

# Pig Liver Sausage as a Source of Hepatitis E Virus Transmission to Humans

Philippe Colson,<sup>1,2</sup> Patrick Borentain,<sup>3</sup> Benjamin Queyriaux,<sup>4,5</sup> Mamadou Kaba,<sup>1,2</sup> Valérie Moal,<sup>6</sup> Pierre Gallian,<sup>7</sup> Laurent Heyries,<sup>3</sup> Didier Raoult,<sup>1,2</sup> and René Gerolami<sup>3</sup>

<sup>1</sup>Pôle des Maladies Infectieuses et Tropicales Clinique et Biologique, Fédération de Bactériologie-Hygiène-Virologie, Centre Hospitalo-Universitaire Timone, <sup>2</sup>Unité de Recherche sur les Maladies Infectieuses et Tropicales Émergentes, Centre National de la Recherche Scientifique Unité Mixte de Recherche 6236–Institut de Recherche pour le Développement 3R198, Facultés de Médecine et de Pharmacie, Université de la Méditerranée (Aix-Marseille-II), <sup>3</sup>Service d'Hépatogastro-Entérologie, Centre Hospitalo-Universitaire Conception, <sup>4</sup>Institut de Veille Sanitaire, Cellule Interrégionale d'Epidémiologie Sud, <sup>5</sup>Institut de Médecine Tropicale du Service de Santé des Armées, Département d'Epidémiologie et Santé Publique, <sup>6</sup>Université de la Méditerranée, Assistance Publique des Hôpitaux de Marseille, Centre de Néphrologie et Transplantation Réanle, Hôpital Conception, and <sup>7</sup>Etablissement Français du Sang Alpes-Méditerranée, Marseille, France

(See the article by Legrand-Abrevanel et al, on pages 835–844, and the editorial commentary by Purcell and Emerson, on pages 819–821.)

**Background.** The source and route of autochthonous hepatitis E virus (HEV) infections are not clearly established in industrialized countries despite evidence that it is a zoonosis in pigs. We investigated the role of figatellu, a traditional pig liver sausage widely eaten in France and commonly consumed raw, as a source of HEV infection.

**Methods.** A case-control study was conducted of 3 patients who presented autochthonous hepatitis E and 15 members of their 3 different families. Anti-HEV immunoglobulin G and immunoglobulin M antibody testing was performed with commercial assays. HEV RNA was detected in serum samples of patients and in pig liver sausages by means of real-time polymerase chain reaction and sequenced by means of in-house sequencing assays. Genetic links between HEV sequences were analyzed.

**Results.** Acute or recent HEV infection, defined by detection of anti-HEV immunoglobulin M antibodies and/or HEV RNA, was observed in 7 of 13 individuals who ate raw figatellu and 0 of 5 individuals who did not eat raw figatellu ( $P = .041$ ). Moreover, HEV RNA of genotype 3 was recovered from 7 of 12 figatelli purchased in supermarkets, and statistically significant genetic links were found between these sequences and those recovered from patients who ate raw figatellu.

**Conclusion.** Our findings strongly support the hypothesis of HEV infection through ingestion of raw figatellu.

Autochthonous hepatitis E virus (HEV) infections are an emerging concern in industrialized countries [1, 2]. HEV was first known as a leading cause of acute hepatitis linked to fecal-oral transmission in tropical and subtropical countries, but hepatitis E has been found to be endemic in Japan, Australia, the United States,

and Europe [1, 2]. Indeed, seroprevalence rates as high as 36% have been recently described, and an increasing number of autochthonous cases have been reported [1, 3].

HEV transmission routes are not fully resolved in developed countries [1, 2, 4]. The virus has been detected in these areas in human sewage and molluscs without evidence of transmission to humans [5, 6], and only anecdotal cases of infection through blood transfusion have been reported [7]. In contrast, a substantial body of data indicates that HEV infection is a porcine zoonosis, as pigs and wild boars are commonly infected [1, 2, 4, 8–18]. Furthermore, hunting and occupational exposure to pigs have been linked with high HEV seroprevalence rates and cases of acute hepatitis E [1, 4, 19, 20]. Regarding the risk of zoonotic foodborne infection, transmission of HEV to humans from deer and

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Reprints or correspondence: Philippe Colson, Pôle des Maladies Infectieuses et Tropicales Clinique et Biologique, Fédération de Bactériologie-Hygiène-Virologie, Centre Hospitalo-Universitaire Timone, 264 rue Saint-Pierre 13385, Marseille CEDEX 05, France (philippe.colson@ap-hm.fr).

