Emergence of Autochthonous Infections with Hepatitis E Virus of Genotype 4 in Europe

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Key Words

Hepatitis E virus · Genotype 4 · France · Laboratory-based surveillance · Zoonosis

Abstract

In Europe, autochthonous hepatitis E is caused by genotype 3 hepatitis E virus (HEV) in almost all cases. A total of 15 infections with genotype 4 HEV were diagnosed in France from May 2009 to April 2012, and all but one of the HEV-4 strains implicated in these infections were genetically related and highly similar to HEV-4 sequences isolated from swine in Belgium. In addition, 5 autochthonous HEV-4 infections have been described in the region of Lazio, Italy, during March and April 2011, and these HEV sequences were 100% identical to one another but showed relatively low similarity (74–85%) to HEV-4 RNA samples collected in France. We report 6 additional HEV-4 infections that were diagnosed from May to July 2012 which represented 50% of the HEV infections

diagnosed during this period in our clinical microbiology laboratory. Five of these HEV-4 strains were associated with autochthonous infections and were clustered together and with the majority of HEV-4 previously described in France, whereas the sixth strain was genetically divergent. Taken together with reports from other teams, these observations indicate that autochthonous infections with HEV-4 are emerging in Europe and have been transmitted by at least two distinct sources.

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Over the last decade, hepatitis E virus (HEV) strains of genotype 3/4 have emerged as causative agents of autochthonous hepatitis E in developed countries, mostly Japan and Europe [1, 2]. A porcine reservoir has been identified for these two HEV genotypes, and food-borne zoonotic transmission is suspected, and demonstrated in a few cases [3–5]. Autochthonous hepatitis E in Japan has involved

Fig. 1. HEV infections diagnosed in the clinical microbiology laboratory of the Marseille university hospitals from January 2011 to October 2012, including with all HEV genotypes and genotype 4 viruses.

HEV-3/4 [2, 6], whereas until recently, HEV-3 had been found in nearly all European cases [1, 2, 4, 7–14]. Indeed, autochthonous infection with HEV-4f was first diagnosed in Germany in 2006–2007 from the report of a single case [15]. Six cases were then diagnosed in France from May 2009 to April 2012 of HEV-4 that were related to subtype 4b and genetically related to one another, although these sequences were found in both the south-east and north-east region and there was no apparent epidemiological link between the cases [10, 11, 16]. Moreover, HEV-4 RNA obtained in France were highly similar to HEV-4 sequences retrieved from swine in Belgium in 2010 [16, 17]. In addition, 5 autochthonous HEV-4 infections have been described in the region of Lazio, Italy, during March and April 2011 [12]. These HEV-4 sequences were retrieved from patients who did not report recent travel to HEV hyperendemic regions and were classified as 4d and 100% identical with one another, although neither an epidemiological link nor a common source was identified. Furthermore, these sequences showed relatively low similarity (74–85%) to HEV-4 RNA that had been described in France, suggesting multiple sources for these infections. Finally, 9 additional HEV-4 infections were identified in France in 2011, 6 of which in patients living in northern France [13]. Pork meat consumption and travel abroad in China were reported in 5 cases and in 1 case, respectively. Phylogenetic analyses revealed that HEV-4 RNA recovered from the 8 autochthonous cases were both clustered together and close to the other HEV-4 sequence described in France, whereas the HEV-4 from the imported case was clustered with sequences obtained in China.

In the Marseille university hospitals, 5 HEV-4 infections were identified from February 2011 to April 2012 (fig. 1) [11, 16]. We describe here 6 additional cases that were diagnosed from May 2012 to July 2012 based on positive testing of anti-HEV IgM and HEV RNA, as previously described [16]. During this 3-month period, these HEV-4 infections represented 50% of the HEV infections (6/12) diagnosed in our laboratory (fig. 1). It is worth noting that all 6 of these patients ate pig liver sausage uncooked; none ate shellfish nor reported any professional exposure to pigs or sewage. Two of the patients were married and reported several potential sources of HEV infection, including keeping pigs at home, manufacturing their own pig liver sausages, and drinking water from their own well. One patient had returned from China 5 weeks ago following a 10-day stay. Based on the analysis of a 424-bp fragment of HEV open reading frame 2 (ORF2), HEV-4 RNA recovered from the serum of this patient showed only 85% nucleotide identity with the HEV-4 RNA from the 5 other patients. This infection was likely acquired in China; the best BLAST matches (96.6–97.8% identity) against the NCBI GenBank nucleotide sequence database were sequences described in humans and swine in China (fig. 2). However, strikingly, similarity was concurrently 97.1% with a sequence from the outbreak of autochthonous HEV-4 infections recently reported in Italy [12]. The 5 other HEV-4 sequences were clustered together and with the 5 HEV-4 previously described in our laboratory [11, 16]. The nucleotide similarity between these 10 sequences was 99.2–100%, and their best hits in GenBank (88.9–94.5% identity) were sequences originating from swine or humans sam-

