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Short communication

Identifications of polyphyletic variants in acute hepatitis suggest an underdiagnosed circulation of hepatitis E virus in Argentina

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ABSTRACT

Background: In recent years, an increasing number of infections with genotype 3 hepatitis E virus (HEV) have been reported in western countries. Data in South America, however, are still scarce. Swine and human variants previously described in Argentina are closely related to a human Austrian one.
Objective: To identify whether HEV is still circulating in Argentina.
Study design: Sera and stool samples from adults and children with unexplained acute liver disease referred to our center during the last six years were prospectively studied. Dual infection with hepatitis A was retrospectively studied in a group of children with fulminant hepatic failure.
Results: Fifteen new cases (13 adults and 2 children), seven of whom required hospitalization, were diagnosed. Nine had detectable HEV RNA, and one had imported genotype 1. Subgenotype 3i HEV-related variants are still circulating. Five autochthonous sequences, related to European, American and Japanese ones, grouped in subgenotype 3a. One case had a subgenotype 3b variant.
Discussion: The polyphyletic variants widespread in Argentina suggest multiple sources of infection. Whether or not their reservoir is swine merits further investigation. Since hepatitis E is still considered rare, differential laboratory testing in unexplained acute liver disease is not routinely performed in Argentina. Broadening awareness of this disease is important in light of the decrease in hepatitis A incidence since universal vaccination was implemented in 2005. The diagnosis of hepatitis E with a combination of serological and molecular tools is needed to better understand its epidemiology and impact on the clinical management of patients with unexplained increased transaminases.

1. Background

Hepatitis E virus (HEV) is the only member of the genus *Hepevirus* in the family *Hepeviridae*.[1] In low endemic regions, imported and autochthonous sporadic hepatitis E cases have been reported, and the close genetic relationship between swine and human variants strongly suggests their zoonotic spread.[2–5]

Although seroprevalence of antibodies to HEV (anti-HEV) has been determined in different population groups in Latin America, and although IgM anti-HEV in acute hepatitis has been found in some countries, the molecular characterization of HEV is scanty [6–9]. Genotype 3 variants grouping in a monophyletic group related to European genotypes have been recovered in adult and pediatric patients and swine in Argentina.[10–12]

2. Objective

This study was conducted to serologically diagnose and molecularly characterize new cases of hepatitis E in patients in Argentina.

3. Study design

3.1. Study population

Samples (sera and feces) from 76 adults (52% female, median age: 40 years; range: 18–74) and 155 children (55% female, median age: 7.3 years) with acute non-A-C hepatitis, referred from hospitals located both in Buenos Aires and environs and in certain Argentine provinces, were studied at the National Reference Laboratory for viral hepatitis from January 2005 to December 2010.

Additional samples from 15 children with fulminant liver failure (FHF) and hepatitis A (8 female, median age: 7.5 years) were also retrospectively studied.[13] Until 2005 hepatitis A was the etiology

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of 68% of the transplanted children with FHF in Argentina, whereas following the implementation of universal vaccination against HAV in that same year, the number of hepatitis A cases has dramatically decreased.[14]

3.2. Serological diagnosis

Until 2006 anti-HEV antibodies were detected with Abbott HEV IgG EIA (Abbott GmbH Diagnostics, Germany), the only commercial ELISA kit available in Argentina at the time. From 2006 to 2009 anti-HEV antibodies were studied with Genelabs HEV IgM (courtesy of Genelabs, Inc., Singapore). Since January 2010 total (Ig M+ IgG) anti-HEV Diapro (HEV Ab EIA, Diapro SRL, Milano, Italy) has been employed.

Due to the low sensitivity of serological tests for diagnosing acute hepatitis in children, their serum samples were routinely tested only for HEV RNA when falling within the period of acute symptoms.[12,15,16]

3.3. HEV RNA detection, characterization and sequence analysis

Samples were tested for HEV RNA by RT-nested PCR using degenerated primers designed within ORF 1 and ORF 2, as previously described.[5,17] In PCR-positive cases, the purified PCR products were sequenced, characterized and analyzed as previously described.[11–13]

4. Results

During the six-year period studied, fifteen patients (13 adults and 2 children) were diagnosed with acute hepatitis E. Seven required hospitalization due to its severity. Additional information on patients is summarized in Table 1; where patients acquired the infection is indicated in Fig. 1.

Fig. 1. Map of Argentina showing where patients acquired HEV infection. Numbers correspond to Table 1.

Table 1
Clinical details and laboratory results of Hepatitis E cases.

