High Proportion of Asymptomatic Infections in an Outbreak of Hepatitis E Associated With a Spit-Roasted Piglet, France, 2013

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Background. On 11 December 2013, 3 clustered cases of hepatitis E were reported on a French coastal island. Individuals had taken part in a wedding meal that included a spit-roasted piglet. The piglet had been stuffed with a raw stuffing partly made from the liver. Investigations were carried out to identify the vehicle of contamination and evaluate the dispersion of the hepatitis E virus (HEV) in the environment.

Methods. A questionnaire was administered to 98 wedding participants who were asked to give a blood sample. Cases were identified by reverse transcription–polymerase chain reaction and serological tests. A retrospective cohort study was conducted among 38 blood-sampled participants after the exclusion of 14 participants with evidence of past HEV infection. Relative risks (RR) and 95% confidence intervals were calculated based on food consumed at the wedding meal using univariate and multivariable Poisson regressions. Phylogenetic analyses were performed to compare the clinical HEV strains. Strains were detected in the liquid manure sampled at the farm where the piglet was born and in the untreated island wastewater.

Results. Seventeen cases were identified, 70.6% were asymptomatic. Acute HEV infection was independently associated with piglet stuffing consumption (RR = 1.69 [1.04–2.73], P = .03). Of clinical strains from the index cases, veterinary and environmental HEV strains were identical.

Conclusions. Our investigation attributed this large HEV outbreak to the consumption of an undercooked pig liver–based stuffing. After infection, the cases became a temporary reservoir for HEV, which was detected in the island’s untreated wastewater.

Keywords. hepatitis E virus; outbreak; asymptomatic infection; pork liver; wastewater.

Hepatitis E virus (HEV) is a nonenveloped RNA virus of the Hepeviridae family. Human HEV is classified into 4 major genotypes, and it has 2 distinct epidemiological profiles [1, 2]. In countries with poor environmental sanitation, HEV1 and HEV2 are transmitted via the fecal–oral route and frequently cause large waterborne outbreaks. In industrialized countries, HEV3 and HEV4 are transmitted zoonotically from different animal reservoirs (eg, pigs, wild boars, deer); the principal reservoir is domestic pigs.

A high seroprevalence has been observed among a pig population in France (31%; 95% confidence interval [CI], 24–38) [3], and close similarities have been reported between human and swine HEV sequences [4, 5]. However, in industrialized countries, most cases remain sporadic with unidentified sources of contamination; only a few small clusters have been attributed to foodborne transmission [6, 7].

HEV has a mean incubation period of 40 days [2]. The symptoms are fever, asthenia, and nausea followed by abdominal pain, vomiting, anorexia, and malaise. Jaundice is reported for about 75% of patients infected by HEV3 or HEV4 [1]. The clinical expression varies from asymptomatic disease to fulminant hepatitis. Chronic hepatitis and cirrhosis have also been described for immunosuppressed individuals [1].

Today, acute HEV infection is diagnosed by detecting serum immunoglobulin M antibodies to hepatitis E (IgM anti-HEV) or by detecting the HEV genome in blood or stools. In industrialized countries, the number of reported hepatitis E cases has increased due to improvements of diagnostic tools and screening strategies [1]. In France, hepatitis E surveillance is based on the activity of the National Reference Centre (NRC), with 1847 cases diagnosed in 2013.

On 11 December 2013, the French Institute for Public Health Surveillance was informed about 3 cases of hepatitis E on a small coastal island west of the French mainland. The individuals lived in the same small hamlet and had symptom onset
between 26 October 2013 and 18 November 2013. On 28 September 2013, the 3 cases had taken part in a wedding meal that included a piglet roasted on a spit. Before being cooked, the piglet had been stuffed with a raw stuffing made from minced offal (liver and heart) mixed with vegetables (tomatoes, zucchinis, eggplants) and wheat semolina.

For the 3 cases, HEV RNA was detected in serum samples using reverse transcription–polymerase chain reaction (RT–PCR). HEV sequences were compared using phylogenetic analysis [5]. The NRC concluded that the strains belonged to the same subtype 3f (genotype HEV3) and were homologous for the ORF2 region.

Investigations were carried out to describe the outbreak and to identify the vehicle and source of contamination. We also investigated untreated and treated wastewater from the island between December 2013 and March 2014 to evaluate the possible dispersion of the HEV in the environment.

