REVIEW ARTICLE
Transmission routes and risk factors for autochthonous hepatitis E virus infection in Europe: a systematic review

H. C. LEWIS[1,2*], O. WICHMANN[3] AND E. DUIZER[4]

[1] *Department of Epidemiology, Statens Serum Institut, Copenhagen, Denmark*
[2] *European Programme for Intervention Epidemiology Training (EPIET), European Centre for Disease Control, Stockholm, Sweden*
[3] *Department for Infectious Disease Epidemiology, Robert Koch Institute, Berlin, Germany*
[4] *National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands*

SUMMARY

Increasing numbers of non-travel-associated hepatitis E virus (HEV) infections have been reported in Europe in recent years. Our objective was to review the evidence on risk factors and transmission routes of autochthonous HEV infection and hepatitis E in Europe in order to develop recommendations for future research, prevention and control. A systematic literature review was performed to identify all primary reports and studies published during 1998–2008 on hepatitis E in humans and animals in Europe by searching Pubmed, reference lists of major articles and international conference proceedings. Each of the 106 included studies was categorized into one of three evidence levels (EL) based on study design and diagnostic methodology. The evidence was generally weak (73 were assigned to EL1, two to both EL1 and EL2, and 30 to EL2), further compounded by the use of poorly validated serological assays in some studies. Only one case-control study was assigned to EL3. Persons with autochthonous hepatitis E infection were on average older than the general population and predominantly male. There was no evidence for one main transmission route of HEV infection or risk factor for hepatitis E. However, zoonotic transmission seemed likely and person-to-person transmission too inefficient to cause clinical disease. Multiple routes of transmission probably exist and should be further investigated through analytical studies and reliable diagnostic kits. Based on current evidence that points to zoonotic transmission from pigs, thorough cooking of all porcine products, prevention of cross-contamination in the kitchen and improved education for occupationally exposed people (e.g. pig farmers, veterinarians and sewage workers) may help prevent HEV infection. Although evidence for parenteral transmission is limited, it is recommended that a risk assessment is undertaken.

Key words: Autochthonous, Europe, hepatitis E, risk factors, systematic review.

INTRODUCTION

Hepatitis E virus (HEV) infection is a major cause of epidemic and acute sporadic hepatitis in many areas of Asia, Africa and Mexico [1], where HEV is considered hyper-endemic. Traditionally, industrialized countries were considered non-endemic, with most HEV infections in these regions being sporadic and considered to be imported. However, in recent years, enhanced surveillance has detected an increasing number of non-travel-associated HEV infections, especially in Europe and Japan [2]. In addition, unexpectedly high anti-HEV immunoglobulin G (IgG) seroprevalence among the general population has

* Author for correspondence: Miss H. C. Lewis, Epidemiologist, World Health Organization Lao PDR Country Office, P.O. Box 343, Vientiane, Lao PDR.
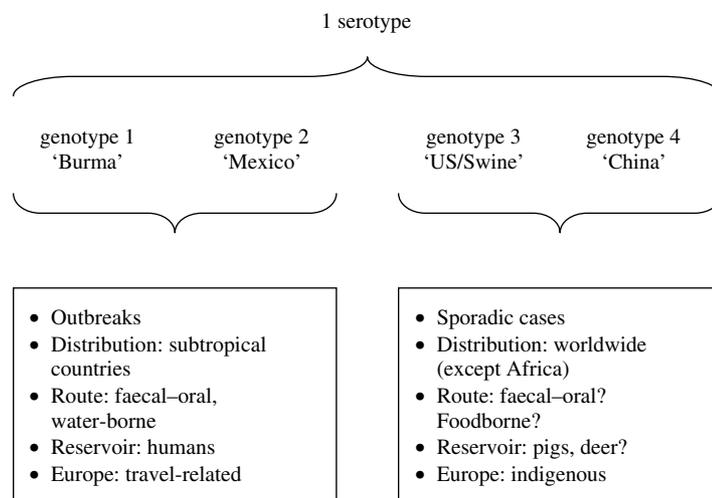(Email: lewish@wpro.who.int)

