MAJOR ARTICLE

Phylogenetic and Case-Control Study on Hepatitis E Virus Infection in Germany

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(See the editorial commentary by Kuniholm and Nelson, on pages 1727–8.)

Background. Hepatitis E is a classic water-borne disease in developing countries. In Germany, hepatitis E virus (HEV) infections are notifiable. The number of non–travel-associated infections has increased in recent years, but the route of transmission in most is unknown. Our objective was to determine risk factors for autochthonous HEV infections in Germany.

Methods. Cases of HEV met clinical definitions and were confirmed by laboratory analysis (defined as detection of HEV by polymerase chain reaction [PCR] or immunoglobulin M by serologic testing). PCR products from blood or stool samples were genotyped for phylogenetic analysis. A case-control study included case subjects with autochthonous HEV infection and matched control subjects who were randomly recruited from a population-based telephone list.

Results. From May 2006 through August 2007, 76 of 96 persons for whom HEV infection had been reported to the routine surveillance system were interviewed. Sixty-six persons had disease that fulfilled the inclusion criteria: 45 (68%) had autochthonous infection, and 21 (32%) had travel-associated disease. Genotypes 3 or 4 were present in 15 of 15 persons with autochthonous infection, and genotype 1 was present in 8 of 9 persons with travel-associated infection. In conditional logistic regression involving 45 case subjects and 135 control subjects, consumption of offal (41% vs. 19%; odds ratio [OR], 2.7; 95% confidence interval [CI], 1.2–6.2) and wild-boar meat (20% vs. 7%; OR, 4.3; 95% CI, 1.2–15.9) were independently associated with autochthonous HEV infection.

Conclusion. Hepatitis E is endemic in Germany and likely exists as a food-borne zoonosis. Implicated meat products should be investigated to provide recommendations for preventive measures.

Hepatitis E is a usually self-limiting disease of varying severity that presents with clinical features similar to those of hepatitis A [1]. Recently, chronic infections have been identified in a number of organ-transplant recipients [2, 3]. The disease is endemic in many developing countries and occurs both in sporadic forms and in epidemics. The case-fatality rate usually ranges between 0.2% and 4% in the general population but can be as high as 8% among pregnant women [1, 4]. Large water-borne outbreaks have been reported, especially from Africa, the Indian subcontinent, and Southeast Asia [4–7]. Industrialized countries where predominantly sporadic cases have been reported (e.g., the United States, the United Kingdom, or the Netherlands) are not considered to be areas of endemicity [8]. However, the number of documented autochthonous infections in industrialized countries seems to be increasing [9]. Still, hepatitis E is regarded by many health care professionals as a typical travel-associated disease. A considerable proportion of autochthonous infections likely remains undiagnosed, and hepatitis of unknown etiology is in fact often caused by hepatitis E virus (HEV) [10].

The relevant reservoir for human HEV infection is largely unknown. However, in both developing and in-

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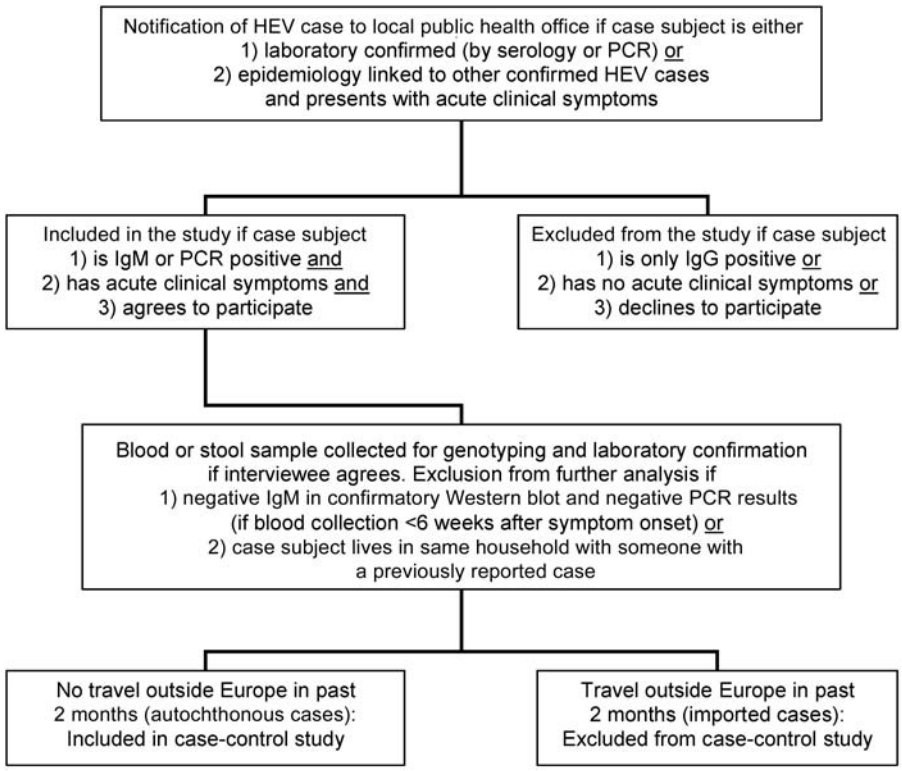

