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A Diffuse Outbreak of Hepatitis E in Mie Prefecture, 2005

Yoko Nakano, Akinori Yamauchi, Takuya Yano, Osamu Nakayama, Haruko Sakai[1], Yuji Nagasaka[2], Seiji Itaba[3], Yoshiki Tabata[3] and Akira Sugiyama*

Mie Prefectural Institute of Public Health and Environmental Sciences, Mie 512-1211; [1]Mie Prefectural Kuwana Health Center, Mie 511-8567; [2]Mie Prefectural Yokkaichi Health Center, Mie 510-8511; and [3]Division of Health and Welfare, Mie Prefecture Government, Mie 514-8570, Japan

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Hepatitis E virus (HEV) causes acute hepatitis, which is transmitted primarily through oral contact with feces (1,2). Pig meat is also a potential source of HEV infection and the consumption of raw pig liver has been suggested to be one of the main transmission routes between pigs and humans (3,4). The virus has a 15- to 60-day incubation period and infected people may be contagious for up to 2 weeks after symptoms appear. The acute phase is self-limiting, but there is a 1 to 2% chance of developing sudden and severe liver damage, resulting in fulminant hepatitis (5).

In late June of 2005, four cases of hepatitis E were reported by three hospitals in the northern district of Mie Prefecture, Japan. To investigate the possible sources of infection in these four patients, we first examined their behavior and living environment. Virological analysis of stored sera was performed by reverse transcriptase-polymerase chain reaction (RT-PCR) for HEV RNA. The amplification products of HEV RNA were then sequenced and their sequences were compared to the genotype I - IV sequences registered in the GenBank database.

Patient 1, a 55-year-old male, complained of general fatigue and loss of appetite on May 13. He visited Hospital X at Inabe-shi on May 16 and tested positive for HEV IgG and IgM antibodies; he was diagnosed with hepatitis E.

*Corresponding author: Mailing address: Department of Microbiology, Mie Prefectural Institute of Public Health and Environmental Sciences, 3690-1, Sakura-cho, Yokkaichi, Mie 512-1211, Japan. Fax: +81-59-329-3004, E-mail: sugiya00@pref.mie.jp

Patient 2, a 66-year-old female, suffered chills and loss of appetite on May 14 and experienced general fatigue on May 16. She was examined at a local clinic on May 18 and, based on indications of liver dysfunction observed at the clinic, was examined at the same hospital visited by Patient 1 on May 20. Her serum was also positive for HEV IgG and IgM antibodies, and she was diagnosed with hepatitis E.

Patient 3, a 59-year-old male, displayed hepatitis symptoms in mid-May and was examined at Hospital Y in Yokkaichi-shi on May 17. His serum was positive for HEV-RNA and he was diagnosed with hepatitis E.

Patient 4, a 54-year-old male, complained of loss of appetite and exhibited jaundice on June 5. This patient stated that he had eaten raw liver from an animal of unknown species. He was under treatment at Hospital Z in Kuwana-shi on June 10. His sera were positive for HEV IgG and IgM antibodies, and he was diagnosed with hepatitis E.

The patients had the following factors in common:
(i) All patients had resided in Japan for the past several years. Due to this absence of a history of foreign travel, the region of infection was limited to Japan.
(ii) There was no obvious contact with HEV infected animals or other HEV vector. Although Patient 4 had eaten raw liver, Patients 1, 2 and 3 had not eaten any wild animal flesh. Thus, no causal food or drink was identified.
(iii) There was no illness in any cohabiting individuals or other such persons.
(iv) The source of drinking water in all cases was municipal water. The residences of the patients all had flush toilets.
(v) None of these individuals kept pet animals in their homes.

(vi) None of the patients had any history of blood transfusion, medical injection or contact with hepatitis patients during the 2 months prior to the onset of illness.
(vii) There were no pork processing or storage plants within a 1-km radius of the residences of these individuals.

