MAJOR ARTICLE

Non–Travel-Associated Hepatitis E in England and Wales: Demographic, Clinical, and Molecular Epidemiological Characteristics

Samreen Ijaz,[1] Eve Arnold,[2] Malcolm Banks,[8] Richard P. Bendall,[4] Matthew E. Cramp,[6] Richard Cunningham,[7] Harry R. Dalton,[5] Tim J. Harrison,[3] Simon F. Hill,[9] Lorna MacFarlane,[10] Rolf E. Meigh,[11] Shuja Shafi,[12] Martin J. Sheppard,[13] Jacquie Smithson,[14] Melanie P. Wilson,[15] and Chong-Gee Teo[1]

[1]Virus Reference Division and [2]Statistics Unit, Centre for Infections, Health Protection Agency, and [3]Windeyer Institute of Medical Sciences, University College London, London, [4]Departments of Clinical Microbiology and [5]Gastroenterology, Royal Cornwall Hospital, Truro, [6]Gastroenterology Unit and [7]Department of Microbiology, Derriford Hospital, Plymouth, [8]Mammalian Virology, Veterinary Laboratories Agency, New Haw, [9]Department of Microbiology, Poole Hospital, Poole, [10]National Public Health Service Microbiology Aberystwyth, Bronglais General Hospital, Aberystwyth, [11]Department of Microbiology, Castle Hill Hospital, Cottingham, [12]Department of Microbiology, Northwick Park Hospital, Harrow, [13]Department of Microbiology, Withybush General Hospital, Haverfordwest, [14]Department of Gastroenterology, Royal Infirmary, Hull, and [15]Department of Microbiology, County Hospital, Hereford, United Kingdom

Between 1996 and 2003, 186 cases of hepatitis E were serologically diagnosed. Of these, 17 (9%) were not associated with recent travel abroad. Patients were >55 years old (range, 56–82 years old) and tended to be male (76%). Two patients presented with fulminant hepatitis. A total of 129 (69%) cases were associated with recent travel to countries where hepatitis E virus (HEV) is hyperendemic. Compared with patients with travel-associated disease, patients with non–travel-associated disease were more likely to be older, living in coastal or estuarine areas, not of South Asian ethnicity, and infected by genotype 3 strains of HEV. The genotype 3 subgenomic nucleotide sequences were unique and closely related to those from British pigs. Patients infected by HEV indigenous to England and Wales tended to belong to a distinct demographic group, there were multiple sources of infection, and pigs might have been a viral reservoir.

Hepatitis E is caused by hepatitis E virus (HEV), an enterically transmitted virus that is closely related to the caliciviruses [1]. In sanitation-poor regions of Asia, Africa, and Central America, sporadic outbreaks of hepatitis E are common, and large epidemics with waterborne transmission occur occasionally [2]. Clinical attack rates are highest among young adults, particularly pregnant women, in whom mortality is also high [3].

In industrialized countries, the disease occurs relatively infrequently and principally affects people who become infected while traveling in areas where HEV is hyperendemic [4]. Cases of hepatitis E in people with no preillness history of recent travel abroad have been reported in developed countries such as New Zealand [5], Australia [6], the United States [7], Greece [8], Austria [9], Italy [10], the United Kingdom [11–13], The Netherlands [14], France [15, 16], Spain [17], Germany [18], Taiwan [19], and Japan [20, 21]. In Japan, deaths resulting from fulminant hepatitis E have been observed [20, 21].

In these countries, HEV subgenomic sequencing studies have revealed a close relationship between the strains infecting humans and those infecting pigs [20, 22–24]. Moreover, experimental data have presented evidence of HEV transmission between pigs and humans [25]. Such findings favor the view that hepatitis E in industrialized countries is a zoonosis transmitted from pigs to humans. In Japan, such transmission may be associated with the consumption of undercooked

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Reprints or correspondence: Dr. Samreen Ijaz, Virus Reference Div., Centre for Infections, Health Protection Agency, 61 Colindale Ave., London NW9 5HT, United Kingdom.

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pig liver [26] and uncooked meats [27, 28], but how humans in Western countries acquire hepatitis E other than from travel to countries where the virus is hyperendemic remains puzzling. Prompted by the findings of the studies of the first known cases [12, 13, 24], we conducted a study of patients with hepatitis E in England and Wales to investigate the demographic, clinical, and molecular epidemiological factors in non–travel-associated disease.