Figure 1. Inclusion and exclusion criteria for enrollment in a case-control study in Germany during 2006–2007 to determine risk factors for autochthonous hepatitis E virus (HEV) infection.

dustrialized countries immunoglobulin G (IgG) antibodies to HEV, as well as viral RNA, have been detected in specimens from wild and domestic animal species [1]. A survey of 41 pig farms in Spain revealed that 34 had IgM-positive pigs [11]. On one farm, 17% of pig stool samples were also positive for HEV by polymerase chain reaction (PCR) analysis. HEV has also been detected in sewage water in Barcelona, Spain; Nancy, France; and Washington, D.C. [12]. In developing countries, genotype 1 is the most prevalent HEV strain among humans. However, in industrialized countries, genotype 3, a strain that has primarily been recovered from pigs and wild boars, has recently also been identified in humans [1, 10–12]. Recovery of similar HEV strains from humans and pigs supports the hypothesis that HEV may exist in some industrialized countries as a zoonosis, but a reservoir common to both humans and pigs remains a possibility [13].

In Germany, HEV infection has been a notifiable disease since 2001. Annually, 17–73 infections have been reported, with an increasing trend since 2002. In 2005, 44% of 54 reported infections were not associated with travel, and the percentage increased to 63% of 73 reported infections in 2007 [14]. However, the source of infection remained unknown in most autochthonous cases. Our study objectives were to prospectively determine epidemiological and molecular characteristics of both travel-associated and autochthonous HEV infections. We aimed to identify possible risk factors for autochthonous HEV infections to elucidate possible routes of transmission and the main source of infection as a basis for preventive measures.

PATIENTS, MATERIALS, AND METHODS

Acute HEV infection is notifiable in Germany. According to the case definition, the infection must be laboratory confirmed by PCR or serologic analysis, or it must be epidemiologically linked to a laboratory-confirmed case and occur in conjunction with at least 1 of the following clinical signs or symptoms: jaundice, fever, abdominal pain, or a $>$2.5-fold increase in transaminase levels. In 2006, we initiated a nationwide enhanced HEV surveillance program in cooperation with all state and local health offices in Germany. From 1 May 2006 through 31 August 2007, all persons with notified HEV infection were contacted by local health authorities. After individuals gave verbal informed consent, they were interviewed by means of a standardized and pretested questionnaire. We collected information about disease onset, symptoms, hospitalization, and occupation. For each person, travel history, specific food items consumed, and exposure to returning travelers, pets, domestic animals, and surface or waste water 2 months before symptom onset were documented. Persons with laboratory-confirmed HEV infection (i.e., those with HEV detected by PCR and/or anti-HEV IgM detected by Western blot or ELISA) and $\geqslant$1 of the clinical signs or symptoms described above were included in this study (figure 1). HEV in-

fections in persons who had not traveled outside Europe ⩽2 months before symptom onset were considered autochthonous (European regions were regarded as areas of nonendemicity). If health authorities were able to contact patients ⩽8 weeks after symptom onset, serum or stool samples were collected after written informed consent was obtained. The study protocol was approved by the Ethics Committee of the Charité University Medicine in Berlin.

Case-control study. We conducted a case-control study that consisted of case subjects with autochthonous HEV infection and matched control subjects. Persons with travel-associated HEV infection and those living in the same household with persons for whom HEV infection was previously reported were excluded from the case-control study. Control subjects were randomly recruited from a group of individuals who participated in a telephone health survey performed 2 years earlier by the Department for Epidemiology and Health Reporting at the Robert Koch Institute. The survey used an initial sample of 5600 participants who were representative of the general adult population in Germany. Control subjects were individually matched to case subjects by age group (in decades), sex, and community size (<20,000, 20,000–100,000, and >100,000 inhabitants). For each case subject, 3 control subjects were selected. Control subjects were recruited and interviewed by Robert Koch Institute staff, using the same questionnaire that was used for the enhanced surveillance program. We formulated the principal hypothesis tested in this study because of a high HEV antibody prevalence among swine farmers and domestic pigs in industrialized countries and because routine HEV surveillance in Germany during 2005 revealed that 6 (26%) of 23 persons with autochthonous infection had had direct contact with domestic pigs [8, 14–16]. To test the hypothesis that close contact with pigs is associated with HEV infection, we estimated that 21 case subjects and 63 control subjects were necessary to show a significant association with 95% confidence and 80% power, assuming that 1% of healthy persons are exposed to pigs.