Stored sera which had been collected at each hospital and dated as closely as possible to the date of onset were used as investigational specimens. The open reading frame 1 (ORF1) region of HEV was targeted for RT-PCR. RNA was extracted using a QIAamp Viral RNA Mini Kit (Qiagen, Tokyo, Japan). Super Script II RnaseH RT (Invitrogen, Tokyo, Japan) was used in RT reactions. TaKaRa Ex Taq (TaKaRa Bio, Inc., Shiga, Japan) was used as a DNA polymerase. First PCR was carried out using HE5-1 as a sense primer and a mixture of HE5-4 and HE5-5 as an antisense primer. Nested PCR was then performed using HE5-2 as a sense primer and a mixture of HE5-3 and HE5-6 as an antisense primer (6). The amplification conditions were 1 cycle of 1 min at 96°C, 30 cycles of 30 sec at 95°C, 45 sec at 55°C, 1 min at 72°C, and 1 cycle of 7 min at 72°C. The amplification product was then ascertained using agarose gel electrophoresis. Amplification products with the expected sizes were obtained from Patients 1, 3 and 4, however, the HEV gene was not detected in the serum of Patient 2.

The amplification products of the ORF1 region were first purified with a MiniElute PCR Purification Kit (Qiagen) and then sequenced using a SequiTherm EXCEL™ II DNA Sequencing Kit-LC (AR Brown Co., Ltd., Tokyo, Japan) and a DNA sequencer LIC-4200 (Aloka, Tokyo, Japan). The sequences were analyzed by the GENETYX version 7 computer software (Genetyx Corp., Tokyo, Japan). BLAST screening was carried out for 326-bp for three specimens of the amplification product (7-10). All three specimens were identified as HEV genotype III. Using Patient 1 as a reference case, Patient 3 showed 98.7% homology and Patient 4 showed 78.9% homology.

The sequences of the 3 specimens were compared to the genotype I - IV sequences registered in the GenBank database. The accession numbers of the reference sequences were M80581, M73218, M74506, AF060668, AF060669, AB074917, AB074918, AB074919, AB074920, AF110387, AF110388, AF110389, AF279122, AF264009, AF264010, AJ315768, AF195064, AF195065, AJ272108, AB097812, AB080575 and AB108654 (6,11-23). The HEVs from Patients 1 and 3 were further differentiated from that of Patient 4 by their classification in a different cluster within genotype III. The HEV from Patient 4 was closely related to genotype III swJ570 (AB073912) isolated in Japan, while that from Patients 1 and 3 was closely related to genotype III sequences isolated in Europe (15,24). Figure 1 shows the analytical results of these strains.

Based on these results, Patients 1 and 3 were again interviewed at the health center, however, no common foods, behavior patterns or other similarities were found; thus, neither the source nor the route of infection could be determined. Given the observance of the extremely high homology of 98.7% between the viruses detected in these two individuals, some mutually unknown point of contact between them was strongly suspected. On this basis, we regarded these cases as a diffuse outbreak. We also attributed the inability to detect the virus from Patient 2 to the long interval of 14 days from the onset of illness to specimen collection. The source of infection derived from our investigation can only be hypothesized to be "consumption of undercooked or raw meat from an unknown animal species."

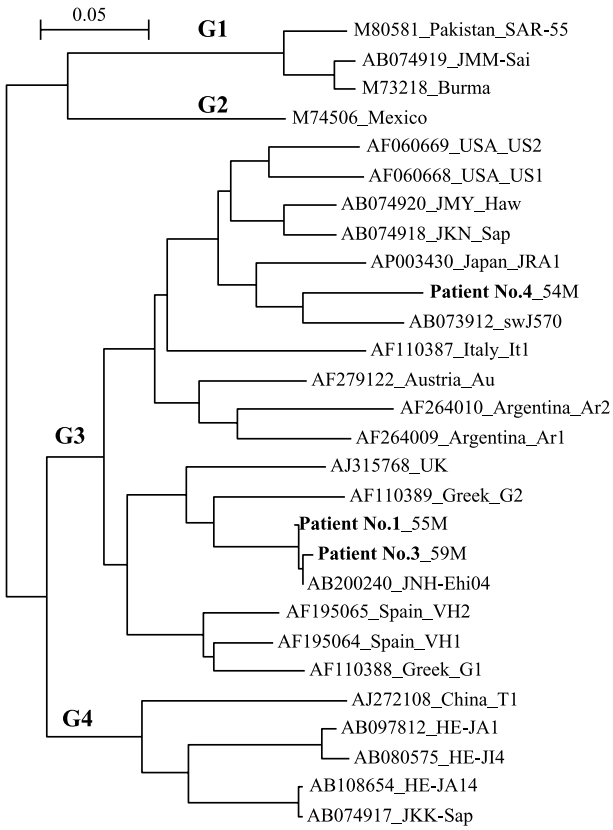

Fig. 1. Phylogenetic tree (neighbor-joining method).

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