58, XJ61, XJ77, XJ155, XJ335, XJ363, XJ364, XJ445, XJ483 and XJ511) (Table S3). Notably, all the nearly complete genome sequences were obtained from *M. arvalis* voles. Genetic analysis of the recovered genome sequences revealed that the viruses sampled from voles shared the lowest levels of sequence diversity, with 0.1–1.5% nucleotide differences in all four segments (Tables S4 and S5). Vole viruses (strains XJ58, XJ61, XJ77, XJ155, XJ335, XJ363, and XJ364) were most closely related to the strains (JMTV_YJ3-3 and JMTV85) at the nucleotide level (up to 99.8% similarity in the S1 segment and 100% similarity in the S2 and S4 segments, Tables S4 and S5), which were identified in *Rhipicephalus microplus* tick sampled from Wenzhou city of Zhejiang province and Jingmen city of Hubei province, respectively (Qin et al., 2014), more than 4000 km away from Xinjiang. The nucleotide differences between these sequences and other tick viruses sampled from other regions in China (Qin et al., 2014) were up to 8.9% (Tables S4 and S5). Additionally, the nucleotide similarities between viruses identified in China and those identified in Africa, Europe, and America ranged from 77.8% to 92.1% (Tables S4 and S5). Finally, all these JMTV are far distant from ALSV (Table S4 and S5), with < 72.0% nucleotide and < 79.2% amino acid identity for the nonstructural genes and < 65.0% nucleotide and < 76.3% amino acid identity for the structural genes, suggesting that ALSV may represent a novel member of Jingmenvirus.

3.3. Phylogenetic relationship between rodent borne JMTV and known JMTV

To better understand the newly identified rodent JMTVs from...
Xinjiang, phylogenetic trees based on all four segment sequences were estimated using the ML method. In the four phylogenetic trees (Figs. 1 and 2), all known JMTVs identified in and outside of China were grouped into three phylogenetic lineages, exhibiting a high genetic diversity. The first group comprises of the viruses sampled from Asia, Africa and South America. The second group contains those identified in Kosovo and Trinidad and Tobago (Emmerich et al., 2018; Samero et al., 2019). Within the first group, viruses were classified into three lineages (Figs. 1 and 2). All Chinese viruses including those identified in this study and sampled from Lao PDR clustered together and formed the phylogenetic lineage I (Qin et al., 2014; Temmam et al., 2019). The second lineage just consisted of one virus which was identified in a monkey sampled from Uganda (Ladner et al., 2016), while the third lineage was comprised of those identified in ticks sampled from Brazil and Guinea (Villa et al., 2017; de Souza et al., 2018).

Within the first lineage, the Chinese JMTVs still exhibited a high diversity (Figs. 1 and 2). Notably, the rodent viruses sampled from Xinjiang were grouped into two distinct sub-lineages in the S1, S2 and S3 trees. The viruses sampled from M. arvalis voles clustered together and showed a closer evolutionary relationship with JMTV_Y3-3 identified in R. microplus from Wenzhou city of Zhejiang province (Qin et al., 2014), while the viruses sampled from Ural field mice (A. uralensis), grey hamsters (C. migratorius), narrow-headed voles (M. gregalis) formed another sub-lineage with other JMTVs identified in R. microplus from Hubei province (Qin et al., 2014). However, all newly identified rodent viruses from Xinjiang clustered together and formed one lineage in the S4 tree.

3.4. Phylogenetic analysis of rodents

Rodents are geographically distributed worldwide, and are highly diverse. To confirm the host species assignment for the rodent JMTV viruses and the transmission of JMTV in relation to host genetic diversity, sequences of cytochrome b gene were amplified from liver tissues of RNA positive rodents captured in Qapqal Xibe Autonomous county of Xinjiang. With these data we compared the phylogenetic relationships between rodents captured in Xinjiang and known rodents. In the tree (Fig. 3), all eight species of rodents captured in this study were closely related to known species. Furthermore, they showed a close phylogenetic relationship with those sampled from Xinjiang and neighboring areas. Particularly, common voles (M. arvalis) captured here were closely related to those also from Yili Autonomous prefecture of Xinjiang and Kazakhstan, while Ural field mice (A. uralensis) clustered together with those sampled from Bole city - a neighboring region of Yili, and Kazakhstan and Uzbekistan.