METHODS

Descriptive Epidemiology
A descriptive study was carried out among individuals who ate the wedding meal. The participants (N = 111) were interviewed by telephone using a standardized questionnaire that covered demographic characteristics, symptoms, and associated medical attention. At-risk exposure involved food consumption during the meal excluding beverages (wine, bottled water). Moreover, a question assessed how thoroughly the pork meat was cooked; the participants described the doneness based on the meat’s color and texture.

Clinical Laboratory Investigations
Here, we use the term “strain” to represent “sequence.” During the interview, participants were asked to visit their general practitioner who would determine whether or not to test for hepatitis E. The blood sample was to be drawn before 31 January 2014.

RT-PCR was performed to identify HEV RNA from each blood serum [8]. When detected, HEV RNA was compared with the outbreak strain by phylogenetic analysis based on the ORF2 region [5]. Serological (IgM and IgG against HEV) tests were also systematically performed on blood sera. Enzyme immunoassays (Wantai Biologic Pharmacy Enterprise, Beijing, China) were carried out according to the manufacturer’s instructions.

Case Definitions
A confirmed case was a wedding meal participant who gave a blood sample from which the outbreak HEV strain was detected. A possible case was a blood sampled participant from whom HEV RNA had not been detected but with serum positive for an anti-HEV IgM test. A symptomatic case (confirmed or possible) had at least 1 symptom compatible with HEV infection, for example, jaundice, asthenia, vomiting, anorexia, abdominal pain, and fever. Participants with evidence of past HEV infection were blood sampled participants from whom HEV RNA had not been detected and who were negative for anti-HEV IgM but positive for anti-HEV IgG. The noncases were the remaining sampled participants.

Analytical Epidemiology
To identify associations between at-risk HEV exposures and acute hepatitis E infection, a retrospective cohort study was conducted. The cohort consisted of sampled participants and excluding participants with evidence of past HEV infection.

Statistical Analyses
A descriptive analysis was carried out for all interviewed participants, for the blood sampled participants, and for cases. A “don’t know” answer was treated as missing.

The attack rate was calculated among the cohort. The associations between acute hepatitis E infection and both at-risk exposures and gender were assessed through univariate and multivariable methods. Poisson regressions with robust variance were performed to estimate the relative risks (RR) and their 95% CI [9]. The finite population correction was used to estimate accurately the variances and the P values associated with exposures and gender [10].

Specific exposures with an estimated P value <.2 and that explained at least 50% of the cases were included in a multivariable model. The least significant factor was dropped from the model in a stepwise fashion until all remaining exposures exhibited a significant association (P < .05). Data were analyzed using Stata, version 12 (StataCorp., College Station, Texas).

Veterinary Investigations
The reseller who provided the animal identified the swine herd where the piglet was born and raised. The piglet came from the farrow-to-finish herd with 120 breeding sows located in mainland Brittany. The herd were reared on a 3-week–based batch system (7 batches of sows with farrowing every 3 weeks and weaning of piglets at 28 days of age). Nine rooms, corresponding to different ages of piglets, were sampled on 18 February 2014. For each sample, at least 200 mL of liquid manure was collected from the pit under the slatted-floor of pigs aged 8, 11, 14, 17, 20, or 26 weeks. HEV RNA was extracted and detected using real-time RT-PCR according to the method described in Barnaud et al [11]. Two positive samples were used for sequencing in the ORF2 region as described in Bouquet et al [4].

Environmental Investigations
Wastewater samples were collected at the island’s 4 wastewater treatment plants. Three treatment plants used a lagoon treatment process (3 serial ponds that were gravity fed), and a fourth plant used an activated sludge process with lagoon-based tertiary treatment.

Untreated wastewater samples were collected at the 4 treatment plants on 19 December 2013; 8, 20, and 27 January 2014; and 29 March 2014. Treated wastewater samples were collected starting on 8 January 2014. Forty milliliters of untreated
and 1 L of treated wastewater samples were concentrated as previously described [12], and viral RNA was extracted using a NucliSENS kit (bioMérieux, France) [13]. HEV RNA was detected using a real-time RT-PCR assay as previously described [14], and positive samples were applied to the nested RT-PCR targeting the ORF2 region for sequencing.