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wild boar meat has been documented using molecular and sequencing assays [21, 22], and several cases of hepatitis E have been reported, mostly in Japan, after consumption of raw or undercooked wild boar or pig meats, in which no remaining food was tested [7, 23–27]. Concurrently, HEV RNA was detected in 1.9%–11% of raw pig liver pieces that were sold in grocery stores as food [28–31]. Nevertheless, despite these findings that indicate the possibility of zoonotic foodborne HEV infection, the source and manner of viral transmission in industrialized countries has not been documented in most cases [1, 2, 4].

In France, >100 autochthonous cases are reported to the national reference center each year [29]. Most of them have occurred in the south, where 7 fatal cases have been reported [32–35]. Moreover, HEV seroprevalence rates range from 3.4% in the north to 16.6% in the south [19, 36]. Figatelli are traditional sausages from Corsica, France (Figure 1A). They are good candidates for HEV transmission because they are made with pig liver (their name means “small livers”) and are commonly eaten uncooked, although their manufacture only includes smoking for a few days. Nearly 10 million kilograms are purchased every year in France, which represents about 30

million sausages. Taken together, the high HEV seroprevalence rate, the incidence and clinical impact of autochthonous hepatitis E, and the absence of a common documented route of HEV transmission in the general population warrant the evaluation of figatelli as a possible source of HEV infection in France. We report evidence based on epidemiological and virological findings that 5 cases of autochthonous acute hepatitis E were linked to ingestion of raw figatelli. Moreover, we detected by real-time polymerase chain reaction (PCR) and sequenced HEV RNA in 7 of 12 figatelli purchased in supermarket groceries in southeastern France.

## PATIENTS AND METHODS

**Study population.** Figatelli was suspected to be the source of autochthonous HEV infections diagnosed in 3 patients at Marseille public hospitals from August 2007 through January 2009. Interviews of these 3 patients (A, B, and C) by their physician to identify previously hypothesized or novel risk factors for HEV infection revealed that they had eaten raw figatelli 4–5 weeks before hepatitis onset. The questionnaire used for this investigation addressed occupation, contact with animals,



**Figure 1.** A, Figatelli purchased in a supermarket in Marseille. B, Real-time polymerase chain reaction curves for the detection of hepatitis E virus in figatelli no. 2 (tested in duplicate) and 4 negative controls. C, Packaging of a figatelli with the new recommendation to cook the sausage. \*Translated from the French language as “product to cook (cook thoroughly).”

food, exposure to water, travel abroad, and transfusion within the 2–9 weeks preceding acute hepatitis, which corresponds to the incubation period of the disease [1]. Subsequently, 15 family members of the 3 patients were interviewed retrospectively, after giving their informed consent, with the same questionnaire used for patients A, B, and C. Anti-HEV antibody and HEV RNA testing was performed on serum samples from the family members of each case patient in the 0–21 weeks following acute hepatitis in that patient.

**Diagnosis of HEV infection.** Immunoglobulin M (IgM) and immunoglobulin G (IgG) anti-HEV antibody testing was performed with EIAGen kits according to the manufacturer's instructions (Adaltis; a test/cutoff optical density ratio of  $\geq 1$  was considered to indicate a positive result) [37]. HEV RNA was detected by means of real-time PCR and sequenced by means of in-house protocols that targeted the open reading frame 2 region of the genome, as described elsewhere [14]. Acute or recent HEV infection was defined by positive testing for the presence of HEV IgM antibodies and/or HEV RNA detection and sequencing in serum samples.

**HEV RNA testing of pig liver sausage.** Twelve figatelli purchased in different grocery stores in southeastern France were tested for the presence of HEV RNA. Slices of the sausages were manually sorted to discard fat. Thereafter, they were mixed in 10% (weight per volume) sterile phosphate-buffered saline in a final volume of 1 mL and were clarified by centrifugation at 10,000 g for 5 min. Two hundred microliters of the clarified material was used for total viral RNA extraction, using the EZ1 Virus Mini kit (version 2.0) on the BioRobot EZ1 workstation (Qiagen). HEV RNA detection and sequencing were then performed as described elsewhere [14].