PATIENTS, MATERIALS, AND METHODS

Patients and samples. In 1996, the Central Public Health Laboratory of the Public Health Laboratory Service (now called the Centre for Infections, Health Protection Agency) introduced a serodiagnostic testing service for hepatitis E. Serum samples were tested using the HEV IgM and IgG ELISAs manufactured by Genelabs Technologies. Patients with suspected acute non-A, non-B hepatitis whose samples were referred between that year and 2003 were evaluated for participation in the study. The patients included in the study were those seropositive for both anti-HEV IgM and IgG. The patients excluded from the study were those seronegative for anti-HEV antibodies, those whose initial samples were seropositive for anti-HEV IgM but not for anti-HEV IgG, and those whose follow-up samples did not show an increase in the level of anti-HEV IgG. Demographic and clinical information about patients (age, sex, history of travel during the 3 months before illness, place of residence, and presenting illness) was obtained from data provided on the referral forms that accompanied the samples. The place of residence was assigned as coastal or estuarine, according to whether the postal code fell within a coastal or estuarine settlement or within 10 km of a coast or estuary, and as being within a dense pig-holding area, if the number of pigs, sows, and gilts was >10/100 hectares of farmed land [29]. Nam Pehchan software was used to assign South Asian ethnicity on the basis of surname [30]; ethnicity was classified as South Asian or non–South Asian. For patients who reported no recent preillness travel, further inquiry was made by review of medical records, correspondence with the referring clinical practitioners, or telephone interviews with the patients. The objective of this inquiry was to establish the outcome of the illness and to determine whether patients had engaged in activities that might have put them at risk for acquiring HEV—that is, coming in contact with people with jaundice, eating shellfish, eating uncooked animal products, keeping pets, and coming in contact with live pigs.

HEV genome amplification. Available serum samples from patients whose travel history could be ascertained were processed for HEV genome detection and characterization. Total RNA was extracted from 200 μL of serum using the QIAamp UltraSens Virus Kit (Qiagen). Reverse-transcription polymerase chain reaction (RT-PCR) was performed as described elsewhere [22]. Briefly, eluted RNA was reverse-transcribed with the antisense degenerate primer 3157 (5′-CCCTTATCCTGCTGAGCATTCTC-3′) by use of SuperScript II (Invitrogen). cDNA of a 304-nt segment of open-reading frame 2 (ORF2), which encodes the viral capsid gene, was then amplified using primers 3156 (5′-AAT[C]TATGCC[A]CAGTACCGGGTTG-3′) and 3157 (5′-CCCTTATCCTGCTGAGCATTCTC-3′) in the first round of PCR and primers 3158 (5′-GTT[C]ATGC[T]TT[C]TGCATACATGGCT-3′) and 3159 (5′-AGCCGACGAAATC[T]AATTCTGTC-3′) in the second round of PCR. PCR cycling conditions for both rounds consisted of 39 cycles of denaturation at 94°C for 1 min, annealing at 42°C for 1 min, and extension at 72°C for 2 min.

Nucleotide sequence analysis. All PCR products were purified using the GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences). Both strands were sequenced using the PCR internal primers and a Beckman CEQ8000 sequencer. The generated nucleotide sequences were assembled and analyzed using DNAstar (version 5.03; Lasergene). Alignments, analyses, and displays of nucleotide sequences were undertaken to determine the phylogenetic relationships between the HEV sequences identified in this study and those retrieved from GenBank. The robustness of the data was determined by bootstrap resampling of the sequence alignment (1000 sets) with SEQBOOT, DNADIST, CONSENSE (PHYLIP package version 3.6) and the neighbor-joining method of NEIGHBOR (PHYLIP package version 3.6). Bootstrap values >70% were included in the maximum likelihood tree.

Statistical analysis. Patients were classified according to their travel history. Logistic regression analysis was performed with SAS (version 8.2; SAS) and was applied to examine possible associations between travel history and demographic factors. The association between travel history and HEV genotype was analyzed separately via a sensitivity analysis that used Fisher's exact test. Where appropriate, Fisher's exact test was used to compare characteristics of patients with non–travel-associated disease.

RESULTS

Travel histories of patients with hepatitis E. Between 1996 and 2003, samples from 478 patients were tested for anti-HEV IgM and IgG. A total of 186 patients fulfilled the serological criteria for the diagnosis of hepatitis E. The age, sex, and ethnic characteristics of these patients are summarized in table 1. Patients who reported recent travel to areas where HEV is hyperendemic ($n = 129$) visited Bangladesh (66), India (30), Pakistan (25), Afghanistan (3), Nepal (2), both India and Bangladesh (1), Nigeria (1), and China (1); these patients were assigned to group T. Patients who reported recent travel to countries where HEV is not hyperendemic ($n = 8$) visited Spain (2), Cyprus (2), Turkey (1), the United States (1), New

Table 1. Travel history and demographic characteristics of 186 patients seropositive for anti–hepatitis E virus (HEV) IgM and IgG.