**Comparison of the Clinical, Veterinary, and Environmental Strains**

Phylogenetic analyses were performed by sequencing a 295nt fragment within the ORF2 region as described in Legrand-Abravanel et al [5]. Nucleotide identity was analyzed using BioEdit software (version 7.0.9.0; http://www.mbio.ncsu.edu/BioEdit/bioedit.html).

**Ethical Approval**

All interviewed participants gave oral consent.

**RESULTS**

**Descriptive Epidemiology**

The 28 September 2013 wedding meal was a buffet with 5 mixed salads, 2 meats (a stuffed piglet roasted on a spit and a barbecued, marinated lamb) served with grilled potatoes and dwarf kidney beans (flageolets) and carrots, 8 flavored goat cheeses, and 6 desserts. The telephone survey was conducted between 23 December 2013 and 14 January 2014.

Of the 111 wedding participants, 98 (88.3%) answered the questionnaire. The median age was 45 years (range, 5–93) and 50 were female (51.0%). Eighty-five (86.7%) of the interviewed participants were residents of the coastal island.

Fifty-two (53.1%) of the interviewed participants had blood samples taken between 18 November 2013 and 24 January 2014 (Figure 1). Forty-six of the sampled individuals were aged ≥18 years and the median age was 55 (range, 5–93). There were 29 females (55.8%) among the sampled participants. Fifty (96.2%) of the interviewed participants were residents of the coastal island.

Fourteen individuals with evidence of past HEV infection were identified. All of them were aged ≥18 years. The median age was 63.5 and there were 7 females (50%). Thirty-two (92.9%) of the participants with evidence of past HEV infection were islanders.

The NRC identified 17 cases including 3 confirmed and 14 possible cases (Figure 1). The confirmed cases were the 3 index cases. The median age of the cases was 52 (range, 8–78) and 14 of them (82.4%) were females. Of the cases, 16 (94.1%) were islanders.

Among the 17 cases, 5 were symptomatic (3 confirmed and 2 possible cases). Thus, 12 (70.6%) were asymptotically infected. The symptoms occurred between 26 October and 1 December (Table 1). There were no immunosuppressed patients and no fulminant hepatitis among the symptomatic cases. Two were hospitalized, and all recovered.

The median age was 48 (range, 8–70) among the asymptomatic cases and 58 (range, 47–78) among the symptomatic cases. The 12 asymptomatic cases were females and 2 of the 5 symptomatic cases were females. The gender distribution was significantly different between asymptomatic and symptomatic cases ($P < 10^{-3}$, Pearson test).

The following 4 food items were consumed during the wedding meal by at least 50% of the cases: salad (rice, tuna, and sweet pepper salad), pork meat, stuffing, and grilled potatoes. The pork meat and the stuffing were consumed by 76.5% and 52.9% of the cases, respectively.

When information was provided about the portion of stuffing consumed, participants often reported intakes of about 1 to 2 tablespoons. Among the pork meat consumers, 23.3% (14/60) reported eating undercooked meat.

**Analytical Epidemiology**

The cohort consisted of 38 participants after excluding 14 participants with evidence of past HEV infection (Figure 1). The attack rate was 44.7%.

Being a female and consuming stuffing were associated with acute HEV infection according to univariate analyses (Table 2). Gender and stuff consumption were the remaining factors included in the final multivariable model. This model showed that acute HEV infection was independently associated with piglet stuffing consumption (RR = 1.69 [1.04–2.73], $P = .03$).

**Veterinary Investigations**

During the farm visit, several risk factors known for HEV spread in swine herds were identified, including mingling at weaning, overdensity in the nursery, no clear separation between piglet batches, and connection of manure pits between rooms [15]. Moreover, drinking water used for the animals was provided by a surface collection pond located at the bottom of the main manure stocking pit.

Of the 9 samples collected and analyzed, all were positive for HEV (data not shown). Quantification of the level contamination was estimated to be between $3.3 \times 10^{4}$ (17 to 20-week-old pigs) and $5.0 \times 10^{6}$ (14-week-old pigs) copies of HEV RNA/g of liquid manure.

**Environmental Investigations**

HEV RNA was detected in the untreated wastewater samples collected at 2 treatment plants on 19 December 2013. At 1 of the 2 plants, untreated wastewater samples were still positive for HEV RNA on 8 and 20 January 2014 and then were negative (limit of detection 1800 RNA copies/L). HEV RNA was not detected in any treated wastewater samples (limit of detection 100 RNA copies/L).