**Study of genetic links between viral nucleotide sequences.** HEV genotype and subtype (as defined by Lu et al [38]) were determined by phylogenetic analysis with a set of published HEV sequences (Figure 2). To test for genetic links between the HEV sequences from patients A, B, and C reported herein and those recovered from the figatelli, we considered 3 main groups of HEV sequences for phylogenetic analysis and identity score analysis. The first group comprised 49 HEV sequences that were previously recovered from patients, including those whose cases are reported here, who received a diagnosis of hepatitis E at our institution in southeastern France. Detailed epidemiological data were not available in most of the cases. However, in addition to the 3 patients whose cases are reported here, 3 other patients were known to have eaten raw figatelli during the incubation period of hepatitis E, whereas consumption of figatelli was excluded in 5 other patients. These 8 patients were not included in the case-control study. The second group of HEV sequences comprised another set of 42 HEV RNA sequences recovered from patients with hepatitis E who were diagnosed in Toulouse in southwestern France [39],

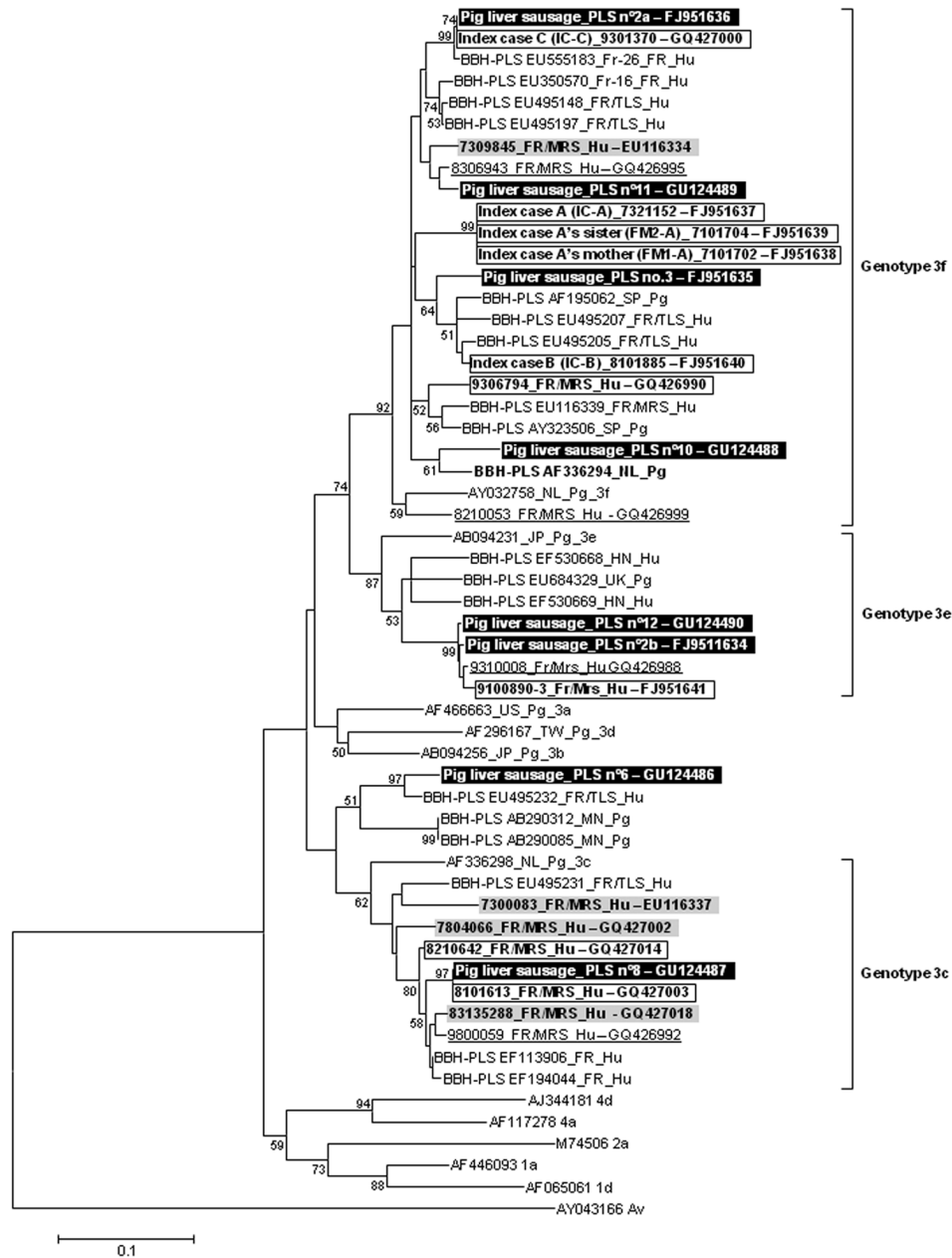
a region where consumption of figatelli is much less common. Finally, the 3 best Basic Local Alignment Search Tool (BLAST) hits (<http://www.ncbi.nlm.nih.gov/BLAST/>) on HEV nucleotide sequences recovered in the present work were also studied. Nucleotide identity scores were analyzed using BioEdit software (version 7.0.9.0; <http://www.mbio.ncsu.edu/BioEdit/bioedit.html>). Regarding the phylogenetic analysis, besides the HEV sequences recovered in the present study and those used to determine the genotype and subtype, only sequences that were clustered in a first-step analysis with those recovered here, as defined by a bootstrap value of  $>50\%$ , were kept in the analysis to build the final tree. GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>) accession numbers for the sequences recovered in this study are shown in Figure 2.

