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

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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.

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

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
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 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 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 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-
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. 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 boldface. 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.
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

**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.
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. Indonesia, South Korea, Taiwan and Vietnam, 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


