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Original Article

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

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Abstract

Hepatitis E virus (HEV) could be classified into four species (A, B, C and D). The host ranges of the four species are distinct. HEV-A infects humans and other mammals, including domestic pigs, goats and wild boars, deer, and camels. The other 3 species, HEV-B, HEV-C, and HEV-D, infect avian, rodent, and bat species, respectively. However, human infections by rat HEV (HEV species C/genotype 1, HEV-C1) have been reported recently, including a case who had visited Taiwan before having the illness in 2019. To investigate whether HEV-C1 is transmitting in rat population and causing human infections in Taiwan, 50 acute-phase human sera samples from HEV suspected patients with HEV-A negative results were re-tested for retrospective review of HEV-C. Besides, rat sera were collected from 3 *Rattus tanezumi* (previously known as *Rattus rattus*) and 47 *R. norvegicus*, which were captured at international airports or harbors. Identifying HEV-C RNA was performed by hemi-nested RT-PCR in human and rat serum samples. Rat sera were also tested for anti-rat HEV antibodies. HEV-C RNA was not detected in either human or *R. tanezumi* samples, but the viral RNA was identified in two *R. norvegicus* sera. Furthermore, 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. This study documents the first detection of HEV-C1 in Taiwan. The seroprevalence and the high homology between HEV-C1 sequences from rats observed in this study might result from viral transmission within certain rodent populations. The risk for indigenous human infection in Taiwan

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should not be ignored because HEV-C1 with zoonotic potential has already been detected in the local rodent population.

Keywords: Hepatitis E virus, *Orthohepevirus C*, Rats, Taiwan

Introduction

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 4 genotypes. Genotype 1 (HEV-C1) can infect rats and shrews and genotype 2 (HEV-C2) infect ferrets and minks. Another two putative novel *orthohepeviruses*, HEV-C3 and HEV-C4, isolated from mouse and vole were also reported previously [3]. In taxonomy, rat HEV shares approximately 50% or less sequence identity with human HEV-A [4].

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 [5]. 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 at least 7 Hong Kong and 1 Canadian additional HEV-C1 cases during 2017–2020 were further detected and subsequently reported [6,7]. In Hong Kong, the discovery of human HEV-C1 infection were already confirmed by the following HEV-C1 infected patients including a case who had visited Taiwan before having the illness in 2019. Although the infections were sporadic, most cases were reported in immunocompromised people with underlying diseases or who were on immunosuppressants [7]. Hong Kong and Taiwan have a similar environment and culture; so the objectives of this study were to assess possible HEV-C occurrence in humans and in rats (animal hosts) in Taiwan by RT-PCR and serological testing methods.

Materials and Methods

Ethical statement: This study was conducted in response to a public health concerned issue. The need for this study approval was exempted by article no. 47 of Communicable

Disease Control Act in Taiwan. The surveillance of vector-borne zoonosis in international ports including rats were implemented with the permission of Taiwan Centers for Disease Control and comply with no. 33 of Regulations Governing Quarantine at Ports in Taiwan [8] (aligned with Article 20 and Annex 1 of the WHO International Health Regulations 2005 [9]). All rats trapping and handling procedures met Taiwanese legal requirements.

Samples: Fifty-two human sera collected from patients suspected of acute hepatitis E (or non-type A, B, C acute hepatitis) from January to April 2019 were previously identified as HEV-A negative. Among them, 2 sera with insufficient volume were excluded in this study. Fifty human sera samples stored at -20°C were all retrospectively examined for further HEV-C RNA. All sera were collected from patients during the period of acute hepatitis (Figure 1). Both commensal rodents *R. tanezumi* (previously known as *R. rattus*) and *R. norvegicus* are hosts for HEV-C1 [1,10]. Fifty total rat leftover sera (3 *R. tanezumi* and 47 *R. norvegicus*) which had been previously collected for surveillance of potential zoonosis and stored at -20℃ were retrospectively sampled from sera of 275 individuals captured for routine pest control in international ports between January 2017 and June 2019. We sampled these stored rat sera with the top 50 volumes because volume of the most leftover sera was insufficient (<0.2 ml) for further HEV-C RNA and serological tests. All rats were trapped from 11 different international airports or harbors (Figure 1).