Laboratory investigations. Blood and stool samples collected from case subjects (all of whom were symptomatic and had laboratory-confirmed HEV infection) were analyzed by the Institute of Medical Microbiology and Hygiene at the University of Regensburg, the German national consulting laboratory for hepatitis E. RNA was extracted from 400 μL of serum and 50 mg of stool and dispensed in 1 mL of distilled water, using the QIAamp DNA Blood Mini Kit (Qiagen) in accordance with the manufacturer's instructions. HEV detection was performed as described previously [17, 18]. In brief, a target sequence of 287 bp in the ORF1 region of HEV was amplified by means of nested PCR and separated using gel electrophoresis. Bands of the expected size were excised, purified using the MinElute PCR Purification Kit (Qiagen), and sequenced with an ABI 3700 DNA analyzer (Applied Biosystems). Sequences were compared with Genbank entries, using the BLAST utility (available at: http://www.ncbi.nlm.nih.gov/blast/Blast.cgi). Phylogenetic trees were generated using Phylip, version 3.67 (available at: http://evolution.genetics.washington.edu/phylip/phylipweb.html). Genotype information of reference sequences were based on the HEV classification scheme of Lu et al. [19]. If sequences of HEV isolates from humans and animals were available, they were integrated into these analyses. In addition to PCR, 20-μL serum specimens were used for IgG and IgM Western blot analysis, using recomBlot HEV IgG/IgM (Mikrogen). Experimental setup and analysis was performed according to the manufacturer's instructions. Subjects with negative results of PCR and an IgM Western blot were excluded from the study if their specimen was collected ⩽6 weeks after symptom onset but remained in the study if they had positive results of an IgG Western blot and >6 weeks had elapsed before collection (figure 1).

Statistical analysis. Data were entered into an Excel 2002 database (Microsoft) and exported to Intercooled Stata, version 9.0 for Windows (StataCorp). Characteristics of persons with autochthonous HEV infection and those with travel-associated infection were compared using the χ^2 test for categorical variables and the Mann Whitney *U* test for continuous variables. A statistically significant difference was defined as a *P* value of <.05. The prevalence of specific exposures was compared between case and control subjects in bivariate matched analysis. Mantel-Haenszel odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Stratified analysis was performed by stratifying the population according to age group (<40 and ⩾40 years), residence (eastern and western Germany), and community size (<20,000 and ⩾20,000 inhabitants). For multivariate analysis, conditional logistic regression models were applied using stepwise backward removal, with inclusion of age and all variables with a *P* value of ⩽.2 in bivariate analysis in the first step. The likelihood ratio test was applied to test different models to be fitted on the basis of maximum likelihood. Different interaction terms were included to test for effect-measure modification.

RESULTS

During the 16-month study period (1 May 2006 to 31 August 2007), 96 cases of HEV infection were reported to the routine surveillance system and met both clinical and laboratory case definitions. Seventy-six individuals (79%) agreed to be interviewed. Serum or stool samples were collected from 47 (62%) and sent to the national consulting laboratory for genotyping and confirmation of the serologic findings. Western blot analysis confirmed the diagnosis of acute HEV infection in 36 samples. Of the 11 IgM-negative samples, 4 were PCR positive, and 2 were collected >6 weeks after symptom onset with anti-HEV IgG antibodies detected by Western blot. The remaining 5 individuals (11%) with negative results of IgM Western blot and PCR were excluded from further analysis despite initially testing positive

Table 1. Demographic and clinical characteristics of persons with autochthonous or travel-associated hepatitis E virus infection, Germany, 2006–2007.