Case	Age/sex	Date	Location	Clinical presentation	Comorbid condition	Hospital admission	Anti-HEV +	HEV RNA/Genotype	Risk factor associated
1	1/M	3/2004	Salta	FHF and death	Acute Hepatitis A	Yes	ND	3a	Not reported
2	28/M	8/2005	BsAs	Jaundice	No	No	IgG	1a	Trip to India
3	29/F	8/2005	Greater BsAs	Anicteric	Previously undiagnosed AIH	No	IgM/IgG	–	Work with children in low sanitary condition
4	46/F	9/2006	Greater BsAs	Anicteric	Not reported	No	IgM	–	Work in a health center
5	34/F	8/2007	Greater BsAs	Anicteric, abdominal pain, pruritis	Not reported	No	IgM	–	Not reported
6	2/M	5/2008	Sgo del Estero	Jaundice	Not reported	Yes	ND	3a	Low sanitary condition
7	52/M	6/2008	Greater BsAs	Jaundice	Previously undiagnosed AIH	Yes	–	3a	Not reported
8	58/M	1/2009	Bs As	Anicteric, astenia	No	No	IgM	3b	Fishing. Wash hands & stuff in river water in Corrientes
9	38/F	1/2009	Bs As	Jaundice	HIV infection	No	IgM	–	Contact with sick people
10	58/M	6/2009	Mendoza	Jaundice, astenia, myalgia	Not reported	Yes	IgM	3i	Trip to Cuba 1 m before
11	74/F	1/2010	BsAs	Anicteric	R.A, dyslipidemia	No	Total	3i	Trip to Santo Domingo 1 m before
12	30/F	3/2010	Greater BsAs	Jaundice	Pregnancy	Yes	Total	–	Not reported
13	45/M	5/2010	BsAs	Anicteric, myalgia, arthralgia	Hypertension	No	Total	3a	Healthcare worker
14	62/M	8/10	Greater BsAs	Jaundice, ascitis	NASH	Yes	Total	3a	Not reported
15	74/M	11/10	Greater BsAs	Astenia, coluria	No reported	Yes	Total	–	Weekends in low sanitary condition. Trip to Miami, EEUU 1 m before

FHF: fulminant hepatic failure. ND: not done BsAs: Buenos Aires. AIH: autoimmune hepatitis. R.A: rheumatoid arthritis. NASH: non alcoholic steatohepatitis.

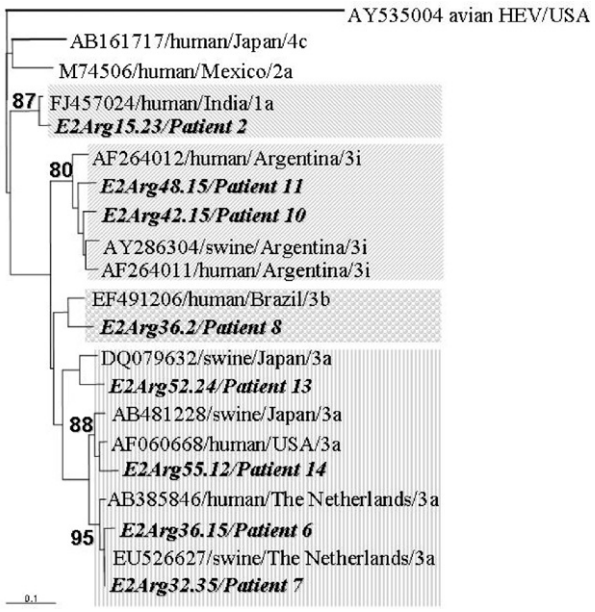

Fig. 2. Phylogenetic tree constructed by means of the neighbor-joining method, based on 98 nt ORF2 sequences. Each reference viral strain is identified by GenBank accession number, species and country of origin, and respective genotype and subtype. Numbers at the nodes indicate bootstrap percentages over 1000 replicates (only values > 70% are shown). The bar indicates genetic distance. Avian HEV is the outgroup.

