Journal of Clinical Virology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

First detection of hepatitis E virus in Central Argentina: Environmental and serological survey

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ARTICLE INFO

Article history:
Received 20 June 2014
Received in revised form 12 August 2014
Accepted 19 August 2014

Keywords:
Hepatitis E virus
Environmental surveillance
Seroprevalence
Argentina
Molecular epidemiology
Subtyping

ABSTRACT

Background: The hepatitis E virus (HEV) is an emergent causative agent of acute hepatitis worldwide, transmitted by fecal-oral route. In Argentina it is considered rare, so differential laboratory testing is not routinely performed. Besides, in Argentina's central area epidemiological and molecular characteristics of HEV are still unknown.
Objectives: Provide evidence of local circulation of HEV by molecular detection on environmental samples and by serological survey in healthy adult population of Córdoba city, Argentina.
Study design: Environmental surveillance was conducted in river and sewage samples collected between 2007 and 2009–2011. Viral detection was performed by RT-Nested PCR of ORF-1 and ORF-2 partial regions. Anti-HEV IgG was determined by EIA in 433 serum samples collected between 2009 and 2010.
Results: HEV was detected in 6.3% of raw sewage samples and in 3.2% of riverine samples. Nucleotide sequencing analyses revealed that all isolates belonged to genotype 3, subtypes a, b and c. The prevalence of IgG anti-HEV was 4.4%. Seroprevalence increased with the age of the individuals (OR: 3.50; 95% CI 1.39–8.87; $p = 0.0065$) and, although the prevalence was higher in low income population, no statistical relation was found between anti-HEV and socioeconomic level.
Conclusions: The environmental findings added to serological results, demonstrate that HEV circulates in central Argentina. Contamination of water with HEV could represent a route of transmission for local populations, which have a high number of susceptible individuals. This fact alerts local health care systems in order to include detection of HEV in the diagnostic algorithm of viral hepatitis.

1. Background

The hepatitis E virus (HEV) (Hepevirus, *Hepeviridae*) is the causative agent of human acute hepatitis E with a worldwide distribution [1,2], responsible for both sporadic cases and large hepatitis epidemics in developing countries [3,4]. It is a single strand, positive sense, RNA non-enveloped virus which is classified into 4 genotypes of mammalian HEV [5]. HEV is mostly transmitted by fecal-oral routes following ingestion of contaminated water or consumption of fruits and vegetables that have been washed with contaminated water [6]. Two epidemiological patterns are observed for HEV infection. In areas of high endemicity, hepatitis E is mainly caused by genotypes 1 (Gnt-1) and 2 (Gnt-2), and primarily transmitted via the fecal-oral route. The second epidemiological pattern occurs worldwide, and consists of sporadic cases of

Abbreviations: HEV, hepatitis E virus; RNA, ribonucleic acid; Nested RT-PCR, reverse transcription reaction followed by a nested polymerase chain; ORF, open reading frame; IgG, immunoglobulin G; anti-HEV IgG, anti-HEV IgG antibodies; EIA, enzyme immunoassay; hIgG, human immunoglobulin G; CO, cut-off; S, sample; HAV, hepatitis A virus.

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http://dx.doi.org/10.1016/j.jcv.2014.08.016
1386-6532/

hepatitis E of zoonotic and/or foodborne transmission, mainly from pigs, caused by genotypes 3 (Gnt-3) and 4 (Gnt-4) [1].

With the exception of Venezuela – where outbreaks of HEV Gnt-1 have been reported, no epidemics of hepatitis E have been documented in South America yet [7]. Sporadic cases E have been documented in Argentina (Metropolitan region), Brazil, Venezuela, Peru, Chile and Uruguay [7] by IgM detection and/or RNA amplification. Until recently, Gnt-3 was the only genotype of HEV detected in autochthonous cases [8–10]. But recent studies placed Gnt-1 as the responsible of an autochthonous case of hepatitis in Uruguay [11], showing more than one genotype circulating in Latin America. Gnt-3 has also been detected in swine and effluent samples from farms and slaughterhouses from Brazil [8,12].

Argentina is considered a low endemic country for hepatitis E [7]. However, seroprevalence data reported are scarce. Previous studies performed ten years ago in the metropolitan region of Buenos Aires showed seroprevalence rates of 0.15% in pediatric population [13], 1.8% in blood donors and 6.6% in HIV infected individuals [14]. Furthermore, human clinical cases of hepatitis E have been diagnosed in the same area [10,15–17]. HEV has also been detected in pigs of commercial farms in many provinces [18]. However, there are no studies on environmental monitoring or serological surveys involving healthy adult population in our country. The lack of epidemiological information is, in part, due to the absence of commercial kits for detection of IgG and IgM anti-HEV until the middle of 2013, when the National Administration of Medicine, Food and Technology (ANMAT) of Argentina approved their use in our country.

