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Hepatitis E Virus Genotype 3 in Sewage and Genotype 1 in Acute Hepatitis Cases, Israel

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Abstract. Hepatitis E virus (HEV) is an emerging infectious agent in developed countries. HEV genotypes 1 (G1) and 3 (G3) have been identified in environmental and clinical samples in Europe. In Israel, the overall prevalence of anti-HEV IgG antibodies was found to be 10.6%; however, reports of HEV infection are scarce. In this study, the presence of HEV in Israel was investigated using 169 sewage samples from 32 treatment facilities and 49 samples from acute hepatitis patients, all collected between 2013 and 2015. Fourteen sewage samples, from Haifa (11/18 samples), Tel Aviv (2/29 samples), and Beer Sheva (1/17 samples), regions with good sanitary conditions and middle-high socioeconomic populations, were HEV positive. Among the patient samples, 6.1% (3/49) were HEV positive, all returning travelers from India. Genotype analysis revealed G1 HEV in patients and G3 HEV sequences in sewage. Evidence that HEV could be establishing itself in our region may justify more active surveillance to monitor its spread.

INTRODUCTION

Hepatitis E virus (HEV) is an emergent causative agent responsible for an estimated 20 million cases of acute viral hepatitis worldwide, each year. Mammalian HEV can be classified into four genotypes: G1 and G2, which infect humans and are responsible for acute hepatitis outbreaks in developing countries, and G3 and G4, which are foodborne viruses and infect both humans and animals including pigs, rabbits, mongoose, deer, and rats. In industrialized countries, both travel-related (G1 HEV infections) and autochthonous infections causing acute hepatic (G1 and G3 HEV) and extrahepatic chronic diseases (mainly G3 HEV related) have been reported.[1,2]

Environmental surveillance has become a very useful population-based tool for monitoring microbial and viral activities. In Israel, a national program for surveillance of the poliovirus in sewage has been ongoing since 1988, and facilitated identification of wild poliovirus in 2013 that activated a major emergency response by the Public Health Services.[3] Unlike the ongoing surveillance for polio, there is no HEV monitoring in Israel. The overall prevalence of anti-HEV IgG antibodies in Israel was recently found to be 10.6%,[4] but in a study that retrospectively assessed acute HEV infections in Israel between 1993 and 2013, only 68 HEV positive cases were identified (~3 per year).[5] HEV has already been identified in sewage and in other environmental samples in several Mediterranean and Middle East countries,[6–8] but not in Israel. With the hypothesis that environmental samples may reflect the prevalence of viral circulation, this study investigated the occurrence of HEV in Israel through molecular screening of raw sewage samples. Blood samples from hospitalized patients, presenting acute hepatitis, were also assessed.

MATERIALS AND METHODS

Raw sewage composite samples ($N = 169$), from 36 treatment facilities and sewage lines that cover more than 102 communities in Israel, collected between 2013 and 2015 within the framework of the national program for surveillance poliovirus 3, were tested. The sewage sampling included sentinel sites that represent both large populations (such as the Shafdan, a sewage treatment facility in the Tel Aviv area) and small populations (such as Tel Sheva). Eight sewage samples collected in Palestine were also available for this study, although the exact sampling location, size, and type of the represented population were unknown. Blood samples ($N = 49$) from patients with acute hepatitis hospitalized during the same study period, found negative for hepatitis A virus, hepatitis B virus, and hepatitis C virus, and for whom diagnostic RNA testing for HEV was requested, were also assessed.

RNA was extracted from 1 mL concentrated sewage samples[9] or 0.5 mL plasma, using EasyMag (bioMérieux SA, Marcy l'Etoile, France); MS-2 coliphage served as an internal control for the extraction.[3] Detection of HEV sequences was performed with the RealStar HEV RT-PCR kit, version 1.0 (Altona Diagnostics GmbH, Hamburg, Germany), according to the manufacturer's instructions. Positive amplification of the internal control confirmed successful real-time polymerase chain reactions (RT-PCRs). The assay, with an estimated 30 IU/mL detection limit, was validated using an external control program (Quality Control for Molecular Diagnostics 2014; Quality Control for Molecular Diagnostics, Glasgow, Scotland) and correctly identified all HEV-positive and HEV-negative samples of the proficiency panel.