**Statistical analysis.** Statistical analysis was performed using Epi Info software (version 3.5.1; <http://www.cdc.gov/epiinfo/epiinfo.htm>). Results for which  $P < .05$  were considered to be statistically significant. Proportions were compared using the  $\chi^2$  test.

## RESULTS

**Intrafamilial case reports and case-control study.** Epidemiological, clinical, and biological characteristics of all individuals studied here are shown in Table 1. Patient A was a 25-year-old man who received a diagnosis of acute hepatitis E in September 2007. The only risk factor for HEV infection that was identified was consumption of raw figatelli 5 weeks before hepatitis onset, during a familial meal shared with his wife, his sister, and his mother (Table 2). The pig liver sausage had been purchased the day before the meal in a supermarket grocery in the Vaucluse department in southeastern France. The mother and the sister (family members FM1-A and FM2-A, respectively) of patient A presented with acute hepatitis 5 days before and 1 week after he did, respectively, and HEV infection was retrospectively diagnosed in serum samples collected outside our institution at hepatitis onset. In contrast, the wife of patient A (family member FM3-A), who was pregnant, did not show clinical or biological evidence of HEV infection. During the meal shared by the 4 family members (which included cooked pork meat), the only dish that was eaten by the 3 case patients and not by the pregnant wife of patient A was raw figatelli. No other risk factor for HEV infection common to the 4 individuals was identified during the incubation period of the disease, apart from contacts with a pet dog. Moreover, the timing of symptomatic onset in the 3 case patients was compatible with a single contamination source, and HEV sequences from the 3 case patients were 100% identical and were classified into genotype 3f, which is the most frequently encountered genotype in France and Western Europe (Figure 2) [32, 33, 39].

Patient B, a 40-year-old man, was one of the investigators. The only risk factor for HEV infection that was identified was



**Figure 2.** Phylogenetic tree constructed by the neighbor-joining method on the basis of partial nucleotide sequences of the 5' end of the open reading frame 2 region of the hepatitis E virus (HEV) genome, with an avian HEV sequence (GenBank accession no. AY043166) as an outgroup, using Molecular Evolutionary Genetics Analysis software (version 4.0; <http://www.megasoftware.net>). HEV sequences recovered from figatelli in the present study are in boldface on a black background, sequences from patients who ate raw figatelli are in boldface and outlined in black, sequences from patients who did not eat figatelli are in boldface on a gray background, and sequences from patients who ate pig liver or cooked figatelli are underlined. The GenBank accession number is indicated at the end of each label. The best Basic Local Alignment Search Tool (BLAST) hits on HEV sequences obtained in the present study are in boldface. Their labels include the identification BBH-PLS (BBH, best BLAST hits; PLS, pig liver sausage [figatelli]), the GenBank accession number, the country of origin, and the host (Hu, human; Pg, pig). HEV sequences recovered from cases of hepatitis E in humans diagnosed in the Timone clinical microbiology laboratory of Marseille are labeled with the laboratory number, the identification FR/MRS\_Hu (FR, France; MRS, Marseille [in southeastern France]; Hu, human) and the GenBank accession number. HEV genotype 3 sequences available from GenBank with previously identified subtypes are labeled with the GenBank accession number, country of origin, host, genotype, and subtype. Bootstrap values were obtained from 1000 resamplings of the data; values of >50% are indicated in the figure. The scale bar represents the genetic distance in substitutions per site. Av, avian; HN, Hungary; JP, Japan; NL, the Netherlands; SP, Spain; TLS, Toulouse (in southwestern France); TW, Taiwan; US, United States; UK, United Kingdom.