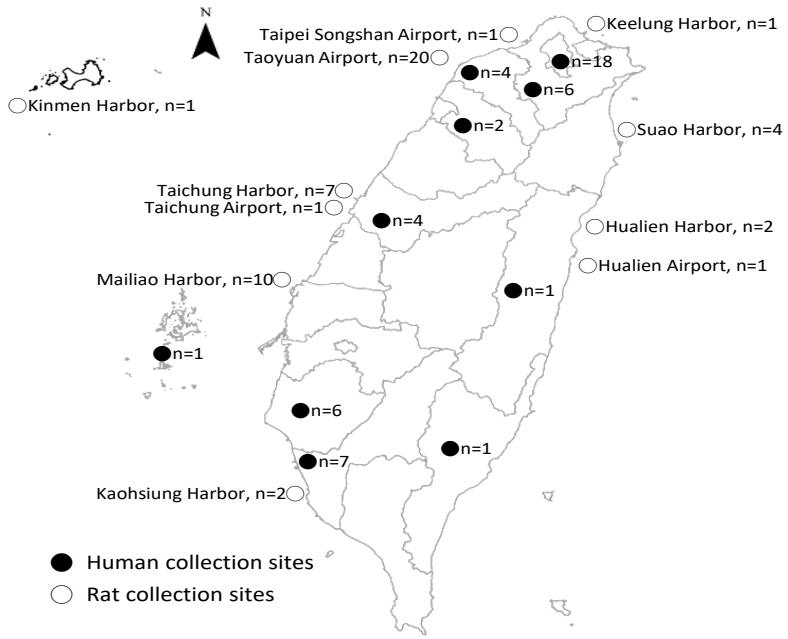

Figure 1. Sample distribution of human and rat sera.

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, 140µL 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 50µl 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-C [11]. 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. A phylogenetic tree was constructed by the neighbor-joining method with the Kimura two-parameter correction model and 1,000 replicates of bootstrap resampling as implemented in MEGA software (version 10.0.5).

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. In brief, the tested serum was not diluted. Both positive and negative controls were reagents provided in the commercial kit. 50µl of each serum sample, positive control and negative control were added into wells of the sandwich ELISA plate. 100µl of HRP-conjugate secondary antibody was added to each of positive control wells, negative control wells and sample wells and incubated for 60 minutes at 37°C. 100µl of chromogen solutions were added to each well for 15 minutes at 37°C. Optical density (O.D.) was read at 450 nm using an ELISA reader within 15 minutes after adding stop solution. Cut-off value was determined as the average O.D. of the negative control wells (n=6) +0.15. 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. tanezumi*, but viral RNA was observed in sera from 2 *R. norvegicus* individuals trapped at Mailiao Harbor in central Taiwan on June 6, 2017 (Figure 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 (Figure 2). In addition, the seropositive rate of anti-HEV antibodies in the 50 rats was 52% (52% (11/21) in 2017; 59% (10/17) in 2018; 42% (5/12) in 2019). The 2 rat sera with HEV-C1 RNA were both negative for anti-HEV antibodies.