Recent travel history	No. (%) of patients	Mean age (range), years	Men, %	South Asian, %
To area where HEV is hyperendemic	129 (69)	34 (9–85)	81	87
To area where HEV is not endemic	8 (4)	58 (27–76)	100	0
None	17 (9)	68 (51–83)	82	0
Unknown	32 (17)	42 (8–82)	56	94

Zealand (1), and Uganda (1). Patients who reported no history of recent travel ($n = 17$) were assigned to group NT. Travel history was unknown for 32 patients.

Patients with non–travel-associated hepatitis E. A summary of the demographic and clinical characteristics of group NT patients is shown in table 2. All patients were white. Ten (59%) were >65 years old; all of these patients were men, and 5 were >75 years old. There were significantly more men (14/17) than women (3/17) ($P = .01$). Fifteen patients presented with jaundice. The other 2 presented with clinical features indicative of fulminant hepatitis; 1 died and 1 required liver transplantation. Three patients lived within dense pig-holding areas, whereas 14 patients did not, and 14 patients lived in coastal or estuarine areas, whereas 3 patients did not. Except for 2 patients (patients NT11 and NT12, who were spouses) who habitually ate mussels and cockles up to the time of illness, no patients reported eating shellfish before their illnesses. None recalled eating raw animal products or being in contact with jaundiced patients or live pigs. Except for 1 patient, who kept a cat, no patients kept pets.

Comparison of demographic characteristics. The demographic characteristics of group NT and group T patients were compared. None of the group NT patients were South Asian ($P < .0001$ for group NT vs. group T patients). Logistic regression identified age and residence in coastal or estuarine areas to be significant risk factors for acquiring non–travel-associated disease, with age being a confounding variable for place of residence. The odds of being in group NT were observed to increase with age at a rate of 16%/year (odds ratio [OR], 1.16 [95% confidence interval {CI} 1.07–1.25]; $P < .0001$). Patients living in coastal or estuarine areas were 75 times as likely as those not living in such areas to be in group NT (OR, 74.9 [95% CI, 6.9–815.9]; $P = .0004$). The difference in the distribution by sex between group NT patients and group T patients was not significant. The proportion of group NT patients living in dense pig-holding areas (3/17) was significantly different from that of group T patients (3/128) ($P = .02$); all 3 group NT patients who resided in dense pig-holding areas also lived in coastal or estuarine areas, compared with 1 of 3 group T patients (table 1).

HEV subgenomic nucleotide sequence characteristics. HEV ORF2 cDNA could be amplified from serum samples from 11 (65%) of 17 group NT patients and from 27 (69%) of 39 group T patients whose serum samples were available for RT-PCR. All sequences derived from serum samples from group NT patients belonged to genotype 3, whereas all sequences derived from serum samples from group T patients belonged to genotype 1 (figure 1). If the unknown genotyping results for the serum samples that were insufficient for amplification and sequencing studies were taken into account, an association between genotype and travel history was significant ($P < .001$). The genotype 3 sequences from group NT patients were unique, clustering with the subgroup composed predominantly of European HEV strains and separated from the subgroup composed of sequences originating primarily in the United States, Canada, Taiwan, and Japan. The distinct clustering by subgroup is supported by the high bootstrap values that were generated. The nucleotide identity between the sequences derived in this study and those reported from British pigs [13, 24] ranged from 82% to 95%, and the amino acid sequence identity ranged from 99% to 100%. The amino acid sequences of UK sw1 and UK sw2 HEV are identical; compared with them, the amino acid sequences from patients NT2–NT7, NT10, and NT11 are identical, and the sequences from patients NT1, NT8, and NT9 have 99% identity.

DISCUSSION

This study reveals demographic differences between patients who acquired non–travel-associated hepatitis E and those who were infected when traveling in countries where HEV is hyperendemic. All patients in group NT were white, whereas the majority of patients in group T were of South Asian ethnicity. Old age and residence in coastal or estuarine areas were significant risk factors for acquiring non–travel-associated disease. The confounding effect of age on the place of residence is consistent with contemporary population census data that show that a high proportion (>20%) of people above state pension age (65 years old for men and 60 years old for women) live along the coast of the United Kingdom [31]. Although the difference in the high proportion of men in both groups was not significant, the proportion of men was significantly higher than the proportion of women in group NT, which indicates that male sex is a risk factor for acquiring non–travel-associated disease. This apparent difference

Table 2. Demographic and clinical characteristics of 17 patients with non–travel-associated hepatitis E.

