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Presence of enteric hepatitis viruses in the sewage and population of Greater Cairo

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Abstract

In Egypt, the disease burden of viral hepatitis is one of the heaviest worldwide. We conducted a survey of hepatitis A virus (HAV) and hepatitis E virus (HEV) in patients and sewage in Cairo. Our data showed that HAV (genotype IB) was predominant over HEV (genotype 3) and was circulating in the population and the environment.

Keywords: Cairo, HAV, hepatitis, HEV, real-time RT-PCR, sewage, viral load, WWTP

Original Submission: 22 July 2010; **Revised Submission:** 18 November 2010; **Accepted:** 28 December 2010
Editor: G. Antonelli
Article published online: 17 January 2011
Clin Microbiol Infect 2011; **17:** 1182–1185
10.1111/j.1469-0691.2011.03461.x

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Hepatitis A virus (HAV) comprises a single serotype and seven genotypes. In Africa and the Middle East, the HAV strains mostly belong to genotypes IA and IB [1–3]. HAV is primarily transmitted by the faecal–oral route. HAV is one of the most resistant enteric viruses in the environment [4]. Hepatitis E virus (HEV) is classified into four genotypes, 1–4, the distribution of which varies around the world [5]. The disease burden of HEV is high in developing countries, where it causes waterborne outbreaks as well as sporadic hepatitis [5]. Little is known about HEV in the environment. In our study, we performed a molecular survey of both viruses in patients suffering from hepatitis and in sewage collected from two waste water treatment plants (WWTPs) of Greater Cairo.

Fifty stool samples were collected from patients with hepatitis hospitalized at the Embaba Fever Hospital in Cairo during July and August 2007. The patients were between 1.5 years and 15 years of age (mean 5.4 years), and presented hepatitis-related symptoms. The level of bilirubin was evaluated for each patient. The stool specimens were collected up to the 16th day after the onset, and were screened for HAV and HEV. The viruses were quantified with real-time RT-PCR: the stool specimens were weighed prior to extraction of the RNA [6].

We collected 76 samples of raw and treated sewage (4 L of each) from two WWTPs, located at Zenin and El-Berka in Greater Cairo, between July 2006 and August 2007. The characteristics of both WWTPs and the sampling procedure from July 2006 through February 2007 have been documented [7]. For July and August 2007, the raw and treated sewage was collected twice during the first and last weeks of the month. Enteroviruses and HAV were concentrated from 2 L of sewage as described previously [8]. The viral particles were concentrated in 3 mL of 0.15 M Na_2HPO_4 (pH 7.4). The remaining 2 L of sewage were decanted prior to concentration of the HEV particles by adsorption/elution as described previously [9]. Viral particles were resuspended in 4 mL of McIlvaine buffer (pH 5) [10]. One millilitre was used for the extraction of the nucleic acids [6].

Three microlitres and 10 μL of RNA were used for the detection of HAV in the clinical and environmental samples, respectively, by RT-PCR with primers H1 and H2 [11]. For the environmental samples, the results were confirmed by

semi-nested PCR with primers H2 and H3 and 1 μL of PCR product. HAV-positive samples were analysed by real-time RT-PCR in triplicate assays [12]. The copy number of the HAV genomic RNA was determined from a standard curve generated with serial dilutions of a plasmid construct of a portion of the 5′-non-coding region of the HM-175 strain amplified with the forward (5′-ATGGTGAGGGGACTTGATACC-3′) and reverse (5′-GCATATGTATGGTATCTCAAC-3′) primers. Ten microlitres of RNA was used for the detection of HEV in the clinical and environmental samples by nested RT-PCR [13]. Additionally, the samples were screened for the presence of HEV by three nested RT-PCRs with primers targeting the ORF1, ORF2 and ORF2/3 regions [14]. The presence of human enteroviruses has already been documented [7]. HAV and HEV were genotyped after sequencing of the PCR product. The HAV viral load (dependent variable) in combination with independent variables (date of collection after onset, bilirubin level and patient age) were modelled with the use of multiple linear regression. Comparisons of percentages were performed with Fisher's exact test (e.g. enterovirus–HAV association). The analyses were performed with the STATA package (Stata Corp LP, College Station, TX, USA).

