

Male patient with acute hepatitis E in Genoa, Italy: figatelli (pork liver sausage) as probable source of the infection

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Hepatitis E virus (HEV) is the major causative agent of epidemic and sporadic acute hepatitis in many areas of developing countries. In Europe, cases of hepatitis E related to genotype 1 are generally considered imported from endemic countries, whereas genotype 3 infections are described as autochthonous because this HEV genotype could be detected in sewage, wild boar meat [1] (Sweden, Italy), or pig liver sausages including figatelli (Corsican raw pork liver dish), and it has never been travel associated [2]. To date, in Italy no cases of acute hepatitis E have been recognized as imported from another European country.

A serum sample collected during the acute phase of hepatitis was sent to Istituto Nazionale Malattie Infettive (INMI) "L. Spallanzani" hospital to carry out molecular investigation. HEV RNA was tested by reverse transcription-polymerase chain reaction with primers derived from a well-conserved region of open reading frame 2 (ORF2) (411 nt long), according to the previously described method [3]. A 44-year-old man (PeGe) from Genoa, a city in the North of Italy, took his vacation in Corsica and in Southern France from the end of August to the beginning of September 2011. During his holiday, he bought uncooked liver sausage (figatelli) in a Corsican supermarket and stored the food at +4°C until September 21, 2011, when he ate uncooked figatelli. At the end of October he experienced general malaise, loss of appetite, and abdominal pain. He was referred to S. Martino Hospital (Genoa, Italy) and admitted to the infectious disease service ward on November

10, 2011, with a provisional diagnosis of acute hepatitis, presenting with fever, fatigue, and dark-coloured urine.

Blood analysis showed alteration of liver enzymes: alanine aminotransferase 2717 IU/L, aspartate aminotransferase 990 IU/mL, increased total bilirubin (4.3 mg/dL) and direct bilirubin (3.3 mg/dL). Normalization of liver enzymes was reached after 45 days. The patient was negative for serum markers of hepatitis B, C, and A viruses, immunoglobulin (Ig) M specific for Epstein-Barr virus and cytomegalovirus, and for screening for autoimmune hepatitis, but tested positive for HEV-specific IgM and IgG, assayed by ELISA immunoassay (RADIM, Pomezia, Italy). Consumption of figatelli was the only identified factor. The patient had not cat or other household pets. He was not a hunter, nor had he had direct contact with pigs, sheep, deer, cattle or goats. He denied having eaten shellfish during the 2-month period before the onset of symptoms. He lived in an urban area, and there was no history of acute hepatitis in any family members. Written consent was obtained from the patient for the use of stored samples to carry out molecular investigation.

Phylogenetic analysis showed that the HEV strain belonged to genotype 3, subtype i (Fig. 1). Comparison of the PeGe ORF2 region with 98 genotype 3 sequences from pig, wild boar and human isolates revealed that the PeGe sequence was most closely related to a sequence obtained from a patient from Marseille, France (GenBank accession number GQ426992), with an identity of 97.7%. PeGe showed 99% identity in a 286-nt overlapping stretch of another strain isolated from south-eastern France, Fr-08V22 (GenBank accession number JF730354.1). To confirm this great similarity between the PeGe and Fr-08V22 strains, the RNA of the French isolated was newly retrotranscribed and cDNA amplified with the primers used to type the PeGe strain. In this stretch of ORF2 region (411 nt), the similarity was 98.1%. In contrast, PeGe was only 78.6% to 91.7% similar to the human and swine HEV strains isolated in Italy [4,5].

The majority of hepatitis E virus cases in Western developed countries are due to infection with genotype 3. Transmission of this genotype has been linked to consumption of undercooked or raw organs from infected swine or wild boar [6]. An outbreak of human cases with acute HEV infections in France was linked to consumption of figatelli. The HEV sequences recovered from figatelli products in local grocery stores were essentially indistinguishable from viral sequences recovered from human patients, thus providing compelling evidence for food-borne transmission. However, all hepatitis E genotype 3 cases in humans observed in Europe have been considered autochthonous (not travel-associated). The acute hepatitis of the PeGe patient represents the first case of HEV genotype 3 infection imported from an industrialized country.