1 serotype

genotype 1   genotype 2   genotype 3   genotype 4
'Burma'   'Mexico'   'US/Swine'   'China'

• Outbreaks
• Distribution: subtropical countries
• Route: faecal–oral, water-borne
• Reservoir: humans
• Europe: travel-related

• Sporadic cases
• Distribution: worldwide (except Africa)
• Route: faecal–oral? Foodborne?
• Reservoir: pigs, deer?
• Europe: indigenous

Fig. 1. Summary of epidemiology and transmission of mammalian HEV.

been reported from some industrialized countries, although the rates varied considerably between reports, ranging from 2·3 to 33% [3–9].

HEV is a non-enveloped, ribonucleic acid (RNA) virus. Its genome is single-stranded, about 7·2 kb in length and contains three open reading frames. HEV is classified in the family Hepeviridae and consists of genotypes (gt) 1–4 that infect humans and other mammals, and a group of avian HEV strains that probably constitute a separate genus [10]. Of interest, the majority of HEV strains from human cases acquired in industrialized countries are different genetically to those isolated from cases in, or imported from, hyper-endemic countries. This geographical difference in genotype distribution contributed to the hypothesis that HEV gt1 and gt2 lead to hepatitis E with a different clinico-epidemiological entity compared to hepatitis E caused by gt3 and gt4 viruses [11, 12] (Fig. 1). In contrast to hyper-endemic regions where HEV gt1 is predominately found during large waterborne outbreaks, autochthonous infections in Europe are usually sporadic and related to gt3. The higher standard of hygienic conditions in industrialized countries makes the waterborne route of HEV transmission unlikely in these regions and might explain the rare occurrence of gt1 infections among autochthonous cases. Thus, it is hypothesized that gt3 is linked to different sources and routes of infection than gt1.

As early as 1990, Balayan and co-workers postulated that HEV could be a zoonosis since a human HEV strain was found to be capable of infecting domestic pigs [13]. In 1997, HEV gt3 strains were detected in swine in the USA [14]. HEV gt3 and gt4 have since been found to be endemic in pig herds worldwide [15, 16]. Both genotypes, whether from pigs or humans, have been shown to be transmissible to non-human primates and a range of other animal species [17, 18]. Very close homology (>90%) has been found between HEV gt3 nucleotide sequences from pigs, wild boar and humans, suggestive of cross-species transmission [6, 19]. As HEV gt3 spreads easily in pig herds but poorly in human communities it has been postulated that humans are not the main host or reservoir [20].

The diagnosis of a HEV infection is mainly dependent on serology. In general, a positive result for anti-HEV immunoglobulin M (IgM) antibodies, or a rise in anti-HEV IgG titre, is indicative of an acute infection. Recent studies have shown that the use of commercially available serological tests (which are mainly based on HEV gt1 antigens) in HEV gt3-endemic regions need further confirmation [6, 21]. Moreover, a comparison of five serological tests showed that the agreement between the tests was moderate at best (kappa ∼0·5) [22]. A couple of IgG enzyme-linked immunosorbent assay (ELISA) commercial kits were withdrawn from the market or replaced by new tests by the manufacturer due to recognition of a high proportions of false-negative results [22, 23]. There are also many reports on the detection of anti-HEV antibodies in serum samples from different animals [17] but these results are often obtained using tests that are developed and evaluated for detecting anti-HEV antibodies in human samples during the acute phase of the disease.

The reliability of these results is therefore questionable.

Detection of a part of the virus, for example HEV RNA, in serum or faecal samples, instead of detection of an immune response against antigens of a virus, is more definite proof for HEV infection. Several reports state that the viraemic phase of HEV in humans is, in general, short and that the virus may disappear from peripheral blood at the height of the disease (reviewed by Jameel [24]). Nevertheless, HEV RNA might be detectable in faecal samples for a longer period of time [25] and recent prolonged infection and shedding has been reported for some immunocompromised patients [26].