Among the 50 individuals with hepatitis, a higher than normal level of bilirubin was detected up to 10 days post-onset for 36 of 39 patients (Table 1). For the remaining 11 patients, the samples were taken on the 15th day and 16th day after the onset, and the bilirubin levels were normal. HAV was detected in 70% (n = 35) of the stool specimens from the patients presenting abnormal bilirubin levels. The HAV isolates all belonged to genotype IB. The viral load varied between 4.1×10^3 and 1.7×10^8 genome copies per gram of stool (Table 1). HAV was no longer detected in stools 2 weeks after the onset. The age of the patients was not related to the viral load or the bilirubin level ($p > 0.05$). The association between the viral load, the collection date and the bilirubin concentration was highly significant (p 0.003, multiple linear regression model). One genotype 3 HEV isolate (GenBank no. GU353342) was detected in samples from a 9-year-old patient. The stool specimen was positive with primers targeting the ORF2/ORF3 overlapping region, and negative with other primer sets and by real-time RT-PCR.

HAV was detected by RT-PCR in 11 of 76 (15%) sewage samples (Table 2). RNA was tested undiluted and at a tenfold dilution. Positive results were obtained for undiluted RNA preparations only, suggesting the efficient removal of PCR inhibitors. The sewage isolates belonged to genotype IB, based on partial sequencing of the VP3–VP1 capsid protein, and were similar to the clinical isolates and the newly described genotype IB variant [15]. The HAV-positive samples were also positive for enteroviruses ($p < 0.0001$, Fisher's exact test). For the two WWTPs, there was a reduction in the viral load between the inlet and the outlet. However, the reduction was not statistically significant for either WWTP. HAV was detected by real-time RT-PCR in eight of 11 samples. The mean viral loads were 4.85 (N = 6) and 2.55 (N = 2) log of genome copies/L for the inlet and the outlet, respectively. For the EB1 and EB2 samples collected in August and September 2006, respectively, HAV was detected in the influent and the effluent of El-Berka during the same day with a 3 log titre reduction (EB3 and EB7; Table 2). All sewage samples were negative for HEV virus by conventional and real-time RT-PCR (data not shown).

The disease burden of hepatitis A and E in Egypt is one of the heaviest worldwide. HAV infection occurs very early in life, with almost 100% seropositivity after the first years of life. In our study using direct detection of the viruses in stool specimens, HAV (N = 35) was largely predominant

TABLE 1. Hepatitis A virus (HAV)-positive and hepatitis E virus (HEV)-positive stool samples from acute hepatitis cases

Isolate	Age (years)	Collection day[a]	Bilirubin (mg/dL)	HAV genotype[b]	HAV viral load[c] (log copy no./g ± SD)
July 2007					
H1	9	7	3.6	IB	3.8 ± 0.3
H2	5.5	2	8.9	IB	7.4 ± 0.2
H3	15	1	17	IB	6.2 ± 0.1
H4	6	1	19	IB	8.1 ± 0.1
H5	4	6	3.6	IB	5.4 ± 0.4
H6	12	3	7.5	IB	6.4 ± 0.4
H7	5	7	3.6	IB	6.1 ± 0.1
H8	14	6	5.9	IB	6.1 ± 0.1
H9	14	4	6.6	IB	3.9 ± 0.1
H10	11	4	6.6	IB	5.6 ± 0.0
H11	2	4	6.1	IB	5.7 ± 0.2
H12	2	2	8	IB	5.5 ± 0.1
H13	10	1	3.6	IB	5.1 ± 0.4
H14	3	5	17	IB	6.1 ± 0.1
H15	5	1	17	IB	8.2 ± 0.2
H16	4.5	1	19	IB	7.6 ± 0.2
H17	4	1	17	IB	6.8 ± 0.0
H18	4	7	6.6	IB	5.5 ± 0.2
August 2007					
H19	6	2	17	IB	6.2 ± 0.3
H20	8	5	19	IB	5.9 ± 0.0
H21	2	8	17	IB	5.0 ± 0.0
H22	2	1	6.6	IB	5.7 ± 0.1
H23	3	10	3.6	IB	4.8 ± 0.1
H24	3	7	8	IB	5.8 ± 0.1
H25	3	1	19.6	IB	5.7 ± 0.0
H26	6	6	5.2	IB	4.7 ± 0.1
H27	4	7	5.7	IB	6.3 ± 0.3
H28	4	4	6	IB	5.6 ± 0.1
H29	9	4	6.1	Negative	Negative
H30	12	8	4.7	IB	4.5 ± 0.1
H31	1.5	10	3.6	IB	3.6 ± 0.1
H32	6	2	8	IB	5.6 ± 0.3
H33	3	5	3.6	IB	5.3 ± 0.1
H34	3	2	8.1	IB	6.9 ± 0.1
H35	3	3	6.5	IB	7.1 ± 0.1
H36	2	3	6.6	IB	6.0 ± 0.1

SD, standard deviation.
[a]Time between symptom onset and sample collection.
[b]Genotype 3 HEV was detected in stool samples from case 29.
[c]The viral load is given per gram of stool.