**Table 1. Epidemiological, Clinical, and Virological Characteristics of Patients with Hepatitis E and Their Family Members**

Family, patient or family member ID	Sex, age in years	Date of HEV testing	Clinical symptoms	ALT level, IU/L	Bilirubin level, $\mu\text{mol/L}$	Prothrombin index, %	Raw figatellu consumption	Time between figatellu consumption and HEV testing, weeks	HEV serological test results, ratio <sup>a</sup>			
									IgG anti-HEV Ab	IgM anti-HEV Ab	Serum HEV RNA test result	HEV genotype
<b>Family A</b>												
Patient A	Male, 25	4 September 2007	Asthenia, fever, abdominal pain, jaundice	5560	87	55	Yes	4	8.0 (positive)	6.8 (positive)	Positive	3f
FM1-A	Female, 49	30 August 2007	Asthenia, fever, abdominal pain	245	18	98	Yes	4	... <sup>b</sup>	... <sup>b</sup>	Positive	3f
FM2-A	Female, 20	8 September 2007	Asthenia, fever, abdominal pain	637	28	96	Yes	5	... <sup>b</sup>	... <sup>b</sup>	Positive	3f
FM3-A	Female, 23	20 September 2007	None	32	12	100	No	7	... <sup>b</sup> (negative)	... <sup>b</sup> (negative)	...	...
<b>Family B</b>												
Patient B	Male, 40	10 September 2008	Asthenia, fever, jaundice	3815	278	100	Yes	7	>10 (positive)	>10 (positive)	Positive	3f
FM1-B	Female, 9	8 December 2008	Asthenia	...	...	...	Yes	19	1.6 (positive)	0.1 (negative)	Negative	...
FM2-B	Female, 11	8 December 2008	Asthenia	...	...	...	Yes	19	2.2 (positive)	1.1 (positive)	Negative	...
FM3-B	Male, 43	8 December 2008	Asthenia	...	...	...	Yes	19	3.8 (positive)	0.1 (negative)	Negative	...
FM4-B	Female, 40	8 December 2008	None	...	...	...	Yes	19	0.8 (negative)	4.7 (positive)	Negative	...
FM5-B	Male, 64	16 December 2008	None	...	...	...	Yes	21	0.1 (negative)	0.1 (negative)	Negative	...
FM6-B	Female, 60	16 December 2008	None	...	...	...	Yes	21	0.3 (negative)	0.5 (negative)	Negative	...
FM7-B	Male, 71	1 December 2008	None	...	...	...	Yes	18	0.1 (negative)	0.04 (negative)	Negative	...
FM8-B	Female, 30	12 September 2008	None	...	...	...	No	7	0.4 (negative)	0.3 (negative)	Negative	...
FM9-B	Female, 65	1 December 2008	None	...	...	...	No	18	0.7 (negative)	0.5 (negative)	Negative	...
FM10-B	Female, 67	5 June 2009	None	...	...	...	No	26	0.2 (negative)	0.3 (negative)	Negative	...
<b>Family C</b>												
Patient C	Female, 55	29 January 2009	Asthenia, pruritus	710	12	100	Yes	5	7.4 (positive)	>10 (positive)	Positive	3f
FM1-C	Female, 20	8 June 2009	Asthenia	...	...	...	Yes	25	0.1 (negative)	0.5 (negative)	Negative	...
FM2-C	Male, 54	3 June 2009	None	...	...	...	No	24	0.9 (negative)	0.2 (negative)	Negative	...

**NOTE.** Ab, antibody; ALT, alanine aminotransferase; FM, family member; HEV, hepatitis E virus; IgG, immunoglobulin G; IgM, immunoglobulin M.

<sup>a</sup> Ratio of the optical density of the serum sample to the cutoff value. A ratio of >1.0 indicates a positive result.

<sup>b</sup> Not performed in the laboratory.

**Table 2. Main Risk Factors for Hepatitis E Virus Infection among Patients with Hepatitis E**

Patient ID	Travel abroad	Blood transfusion	Contact with a pet animal	Direct exposure to wild or farm animals	Exposure to contaminated water	Alimentary risk factors			Drinking water source or well water
						Ingestion of pig liver or derived food product	Ingestion of wild boar or other wild animal	Ingestion of shellfish	
Patient A	No	No	Yes (dog)	No	No	Yes (figatellu)	No	No	No
Patient B	No	No	No	No	No	Yes (figatellu)	No	No	No
Patient C	No	No	No	No	No	Yes (figatellu)	No	No	No

consumption of raw figatellu during a familial meal 7 weeks before hepatitis onset (Table 2). The figatellu had been purchased in a supermarket in Corsica, France. Eight adults and 2 children from the same family shared the meal that included the pig liver sausage. These family members were tested 7–26 weeks later (Table 1; Figure 3). Among these persons, 2 (20%) tested positive for anti-HEV IgM antibodies. Both persons had eaten raw figatellu, and asthenia was retrospectively reported by one of them. No other common risk factor for HEV infection was found in these 2 family members. On the contrary, the mother (family member FM10-B), the aunt (family member FM9-B), and the pregnant wife (family member FM8-B) of patient B, none of whom ate the sausage, tested negative for acute or recent HEV infection. The HEV sequence recovered from the serum sample from patient B was of genotype 3f, but it was not clustered with the HEV sequences recovered from family A.

