SCIENCE CHINA
Life Sciences

•LETTER TO THE EDITOR•

May 2019 Vol.62 No.5: 701–704
https://doi.org/10.1007/s11427-018-9435-7

Novel hepacivirus in Asian house shrew, China

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Received September 25, 2018; accepted October 29, 2018; published online January 28, 2019

Citation: Guo, H., Cai, C., Wang, B., Zhuo, F., Jiang, R., Wang, N., Li, B., Zhang, W., Zhu, Y., Fan, Y., et al. (2019). Novel hepacivirus in Asian house shrew, China. Sci China Life Sci 62, 701–704. https://doi.org/10.1007/s11427-018-9435-7

Dear Editor,

Hepatitis C virus (HCV) is a leading global cause of various liver diseases, including chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. The genome of HCV is monopartite, single-stranded, positive RNA, about 10 kb in size. HCV is the prototype species of the *Hepacivirus* genus, which contains 14 species according to the update from the International Committee on Taxonomy of Viruses (Smith et al., 2016). Prior to 2005, humans were thought to be the only host of HCV; however, after that, genetically diverse hepaciviruses were detected or isolated from dogs, cows, horses, primates, bats, and rodents.

Asian house shrews (*Suncus murinus*, also called Asian musk shrews) are small insectivore mammals belonging to the family Soricidae, order Eulipotyphla. They are widely distributed in southeastern Asia, Africa, coastal Arabia, islands in the Indian Ocean. Many types of viruses have been found in Asian house shrews, including coronaviruses, hantaviruses, severe fever with thrombocytopenia syndrome virus, hepatitis E virus, phleboviruses, adenoviruses, and arenaviruses, suggesting that these mammals play an important role as a reservoir for viruses.

Here, we report a highly diverse group of hepaciviruses discovered in the Asian house shrews captured in Shenzhen city, China. For virus screening, we captured 86 Asian house shrews at 7 districts in Shenzhen city, Guangdong province, China from 2013 to 2015 (Table S1 in Supporting Information). All shrews were humanely sacrificed, and their intestines, lungs, and livers were collected and preserved at –80°C. All procedures were carried out with approval from the Animal Ethics Committee of the Wuhan Institute of Virology (approval number: WIVA05201202).

RNA was extracted from liver tissues and analyzed for the presence of hepacivirus by reverse-transcription nested polymerase chain reaction (RT-PCR) with degenerate primers targeting the NS3 gene (Drexler et al., 2013). Quantitative RT-PCR (qRT-PCR) and specific PCR were performed with primers designed based on the viral sequences obtained in this study (Table S2 in Supporting Information).

Using degenerate primers, hepacivirus sequences were detected in four (4.7%) liver samples (Table S1 in Supporting Information). Use gene-specific primers, 30 more liver samples were found positive for hepacivirus (Table S1 in Supporting Information) (GenBank accession nos. MF775331–MF775364). We named these newly discovered viruses as *Suncus murinus* hepacivirus (SmHCV). SmHCVs were detected out at five sites in Shenzhen city, while more than two thirds positive samples came from the Bao'an and