Fig. 2. Phylogenetic tree based on a partial (424 nucleotides; nucleotides 6,553–6,974 of the HEV genome accession No. AB291961) nucleotide sequence of ORF2 of the HEV genome. The HEV sequences collected in the present study are indicated by black boxes and a black background. The HEV sequences of genotype 4 obtained earlier in our laboratory are indicated by black boxes and a white background. The HEV sequences with the highest BLAST scores using the sequences from this study as queries are indicated in bold-face. Sequence E2105 ITA Hu 2011 was kindly provided by Drs. Anna Rosa Garbuglia and Maria R. Capobianchi [12]. The nucleotide alignments were performed using ClustalX v2.0 (<http://www.clustal.org/download/current/>). The tree was constructed using the MEGA v5.0 software (<http://www.megasoftware.net/>) with the neighbor-joining method, as described previously [16]. The branches with bootstrap values, obtained from 1,000 resamplings of the data, >50% are labeled on the tree. The avian HEV sequence AY043166 was used as an outgroup. The scale bar indicates the number of nucleotide substitutions per site. The HEV sequences are labeled with the GenBank accession numbers, countries of origin, host species, and collection or submission dates. Av, Avian; BBH, best BLAST hit; CHN, China; FRA, France; Hu, human; JPN, Japan; MAR, Marseille; Sw, swine; Rab, rabbit; Ref, reference sequences.

pled in China from 2006 through 2011 (fig. 2). In addition, another fragment (303 nucleotides) of HEV ORF2 obtained from 2 autochthonous cases identified in our laboratory from May 2012 to July 2012 were clustered with the 14 HEV-4 sequences previously retrieved from

autochthonous cases in France, and showed high levels of similarity (98.6–100%) to these sequences [10, 13]. Then, the best matches in GenBank (94% identity) were sequences originating from swine, *Macaca mulata* and humans sampled in China (fig. 3). No other cases of

Fig. 3. Phylogenetic tree based on a partial (204 nucleotides; nucleotides 6,025–6,326 of the HEV genome accession No. AB291961) nucleotide sequence of ORF2 of the HEV genome. The phylogenetic analysis is as described in figure 2, except that the avian HEV sequence AM943646 was used as an outgroup. The sequences are labeled as described above with the following exceptions: the sequences from this study are indicated by a black box with a black background and the abbreviations are the same, with the addition of ITA, Italy; TWN, Taiwan; and VNM, Vietnam.

HEV-4 infection were detected in our center from August 2012 to June 2013.

Worldwide, HEV-4 strains have been mostly described in Japan and China in humans and swine [18–20]. Several HEV-4 subtypes have been described in Japan, including

subtypes b and f, in addition to HEV-4 transmission through pig meat consumption [3, 21]. In China, HEV-4 has emerged over the last decade and overtaken HEV-1 as the predominant strain in humans and swine [19, 20, 22]. The HEV molecular epidemiology has been found to be

complex in this country, as several HEV-4 subtypes, including subtypes b and d, have been detected, and sequences recovered from humans were found (although inconsistently) to share a high level of nucleotide similarity with those found from swine in the same geographical area [19, 20, 22]. Otherwise, HEV-4 have been collected in India, Indonesia, South Korea, Taiwan and Vietnam [18, 20, 23–27], including in autochthonous hepatitis E, and an epidemiological link with pigs or wild boars has been observed in cases diagnosed in Bali and South Korea [14, 23, 25]. In addition, HEV-4 infection was reported in 2010 in England in a patient who returned from a 2-month stay in India 1 month before presenting jaundice [28]. HEV-4 collected in Japan and France have been associated with higher liver cytolysis and a lower prothrombin index relative to HEV-3, and more severe illness was described in Japan for HEV-4 compared to HEV-3 [29, 30]. However, further studies are needed to determine if differences in the hepatitis E presentation and outcome might be linked to the HEV genotypic patterns or the host or both.