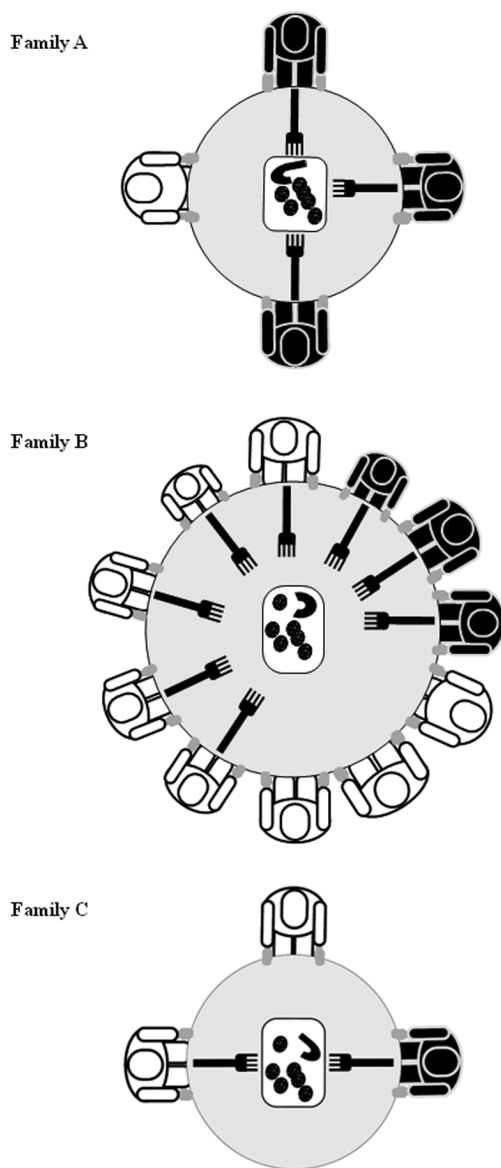
Patient C was a 55-year-old woman who received a diagnosis of acute hepatitis E in January 2009. She had eaten raw figatellu once, 5 weeks before the onset of clinical hepatitis. The figatellu had been purchased in a supermarket in Marseille (in southeastern France). The daughter of patient C, who also ate the figatellu during the same meal, reported asthenia but tested negative for anti-HEV IgM and IgG antibodies in a blood sample that was collected 20 weeks after the onset of hepatitis in her mother (Table 1; Figure 3). The husband of patient C did not eat the sausage. He reported no clinical symptoms and tested negative for anti-HEV antibodies. No other common risk factor for HEV infection was identified in this family. The HEV sequence recovered from the serum sample from patient C was classified into genotype 3f and was not clustered with those recovered from members of families A or B.

In the whole population studied here, the consumption of raw figatellu was statistically significantly linked to HEV infection. Thus, all 7 patients who tested positive for HEV RNA and/or anti-HEV IgM antibodies, indicating acute or recent HEV infection, had eaten raw figatellu, compared with 6 of 11 (55%) persons with no such virological markers ( $P = .041$ ) (Table 1; Figure 3). In addition, the proportion of persons who ate raw figatellu was statistically significantly higher among pa-

tients with positive anti-HEV IgG antibody test results (6 of 6 persons) than among persons with negative anti-HEV IgG antibody test results (5 of 10 persons) ( $P = .043$ ). Moreover, asthenia was statistically significantly more frequent among persons who ate raw figatellu (9 of 13 persons) than among those who did not eat raw figatellu (0 of 5 persons;  $P = .015$ ). Hepatitis onset occurred 5–7 weeks following consumption of the sausage (mean, 5.4 weeks). The clinical attack rate was 36%, and 5 (71%) of 7 individuals with virological markers of acute or recent HEV infection reported clinical symptoms to a physician during acute hepatitis. In all PCR-documented cases of hepatitis E, clinical and biological outcomes were favorable within a maximal duration of 8 weeks.

**HEV detection in pig liver sausages.** We tested 12 figatelli purchased in different grocery stores in southeastern France for the presence of HEV RNA to investigate whether they may represent sources of HEV transmission through ingestion. Two sausages (no. 1 and 2) were purchased in the same supermarket and from the same manufacturer as the one eaten by patient B, and the other 10 sausages (no. 3–12) were randomly purchased in supermarkets in Marseille. Scraps of sausage that had been eaten by patients A, B, and C could not be obtained. HEV RNA was detected in 7 (58%) of the 12 figatelli by use of real-time PCR (Figure 1B) and sequencing. These results were reproduced at least twice in distinct manipulations. On the basis of the real-time PCR results, we estimated that figatelli might contain  $10^3$ – $10^6$  HEV RNA copies per slice.