**Phylogenetic Analyses of the HEV Sequences**

Two positive samples of liquid manure collected at the pig farm were used for sequencing in the ORF-2 region. The 2 sequences
were 100% identical. The ORF2 region was also successfully amplified from the 2 untreated wastewater samples collected on 19 December 2013 and 20 January 2014 at the same plant; the two sequences were 100% identical.

The 3 clinical strains and the strains recovered from the local wastewater plants and the manure from the pig farm belonged to subtype 3f. The sequences formed a distinct cluster in the phylogenetic tree (Figure 2) and were 99.0% to 99.6% identical.

**DISCUSSION**

This investigation confirmed a large outbreak of HEV due to foodborne transmission. Epidemiological and veterinary results suggest that this outbreak was due to contaminated spit-roasted piglet. In industrialized countries, acute HEV infections are an emerging concern, and clusters of cases that are linked to HEV foodborne transmission remain rare and small. To our knowledge, only 4 previously published
investigations revealed clinical HEV strains homologous to strains recovered from food leftovers. Two were HEV clusters (4 and 2 cases, respectively) that were attributed to uncooked deer meat and raw pork liver sausage using phylogenetic analyses [6, 7]. The 2 remaining studies were case reports incriminating consumption of wild boar and pork meat [16, 17]. In addition to these 4 documented investigations, 2 studies strongly suspected pig liver and intestines [18] and pig liver sausages [19] as food vehicles for acute hepatitis E infection. A larger outbreak (33 cases) was also reported among the passengers of a cruise ship [20]. In that investigation, tainted shellfish was the suspected contamination vehicle based only on epidemiological links.

The detection of HEV RNA in the swine herd environment where the consumed piglet was born and reared is consistent with a previous publication reporting an important HEV carriage among slaughter-aged pigs [3]. According to that study, 24% (95% CI, 17–31) of the French breeding farms had at least 1 pig with a liver positive for HEV RNA. Moreover, the investigated swine herd had some well-known risk factors for HEV spread. Concerning our investigation, the homology between HEV RNA sequences recovered from the farm environment and from the 3 confirmed cases is remarkable. This finding incriminates the roasted piglet as the vehicle for HEV infection, although it remains insufficient to clearly distinguish the roles of pork meat and piglet stuffing.

Our investigation included a cohort of 38 individuals with a clearly determined serological status for HEV infection following blood sampling of almost half of the wedding attendees. The cohort study findings are consistent with the veterinary results and suggest that the outbreak is attributable to the consumption of the piglet stuffing tainted with HEV (P = .03).

It is noteworthy that cooking at 71°C for 20 minutes is required to fully inactivate the virus [1, 11]. Since 23.3% of the meat consumers reported undercooked meat, the stuffing, partly made from the piglet’s liver, was likely undercooked, too. Thus, our hypothesis about the vehicle of the infection is consistent with previous studies that incriminated or suspected some undercooked pig liver–based food as the vehicle for HEV contamination [7, 19]. Furthermore, our hypothesis is consistent with a French publication in which a frequent HEV contamination in food containing raw pork liver was reported [21].

The large number of cases, their clinical description, and the cohort blood sample results enabled us to determine that 70.6% (12/17) were asymptotically infected. This finding confirms the results from another study, suggesting the importance of subclinical hepatitis E infections in industrialized countries [22]. In particular, our findings are similar to the proportion of asymptomatic cases (66.7%) reported in a large outbreak attributed to genotype HEV3 [20]. This high proportion of asymptomatic cases could, in part, explain the discrepancies observed in France between the limited number of acute hepatitis E infections diagnosed by the NRC and the relatively high frequency of viremic HEV infections in blood donors (1/2218) [23].

Of the sampled individuals aged ≥18 years, 30.4% (14/46) had serological evidence of past hepatitis E infection. Thus, our investigation suggests that hepatitis E infection may be relatively common in western France. This proportion remains lower than that of the blood donors aged >18 years (52.5%) who were positive for anti-HEV IgG in southwestern France using the same assay [24, 25].