Environmental surveillance using molecular technology is an additional tool to determine the epidemiology of different viruses circulating in a given community [19,20]. Previous studies in our area have shown a correlation between virus detected in sewage and clinical cases [18], showing that this type of study is very useful for virus monitoring.

Herein, we present the data from the first study of environmental surveillance and serological survey of HEV in Córdoba, Argentina.

2. Objectives

The aim of this study was to provide evidence of HEV circulation in central region of Argentina. For that, molecular detection of HEV was performed on environmental samples, as well as detection of IgG-anti HEV in healthy adult population of Córdoba city.

3. Study design

3.1. Environmental samples collection

Wastewater samples ($n = 48$) were monthly collected in the years 2007, 2009, 2010 and 2011 from the main pipe that enters the treatment plant which receives sewage discharges from about 61% of the population of Córdoba city (1,330,023 inhabitants, census 2010). Samples of the Suquía River ($n = 31$) were collected seasonally during 2010 in eight sampling points that cover the whole of its course across Córdoba city. During spring, point 7 could not be sampled (Fig. 1).

For each sample, 1500 mL of water were collected in sterile plastic bottles, stored at 4 °C and transported to the laboratory for immediate analysis.

3.2. Sample concentration, viral extraction and reverse transcription

Samples were concentrated 100× by centrifugation and polyethyleneglycol precipitation (10). RNA was extracted from 140 μL of concentrated samples using a QIAamp® Viral RNA Kit (Qiagen GmbH, Germany). Then, reverse transcription was performed adding 10 μL of extracted RNA to 10 μL of mix containing: 1 μL Reverse Transcriptase (ImPromII – Reverse Transcriptase – Promega, Madison WI, USA), 0.5 μL RNase Out (RNase Out Recombinant Ribonuclease Inhibitor, 40 U/μL – Invitrogen, CA, USA), 4 μL buffer 5× (ImPromII – Reverse Transcriptase – Promega, Madison WI, USA), 2.4 μL $MgCl_2$ 25 mM, 1 μL random primers (10 pmol/μL) (Promega, Madison WI, USA), 1 μL dNTPs 10 mM and 0.1 μL free RNase water (final volume of 20 μL).

3.3. PCR, Nested-PCR and molecular analyses

During this study, two Nested-PCR assays were performed, targeting ORF 1 and ORF 2 regions, following protocols previously described [21,22]. Amplification of ORF 2 was utilized as screening, and positive specimens were processed for ORF 1 detection.

Specific PCR products of 418 bp and 348 bp respectively were sequenced directly in both directions by Macrogen automatic sequencing service, Korea. Phylogenetic analyses were performed using MEGA software v5.0 [23]. Phylogenetic trees were constructed with neighbor-joining method and Kimura two-parameter as model of nucleotide substitution. Bootstrap values were determined with 2000 resamplings of the datasets. A consensus tree was generated and bootstrap values greater than 50% provide significant evidence for phylogenetic grouping.

3.4. Nucleotide sequence accession numbers

Nucleotide sequences analyzed in this work were deposited at GenBank under accession numbers KF751218–KF751221 for ORF 2 genomic region and KF765479 for ORF 1 genomic region (see Table 1).

3.5. Serum samples

A retrospective study was carried out with 433 serum samples from individuals who attended health care centers of Córdoba city during September 2009 and September 2010. The enrolled individuals were classified into three groups according to age (range 18–78 years old: younger than 30 years old, 31–45 years old and older than 46 years old) and two groups according to socioeconomic level (low-income population and middle/high-income populations) following a classification provided by the Municipality of Córdoba, which is based on the economic, social and educational level of each person [24]. The location of collected samples is shown in Fig. 1.

3.6. Serological test

A third generation enzyme immunoassay (EIA) for the determination of IgG specific antibodies against HEV (Diapro, Milan, Italy) was used. EIA microplates were coated with HEV-specific synthetic antigens encoding for conservative and immunodominant determinants derived from ORF2 and ORF3 of all genotypes. This EIA was performed strictly following the manufacturer's instructions. Test results were interpreted as ratio of the sample (S) and the cut-off (CO) (S/CO). Samples with ratio below 0.9 were considered negative, between 0.9 and 1.1 as equivocal result and above 1.1 were considered positive results.

Please cite this article in press as: Martínez Wassaf MG, et al. First detection of hepatitis E virus in Central Argentina: Environmental and serological survey. J Clin Virol (2014), http://dx.doi.org/10.1016/j.jcv.2014.08.016