**Genetic relationship between HEV sequences recovered from figatellu and those from patients who ate figatellu.** Eight HEV sequences were recovered from the 7 figatelli. They were classified into genotype 3, with 3 sequences being most closely related to subtype f, 2 sequences to subtype e, and 1 sequence to subtype c; 2 sequences could not be classified into any subtype. It is noteworthy that 2 distinct HEV sequences (genotypes 3f and 3e) were recovered from figatellu no. 2 (Figure 2). The most similar sequences found for 2 of the 8 HEV sequences recovered from the figatelli were recovered from 2 patients who ate raw figatellu and in whom hepatitis E was diagnosed in our laboratory; the identity scores were 99.4% in both cases. One of the 2 patients was patient C, and the second patient was a



**Figure 3.** Acute or recent hepatitis E virus (HEV) infections and consumption of uncooked figatellu among members of the 3 families. Individuals with acute or recent HEV infection, defined by positive testing for anti-HEV immunoglobulin M antibody and/or HEV RNA, are shown in black, whereas other family members are shown in white. Forks indicate consumption of raw figatellu.

kidney transplant recipient who was known to frequently eat uncooked figatelli and from whom HEV sequences that were most closely related to genotypes 3c and 3e had been recovered concurrently in 3 sequential serum samples (GenBank accession no. FJ951641 and GQ427003) (Figure 2). HEV sequences recovered from patients who ate cooked pig liver were the most similar to 3 other sequences recovered from the figatelli. The highest nucleotide identity scores between each HEV sequence recovered from patients who ate raw figatellu and HEV se-

quences recovered from figatelli were 92.2%–99.4% (mean score, 96.5%; median score, 96.3%).

Scores of >98% between HEV sequences recovered from figatelli and those recovered from humans were statistically significantly more frequent for cases of hepatitis E diagnosed in Marseille in southeastern France (6 cases) than for cases diagnosed in Toulouse in southwestern France (0 cases;  $P = .023$ ), where consumption of figatellu is far less common than in southeastern France. Such high levels of identity with HEV sequences recovered from figatelli were also statistically significantly more frequent for sequences recovered from individuals who ate raw figatellu (4 cases) than for sequences recovered from individuals in Toulouse who received a diagnosis of hepatitis E (0 cases;  $P < 10^{-3}$ ) or for sequences corresponding to the best BLAST hits on those recovered from figatelli (1 case;  $P < .005$ ). Upon phylogenetic analysis, 3 (43%) of the 7 HEV sequences recovered from humans who ate raw figatellu were clustered with sequences recovered from figatelli with a bootstrap value of >90%, compared with 1 (2.4%) of 42 sequences from other patients in Marseille who received a diagnosis of hepatitis E ( $P > .001$ ) and 1 (2.4%) of 42 sequences from patients with cases that were reported in Toulouse ( $P > .001$ ).

## DISCUSSION

The present study provides strong evidence that autochthonous HEV infections in southeastern France were linked to consumption of raw figatellu. This was first suggested by a case-control study conducted with 3 families that showed a statistically significant correlation between consumption of raw figatellu and acute or recent HEV infection (Figure 3). The timing of clinical symptom onset was compatible with infection through consumption of raw figatellu in all cases. In addition, the anti-HEV IgG seroprevalence rate was 54% among individuals who ate raw figatellu, which is far higher than that found among blood donors in Marseille in a preliminary study (8%; Pierre Gallian et al, unpublished data, July 2008) and those found in 2 other French studies (3.2% in the northern part of France [36] and 16.6% in southwestern France [19]). Transmission of HEV between members of each family is very unlikely considering the chronology of hepatitis onset in family A, and previous findings indicated that secondary transmission of HEV is very rare [40, 41].

In a second step, we detected HEV RNA in 7 of 12 figatelli purchased in supermarkets, at titers of up to  $\sim 10^6$  copies per slice of sausage. The recovery of 2 HEV sequences of different subtypes of genotype 3 from the same figatellu might be explained by a mix of several HEV-infected pig livers in the manufacture of the sausage or by co-infection with 2 HEV strains of a single pig, the liver of which was used. Indeed, different HEV genotypes have been recovered previously from pigs at

the same farm [10, 42, 43], and co-infection of a pig with 2 different genotypes has also been observed [10, 44].

