The First Documented Detection in Taiwan of The Hepatitis E Virus in Rats

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Short report

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Abstract

Background: Human infections by rat HEV (HEV-C1) have been serially reported, including a case who had visited Taiwan before having the illness in 2019. The objective of this study was to investigate whether HEV-C1 is circulating and causing human infections in Taiwan.

Methods: Fifty acute-phase human sera samples from HEV suspected patients with the negative results were randomly chosen for retrospective review. Rat sera were collected from 3 *Rattus rattus* and 47 *R. norvegicus*, which were captured at international airports or harbors. Identifying HEV-C1 RNA was performed by hemi-nested RT-PCR in human and rat serum samples. Rat sera were also tested for anti-rat HEV antibodies.

Results: HEV-C1 RNA was not detected in either human or *R. rattus* samples, but the viral RNA was identified in two *R. norvegicus* samples. The 2 rat HEV strains shared identical partial sequences in the RNA polymerases gene. In serology, anti-HEV antibodies were detected in 52% (26/50) of the trapped wild rats.

Conclusions: This study documents the first detection of HEV-C1 in Taiwan. The high homology between HEV-C1 sequences from rats observed in this study might result from viral circulation and transmission within certain rodent populations. The risk of indigenous human infection in Taiwan should not be ignored because of the domestic detection of HEV-C1 RNA.

Background

Hepatitis E virus (HEV) contains a positive-sense, single-stranded RNA genome and is a member of the family *Hepeviridae*, genus *Orthohepevirus*. Within the genus *Orthohepevirus*, HEV is further classified into four species, *Orthohepevirus A*, *B*, *C* and *D* (HEV-A, B, C and D, respectively). The host ranges of these four species are distinct. HEV-A infects humans and other mammals, including domestic pigs and goats, wild boars, deer, and camels. The other 3 species, HEV-B, HEV-C, and HEV-D, infect avian, rodent, and bat species, respectively [1]. HEV-C was first identified in Norwegian rats (*Rattus norvegicus*) trapped in the manholes of a sewer system in Germany in 2010 [2]. Moreover, HEV-C is further classified into genotype 1 (HEV-C1), which can infect rats and shrews, encompassing genotype 2–4 [3, 4]. In taxonomy, rat HEV shares approximately 50% or less sequence identity with human HEV-A [5].

The role of rats in human HEV infections is controversial. Rat HEV was experimentally observed to be unable to infect primates in a rhesus monkey model [6]. However, recent HEV-C1 infections in humans challenged the previously known host range of HEV-C1. HEV-C1 was discovered in plasma, stool and liver tissue from a liver transplant recipient in Hong Kong in 2017, and an additional 10 Hong Kong and 1 Canadian HEV-C1 cases during 2017 ~ 2020 were detected and subsequently reported [7, 8, 9, 10]. In Hong Kong, the discovery of human HEV-C1 infection was borne out by the following HEV-C1 infected patients, although the infections were sporadic, with most cases being reported in immunocompromised people with underlying diseases or who were on immunosuppressants [11]. Hong Kong and Taiwan have

a similar environment and culture, so the objective of this study was to assess possible disease occurrence in Taiwan using RT-PCR and serological testing methods.

Methods

Samples

Fifty human sera samples stored at -20°C were retrospectively examined. All sera were collected from suspected HEV patients during the period of acute hepatitis in 2018 (**Figure 1**). Additionally, these sera samples were previously identified as HEV-A negative. Both commensal rodents *R. rattus* and *R. norvegicus* are hosts for HEV-C1 [1]. Fifty total rat sera stored at -20°C were retrospectively sampled from 3 *R. rattus* and 47 *R. norvegicus* individuals between January 2017 and June 2019. All rats were trapped from 11 different international airports or harbors in Taiwan for surveillance of potential zoonosis.

RT-PCR analysis

RNA was manually extracted from human and rat serum samples using the QIAamp Viral RNA Mini Kit (Cat. no. 52906, QIAGEN, Hilden, Germany) following the instructions of the manufacturer. Briefly, 140 µL of human or rat serum was applied to a spin column for RNA to bind onto the column. Ethanol was added to the flow-through and RNA and bound to the membrane when the sample was passed through a spin column. After washing, the RNA was eluted in 50 µL of nuclease-free water. Extracted RNA was further tested for the presence of HEV RNA using a broadly reactive nested RT-PCR assay that detects largely divergent HEV variants, including HEV-C1 [12]. Samples positive for nested RT-PCR were tested again using a hemi-nested RT-PCR (an interior primer of the nested RT-PCR was excluded in the following hemi-nested RT-PCR) to avoid false positivity.

Amplicons of the hemi-nested RT-PCR were directly sequenced on both strands using the ABI 3730 XL DNA Analyzer (Applied Biosystem Inc., Foster City, California, USA). The sequences were aligned with other HEV sequences using CLUSTAL W [13]. A phylogenetic tree was constructed by the neighbor-joining method [14] with the Kimura two-parameter correction model and 1,000 replicates of bootstrap resampling as implemented in MEGA 10 (version 10.0.5) [15].

Serological assays

HEV-C serological testing of rat sera was performed using a commercial Qualitative Rat Hepatitis E Virus Antibody (Anti-HEV) ELISA kit (Cat. no. MBS9357409, MyBiosource, California, USA). Interpretation of the results was performed according to the manufacturer's instructions. This kit detects both IgG and IgM. Detected immunoglobulin types were not further differentiated.