The clinical presentation of hepatitis E in humans is indistinguishable from hepatitis A. Commonly reported symptoms include jaundice, anorexia, abdominal pain and hepatomegaly accompanied by fever, nausea and vomiting [27]. Hepatitis E is usually self-limiting but very recently chronic infections have been described in organ transplant recipients in France [28, 29] and The Netherlands [26]. Fulminant and even fatal hepatitis E caused by gt3 have been reported in Japan [30] and in Europe [31–35], often in older males with underlying chronic liver disease. In contrast, in hyper-endemic gt1 and gt2 areas, pregnant women are at higher risk for severe disease and death [36, 37]. This feature has not yet been reported for HEV gt3 infections.

In Europe, acute HEV infection is diagnosed in 5–15% of patients with acute hepatitis for whom hepatitis A, B and C has been ruled out [6, 38–41]. Requests for HEV testing in patients without travel history continue to be infrequent and consequently the actual number of hepatitis E cases is underestimated.

In industrialized countries, little is known about possible sources and transmission routes for endemic human HEV infections. Research is hindered because outbreaks are rarely detected, many infections remain asymptomatic and there is a long and variable incubation period. Narrative, non-systematic reviews have commented on the zoonotic potential and other transmission routes of HEV [11, 42]. However, none have documented a systematic review process or specifically focused on Europe. Our objectives were to determine risk factors for HEV infection and disease and the likely routes of HEV transmission in Europe. We systematically reviewed the scientific literature to give an overview of the evidence available from the last ten years and make recommendations for future research, and prevention and control.

METHODS

Literature search and data extraction

A systematic review of the literature was performed to identify all primary reports and studies that have been published between January 1998 and September 2008, conducted on: (i) HEV infection or anti-HEV antibody prevalence in humans, or (ii) HEV or anti-HEV antibody prevalence in food, animals or the environment. The primary objective was to review and report on the current knowledge and evidence that exists on risk factors for hepatitis E and the most likely routes of HEV transmission in Europe. Thus, the review was limited to the occurrence of HEV infection and disease in Europe, and three report categories were included: (i) original studies published in peer-reviewed scientific journals, (ii) abstracts in conference proceedings, and (iii) correspondence with scientific experts.

For the first category, an electronic search of the United States National Library of Medicine and the National Institutes of Health Medical Database (Pubmed) from its inception was conducted in September 2008. Search words used were: ('HEV' or 'hepatitis E') and ('zoonosis' or 'zoonotic' or 'epidemiology' or 'food' or 'environment' or 'animal' or 'pig' or 'meat' or 'rodent' or 'indigenous' or 'autochthonous' or 'industrialized'). Articles from 1998 onwards were picked out manually. To verify the search, reference lists of the five most recent literature reviews and five most cited primary articles investigating the zoonotic potential of hepatitis E were hand-searched and relevant citations were added to the database. For the second category, a hand-search included eight international conference proceedings held between 2006 and 2008: International Conference on Emerging Infectious Diseases (ICEID), Med-Vet-Net annual scientific meetings, International Meeting on Emerging Diseases and Surveillance (IMED) and International Congress on Infectious Diseases (ICID). Finally, when a published study could not be located, we contacted topic-related scientists of relevant conference abstracts to gather more detailed information.

Abstracts were examined to determine if the inclusion criteria were met. A relevance tool was then applied to the selected reports: (1) countries are

contained entirely within continental Europe (i.e. excluding Turkey and the Russian Federation); (2) abstract is in English, French, German or Dutch; (3) diagnosis of hepatitis E or HEV infection was laboratory-confirmed using recognized tests; (4) report has relevance to transmission of, or risk factors for, autochthonous HEV infection (including reports with isolation of HEV gt3). If one of the four criteria was not met, the report was excluded.