Finally, some HEV RNA sequences that were recovered from patients who ate raw figatellu were the most similar to sequences that were recovered from figatelli, with identity scores of >99% at the nucleotide level. As a comparison, the nucleotide divergence is 12.6%–19.8% at the subtype level and 2.0%–10.1% at the isolate level, when comparing the 5' end of the open reading frame 2 region that is targeted by our sequencing assay [38]. A statistically significant genetic relationship was also observed between sequences recovered from a figatellu and sequences recovered from patients who ate raw figatellu. Moreover, the former sequences were statistically significantly closer to those recovered from humans who presented with autochthonous hepatitis E in southeastern France than those recovered from humans with hepatitis E in southwestern France, where consumption of raw figatelli is far less common. Similarly, it is striking that most cases of autochthonous hepatitis E reported in France have occurred in its southern part, where most pig liver sausages are manufactured and eaten [32, 33, 35].

Our finding that figatellu represents a source of HEV infection in humans makes sense. Although transmission routes of autochthonous hepatitis E in industrialized countries remain unknown in most cases, it has been established that pigs are major HEV reservoirs [1, 2, 4]. Thus, pig farms very frequently contain infected pigs, and within farms, prevalence rates of IgG anti-HEV antibodies and HEV RNA detection of >50% have been commonly reported [8, 11, 12]. In France, an ongoing national survey indicates high seroprevalence rates, with >90% of growing farms having seropositive pigs and seroprevalence rates within farms of 2.5%–80% [29]. Moreover, HEV sequences that were recovered from pigs and from humans with autochthonous hepatitis E in industrialized countries belong to genotypes 3 or 4 and have been found to be highly similar or even identical in some cases [1, 4, 8, 38, 45]. Several studies have shown that pigs mostly carry HEV at ~3 months of age, but HEV RNA has been detected at rates as high as 41% in feces and serum samples from 6-month-old pigs, at the age when they are led to slaughterhouses [9–11]. Moreover, HEV RNA has been previously detected in 1.9% of raw pig liver pieces sold in grocery stores as food in Japan, 3% of pieces in France, 6.5% of pieces in the Netherlands, and 11% of pieces in the United States, with viral loads as high as  $10^7$  copies per gram of liver [28–31].

These data raise the question of whether HEV transmission might occur following ingestion of pig-derived food products. Two studies have reported cases of acute hepatitis E after consumption of raw or undercooked pig meats [7, 28]. These cases have occurred in Japan, where eating habits include the consumption of raw liver from mammals [23]. In 2 studies, epi-

demiological investigations and phylogenetic analyses strongly argued that pig livers are a source of HEV infection, although no ingested meat could be retrospectively tested [7, 28]. Yazaki et al [28] reported that 9 patients developed hepatitis E within 2–8 weeks of consumption of grilled or undercooked pig liver. In addition, HEV sequences recovered from pig livers that were purchased afterward in grocery stores were closely related or identical to those recovered from the patients. More recently, Matsubayashi et al [7] described the investigation of a transfusion-associated HEV infection, which revealed that among 13 family members who enjoyed grilled pork liver and/or intestines, 2 presented PCR-documented hepatitis E and 6 others showed serum markers of HEV infection. Moreover, HEV RNA sequences from the 2 case patients showed 99.3% identity with that previously recovered from a pig liver [28].

Our study extends these findings to a French-manufactured pork product, figatellu, which is a very good candidate for a source of HEV transmission. First, it is made with pig liver, in which viruses multiply and can be found at high titers [28]. Second, this sausage is only smoked for a few days while it is being manufactured and is commonly eaten raw, which may contribute to its potential infectivity. In our study, all patients who developed hepatitis E had eaten the figatellu uncooked, which points out the risk for enteric transmission of pathogens that is associated with eating raw food [46, 47]. This is a major issue, since it has been shown that HEV in contaminated commercial pig livers can be effectively inactivated if the meat is cooked properly (at an internal temperature of 71°C for 5 min or boiled in water for 5 min), whereas incubation at 56°C for 1 h can be insufficient to inactivate the virus [31, 48].

In conclusion, the present findings strongly support the hypothesis of HEV transmission through ingestion of raw pig liver sausage. These preliminary data prompt further large-scale studies to assess the prevalence of HEV in these food products and of HEV infection through this transmission route. In any case, consumption of raw figatellu represents a public health concern, considering the high frequency of consumption of this sausage in France and the high mortality rate observed for symptomatic hepatitis E in developed countries, which was 8%–11% in some studies and might increase to 70% for individuals with underlying liver diseases [1, 49]. Therefore, our findings were influential in convincing the French public health authorities to compel manufacturers of pig liver sausages to note on the packaging that the sausages must be cooked thoroughly, starting in May 2009 (Figure 1C) [50].

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