Variable	Autochthonous infection (n = 45)	Travel-associated infection (n = 21)	P
Male sex	34 (76)	13 (62)	.25
Age, years	46 (6–84)	37 (8–63)	.03[a]
Community size			
<20,000 inhabitants	23 (51)	1 (5)	
20,000–100,000 inhabitants	12 (27)	6 (28)	
>100,000 inhabitants	10 (22)	14 (67)	<.001[b]
Clinical manifestations			
Fatigue	40 (89)	18 (86)	.71
Jaundice	31 (69)	15 (71)	.83
Dark urine	32 (71)	13 (62)	.7
Nausea	26 (58)	12 (57)	.96
Pruritus	20 (44)	10 (48)	.81
Abdominal pain	19 (42)	12 (57)	.45
Fever	17 (38)	8 (38)	.4
Headache	14 (31)	10 (48)	.4
Vomiting	12 (27)	6 (29)	.69
Hospitalized for HEV infection			
Overall	35 (78)	13 (62)	.18
Duration, days	10 (1–28)	7 (4–26)	.37[a]
Workdays missed, number	21 (0–62)	19 (0–35)	.43[a]
Hepatitis E virus genotyping			
Genotype 1	0 (0)	8 (89)	
Genotype 3 or 4	15 (100)	1 (11)[c]	<.001

NOTE. Data are no. (%) of persons or median value (range).

[a] By the Mann-Whitney U test.
[b] By the χ^2 test for trend.
[c] Imported from the United States.

for IgM by ELISA at a local laboratory before notification. Five additional individuals who were living in households with a person for whom HEV infection was previously identified were excluded. In total, 66 individuals remained in the final analysis: 45 (68%) had autochthonous HEV infection, and 21 (32%) had travel-associated HEV infection.

Characteristics of travel-associated and autochthonous HEV cases. The majority of persons with travel-associated infection and autochthonous infection were male (62% and 76%, respectively). Individuals with travel-associated disease were significantly younger (median age, 37 years) than those with autochthonous infection (median age, 46 years; $P = .03$). Although the majority (67%) of persons with travel-associated infection were living in cities with >100,000 inhabitants, 51% (95% CI, 36%–66%) with autochthonous disease resided in communities with <20,000 inhabitants ($P < .001$) (table 1). According to 2004 census data, 42% of Germans live in communities with <20,000 inhabitants. Persons with travel-associated infection and those with autochthonous infection did not differ with respect to clinical symptoms (table 1). Jaundice was observed in 71% of individuals with travel-associated infection and 69% with autochthonous infection. Sixty-two percent with travel-associated infection and 78% with autochthonous infection required hospitalization (median durations, 7 and 10 days, respectively). One female was in the third trimester of pregnancy during infection but did not experience a complicated course of hepatitis E. No deaths occurred.

Most individuals with travel-associated HEV infection (11 [52%]) had traveled to the Indian subcontinent. Africa (5 persons [24%]), Southeast Asia (3 [14%]), the Caribbean (1 [5%]), and the United States (1 [5%]) were other travel destinations. The majority (11 [52%]) were traveling as tourists, and 6 (29%) were visiting friends or relatives. Most travelers stayed overnight in rather simple accommodations: 9 (43%) stayed in guesthouses, 4 (24%) stayed in low-budget hotels, and 4 (24%) stayed with friends or family. Risk behaviors for enteric diseases included eating fresh salad (71% of case subjects), consuming drinks with ice cubes (38%), and drinking tap water (29%).

Phylogenetic analysis of imported and autochthonous HEV strains. HEV PCR products were detected in 29 cases, and genotyping was successfully performed in 24 cases. Eight (89%) of 9 imported HEV isolates were genotype 1. One genotype 3 strain

Table 2. Genotype, Genbank accession number, and travel history associated with hepatitis E virus isolates sequenced in this study, Germany, 2006–2007.

Isolate	Genotype	Accession number	Travel outside Europe during 6-week incubation period[a]	
			Destination	Duration, weeks
Travel associated				
V0607258	1	EU879097	Nepal	5
V0609824	1	EU879101	India	3
V0610936	1	EU879104	India	6
V0616473	1	EU879105	India	4
V0616932	1	EU879107	India	4
V0616998	1	EU879108	Pakistan	4
V0707613	3	EU879113	United States	4
V0711187	1	EU879115	India	2
V0714305	1	EU879119	Ethiopia	6
Autochthonous				
V0607568	3	EU879098	. . .	. . .
V0609076	3	EU879099	. . .	. . .
V0609821	3	EU879100	. . .	. . .
V0609825	3	EU879102	. . .	. . .
V0609890	3	EU879103	. . .	. . .
V0616823	3	EU879106	. . .	. . .
V0703163	3	EU879109	. . .	. . .
V0705397	3	EU879110	. . .	. . .
V0706586	3	EU879111	. . .	. . .
V0707060	3	EU879112	. . .	. . .
V0710246	3	EU879114	. . .	. . .
V0711277	3	EU879116	. . .	. . .
V0713286	3	EU879117	. . .	. . .
V0714229	3	EU879118	. . .	. . .
V0716883	4	EU879120	. . .	. . .