For each report or study included, the following information was extracted (as applicable to study): type of study, location (country and setting), study population (number of cases, patients or risk groups, etc.), demographics (age and sex), laboratory tests utilized, positivity rates of humans and animals tested by serological tests and for RNA, HEV genotype, risk factors questioned about, and risk factors found.

Assessment of level of evidence and critical appraisal

Each identified study was categorized to an evidence level (EL) regarding the route of transmission of HEV in Europe, taking both study design and diagnostic methodology into account and using the risk assessment template developed by Palmer et al. [43] as follows:

Evidence level 1

- Single human case report confirmed by detection of anti-HEV IgM or HEV RNA, or,
- human hepatitis E case series diagnosed by detection of anti-HEV IgM or HEV RNA without specific exposures by subgroup, or,
- serosurveys carried out on acute non-A–C hepatitis patients (without comparison of risk groups) with a proportion of cases confirmed by detection of anti-HEV IgM or HEV RNA, or,
- animal or environmental studies finding HEV gt3 RNA in samples with <100% nucleotide sequence relatedness to human HEV.

Evidence level 2

- Enhanced surveillance with hepatitis E case series confirmed by detection of anti-HEV IgM or HEV RNA and cases with specific exposures by subgroup, or
- serosurvey data comparing risk groups vs. non-risk groups. The limitations of the diagnostic kits used in some of these studies were taken into account by limiting data interpretation to comparative data

Table 1. *Inclusion and exclusion criteria used for this review and number of reports identified*

Criteria	n
Potential relevant reports identified by the search of Pubmed	1884
Application of inclusion criteria	
(i) Published after 1998 (−750)	1134
(ii) Relevant study type (−580)	554
Application of exclusion criteria	
(i) Acquired infection outside Europe (−444)	110
(ii) Publication language not English/French/German/Dutch (−4)	106
(iii) No recognized diagnostic tests (−1)	105
(iv) No relevance to transmission/risk factors for hepatitis E virus infection or disease in Europe (−13)	92
(v) Duplicate results (−3)	89
Add conference proceedings (+7)	96
Add reports found in reference lists/from correspondence with authors (+10)	106
Total	106

and consideration not being given to the absolute levels of seroprevalence found.

Evidence level 3

- Analytical epidemiological study (case-control study or cohort study), or
- single human case reports with 100% RNA sequence identity between source and human HEV.

RESULTS

Of 1884 de-duplicated citations published between 1998 and 2008, 110 reports or studies were relevant primary papers or conference proceedings that were suitable for inclusion (Table 1).

Identification of relevant studies by evidence level

Evidence level 1

For HEV in humans, 37 reports pertaining to HEV reservoir and transmission routes can be divided into single case reports (n = 16) or case-series (n = 18) with the remaining three studies adding molecular sequencing results (Table 2). Even though these reports provide only a weak level of evidence to evaluate risk factors for infection or disease, the majority do provide descriptive epidemiological information and

Table 2. *Case reports and case series on autochtonhous hepatitis E infections in Europe, published 1998–2008 (Evidence level 1)*