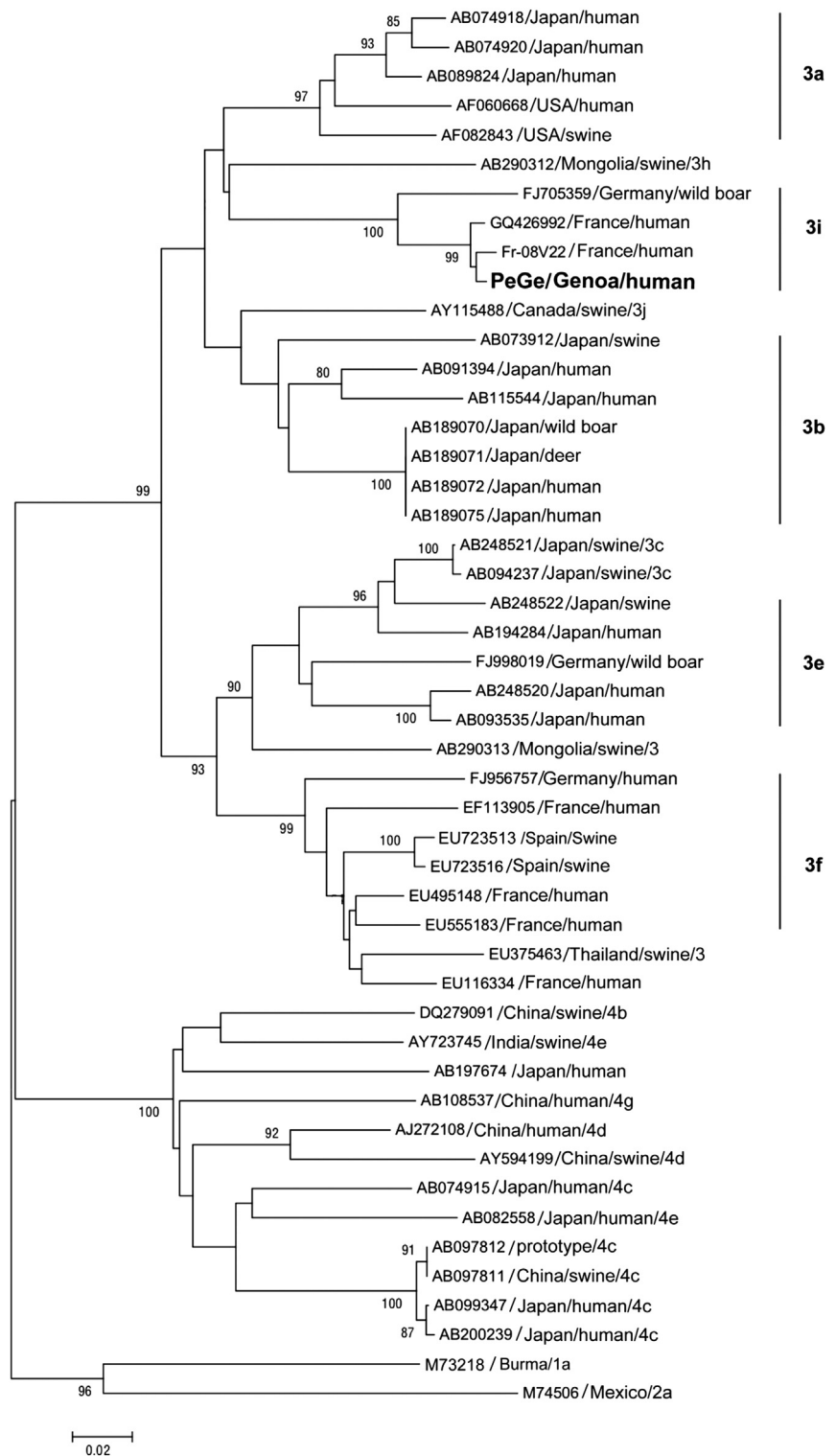


FIG. I. Phylogenetic analysis of hepatitis E virus based on partial ORF2 sequence region (304 nt). The phylogenetic tree was constructed by the neighbour-joining method, using the Jukes-Kantor correction model and 1000 replicates of bootstrap resampling as implemented in the MEGA 5 software (version 5.1.0). Bootstrap values of >70% are indicated at the respective branches. The scale bar represents the genetic distance in substitution per site. HEV genotype 3 sequences available from GenBank with previously identified genotypes are labelled with GenBank accession number, country of origin, host. The virus from the case described in this study (PeGe) is indicated in bold. PeGe sequence GenBank accession number: KF751185; Fr-08V22 sequence GenBank accession number: KF751184.

Although we were unable to provide direct evidence that the French figatelli was the source of infection because there were no leftovers, it seems reasonable to speculate that PeGe contracted HEV from figatelli based on the following evidence: 1)

the 3i strain isolated from PeGe was closely related only to 2 French strains circulating in the restricted area of the South of France where PeGe bought the figatelli, 2) a high frequency (44%) of pig-liver sausage contamination with HEV-RNA has

been described in a recent study [7], and 3) the recovery of infectious virus from pork liver sausages produced in France [8]. In addition, the viral subtype (3i) has never been described in Italy, and its similarity with French strains strengthens the hypothesis that the French food represents the most probable source of contamination. The patient, unaware that he had contracted an HEV infection, continued to have regular contacts with family members (for instance, they had lunch and dinner together and had close contact with children), but no seroconversions were noted in his family after 1 year, confirming that person-to-person transmission of HEV occurs infrequently, as previously suggested [9]. This case underscores the importance of testing for HEV infection not only in people with acute hepatitis symptoms returning from countries where hepatitis E is endemic (Southeast Asia, Africa), but also in patients who have visited industrialized countries in a time frame compatible with the incubation period, if risk factors for acquiring HEV infection are met. Furthermore, regarding the source of contamination, European food safety authorities underscored recently the urgent need for studies on HEV circulation in food. Martin-Latil S et al. test and report new tools that can be used in this situation [10]. In summary, although hepatitis E is mainly an autochthonous disease in Europe, its epidemiological feature is important for public health, and imported HEV infection also should be considered for patients travelling to developed countries.

Transparency declaration

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