Overall, previous data indicate the emergence of autochthonous HEV-4 infections in Europe, originating from different sources, and these findings create an incentive to implement a surveillance of HEV-4 infections on that continent to aid in documenting the presence of this genotype in European pigs or wild boars and identify the possible importation of HEV-4 from Asia.

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Disclosure Statement

The authors have no conflicts of interest to disclose.

References

- Kamar N, Bendall R, Legrand-Abravanel F, Xia NS, Ijaz S, Izopet J, Dalton HR: Hepatitis E. *Lancet* 2012;379:2477–2488.
- Kaba M, Moal V, Gerolami R, Colson P: Epidemiology of mammalian hepatitis E virus infection. *Intervirology* 2013;56:67–83.
- Matsubayashi K, Kang JH, Sakata H, Takahashi K, Shindo M, Kato M, Sato S, Kato T, Nishimori H, Tsuji K, Maguchi H, Yoshida J, Maekubo H, Mishihiro S, Ikeda H: A case of transfusion-transmitted hepatitis E caused by blood from a donor infected with hepatitis E virus via zoonotic food-borne route. *Transfusion* 2008;48:1368–1375.
- Colson P, Borentain P, Queyriaux B, Kaba M, Moal V, Gallian P, Heyries L, Raoult D, Gerolami R: Pig liver sausage as a source of hepatitis E virus transmission to humans. *J Infect Dis* 2010;202:825–834.
- Purcell RH, Emerson SU: Hidden danger: the raw facts about hepatitis E virus. *J Infect Dis* 2010;202:819–821.
- Purcell RH, Emerson SU: Hepatitis E: an emerging awareness of an old disease. *J Hepatol* 2008;48:494–503.
- Lewis HC, Wichmann O, Duizer E: Transmission routes and risk factors for autochthonous hepatitis E virus infection in Europe: a systematic review. *Epidemiol Infect* 2010;138:145–166.
- Legrand-Abravanel F, Mansuy JM, Dubois M, Kamar N, Peron JM, Rostaing L, Izopet J: Hepatitis E virus genotype 3 diversity, France. *Emerg Infect Dis* 2009;15:110–114.
- Bouquet J, Tesse S, Lunazzi A, Eloit M, Rose N, Nicand E, Pavio N: Close similarity between sequences of hepatitis E virus recovered from humans and swine, France 2008–2009. *Emerg Infect Dis* 2011;17:2018–2025.
- Tesse S, Liouze B, Fornecker L, Wendling MJ, Stoll-Keller F, Bigaillon C, Nicand E: Circulation of genotype 4 hepatitis E virus in Europe: first autochthonous hepatitis E infection in France. *J Clin Virol* 2012;54:197–200.
- Colson P, Swiader L, Motte A, Ferretti A, Borentain P, Gerolami R: Circulation of almost genetically identical hepatitis E virus of genotype 4 in France. *J Clin Virol* 2012;55:181–183.
- Garbuglia AR, Scognamiglio P, Petrosillo N, Mastroianni CM, Sordillo P, Gentile D, La SP, Girardi E, Capobianchi MR: Hepatitis E virus genotype 4 outbreak, Italy, 2011. *Emerg Infect Dis* 2013;19:110–114.
- Jebblaoui A, Haim-Boukobza S, Marchadier E, Mokhtari C, Roque-Afonso AM: Genotype 4 hepatitis E virus in France: an autochthonous infection with a more severe presentation. *Clin Infect Dis* 2013;57:e122–e126.
- Bouamra Y, Moal V, Colson P: Map of autochthonous infections with hepatitis E virus of genotype 4 in Europe and Asia (outside Japan and China). URL: <http://maps.google.com/maps/ms?ie=UTF&msa=0&msid=200914559094835369589.0004c30d23e82dd74fa30>.
- Wichmann O, Schimanski S, Koch J, Kohler M, Rothe C, Plentz A, Jilg W, Stark K: Phylogenetic and case-control study on hepatitis E virus infection in Germany. *J Infect Dis* 2008;198:1732–1741.
- Colson P, Romanet P, Moal V, Borentain P, Purgus R, Benezech A, Motte A, Gerolami R: Autochthonous infections with hepatitis E virus genotype 4, France. *Emerg Infect Dis* 2012;18:1361–1364.
- Hakze-van der Honing RW, van CE, Antonis AF, van der Poel WH: First isolation of hepatitis E virus genotype 4 in Europe through swine surveillance in the Netherlands and Belgium. *PLoS One* 2011;6:e22673.
- Lu L, Li C, Hagedorn CH: Phylogenetic analysis of global hepatitis E virus sequences: genetic diversity, subtypes and zoonosis. *Rev Med Virol* 2006;16:5–36.
- Zhu YM, Dong SJ, Si FS, Yu RS, Li Z, Yu XM, Zou SX: Swine and human hepatitis E virus infection in China. *J Clin Virol* 2011;52:155–157.
- Purdy MA, Khudyakov YE: The molecular epidemiology of hepatitis E virus infection. *Virus Res* 2011;161:31–39.
- Yazaki Y, Mizuo H, Takahashi M, Nishizawa T, Sasaki N, Gotanda Y, Okamoto H: Sporadic acute or fulminant hepatitis E in Hokkaido, Japan, may be food-borne, as suggested by the presence of hepatitis E virus in pig liver as food. *J Gen Virol* 2003;84:2351–2357.
- Liu P, Li L, Wang L, Bu Q, Fu H, Han J, Zhu Y, Lu F, Zhuang H: Phylogenetic analysis of 626 hepatitis E virus isolates from humans and animals in China (1986–2011) showing genotype diversity and zoonotic transmission. *Infect Genet Evol* 2012;12:428–434.