[a] Onset of symptoms occurs 2–8 weeks after infection.

was isolated from a traveler returning from the United States (GenBank accession number EU879113) (table 2). In contrast, 14 (94%) of 15 autochthonous HEV isolates were genotype 3, and the majority of these viruses had close nucleotide identity to strains isolated from pigs in the Netherlands. The isolates clustered predominantly in subtype 3a and 3f (figure 2). Genotype 4 was detected in 1 autochthonous case (GenBank accession number EU879120).

Case-control study of risk factors for autochthonous HEV infection. Forty-five case subjects with autochthonous infection and 135 individually matched control subjects were included in the case-control study. Because of accurate matching, 76% of subjects in each group were male, 51% were living in communities with <20,000 inhabitants, and 33% were ⩾50 years old. Twelve of 16 German provinces reported 1–6 autochthonous cases of HEV infection during the study period, without clear geographic clustering. However, 20 (44%) of the case subjects and 20 (15%) of the control subjects were living in eastern Germany ($P = .001$). In bivariate analysis, the consumption of raw or undercooked beef, wild-boar meat, and offal was significantly associated with autochthonous HEV infection (table 3). More-specific questions about offal consumption revealed that cattle liver did not have a significant association with infection, pig liver had an important but nonsignificant association, and other offal (i.e., kidney and intestine) had a significant association (table 3). Pet ownership was inversely associated with infection. Analysis of occupations did not reveal a significant difference between the groups. Only 1 case subject, a sewage worker, had obvious professional contact with a potential HEV source.

In conditional logistic regression, consumption of offal and wild-boar meat was independently associated with autochthonous HEV infection (table 4). The creation of a summary variable (consumption of offal or wild-boar meat) showed that 53.3% of case subjects and 23.7% of control subjects consumed

