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Foodborne Transmission of Hepatitis E Virus from Raw Pork Liver Sausage, France

To the Editor: The number of sporadic autochthonous cases of acute hepatitis E is increasing in many industrialized countries (1). These cases involve hepatitis E virus (HEV) genotypes 3 and 4, which are zoonotic. Although risk for foodborne transmission from pork is now recognized, we report here direct HEV transmission

through ingestion of raw pig liver sausages (figatellu [plural: figatelli]) in southeastern France.

The index case-patient was a 45-year-old woman from Hyères (southeastern France) who had no underlying medical condition. She visited her general practitioner on December 17, 2013, reporting 3 days of weakness. Acute hepatitis was diagnosed 2 days later on the basis of elevated liver enzymes (alanine aminotransferase 1,265 IU/L [reference <35 IU/L]) and bilirubin (65 μ mol/L [reference <17 μ mol/L]). Serum markers for acute hepatitis A, B, and C; cytomegalovirus; and Epstein-Barr virus were negative. Jaundice appeared on December 19, and the patient was referred to the Medical Unit of Hyères for additional investigations. A serum sample collected on December 20 tested positive for HEV RNA; viral load was 3.3 \log_{10} IU/mL (Ceeram, La Chapelle sur Erdre, France), and IgM and IgG against HEV were found (Wantai, Beijing, China), which led to the diagnosis of acute hepatitis E. The HEV genotype was 3f, as determined from the phylogenetic analysis of a portion of the open reading frame (ORF) 2 (2). The index case-patient recovered by the end of January; HEV viremia was undetectable on January 17, 2014.

The index case-patient and her family regularly ate figatelli (raw pork liver sausages) made in Corsica. The patient had most recently eaten figatelli at a lunch with 8 family members on October 28, 2013, seven weeks before illness onset. After receiving informed consent, we conducted laboratory investigations of samples from the other family members; tests included HEV serology and HEV RNA detection in serum and fecal samples. Samples were obtained from family members during January 8–21, 2014 (41–54 days after the lunch). Positive HEV IgM and detectable HEV RNA were found in the serum of the index case-patient's daughter, who was asymptomatic. Because

the sample was tested 10 weeks after the family lunch, the daughter's HEV viral load was too low to enable sequence characterization and clustering of HEV strains. Three other family members were IgG positive for HEV, indicating previous HEV infection. Leftover sausages had been kept frozen and were available for HEV testing.

HEV RNA was detectable from the leftover sausages, and HEV sequences were amplified in 2 different genomic regions (ORF1: RNA-dependent RNA polymerase and ORF2), as described previously (2). Comparison with the index case-patient's sequences showed 100% nt identity for both regions (Figure). Samples of food and samples from the index case-patient were analyzed in 2 independent laboratories to avoid any cross-contamination. The level of contamination of the figatellu was $\approx 4.8 \times 10^4$ copies of HEV RNA/g of sausage (3).

Figatellu, a dried sausage, contains 30% pork liver and no heating step occurs during its manufacture. Usually deep cooking is recommended on the package, but consumers might not follow the cooking recommendation; also, figatelli can be sold in small local shops with no label. In the instance reported here, the figatellu was sold without any warning label and was eaten raw.

That HEV was transmitted through ingestion of contaminated food is supported by the following evidence. First, 3 case reports have provided direct evidence of HEV transmission through ingestion of contaminated animal food products with identical or near identical sequences between the patients and the contaminated food they ate. Two cases occurred in the early 2000s in Japan through consumption of grilled wild boar (4) or sashimi of Sika deer (5); the third, reported recently in Spain, was transmitted through ingestion of pig meat (6). Second, HEV widely infects domestic pigs and wild boar (7). Third, swine and human HEV strains have genetic similarities and, in