- 23 Wibawa ID, Suryadarma IG, Mulyanto, Tsuda F, Matsumoto Y, Ninomiya M, Takahashi M, Okamoto H: Identification of genotype 4 hepatitis E virus strains from a patient with acute hepatitis E and farm pigs in Bali, Indonesia. *J Med Virol* 2007;79:1138–1146.
- 24 Yun H, Kim JS, Lee HJ, Jeong SH, Kim JS, Park SJ, Hwang SG, Kang SK, Jee Y, Kim JH: The complete genome sequence and molecular analysis of human hepatitis E virus genotype IV identified from a Korean patient. *Arch Virol* 2010;155:1003–1008.
- 25 Kim YM, Jeong SH, Kim JY, Song JC, Lee JH, Kim JW, Yun H, Kim JS: The first case of genotype 4 hepatitis E related to wild boar in South Korea. *J Clin Virol* 2010.
- 26 Hijikata M, Hayashi S, Trinh NT, Ha ID, Ohara H, Shimizu YK, Keicho N, Yoshikura H: Genotyping of hepatitis E virus from Vietnam. *Intervirology* 2002;45:101–104.
- 27 Wu JC, Chen CM, Chiang TY, Sheen IJ, Chen JY, Tsai WH, Huang YH, Lee SD: Clinical and epidemiological implications of swine hepatitis E virus infection. *J Med Virol* 2000;60:166–171.
- 28 Rolfe KJ, Curran MD, Mangrolia N, Gelson W, Alexander GJ, L'Estrange M, Vivek R, Tedder R, Ijaz S: First case of genotype 4 human hepatitis E virus infection acquired in India. *J Clin Virol* 2010;48:58–61.
- 29 Mizuo H, Yazaki Y, Sugawara K, Tsuda F, Takahashi M, Nishizawa T, Okamoto H: Possible risk factors for the transmission of hepatitis E virus and for the severe form of hepatitis E acquired locally in Hokkaido, Japan. *J Med Virol* 2005;76:341–349.
- 30 Inoue J, Takahashi M, Mizuo H, Suzuki K, Aikawa T, Shimosegawa T, Okamoto H: Nucleotide substitutions of hepatitis E virus genomes associated with fulminant hepatitis and disease severity. *Tohoku J Exp Med* 2009;218:279–284.