Our investigation revealed that among the cases, most were female. This finding is contrary to the results of Said et al.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Symptomatic Confirmed Cases (n = 3)</th>
<th>Symptomatic Possible Cases (n = 2)</th>
<th>Total (N = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jaundice</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Anorexia</td>
<td>2</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Vomiting</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Fever</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

N = 5.

Table 1. Clinical Description of the Symptomatic Cases for an Outbreak of Hepatitis E in France in 2013

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Exposed Cases</th>
<th>Total</th>
<th>AR (%)</th>
<th>Unexposed Cases</th>
<th>Total</th>
<th>AR (%)</th>
<th>Relative Risk</th>
<th>95% Confidence Interval</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Being a female</td>
<td>14</td>
<td>22</td>
<td>63.6</td>
<td>3</td>
<td>16</td>
<td>18.8</td>
<td>3.39</td>
<td>1.50–7.67</td>
<td>.004</td>
</tr>
<tr>
<td>Stuffing</td>
<td>9</td>
<td>14</td>
<td>64.3</td>
<td>8</td>
<td>22</td>
<td>36.4</td>
<td>1.77</td>
<td>1.05–2.97</td>
<td>.03</td>
</tr>
<tr>
<td>Pork meat</td>
<td>13</td>
<td>24</td>
<td>54.2</td>
<td>4</td>
<td>12</td>
<td>33.3</td>
<td>1.62</td>
<td>.83–3.19</td>
<td>.15</td>
</tr>
<tr>
<td>Rice, tuna, and sweet pepper salad</td>
<td>6</td>
<td>12</td>
<td>50.0</td>
<td>6</td>
<td>18</td>
<td>33.3</td>
<td>1.50</td>
<td>.77–2.93</td>
<td>.22</td>
</tr>
<tr>
<td>Grilled potatoes</td>
<td>11</td>
<td>27</td>
<td>40.7</td>
<td>5</td>
<td>9</td>
<td>55.6</td>
<td>0.73</td>
<td>.42–1.29</td>
<td>.27</td>
</tr>
</tbody>
</table>

N = 38.

Attack rates and relative risks by at-risk exposure and gender. Where the sum of exposed and unexposed does not equal 38, there are "don’t know" answers which were treated as missing. Abbreviation: AR, attack rates.

* Relative risk, confidence interval, and P value were assessed using univariate Poisson regression with robust variance and finite population correction.
who investigated a larger hepatitis E outbreak and found an excess of infected males [20]. However, our significant association between being female and acute hepatitis E infection may be a random association. Second, of symptomatic cases, 60% were males compared with 0% male asymptomatic cases. This finding is consistent with research suggesting that more men develop symptomatic hepatitis [17]. However, this result should be considered with caution since it is due to small numbers of symptomatic cases (n = 5).

Although previous investigations detected HEV in urban sewage [26, 27], our work is a rare example of an investigation where a clinical strain was isolated from the wastewater related to clustered HEV cases. After infection, the 17 cases became a temporary reservoir for HEV, and HEV RNA was detected in the wastewater samples until 20 January 2014. Although the HEV RNA concentrations in treated wastewater samples were below the detection limit (100 RNA copies/L), the virus could have been dispersed into the water environment through the wastewater effluents contaminated by the cases. It is notable that there were only 4 general practitioners on the island. All were alerted of the outbreak and none reported secondary cases that may have been attributed to HEV dispersion in the environment.

Our investigation had 2 principal limitations. First, we had only indirect laboratory evidence. Since there was no leftover food, the clinical HEV strains could be compared only with strains recovered from the pig farm environment. The second limitation concerns analytical results. Although we found a significant association between acute hepatitis E infection and stuffing consumption, the RR remains weak (1.69 [1.04–2.73]). The RR may be underestimated due to nondifferential misclassification errors mostly linked to late telephone interviews and to very small intakes of stuffing (1 to 2 tablespoons).

In conclusion, our investigation attributed the HEV outbreak to consumption of undercooked stuffing partly made from a piglet’s liver. Our results illustrate and confirm the need to thoroughly cook pig liver–based food.

Notes
Acknowledgments. We acknowledge the investigation team of the West Regional Epidemiology Unit who interviewed the wedding participants; the general practitioners of the coastal island who prescribed the blood samples and the biologists who collected the samples; and Philippe Le Mehaute (water company SAUR) who helped Ifremer with environmental investigations.

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