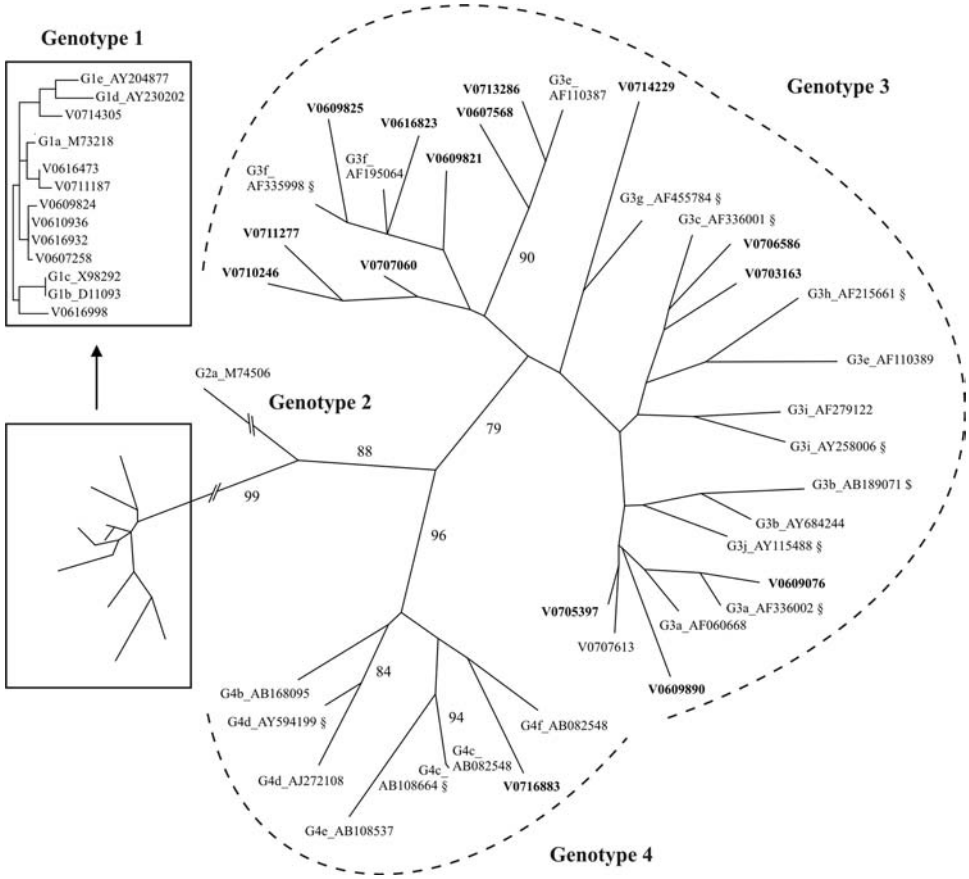

Figure 2. Phylogenetic tree of autochthonous and travel-associated hepatitis E virus (HEV) isolates recovered in Germany during 2006–2007. Strains denoted by "V" are isolates from 24 HEV-infected case subjects enrolled in the study. Autochthonous strains are printed in *bold*. Genbank accession numbers are listed in table 2. The designation of the reference sequences includes the genosubtype based on the classification scheme created by Lu et al. [18] and the Genbank accession number. Bootstrap values of >70% are indicated at the respective branches. §, strains isolated from pigs; $, strains isolated from deer; no symbol, strains isolated from humans.

≥1 of these food items during the 2 months before symptom onset (OR, 3.5; 95% CI, 1.8–7.7). Pet ownership was inversely associated with HEV infection. Of the 21 case subjects who had not consumed offal or wild-boar meat, none were living on a farm, none had had other professional contacts to animals, 2 had visited a petting zoo but did not have direct animal contact, 1 had hunted for sport, and 2 had rodents as pets in their households. Of interest, 14 (67%) of the 21 case subjects mentioned above had consumed raw or undercooked pork meat or pork sausage during the 2 months before symptom onset, which was, however, not significantly more often than that reported by control subjects.

DISCUSSION

Reports of autochthonous hepatitis E are increasing in industrialized countries [9]. The disease is usually mild and self-limiting, but fulminant and even fatal courses have recently been described in France and the United Kingdom [9, 20]. The main source of infection and the route of transmission in industrialized countries are unknown. However, the detection of genotype 3 strains in humans that are similar to strains found in pigs and wild boars suggests that these animals could be relevant reservoirs [1, 11–13]. The present study, which to our knowledge is the first large-scale case-control study on hepatitis E in an industrialized country, provides another important piece of evidence. Fifty-three percent of case subjects had consumed either of the 2 food items (offal or wild-boar meat) that were identified as independent risk factors in the multivariate analysis. Therefore, it is very likely that meat or offal consumption is a major route of HEV transmission in Germany. In contrast, direct animal contact and contact with waste or surface water seems to play no major role.

The results of this study are in accordance with several other recently published findings from seroprevalence studies and case series. Studies involving several hundred Swedish pig farmers and Swiss sewage workers did not show significantly different prevalences of anti-HEV IgG antibodies, compared with population-based, nonexposed control groups [21, 22]. In Japan, HEV has been linked to consumption of uncooked deer and

Table 3. Prevalence and bivariate analysis of exposures and potential risk factors among case subjects with autochthonous hepatitis E virus infection and matched control subjects, Germany, 2006–2007.