For HEV genotyping, RNA from HEV-positive samples was subjected to RT-PCR, using several primers located in different locations within open reading frame 1 (ORF1) of HEV, followed by nested PCR using semi-nested primers.[10,11] Samples with nested PCR products were sequenced using the forward 5′-CTGGCATYACWACTGCYATTGAGC-3′ and reverse 5′-TACCAVCGCTGRACRTC-3′ primers (nucleotides 56–79 and 353–334 of the Burmese isolate accession number D10330).[12] All identified nucleotide sequences and 40 prototype sequences from GenBank (representing HEV G1–G4 sequences with avian HEV used as an out-group) were aligned using Sequencher 5.0 (Genecodes, Anne Arbor, MI), and clustered with the Clustal W algorithm (bootstrap value of 1,000).[13] Phylogenetic trees were produced by NJplot.[14] All of the sequences determined in this study were deposited in the GenBank database (KU315218-KU315226).

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RESULTS

RT-PCR of internal control was successful in 94.7% (160/169) of the samples; 8.8% (14/160 samples) were HEV positive (Table 1, Figure 1). Fourteen sewage samples, from Haifa (61.1%, 11/18 samples), Tel Aviv (6.9%, 2/29 samples), and Beer Sheva (5.9%, 1/17 samples) were HEV positive. Clearly, most of the positive samples were from the region of Haifa (78.6%, 11/14 of all positive sewage samples), covering a population of 555,000 individuals in northwestern Israel. Two of the 14 positive sewage samples were identified in the Tel Aviv metropolitan sewage facility, servicing a population of 1,119,000 and one in Beer Sheva in the south, a facility which covers both Jewish and Bedouin communities, and services a population of 264,000 individuals. No positive samples were found in the southern Israel line connected to the Beer Sheva facility that serves the Bedouin community (Tel Sheva). The eight facilities from Palestine were all negative for HEV. Although not thoroughly assessed, no clear seasonal pattern was identified in samples positive for HEV. In parallel, 6.1% (3/49) of patients presenting acute hepatitis were HEV-RNA positive (these were also found to be anti-HEV IgG and IgM positive). These were all travelers returning from India, a country endemic for HEV. Samples from the three HEV-positive patients (one of whom was represented by two consecutive samples) and five of the HEV-positive sewage samples were successfully sequenced. Phylogenetic analysis (Figure 2) revealed clustering of sewage samples within other G3 isolates (closest to G3f and G3e sequences), and of the clinical samples with G1 HEV sequences, with no defined G1 subtype cluster. The two consecutive plasma samples from one of the patients hospitalized with acute hepatitis, clustered together. The mean pairwise distance between the G1 sequences detected in this study was 3%, and between the G3 sequences, was 9.8%.

DISCUSSION

The results of this study are the first demonstration of HEV circulation in environmental samples in Israel. Fourteen of the 160 sewage samples with successful PCR results were HEV positive; all the positive samples were from sewage facilities and lines that serve Israeli populations (the few Palestinian sewage samples were negative) of middle-high socioeconomic status and are located in regions with good sanitary conditions.[15] This is in clear contrast to the concentration of positive poliovirus sewage samples in rural, low socioeconomic, poor sanitary regions in Israel.[3] Most of the positive samples were identified in the region of Haifa, northwest of Israel, but the sequences of the virus were also detected in sewage samples from treatment facilities located in other areas. No registered HEV outbreaks have ever been documented in the region of Haifa or in any other part of Israel. The few HEV cases ever reported included several pregnant women with no history of travel to endemic countries[5] and travelers returning from developing countries.[16] Except for one sequence-confirmed HEV G1 case (pregnant woman infected in India), the HEV genotype in both of these studies is unknown. Here, the three patients presenting HEV-related acute hepatitis were travelers returning from an endemic HEV region (India) and G1 HEV infection was confirmed. On the other hand, sewage samples contained G3