Patient	Sex/age in years at onset of illness	County of residence	Geographical location of residence	Residence in dense pig-holding area	Presenting illness	Course of illness
NT1	M/62	Cornwall	Coastal	No	Acute hepatitis	Uneventful recovery
NT2	M/77	Pembrokeshire	Coastal	No	Acute hepatitis	Uneventful recovery
NT3	**M/71**	**Pembrokeshire**	**Coastal**	**No**	**Acute hepatitis**	**Uneventful recovery**
NT4	**M/71**	**Devon**	**Coastal**	**No**	**Acute hepatitis**	**Uneventful recovery**
NT5	M/82	Dorset	Coastal	No	Acute hepatitis	Uneventful recovery
NT6	**M/51**	**Dorset**	**Coastal**	**No**	**Acute hepatitis**	**Uneventful recovery**
NT7	**M/74**	**East Yorkshire**	**Estuarine**	**Yes**	**Acute hepatitis**	**Uneventful recovery**
NT8	**M/78**	**East Yorkshire**	**Coastal**	**Yes**	**Fulminant hepatitis**	**Died**
NT9	**M/70**	**East Yorkshire**	**Estuarine**	**Yes**	**Acute hepatitis**	**Uneventful recovery**
NT10	**M/79**	**Middlesex**	**Inland**	**No**	**Acute hepatitis**	**Uneventful recovery**
NT11	**F/56**	**Powys**	**Estuarine**	**No**	**Acute hepatitis**	**Uneventful recovery**
NT12	M/58	Powys	Estuarine	No	Acute hepatitis	Uneventful recovery
NT13	**F/58**	**Cornwall**	**Coastal**	**No**	**Acute hepatitis**	**Uneventful recovery**
NT14	M/81	Cornwall	Estuarine	No	Acute hepatitis	Died of unrelated causes
NT15	**F/64**	**Cornwall**	**Coastal**	**No**	**Acute hepatitis**	**Uneventful recovery**
NT16	F/56	Herefordshire	Inland	No	Fulminant hepatitis	Liver transplantation required; recovering
NT17	**M/59**	**Herefordshire**	**Inland**	**No**	**Acute hepatitis**	**Uneventful recovery**

NOTE. Bold denotes patients for whom serum samples were positive for hepatitis E virus RNA. The case history of patient NT1 has been reported in detail elsewhere [16]. Patients NT11 and NT12 were spouses.

may, however, be due to the small sample size of that group. A difference was also found in the molecular epidemiological characteristics of the HEV genotypes infecting the 2 groups. Subgenomic HEV nucleotide sequencing showed that group NT patients were exclusively infected by genotype 3 strains of HEV, whereas group T patients were exclusively infected by genotype 1 strains of HEV. The differences between the groups point to the coendemicity in England and Wales of 2 epidemiological forms of hepatitis E, with the non–travel-associated form connoting indigenous infection and the travel-associated form connoting imported infection.

The perception that hepatitis E is exclusively a travel-associated disease probably leads to its underdiagnosis in nontravelers. We estimate that, in Cornwall, 20% of the cases of unexplained hepatitis, at most, are investigated for hepatitis E, despite local interest in the disease (H.R.D. and R.P.B., unpublished data). Throughout the United Kingdom, the rate of testing may be even lower, which is consistent with the finding in the present study that only 9% of patients with hepatitis E were nontravelers.

Risk factors for acquiring non–travel-associated hepatitis E in industrialized countries are being identified. High anti-HEV antibody seroprevalences were found in pig veterinarians, farm workers who come into contact with pigs [32], and vagrants exposed to rodents [33]. In Japan, consumption of inadequately cooked pig liver [26] and inadequately cooked or raw deer and boar meat [27, 28] has been associated with clusters of cases or common-source outbreaks. Moreover, there are reports of the recovery of infectious HEV and the viral genome from slaughterhouse sewage in Spain [17] and from urban sewage in Spain, France, and the United States [34] and of hepatitis E in a French wastewater worker [15], all of which point to the potential of HEV to transmit through sewage-contaminated water. These reports suggest that, in industrialized countries, HEV may be transmitted along foodborne, waterborne, and zoonotic routes.