Variable	Case subjects, no. (%) (n = 45)	Control subjects, no. (%) (n = 135)	P	OR[a] (95% CI)
Underlying liver infection[b]	3 (6.7)	2 (1.5)	.10	4.5 (0.75–26.93)
Farm residence or employment	2 (4.4)	6 (4.4)	>.99	1.0 (0.19–5.32)
Manure used for fruits/vegetables grown in backyard	1 (2.2)	10 (7.4)	.24	0.3 (0.04–2.29)
Professional or amateur hunter	2 (4.4)	3 (2.2)	.45	2.0 (0.33–11.97)
Occupational exposure to animals				
Overall	2 (4.4)	7 (5.2)	.85	0.9 (0.18–4.13)
Slaughter house employment	0 (0.0)	1 (0.7)	.46	NA
Direct contact with pigs	1 (2.2)	3 (2.2)	>.99	1.0 (0.10–9.61)
Household pet ownership				
Overall	13 (28.9)	72 (53.3)	.004	0.3 (0.14–0.69)
Dogs	8 (17.8)	33 (24.4)	.33	0.6 (0.26–1.57)
Cats	4 (8.9)	36 (26.7)	.01	0.2 (0.06–0.73)
Rodents	2 (4.4)	22 (16.3)	.06	0.3 (0.06–1.08)
Domestic animal ownership				
Overall	1 (2.2)	9 (6.7)	.24	0.3 (0.04–2.29)
Pigs	0 (0.0)	4 (3.0)	>.99	NA
Cattle	1 (2.2)	3 (2.2)	.78	0.7 (0.07–7.36)
Chicken	0 (0.0)	7 (5.2)	>.99	NA
Risk behavior during past 2 months				
Blood transfusion	0 (0.0)	0 (0.0)	>.99	NA
Travel within Europe	10 (22.2)	26 (19.3)	.44	1.2 (0.52–2.78)
Close contact with a recent traveler	2 (4.4)	5 (3.7)	.81	1.3 (0.20–8.03)
Visited a farm or petting zoo	6 (13.3)	33 (24.4)	.63	1.1 (0.75–1.61)
Drank water from a well	1 (2.2)	4 (3.0)	.55	0.6 (0.12–3.09)
Contact with surface water	6 (13.3)	22 (16.3)	.62	0.8 (0.28–2.13)
Contact with waste water	1 (2.2)	1 (0.7)	.44	3.0 (0.19–47.96)
Any consumption of meat products	44 (97.8)	132 (97.8)	>.99	1.0 (0.10–9.61)
Food products consumed during past 2 months				
Mussels or shellfish	14 (31.1)	43 (31.9)	.92	1.0 (0.45–2.05)
Poultry, undercooked	5 (11.9)	4 (3.0)	.05	4.2 (0.99–18.04)
Pork products, raw or undercooked[c]				
Overall	33 (78.6)	89 (66.4)	.11	2.0 (0.86–4.81)
Pork meat, undercooked	5 (11.1)	6 (4.5)	.12	2.7 (0.77–9.49)
Beef, raw or undercooked	13 (31.0)	19 (14.1)	.02	2.6 (1.15–5.99)
Wild boar, cooked or undercooked	9 (20.0)	9 (6.7)	.01	4.9 (1.45–16.44)
Wild-animal meat (other than wild boar)	8 (18.2)	31 (23.0)	.45	0.7 (0.29–1.73)
Offal, cooked or undercooked[d]				
Overall	18 (40.9)	25 (18.5)	.004	3.1 (1.43–6.61)
Cattle liver	4 (8.9)	15 (11.1)	.68	0.8 (0.25–2.46)
Pig liver	9 (20.5)	15 (11.1)	.15	1.9 (0.80–4.63)
Other (kidney, gut)	10 (22.2)	5 (3.7)	.001	7.1 (2.20–22.65)

NOTE. CI, confidence interval; NA, not applicable; OR, odds ratio.

[a] By the Mantel-Haenszel test.
[b] Due to hepatitis B virus or hepatitis C virus.
[c] Including raw sausage and raw minced pork.
[d] Including liver, kidney, and gut.

inadequately cooked wild-boar meat and liver [23–25]. However, evidence for food-borne HEV transmission in Japan has, to date, been derived only from case reports, local outbreaks, and small, nonsystematic studies [13]. Even though consumption of food sashimi style is not a habit among Europeans [13], the possibility of food-borne transmission still exists if meat or liver is

Table 4. Final multivariate model of risk factors for autochthonous hepatitis E virus infection, Germany, 2006–2007.

Risk factor	Case subjects, no. (%) (n = 45)	Control subjects, no. (%) (n = 135)	P	OR[a] (95% CI)
Pets in the household	13 (28.9)	72 (53.3)	.04	0.4 (0.17–0.95)
Consumed in the previous 2 months				
Wild boar, cooked or undercooked	9 (20.0)	9 (6.7)	.03	4.3 (1.15–15.85)
Any offal, cooked or undercooked[b]	18 (40.9)	25 (18.5)	.02	2.7 (1.15–6.24)

NOTE. The model was determined by means of conditional logistic regression. All values are adjusted for age. OR, odds ratio; CI, confidence interval.

[a] By the Mantel-Haenszel test.

[b] Including liver, kidney, and gut.

not prepared properly. In the Netherlands, 6.5% of 62 commercial porcine livers were recently shown to be positive for HEV RNA [26]. In Germany, 5.3% of 189 wild-boar serum specimens tested positive for HEV by PCR [27]. Sequence analysis of isolated HEV strains revealed genotype 3 in both studies, which is the predominant strain detected in humans in Europe and was the most common strain recovered in our study population. Our study provides strong epidemiological evidence that consumption of presumably undercooked offal or wild-boar meat or cross-contamination of other food during preparation is likely a major route of HEV transmission in Europe.