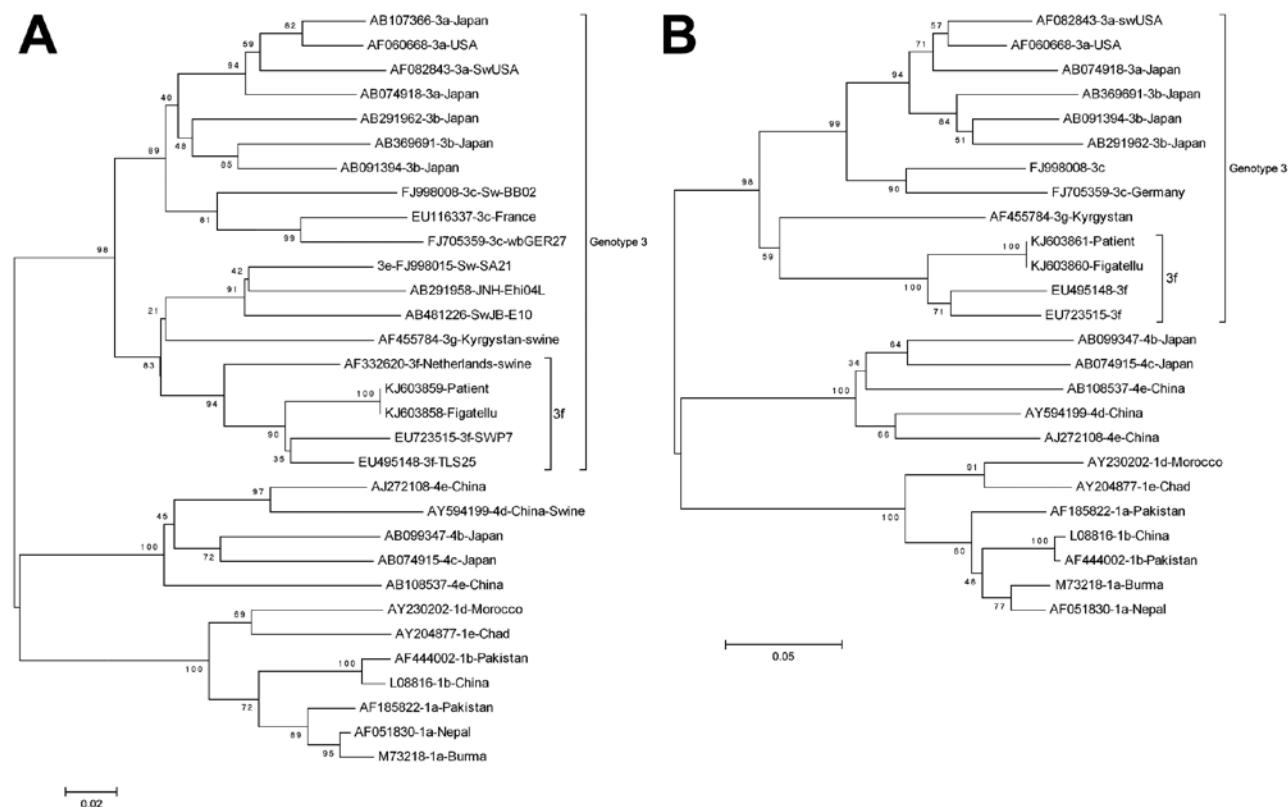


Figure. Phylogenetic analysis of partial open reading frame (ORF) 2 and ORF1 sequences of hepatitis E virus (HEV). Phylogenetic trees were constructed in MEGA6 software (<http://www.megasoftware.net>) by using the neighbor-joining method from a Kimura 2-parameter distance matrix based on partial nucleotide sequences of ORF2 (A) and ORF1 (B). Bootstrap values obtained from 500 resamplings are shown. Sequences were retrieved from the serum of a 45-year-old woman in France in whom hepatitis E was diagnosed in December 2013 and from frozen leftovers of the figatellu she had eaten in October 2013. Sequences obtained from the figatellu (GenBank accession nos. KJ603858 and KJ603860) were 100% nt identical to sequences obtained from the index case-patient (GenBank accession nos. KJ603859 and KJ603861). Reference sequences are indicated by their GenBank accession numbers. Scale bars indicate nucleotide substitutions per site.

some cases, are indistinguishable (1). Fourth, in Marseille, France, a case-control study identified ingestion of figatellu as a risk factor for HEV infection. Genetic similarities were found between sequences isolated from patients with autochthonous hepatitis E and nonrelated figatelli purchased in the same region (8). Finally, infectious virus, replicating in a 3-dimensional culture system, was identified in a HEV RNA-positive figatellu (9).