The results of the present study further suggest that, in England and Wales, several HEV transmission routes maintain the endemicity of hepatitis E. A co-occurrence of hepatitis E in spouses (patients NT11 and NT12) who reported habitual shellfish consumption implicates that activity as a risk factor, which is consistent with previous anecdotal reports [35]. The 15 additional patients who acquired non–travel-associated hepatitis E did not report similar behavior, so other modes of transmission are implied in those infections. A substantial proportion of these patients were elderly men. It is clear that this subset of patients was not infected from a common source, because the HEV subgenomic nucleotide sequences indicated a multiplicity of subgenomic strains. In light of the distinct demographic profile of these patients, it is possible that they had a common route of infection. Our inquiry was not sufficiently exhaustive to uncover all risk factors. For example, a dietary history was not obtained, nor were the patients asked about cooking practices, the source of their drinking water supply, and their bathing habits. The possibility that older people are more susceptible to infection or are more likely to manifest symptomatic disease also needs to be entertained. An association between older age and increased exposure to infection has been reported in India [36].

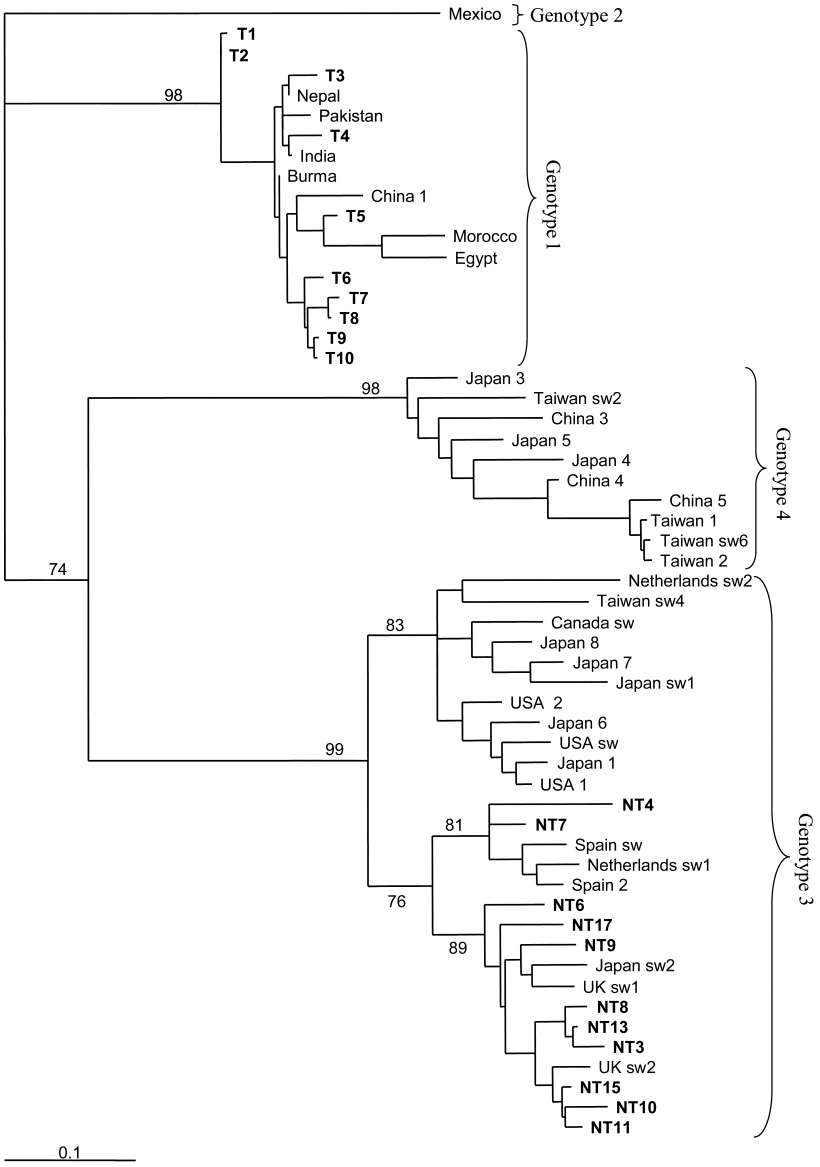

Figure 1. Maximum likelihood dendrogram featuring nucleotide sequences (in bold) derived from open-reading frame 2 of hepatitis E virus (HEV). Serum samples were from patients with travel-associated (denoted by "T") and non–travel-associated (denoted by "NT") hepatitis E. Sequences from group T patients are illustrative only and do not represent all sequences that were generated. Representative GenBank HEV sequences derived from pigs (denoted by "sw") and from humans are also shown. Numerals at the nodes are bootstrap values, expressed as percentages, obtained from 1000 resamplings of the data. The sequences from group NT and group T patients are assigned under GenBank accession nos. AJ879566–AJ879574, AY362357, and AY582797. Prototypic HEV GenBank sequences are as follows: Mexico, M74506; Nepal, AF051830; Pakistan, AF185822; India, AF124407; Burma, M73218; China 1, M94177; Morocco, AF065061; Egypt, AF051351; Japan 3, AB082557; Taiwan sw2, AF117281; China 3, AJ272108; Japan 5, AB082559; Japan 4, AB082558; China 4, AF151962; China 5, AF151963; Taiwan 1, AF117275; Taiwan sw6, AF302068; Taiwan 2, AF296162; Netherlands sw2, AF336298; Taiwan sw4, AF296166; Canada sw, AY115488; Japan 8, AB082562; Japan 7, AB082561; Japan sw1, AB073910; USA 2, AF060669; Japan 6, AB082560; USA sw, AF082843; Japan 1, AB082563; USA 1, AF060668; Spain sw, AF195063; Netherlands sw1, AF336295; Spain 2, AF195061; Japan sw 2, AB073911; UK sw1, AF503511; and UK sw2, AF503512.

We have shown elsewhere that >80% of pigs in the United Kingdom are seropositive for anti-HEV antibody [24], suggesting that HEV is endemic in pigs bred there. Our study reveals that the genotype 3 sequences generated from patients with non–travel-associated disease constitute a subgroup composed primarily of European HEV sequences. Some of these cluster with sequences originating in British pigs, and they are distinct from the sequences in the other genotype 3 subgroup, which is composed predominantly of non-European human and swine HEV sequences (figure 1). No segregation of the