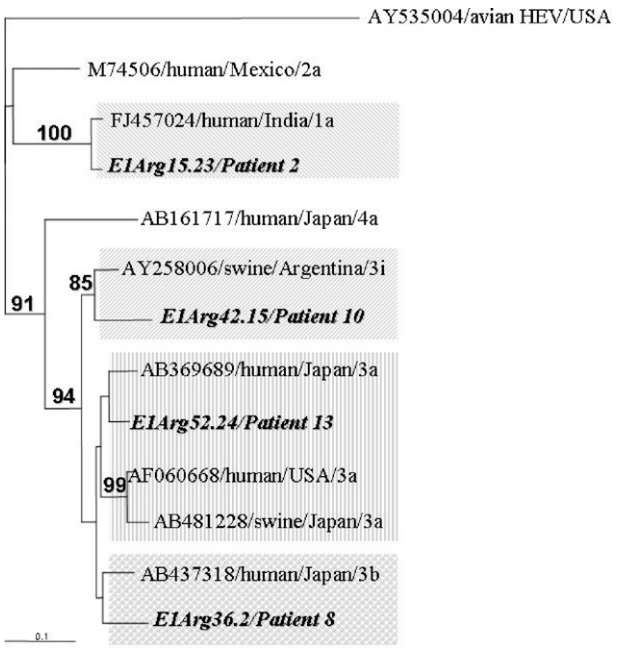

Fig. 3. Phylogenetic tree constructed by means of the neighbor-joining method, based on 252 nt ORF1 sequences. Each reference viral strain is identified by GenBank accession number, species and country of origin, and respective genotype and subtype. Numbers at the nodes indicate bootstrap percentages over 1000 replicates (only values > 70% are shown). The bar indicates genetic distance. Avian HEV is the outgroup.

Nine cases were nested RT-PCR HEV RNA-positive for the ORF 2 region. In five of them, an ORF1 fragment was also sequenced. The lower sensitivity for ORF 1 has been previously reported.[18–19]

The genotype and subtype were assigned one by one by means of a careful phylogenetic analysis of the best sequences for each case, taking into account the highest hits in GenBank.[20]

The length of the ORF2 fragments varied between 86 and 148 nucleotides, while that of ORF1 was between 131 and 379 nucleotides. Phylogenetic trees were made with the length permitting the inclusion of the largest number of cases (Figs. 2 and 3). The short length of a fragment resulted in low bootstrap values.

Patients with non-detectable HEV RNA had an evolution of between 20 days and 2 months following the onset of symptoms.

Patient 2 was diagnosed as a case imported from India, the first, to our knowledge, to be characterized in South America.

Liver biopsy in patient 7 revealed acute cholestatic hepatitis in addition to signs of previously undiagnosed autoimmune cirrhosis. The absence of IgM anti-HEV has been previously reported.[21–22]

Patient 8 lived in Buenos Aires but could have acquired the infection in the province of Corrientes (north-east). The sequences showed 91 and 90% homology with Brazilian and Japanese 3b ones.

Patients 10 and 11 had traveled to tropical Caribbean countries. Nevertheless, they showed genotype 3i variants closely related to previously reported autochthonous ones.[10–12]

Patients 1, 6, and 7 had HEV variants that grouped in subgenotype 3a and were related to European ones (97–100% homology). The 3a variants characterized in patients 13 and 14 were more closely related to American and Japanese ones (92–97% homology) than to each other (79%).

5. Discussion

The above results show that HEV has continued circulating in Argentina during the last six years. The different subgenotypes and polyphyletic variants found suggest multiple sources of infection.

Subgenotype 3i, the first described in humans and swine in Argentina, is still circulating. In this study, we found this subgenotype not only in Buenos Aires, but also in the province of Mendoza (center-west). The precedent of having traveled abroad would support the assumption of an imported case when no molecular characterization is available or the molecular epidemiology is obscure.[23]

Subgenotype 3b could be circulating in the province of Corrientes, which shares a border with Brazil, where a fatal case in a child infected with a 3i variant has been reported.[8,9,12]

Subgenotype 3a, related to European strains, was first found in 2004 in a fatal HAV + HEV infection in a pediatric patient in northwest Argentina, appearing in 2008 in the center-north and Buenos Aires. A temporal rather than geographical association of the variants has been previously reported.[5] The fragment characterized in ORF 2 is too small to permit any inferences regarding its high identity with a swine strain from the Netherlands.[24] The larger sequence or additional ORF 1 needed to confirm this high identity could not be achieved. The two new 3a variants found in 2010 were unrelated.

Extending the characterization of swine herds in Argentina could contribute to clarifying whether this reservoir can explain the different variants in locally acquired infections.

As a National Reference Laboratory, at times we receive samples, usually many days after the onset of symptoms, from "special" patients with undiagnosed hepatitis: hospitalized people, HIV-positive patients, healthcare workers, pregnant women, and tourists. Routine hepatitis E testing in all patients with unexplained acute liver disease is not done in Argentina. The National Notifiable Diseases Surveillance System (SINAVE) assumes that unspecified hepatitis cases are hepatitis A rather than any other form of hepatitis, notwithstanding the dramatic decrease in its incidence rate since universal vaccination was implemented in 2005.[14] The optimal diagnosis of hepatitis E infection may be too expensive to become widespread.[1,19,25–27]

Although Argentina is considered a low endemic country for hepatitis E, its incidence is underestimated.[28] In our view, HEV testing should be included in differential diagnosis, not only

for surveillance purposes and to clarify HEV epidemiology in Argentina, but also because of its impact on the clinical management of patients with unexplained increased transaminases, which may unnecessarily progress to liver biopsy, an empirical trial of steroids or the withdrawal of presumed toxic drugs.[29]

Conflict of interest statement

None.

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