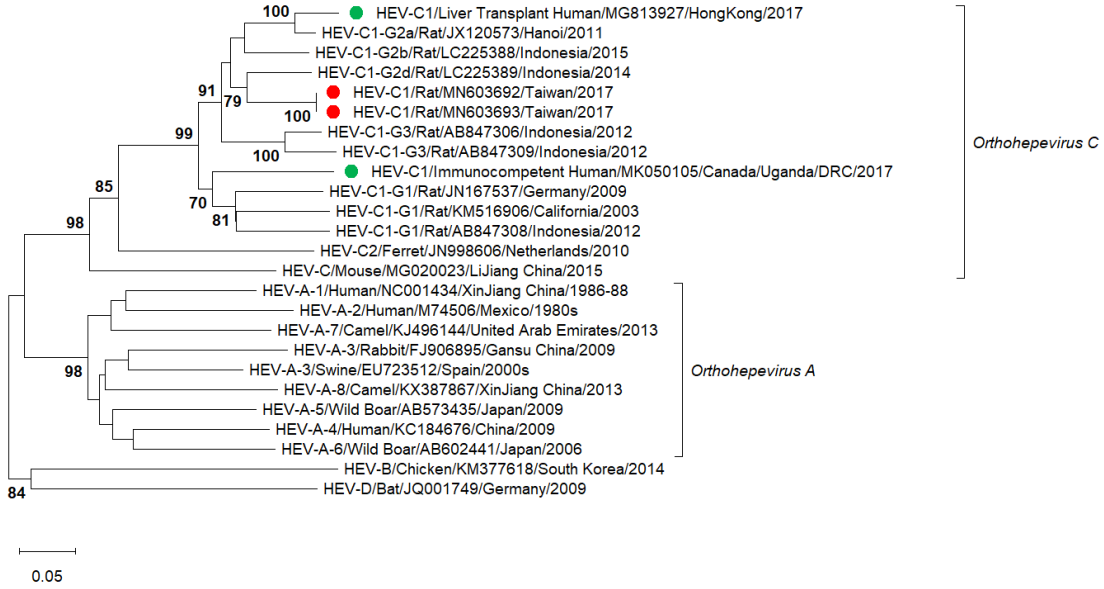

Figure 2. Phylogenetic relationships of HEV detected in this study and reference strains using sequences of RNA-dependent RNA polymerase. Avian and bat HEV, classified as genus *Orthohepevirus B* and *Orthohepevirus D*, were used as the outgroup virus. Bootstrap values (>70%) are shown as percentages 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.

Discussion

This study identified HEV-C 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 [7]. 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% to 22.8%) in previous studies in Europe and Asia in the 2010s [10,12,13].

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 neighboring 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 transmitting within *R. norvegicus* 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 18.1% in Indonesia [14], 12.9% in Vietnam [15], 23.3% in China [16], 24.5% in Germany [17], 27.9% in Japan [18], and 31.2% in Lithuania [19]. 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 [20]. 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 [21]. However, high seropositivity of HEV-C antibody among rats with the detection of HEV-C RNA in Taiwan indicated that HEV-C had been pre-existing in Taiwan and might be transmitting in local rat populations.

Since 2010, HEV-C1 RNA and antibodies have gradually been detected in rats on the Eurasian continent, North American continent, and the Indonesian archipelago [5,15-20]. 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 documents the detection of HEV-C1 in Taiwan. The high homology between HEV-C1 sequences of rats observed in this study might result from viral transmission within certain rodent populations. In this study, no HEV-C RNA was found among 50 human samples in the spring season of 2019. The conclusion for human infection could not be made based on the limited sample size within a short time-period. Therefore, human surveillance of rat HEV should be continuously included in the laboratory diagnosis of reported HEV patients.

In this study, although no human cases of HEV-C were found, HEV-C were detected in its host population (*R. norvegicus*) with high serological prevalence rates and 2 HEV-C viral RNA. In Taiwan, this rat species is very common in city and rural areas with high densities. The usual HEV causing human infection is transmitted mainly through the fecal-oral route. Therefore, environmental hygiene and five keys to food safety were highly recommended in prevention of HEV-C infection, especially immunocompromised people with underlying diseases or on immunosuppressants [22]. They are Choose (Choose safe raw materials), Clean (Keep hands and utensils clean), Separate (Separate raw and cooked food); Cook (Cook thoroughly), and Safe Temperature (Keep food at safe temperature). In addition, surveillance of rat HEV should be included in the human reported cases of acute viral hepatitis with history of contact with rodent and/or their excreta as well as in the rat surveillance of airports and harbors.

Acknowledgements

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