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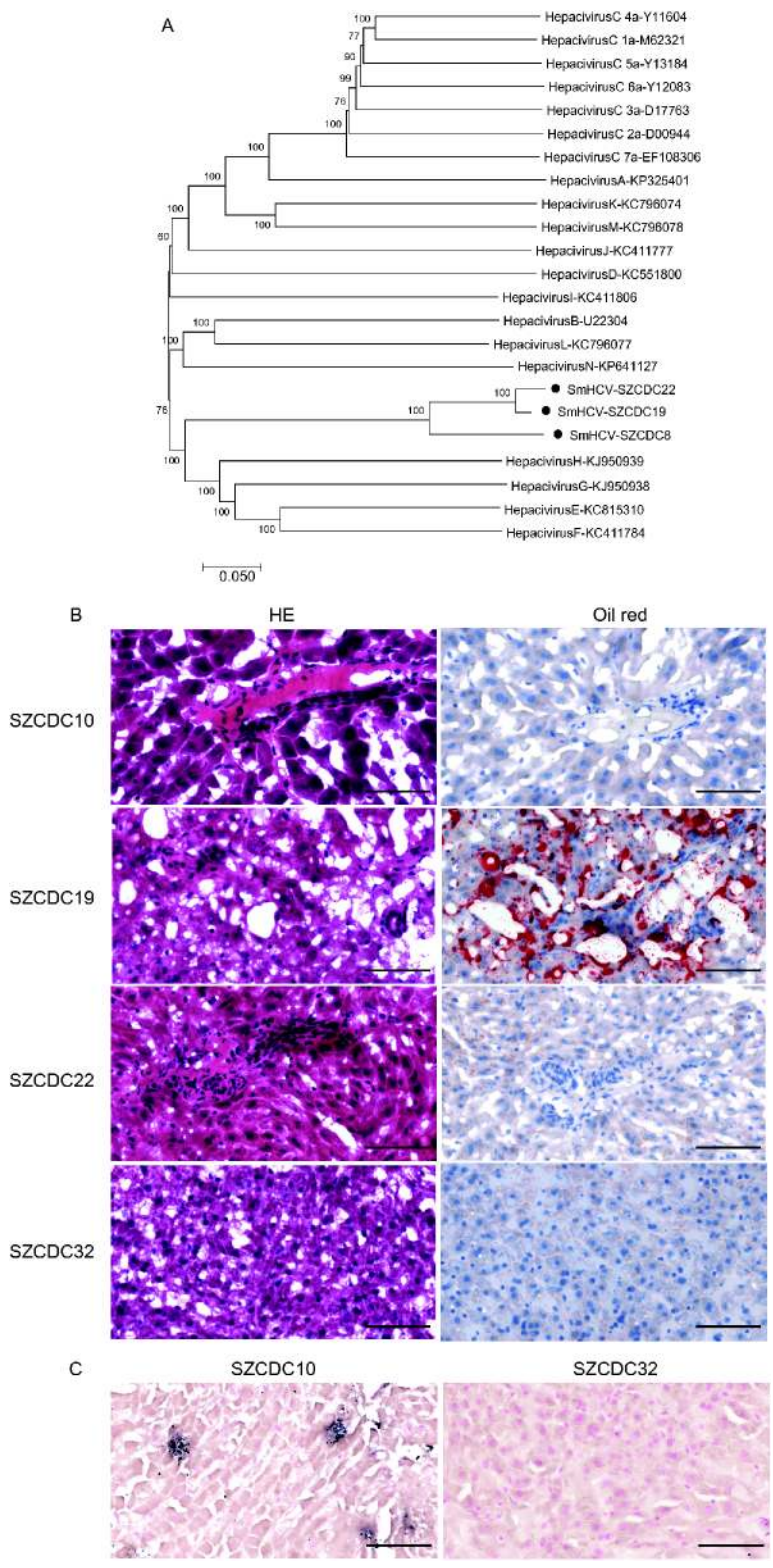

Figure 1 Sequence analysis, pathogenesis, and viral RNA detection of novel hepaciviruses in Asian house shrews. A, Phylogenetic tree of SmHCVs based on nucleotide sequence from NS3 to NS5 regions. Neighbor-Joining tree was produced using MEGA7 software with the p-distance method (https://www.megasoftware.net). Bootstrap value (%) was 1,000 replicates. The scale bar indicates nucleotide substitutions per site. The sequences marked with the black circle were obtained in this study. B, Histopathology and SmHCV RNA detection in the liver samples of Asian house shrew. Cryostat sections of liver tissue were stained with hematoxylin-eosin (HE) and Oil red staining. Inflammatory cell infiltration caused by SmHCV in SZCDC10. Severe steatosis and piecemeal necrosis caused by SmHCV in SZCDC19. Inflammatory cell infiltration caused by SmHCV in SZCDC22. Liver sections without virus infection in SZCDC32. C, Dark blue indicates viral RNA detected in liver tissue of SZCDC10 by RNA probe targeting viral genomic NS3 gene labelled with digoxigenin (DIG). The cryostat sections were scaned on Pannoramic MIDI and pictures were taken by Pannoramic Viewer 1.15.3. Scale bars: 100 μm.

Nanshan districts. More importantly, we found SmHCV positive samples collected in three independent years at Luohu district, though only small amount of samples were collected in year 2013 and 2014.

The detected SmHCV sequences exhibited 77.2%–100% nt identity among themselves and 44.9%–58.1 % nt identity with known rodents hepaciviruses. Analysis of the phylogenetic neighbor joining tree based on the 167 bp sequences of the NS3 region revealed that these sequences formed an independent branch (Figure S1A in Supporting Information). Meanwhile, the sequences from the Longhua and Dapeng were located in separate branch while the sequences from the Bao'an, Luohu and Nanshan crossed together in different branches (Figure S1B in Supporting Information)**.** These results demonstrated that diverse SmHCVs were circulated in Asian horse shrews in Shenzhen city.