A clear advantage to our study was that hepatitis E is a notifiable disease in Germany and was thus reported from all local health authorities in districts where the disease was diagnosed. This might have contributed to some different demographic features of our study population, compared with those reported in studies from the United Kingdom, which were solely based on data from 2 reference laboratories [28, 29]. There, the majority of persons with autochthonous cases were more likely to live in coastal or urban areas, whereas the majority of our cases were detected in persons living in rural areas [28, 29]. In our study population, the median age of persons with autochthonous infection was, as in both studies from the United Kingdom, higher than that of persons with travel-associated disease. This is because young people tend to travel more frequently and more often under decreased hygienic conditions. But autochthonous hepatitis E in Germany affected all age groups, with only 33% aged $\geqslant$50 years, which clearly contrasts with findings from the United Kingdom [28]. Of interest, although authors of the latter study assumed that pet ownership was the only common risk factor among persons with autochthonous HEV infection (60% of the study subjects owned pets, but no control group was included), this factor was inversely associated with HEV infection in our study [28]. A possible explanation for our finding is that pet owners might have different meat-consumption habits. However, the association also remained significant in the multivariate analysis and could have been due to residual confounding by factors such as unmeasured food-consumption habits. Because cat ownership was the most protective factor, another explanation might be a higher prevalence of preexisting immunity that resulted from subclinical infection following frequent preparation of raw liver for use as cat food. This hypothesis could be addressed by a future study to assess anti-HEV seroprevalence in a population with and without cat owners.

Previous serology-based studies have suggested that rodents might serve as a reservoir for HEV [30, 31]. In our population, ownership of pet rodents and professional exposure to sewage were not significantly associated with HEV infection. Assessment of accidental exposure to wild rodents or their feces during the 2-month incubation period seemed unrealistic to us. Thus, it was not specifically addressed in the questionnaire, and we cannot exclude the possibility that a few cases were infected through wild-rodent exposure. A lower frequency of exposure to wild rodents or rodent stool might be associated with cat ownership and could serve as another explanation for the finding described above. To date, HEV RNA has never been detected in rodent serum or stool specimens, and thus the genotype of HEV circulating in this population is unknown. In addition, the role of rodent reservoirs in the transmission of HEV to humans is unclear and warrants further study.

During the study period, more individuals with autochthonous than travel-associated HEV infection were reported and included in the study. In both groups, bivariate and multivariate analysis revealed that travel to other European countries was not associated with an increased risk of HEV infection. This finding shows that hepatitis E is endemic in Germany, and physicians need to consider hepatitis E as a differential diagnosis in patients with acute liver disease, regardless of their recent travel history. It is noteworthy and relevant to travel medicine that >50% of travel-associated infections were acquired on the Indian subcontinent. In India, hepatitis E is a classic water-borne infection caused by HEV genotype 1, which is also reflected by the genotype distribution in our study population. A large proportion of the HEV-infected travelers revealed that they engaged in high-risk behavior for enteric diseases during travel.

One HEV isolate consistently clustered with genotype 4 reference sequences. To our knowledge, this is the first time that genotype 4 has been detected in a person outside of Asia [19]. This

individual had not traveled outside of Germany ≤2 months before symptom onset. Because we cannot exclude the possibility that food from abroad might have been the source of infection in this case, further genotyping studies are needed to investigate whether genotype 4 virus is also endemic in central Europe.

Because of the relatively long incubation period of hepatitis E, we asked individuals to specify food items that they had consumed during the previous 2 months. Yet, we did not have the impression that responses to this question were much affected by problems of recall, since the consumption of distinct food items such as wild-boar meat or offal were usually well remembered by both case and control subjects. It was probably more difficult to recall whether some of the meat products eaten were undercooked. The assumption that it only happens occasionally that meat contains an infective concentration of HEV [26] and is not properly cooked at the same time can explain why the role of undercooked meat is difficult to detect by means of case-control studies, but it can also explain the low number of annually reported infections. During the incubation period, 67% of 21 case subjects who had not consumed offal or wild-boar meat and 79% of all case subjects consumed raw or undercooked pork products. However, because consumption of raw pork meat or sausages is common in Germany, this exposure was not significantly more common among case subjects than among control subjects.

We conclude that, as in many other industrialized countries, hepatitis E is endemic in Germany and that more infections were autochthonous than travel associated. Results of the case-control study show that a substantial proportion of autochthonous cases in Germany may be due to consumption of presumably undercooked meat products, such as wild-boar meat or offal. When these results are considered alongside phylogenetic findings, it is very likely that the disease exists as a food-borne zoonosis. It is also likely that hepatitis E notification detects only the tip of the iceberg, because many HEV infections may have an asymptomatic or mild clinical course and remain undiagnosed. Incriminated meat products such as wild-boar meat and offal should be investigated for HEV to provide data on the contamination of these products. A study has shown that HEV can be inactivated in contaminated liver if the meat is thoroughly cooked [32]. Recommendations for proper cooking of wild-boar meat and offal and for hygienic precautions to avoid cross-contamination should be given to consumers, especially persons with chronic liver disease and recipients of an organ transplant.

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