TABLE 1
Participating STFs or sewage lines and RT-PCR results on sewage samples

Region	STFs/lines	Population equivalents served by STFs/lines	No. of municipalities served by STFs/lines	No. of HEV-positive samples/total collected 2013–2015				Total no. of HEV-positive samples	Total no. of samples (no. of PCR failures)
				Q1 (January–March)	Q2 (April–June)	Q3 (July–September)	Q4 (October–December)		
Beer Sheva	Beer Sheva,* Tel Sheva	264,000	4	0/3	1/5	0/4	0/5	1	17
South	Ashdod, Kiryat Gat, Rahat, Arara, Shoket, Kseifa	434,400	15	0/0	0/7	0/8	0/5	0	20 (2)
Central	Ayalon, Baka, Horashim, Tnuvot	413,100	15	0/4	0/2	0/4	0/4	0	14 (2)
Jerusalem	Sorek, Og, Kidron	1,018,000	13	0/8	0/1	0/0	0/6	0	15 (1)
Northeast	Manda, El Hamra, Zfat, Tel Adashim	204,800	12	0/7	0/0	0/4	0/4	0	15 (1)
Northwest	Eron, Akko	217,000	15	0/5	0/1	0/2	0/3	0	11
Haifa metropolitan	Haifa,* Nesher, Kryaot,* Tirat HaCarmel*	555,000	13	4/7	4/7	1/1	2/3	11	18
Tel Aviv metropolitan	Shafdan,* line B,* line C	1,119,000	19	1/8	0/7	1/8	0/6	2	29 (1)
Palestine	8 facilities	NA	NA	0/10	0/0	0/3	0/7	0	20 (2)

NA = not available; RT-PCR = reverse transcription polymerase chain reaction; STF = sewage treatment facility.
*STFs or lines where HEV-positive samples have been identified.

FIGURE 1. Sewage facilities and sampling sites in Israel.

HEV sequences, closely related to subtypes G3f and G3e, the most commonly identified HEV G3 subtypes in Europe.[7] To the best of our knowledge, this is the first documentation of G3 HEV in Israel. Interestingly, sewage samples with G1 HEV were not identified.

Patients presenting acute hepatitis, resulting from G1 or G3 HEV infection, and sewage samples positive for one of these HEV genotypes, have been documented in several Mediterranean countries. In Italy and Spain, both G1 and G3 HEV were identified in clinical and sewage samples. In Spain, 32% (29/91) sewage samples were either G1 or G3 positive. In Italy, most (18/19) of the 16% (19/118) positive sewage samples were HEV G1. In Tunisia, the presence of both genotypes in sewage samples was also reported.[6,8,17,18] In Egypt, where the disease burden of viral hepatitis is one of the heaviest worldwide, high prevalence of the virus was reported and cases of HEV G1 or G3 acute hepatitis have been identified.[19,20] However, in the same study, only one of the 76 sewage samples assessed for HEV RNA sequences was found positive.[19] These unexpected results were ascribed to technical difficulties in environmental sample processing.