4. Discussion

Since its discovery in 2010 (Qin et al., 2014), JMTV has been identified not only in multiple Chinese regions (Shi et al., 2016; Jia
Fig. 3. Phylogenetic relationships among voles, hamsters, gerbil and mice captured in China and other known rodents obtained from the GenBank. The ML trees were constructed based on mt-cyt b gene. The sequence of *Murina leucogaster* was used as an outgroup. The highlighted colour in tree indicated the new identified hosts in this study. The bootstrap support values greater than 70% are shown at relevant nodes. Scale bar represents number of nucleotide substitutions per site.
et al., 2019), but also in Asia, Africa, America and Europe (Qin et al., 2014; Ladner et al., 2016; Shi et al., 2016; Villa et al., 2017; de Souza et al., 2018; Emmerich et al., 2018; Jia et al., 2019; Samero et al., 2019; Temmam et al., 2019), indicating a worldwide distribution. Additionally, JMTVs and JMTV-like viruses exhibit a high genetic diversity (Ladner et al., 2016; Shi et al., 2016; Wang et al., 2019). In this study, the newly identified rodent JMTVs from Qapqal Xibe Autonomous county of Xinjiang also show a high genetic diversity, especially up to nearly 10% nucleotide difference in the both the S2 and S4 segments. Interestingly, although viruses (Xinjiang-Au-9; Xinjiang-Mg-26; Xinjiang-Au-445; Xinjiang-Au-483; and Xinjiang-Cm-511) were sampled from different rodent species, they exhibited high similarity (Figs. 1 and 2). Considering the hosts were captured from the same trapping location, this may suggest common transmission of these viruses between these different rodent species.

During a long-term of evolutionary history, some of mammal RNA viruses establish a specific association with their mammal hosts and show a geographic clustering pattern such as arenaviruses and hantaviruses (Li et al., 2015; Guo et al., 2013). However, generally, some of arthropod RNA viruses have lost the ability to transmit in vertebrates such as flaviviruses and phleboviruses (Elliott and Brennan, 2014; Bradley and Andrew, 2015; Zhang et al., 2012). To date, JMTVs have been identified in both arthropods and mammals (Qin et al., 2014; Ladner et al., 2016; Villa et al., 2017; de Souza et al., 2018; Jia et al., 2019; Samero et al., 2019; Temmam et al., 2019). Herein, JMTV is also identified in multiple species of rodents. All these data indicate that JMTVs have a broad range of hosts (Qin et al., 2014; Ladner et al., 2016; Villa et al., 2017; de Souza et al., 2018; Emmerich et al., 2018; Jia et al., 2019; Samero et al., 2019; Temmam et al., 2019). These data also suggest that no observable variation in genome structure is needed for the transmission of JMTV in between arthropods and mammals. Although JMTVs show a geographic cluster pattern in a large geographical scale, such as between different continents, the viruses identified in Xinjiang are closely related to those identified in Hubei and Zhejiang provinces of China (Qin et al., 2014), which are very distant from Xinjiang (about 4000 km apart). Additionally, one single lineage of viruses is just identified in M. arvalis voles. In sum, all these data suggest a complex evolutionary history of JMTV.

Rodents are geographically distributed worldwide, being the most diverse mammals. Additionally, they often live in close proximity to humans or domestic animals. They serve as one of the most important reservoirs for a broad range of human pathogens, and they play a key role in the transmission of zoonotic diseases in humans such as hemorrhagic fever with renal syndrome and plague (Meerburg et al., 2019; Temmam et al., 2019), over the past decade, more and more viruses have been identified in rodents sampled around the world including coronavirus, rotavirus, paramyxovirus, and orthopoxvirus (Wang et al., 2015; Oldal et al., 2015; Li et al., 2016; Berto et al., 2018). In addition to the high prevalence of JMTV in a broad range of arthropods (Qin et al., 2014; Villa et al., 2017; de Souza et al., 2018; Jia et al., 2019; Samero et al., 2019; Temmam et al., 2019), previous studies also revealed the presence of JMTV in cattle and monkey (Qin et al., 2014; Ladner et al., 2016; de Souza et al., 2018). Recently, JMTVs including ALSV are found to be associated with human disease (Jia et al., 2019; Wang et al., 2019). In this study, JMTV was identified in eight species of rodents sampled from Qapqal Xibe Autonomous County of Xinjiang, China, with a high detection rate (25.6%). Furthermore, JMTV was also identified in a broad range of tissue samples. Hence, our data indicate that rodents are one of the natural hosts of JMTV. In addition, as shown in Figs. 1 and 2, rodent JMTVs were closely related to those identified in arthropods from China (Qin et al., 2014; Jia et al., 2019). The close relationship between rodent and tick JMTVs suggests a possibility of horizontal transmission between arthropods and rodents. As rodents are also a natural host of ticks, rodents may play an important role in the evolution and transmission of JMTV. However, as the current investigation was performed only in a small region of Xinjiang, a large-scale surveillance is needed to fully understand the circulation and transmission of JMTV in rodents and between rodents and arthropods.

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Credit author statement

I declare that the submitted article was finished independently by the tutor's guidance, all results and data obtained in this study were authentic and repeatable, with none of the material has been published or is under consideration elsewhere.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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