sequences found in the present study according to the county of residence is apparent, however. The sequences from group NT patients are very closely related to HEV sequences found in British pigs. In a study published elsewhere of another patient with non–travel-associated hepatitis E in the United Kingdom, we found that the ORF2 nucleotide sequence derived from that patient had 95% identity with the UK sw2 sequence, and the amino acid sequence derived from that patient had 100% identity with the UK sw2 sequence [13]. Such relatedness is consistent with pigs being the reservoir of infection, although the possibility that there is a reservoir common to pigs and humans cannot be excluded. The finding that a higher proportion of patients with non–travel-associated disease resided in dense pig-holding areas, compared with those with travel-associated disease, suggests that contact with or proximity to pigs is a risk factor for acquiring non–travel-associated disease; the confounding effect of living in coastal or estuarine areas requires further investigation involving larger sample sizes. The growing evidence for a transmission link between pigs and humans reinforces the hypothesis that routes of HEV infection in industrialized countries are different from those in developing countries. In India, where the anti-HEV seroprevalence in pigs is high, neither genotype 3 nor genotype 4 strains of HEV have been detected in humans, even though strains of these genotypes circulate widely in pigs [37].

Fulminant hepatitis has been observed in non–travel-associated disease, as exemplified by patients NT8 and NT16. We were unable to determine whether the frequency of fulminant hepatitis in non–travel-associated disease was different from that of travel-associated disease, because the design of the study precluded inquiry into the outcome of travel-associated disease. In Japan, whether fulminant hepatitis E is common is controversial [20, 21]. No controlled studies examining the relative frequencies of this complication in different epidemiological forms of hepatitis E or the relationship between susceptibility to severe liver disease and age have been reported in Japan or elsewhere.

Patients in England and Wales who acquire non–travel-associated disease have characteristics that contrast strikingly with those of patients infected when traveling in countries where HEV is hyperendemic. Patients with non–travel-associated disease tend to be male, old, live near the coast or estuaries, and be infected by genotype 3 strains of HEV that, although originating in multiple sources, are closely related to British strains of swine HEV. Prospective in-depth epidemiological studies based on structured interviews with patients are ongoing to define the routes of transmission, which may enable sources of infection to be identified. To establish that pigs are indeed the source, it would be necessary to sample the environment for the presence of HEV—for example, water used for irrigation and drinking [38], wastewater [39], pig products [26], and shellfish [40]—and to complement this data with comparative analyses of HEV genomic sequences generated from the samples and with transmission studies [25].

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