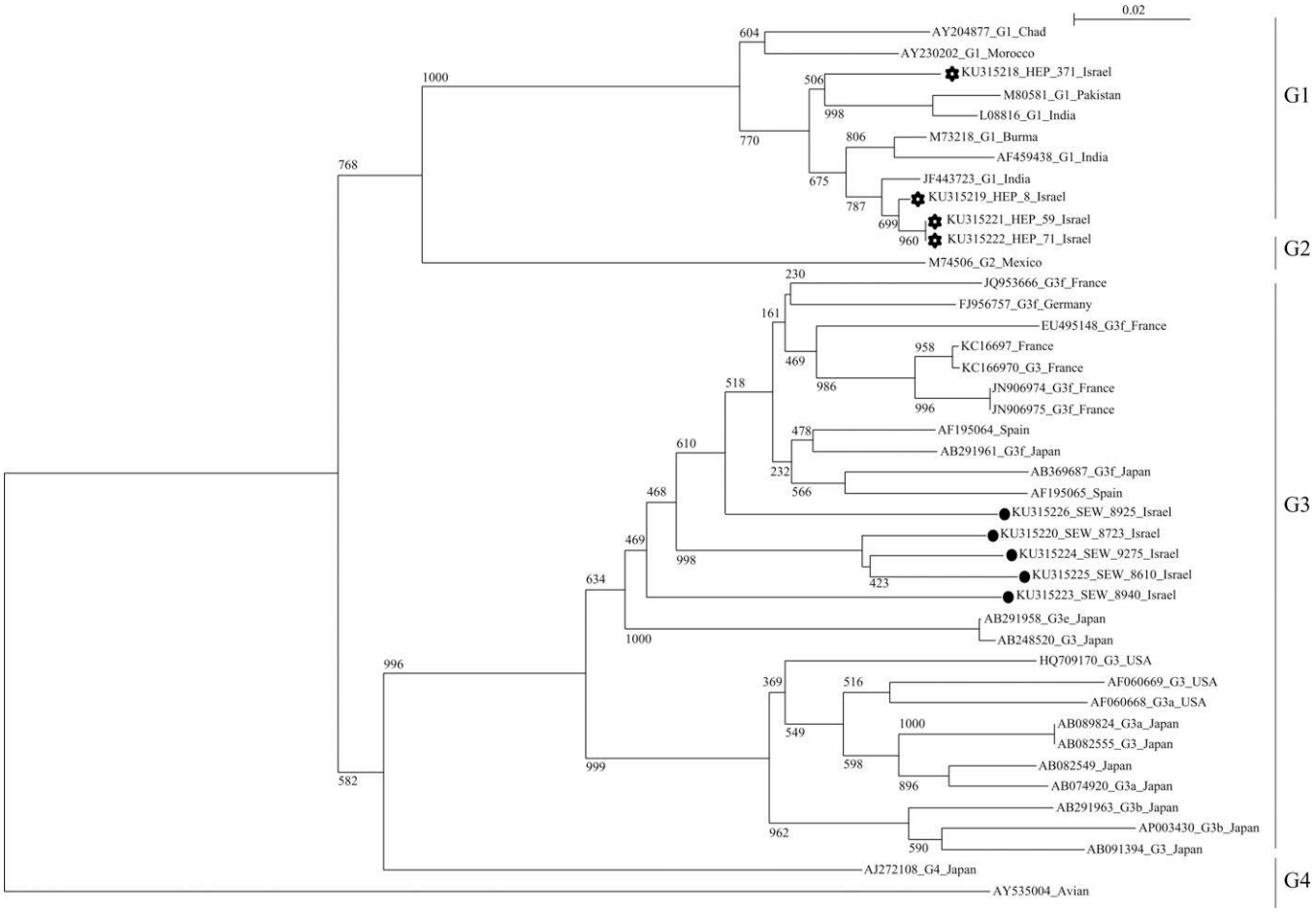

FIGURE 2. Phylogenetic analysis of hepatitis E virus (HEV) open reading frame 1 (ORF1) from sewage and clinical samples identified in Israel and from 33 prototype sequences obtained from GenBank. A phylogenetic tree reconstructed by Clustal W pairwise alignment, with 310 nucleotides ORF1 sequences. The prototype sequences are identified by the GenBank accession number, the HEV genotype and subtype (if known) and the name of the country of origin. The Israel-identified sewage samples are designated "SEW" (●) and the patient samples are designated "HEP" (✿). An avian HEV was included as an out-group. Bootstrap values are indicated for the major nodes, as data obtained from 1,000 replicates.

Identification of HEV G3 in more than 8% of the sewage samples assessed, with only few clinical cases with HEV G1 subtype, raises the possibility of subclinical HEV G3 infection, especially since hepatitis is a reportable disease in Israel. The high HEV seroprevalence rates identified in the healthy population in Israel (10.6%), using an enzyme-linked immunosorbent assay adequate for diagnosis of IgG antibodies against all HEV genotypes, also support this possibility. Circulation of the virus in the environment, without apparent clinical cases, may also result from reduced awareness for this virus and lack of tools for identification of HEV infection. In such cases, such as poliovirus circulation in highly immune populations, environmental surveillance can become a very useful tool for public health services to monitor the presence of viral activity. Extrahepatic manifestations of autochthonous HEV chronic infections, including neurological disorders such as Guillain-Barrè, kidney injuries, and hematological disorders, have also been reported and attributed mainly to genotype 3 of HEV.[2] The consistent identification of HEV G3 in sewage samples from Haifa may suggest persistent fecal HEV G3 excretion as a consequence of chronic HEV infections other than hepatitis, and raise the need to include HEV infection in the differential diagnosis of systemic diseases with unknown etiology especially in this region of Israel.

Israel is characterized by unique aspects with regard to HEV. It is a country of immigration from countries in Africa, Asia, and the former Soviet Union, which are endemic for HEV, mainly G1. It is also the temporary home for many refugees from African countries such as Sudan and Eritrea, endemic for HEV G1. It is located in the vicinity of countries endemic for HEV G1 such as Egypt. Taking all this into account, identification of sewage samples positive for G3 HEV (and not for HEV G1) was surprising.

In conclusion, our results indicate that HEV may be establishing itself in the region and calls for the attention of health providers and physicians with regard to this emerging pathogen. As G3 HEV infects human and animals, the possibility of zoonotic transmission should also be assessed.

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