To further delineate the genetic information of these SmHCVs, nearly complete or partial genomic sequences (9616, 7765, and 7343 bp) were obtained from three samples SmSZCDC22, SmSZCDC8, and SmSZCDC19 which had higher viral genome RNA copies than others (Figure S2 in Supporting Information). Pairwise comparison showed that these three strains share 81%–96% identities with each other. The nearly complete genome sequence of SmHCV-SZCDC22 shares 29%–31% nt identity with known hepacivirus and its predicted polyprotein shares highest identify of 31% with rodent hepacivirus G. The phylogenetic tree based on obtained genome sequences (NS3 to NS5B region) showed these hepacivirus strains detected in Asian house shrews formed an independent branch (Figure 1A). The amino acid p-distances of conserved regions of 977–1418 and 2694–3108 (relevant to positions 1123–1566 and 2536–2959 of *Hepacivirus* C1a, M62321) of SmHCV-SZCDC22 ranges 0.52–0.59 and 0.44–0.60 with known species in *Hepacivirus* genus, respectively, which meet the demarcation (Amino acid p-distances of greater than 0.25 in the region 1123–1566 and greater than 0.3 in the region 2536–2959) for a new species in *Hepacivirus* genus (Table S3 in Supporting Information).

The viral RNA copies in different tissues were quantified by qPCR, with a positive control from the partial genome RNA transcript by DNA template using a MAXIscript Kit (Applied Biosystems, Foster City, CA, USA) *in vitro*. The viral RNA load ranged from 3×10^2 to 4×10^7 copies/g liver tissue (Figure S3A in Supporting Information). To further determine the viral tissue tropism, viral RNA from three types of tissues of 10 animals was quantified by qPCR. The viral RNA was detected in all three tissues of the selected positive samples. Moreover, the viral load was higher in the liver than other two tissues and could reach 4×10^7 copies/g tissues (Figure S3B in Supporting Information). Interestingly, SmHCV RNA was also detected in the intestine, which may represent the possibility of fecal spreading.

To further evaluate the pathogenesis of these hepaciviruses, liver sections of four shrews were checked by staining with hematoxylin-eosin (HE) and oil red staining. These four liver samples were tested negative for *Hepatovirus*, *Hepadnavirus*, and *Hepevirus* based on PCR (Table S1 in Supporting Information). Inflammatory cell infiltration was observed in samples SZCDC10 and SZCDC22, severe steatosis and piecemeal necrosis were present in sample SZCDC19 (Figure 1B). A digoxigenin (DIG)-labelled RNA probe targeting viral genomic NS3 gene has succeeded to detect the viral RNA in liver tissue of SZCDC10 (Figure 1C).

In this study, highly diverse hepacivirus (SmHCV) sequences were detected in Asian house shrews. The viral RNA could be detected in samples collected from 2013 to 2015, suggesting that these SmHCVs had a wide distribution in Shenzhen city and had been circulated for a long time in Asian house shrews. The obtained genomic sequence of SmHCVs share low similarity with known hepaciviruses. Analysis of amino acid p-distances of the conserved regions suggests these viruses should be classified as a new species in the *Hepacivirus* genus (Smith et al., 2016).

SmHCVs could be detected in the liver, intestine, and lung tissues with high virus concentrations and showed wide tissue tropism. Histology analysis demonstrated that SmHCVs cause inflammatory cell infiltration, steatosis, and cirrhosis in the target tissues. Due to the pathogenesis of SmHCV in liver tissue, Asian shrews could be a potential animal model for hepacivirus study as well as transgenic mice.

Recently, several studies have reported *Hepacivirus*- or *Pegivirus*-related sequences in small wild mammals (rodents and bats) and domesticated animals living in close contact with humans (dogs and horses) (Drexler et al., 2013, Pybus and Thézé, 2016. In this study, we found the Asian house shrew is another group of animal hosts of hepacivirus. Shenzhen is a highly populated and rapid urbanization city with a high chance of close contact with wild animals. With the high diversity of SmHCVs presented in this region, there should be a high chance of virus transmission from animals to humans. Thus, long-term surveillance should be conducted in the future. As the fourth most commonly reported infectious disease in China, HCV infection maybe get more complicated because of novel hepaciviruses discovery (Qin et al., 2015). In addition, our investigation was just based on small sample numbers in Shenzhen city. Considering geographically wide distribution of Asian shrews, we believe there should be more hepaciviruses to be discovered in the future.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

Acknowledgements *This work was supported by the National Natural Science Foundation of China (81290341), and the Basic Research Project of Shenzhen Science and Technology Innovation Program*

(JCYJ20150402102519532). We also thank Juan Min in the Core Facility and Technical Support of the Wuhan Institute of Virology for her help in imaging data collecting.

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SUPPORTING INFORMATION

Figure S1 Neighbor-joining phylogenetic tree of *hepaciviruses*.

Figure S2 The genome organization of SmHCVs obtained in this study.

Figure S3 SmHCV RNA quantification by qPCR in the liver tissues of the Asian house shrews.

Table S1 Detection of hepacivirus in Asian House shrews captured in Shenzhen city

Table S2 Primers used in this study for viral RNA detection and quantification

Table S3 The p-distance vaules of pp977-1418 and pp2694-3108 of SmHCV-SZCDC22 comparasion with other hepaciviruses

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