In the present study, the homology between sequences recovered from the index case-patient and those recovered from leftovers of figatellu provides additional proof of HEV foodborne transmission in a Western country. In France, information

about the risk for HEV transmission through the ingestion of such delicatessen was published by French authorities in 2010 (10), but the present case demonstrates that public education and warning, or larger and more explicit labels on the package, must be improved to reduce the risk for HEV exposure.

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***Burkholderia pseudomallei* in Water Supplies, Southern Thailand**

To the Editor: Melioidosis is an infectious disease caused by the environmental gram-negative bacillus *Burkholderia pseudomallei*, which is present in northern Australia and across much of Asia (1,2). In Thailand, melioidosis is highly endemic to the northeast, where most infected persons are agricultural farmers with repeated environmental exposure (3). Melioidosis is infrequently reported from southern Thailand, although a cluster of 6 cases occurred in Phangnga Province after the December 2004 tsunami (4). Given the infrequency of reported cases, a cluster of 11 persons with melioidosis on Koh Phangan (an island in the Gulf of Thailand) during January–March 2012 (5) led to an investigation. Three case-patients were foreign tourists; 8 Thai case-patients were from 7 different villages throughout the island, and none were agricultural workers (5). Three cases were fatal; water inhalation was suspected as a route of infection in a fatal case in a neonate who was born in a birthing pool outside of a hospital (online Technical Appendix Table 1, <http://wwwnc.cdc.gov/EID/article/20/11/14-0832-Techapp1.pdf>). The lack of history for environmental exposure, such as farming, led to the hypothesis that water was the source of infection. After a request by Koh Phangan Hospital and the Thai Ministry of Public Health, an environmental survey was conducted for *B. pseudomallei* in water supplies on the island.

In March 2012, we randomly collected water from accessible water supplies in local residences and hotels from all 14 villages on Koh Phangan. A total of 190 samples were collected (range 10–18 samples per village, Figure) for culture, genotyping, and analysis (online Technical Appendix).

Isolates from 3 persons who died (a single bacterial colony saved from each person) from Koh Phangan were also available for genotyping and analysis. 26 (14%) of 190 samples were culture positive for *B. pseudomallei*. The positivity rate did not differ by source of the water sample: spring (5 [28%] of 18 samples), well (17 [13%] of 127), and tap water (4 [9%] of 45; $p = 0.16$, Fisher exact test). Of the 26 samples, 16 (62%), 9 (34%), and 1 (4%) were from local residences, hotels, and an ice cream shop, respectively. Positive water samples were distributed across the island (Figure). The median quantitative *B. pseudomallei* count was 30 CFU/L (range <10–11,300 CFU/L). The quantitative count did not differ by sample source ($p = 0.16$, Kruskal-Wallis test), and the sample with the highest quantitative count (11,300 CFU/L) was from well water. Of the 26 samples, only 1 was from a source that was consumed as drinking water.

We identified 12 multilocus sequence types (STs): 10 STs from water samples and 2 different STs from 3 clinical isolates (online Technical Appendix). The most frequent ST (ST1117, 10 isolates) was widely distributed across the island (Figure; online Technical Appendix Table 2). Phylogenetic analysis showed 12 genetically diverse STs identified on Koh Phangan and separate clusters of the clinical and environmental isolates (online Technical Appendix Figure).

Public tap water contaminated with *B. pseudomallei* has been reported previously in northeastern Thailand (6). The country's National Tap Water Quality Assurance Program does not include *B. pseudomallei* (7), a situation that warrants review. A combination of filtration and chlorination is recommended for treatment of village tap water systems in Thailand, but recent studies report that the quality of village tap water is suboptimal (8). Chlorination with sufficient contact time and free available chlorine can kill *B. pseudomallei* (9,10).