Results

HEV-C RNA was not detected among sera collected from the 50 hepatitis patients or the 3 *R. rattus*, but viral RNA was observed in sera from 2 *R. norvegicus* individuals trapped at a harbor on June 6, 2017 (Fig. 1). The two detected partial sequences with a length of 382 base pairs shared 100% genetic identity in the RNA-dependent RNA polymerases gene of HEV. Nucleotide sequences analyzed in this study were submitted to GenBank with accession numbers of MN603692 and MN603693. Phylogenetically, both rat HEV strains were further grouped together with a HEV-C1-G2 variant isolate (HEV species C/genotype 1/genetic group G2, GenBank accession no. LC225389), which was discovered in Indonesia in 2014. The two sequences obtained in this study were also phylogenetically close to isolates of human HEV-C1 infections, including the first discovered strain from a liver transplant receipt and another strain detected from an immunocompetent adult (Fig. 2). In addition, the seropositive rate of anti-HEV antibodies in the 50 rats was 52% (59%, 42% and 52% in 2017, 2018 and 2019, respectively). The 2 rat sera with HEV-C1 RNA were both negative for anti-HEV antibodies.

Discussion

This study identified HEV RNA in a local rat population in Taiwan with a detection rate of 4%. Additionally, anti-HEV antibodies were detected in 52% of trapped wild rats. This RNA result is similar to the Hong Kong study [10]. The detection rates of HEV-C RNA in *R. norvegicus* were 4.3% and 4.4% in Taiwan and Hong Kong, respectively. Moreover, this finding is consistent with the detection rates of HEV RNA (1.7–22.8%) in previous studies in Europe and Asia in the 2010s [16, 17, 18]. This finding implies that Taiwan is likely to have a risk of human HEV-C infection, especially given that ongoing sporadic transmission throughout 2017–2020 has already been observed in Hong Kong. The high homology between these 2 rat HEV sequences in this study is not surprising because both rats belonged to the same species, *R. norvegicus*, and were trapped simultaneously in the same harbor, indicating that HEV-C1 might be circulating and transmitting within rat populations in Taiwan.

Overall, we observed that 52% of wild rats were seropositive for rat HEV in international ports in Taiwan. This prevalence rate is higher than that of early studies, including 4.1–18.1% in Indonesia [19, 20], 12.9% in Vietnam [21], 23.3% in China [22], 24.5% in Germany [23], 27.9% in Japan [24], and 31.2% in Lithuania [25]. However, the seropositive rates between studies might be difficult to directly compare due to the lack of identical antigens and detection antibodies used in the ELISA tests. The limited agreement between different ELISA tests for determination of anti-HEV antibodies has also been mentioned previously [26]. Moreover, one report described HEV-positive ELISA results in cattle serum containing HEV neutralizing IgG in the absence of HEV genomes throughout the cattle's life. Antibodies induced by unknown etiological agents that generate cross-reacting antibodies might also increase the difficulty of explaining the serological results [27].

Since 2010, HEV-C1 RNA and antibodies have gradually been detected in rats on the Eurasian continent, North American continent, and the Indonesian archipelago [6, 19, 20, 21, 22, 23, 24, 25]. This study further documents the first detection of HEV-C1 in rats in Taiwan, a geographically isolated island. Phylogenetically, detected rat HEV could also be traced back to the HEV-C1-G2 variant strains discovered

in Indonesia in 2014. This possibility indicates that the viruses could have crossed geographic boundaries to achieve transmission. It also reflects the importance of sanitation control of vectors and reservoirs in ships, aircraft and international ports. This study might be insufficient to entirely clarify the prevalence of HEV-C in humans and rats in Taiwan due to the limited sample size. However, the risk for indigenous human infection in Taiwan should not be ignored because HEV-C1 RNA with zoonotic potential has already been detected in the local rodent population.

Conclusions

This study documents the first detection of HEV-C1 in Taiwan. The high homology between HEV-C1 sequences of rats observed in this study might result from viral circulation and transmission within certain rodent populations. The risk of indigenous human infection in Taiwan should not be ignored given the domestic detection of HEV-C1 RNA.

Declarations

Ethics approval and consent to participate

The need for approval was waived by article no. 47 of Infectious Disease Control Act.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and analyzed in the current study are available in the GenBank repository, <https://www.ncbi.nlm.nih.gov/genbank/>.

Competing interest

The authors declare that they have no competing interests.

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Author contributions

Pai-Shan Chiang prepared the manuscript and collected data. Wei-Lun Huang collected data. Han-Hsuan Chung edited the manuscript and performed data analysis. Jyh-Yuan Yang edited the manuscript. Hwa-Jen Teng directed the roll out and edited the manuscript.

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Figures

Figure 1

Sample distribution and findings of human and rat sera.

Figure 2

Phylogenetic relationships of HEV detected in this study and reference strains using sequences of RNA-dependent RNA polymerase. Avian and bat HEV, classified as genus *Orthohepevirus B* and *Orthohepevirus D*, were used as the outgroup virus. Bootstrap values (>70%) are shown as percentages derived from 1,000 sampling at the nodes of the tree. Scale bar denotes the number of nucleotide substitutions per site along the branches. Red dots indicate viruses detected and sequenced in this study. Green dots indicate viruses isolated from human cases of rat HEV.

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