Country	Diagnostic marker	No. HEV cases (any marker)	No. HEV RNA positive	Genotype	Age, sex	Estimated no. autochonthous cases (gt3 or non-travel)	Risk factors reported (Y/N)*	Comorbidity/ pre-disposing factors reported (Y/N) *	Ref.
Austria	IgM, IgG, RNA	1	1	gt3	65 yr, male	1	N	Y	[113, 114]
England	IgM, IgG, RNA	1	1	gt3	59 yr, male	1	Y	Y (biopsy)	[79]
	IgM, IgG, RNA	13	8	8 × gt3	Median 71 yr 46–85 yr, 85 % male	13	Y	Y	[39]
	IgM, RNA	28	16	15 × gt3	Mean 61·3 yr, M:F ratio 4·6	28	Y	Y	[49, 54]
	IgM, IgG, RNA	4	2	n.t.	61 yr, female; 62 yr, male; 82 yr, male; 19 yr, male†	3	Y	N	[115]
	IgM, IgG, RNA	8	2	1 × gt3 & 1 × gt1	36–78 yr, 50 % male	8	Y	N	[85]
	IgM, IgG, RNA	2	2	2 × gt3	40's yr, male; 65 yr, male	2	Y	Y	[95]
	IgM, RNA	1	1	gt3	58 yr, female	1	Y	N	[72]
	IgM, RNA	1	1	gt3	61 yr, male	1	N	N	[116, 117]
	IgM, IgG (1 × IgG only)	4	n.t.	n.t.	44 yr, female; 51 yr, female; 70 yr, male; 71 yr, female	4	N	Y	[118]
France	RNA	14	14	12 × gt3	28–67 yr, 11 male	14	Y	Y	[29]
	IgM, RNA	1	1	n.t.	52 yr, male	1	Y	Y (previously)	[28]
	IgM, IgG, RNA	1	1	gt3	72 yr, female	1	Y	Y	[87]
	IgM, RNA	1	1	gt3	7 yr, male	1	Y	Y	[55]
	IgG, RNA	1	1	gt3	35 yr	1	Y	N	[77]
	IgG, RNA	3	1	n.t.	52 yr, male; 47 yr, male†; 46 yr, female†	1	Y	N	[90]
	IgM, RNA	2	2	n.t.	1 male, 1 female (couple)	2	Y	N	[88]
	IgM, IgG, RNA	1	1	gt3	51 yr, female	1	Y	N	[119]
	RNA	7‡	7	5 × gt3	mean 65 yr ± 11, 5 male	7	Y	Y	[35]
	IgG, RNA	11‡	6	n.t.	mean 61 yr ± 13, 8 male	11	Y	Y	[120]
	IgG, RNA	23‡	18	14 × gt3	Mean 54·4 ± 16·6 yr†, 17 (74 %) male	20	Y	Y	[53]
	RNA	1‡	1	gt3	45 yr, male	1	Y	Y	[81]
	IgM, IgG, RNA	3	3	nt	41 yr, male and his wife and child	3	Y	N	[90]
	IgG, RNA	1	1	nt	28 yr, male	1	Y	Y (therapy)	[78]
	IgG, RNA	1	1	gt3	41 yr, female	1	Y	N	[121]
Germany	IgM, IgG, RNA	1	1	gt3	50 yr, male	1	N	N	[69]
	IgG, IgM	1	n.t.	n.t.	n.a.	1	Y	Y	[91]
Hungary	IgM, IgG, RNA	1	1	gt3	60 yr, male	1	Y	N	[70]
Italy	IgM, IgG (1 × IgM only)	2	n.t.	n.t.	45 yr, female; 60 yr, female	2	N	N	[122]
Spain	IgG, RNA	11	3	2 × gt3, 1 × gt1	Mean 55 yr† (range 28–71); 6 (67 %) male	7	Y	Y	[86]
	RNA	1	1	gt3	62 yr, male	1	Y	Y	[80]
The Netherlands	IgM, IgG, RNA	2	2	2 × gt3	2 Females	2	Y	Y	[26]
	IgG, RNA	2	2	n.t.	78 yr, female; 57 yr, male	2	Y	Y	[123]
	IgM, IgG, RNA	3	1	gt3	80 yr, female; 82 yr, female; 84 yr, male	3	Y	N	[124]

HEV, Hepatitis E virus; IgM/G, immunoglobulin M/G; n.t., not tested; n.a., not applicable as not covered by article; RNA, ribonucleic acid; yr, years.

* Reported risk factors and chronic comorbidity/pre-disposing factors are summarized in Appendix Table 1.

† Series included travel-related cases.

‡ Possible duplication of cases between reports.

description of the infecting HEV. In fact, in 23 of the 34 reports, information on the infecting genotype was given: of 75 HEV strains identified, 73 belonged to gt3. On most occasions, clinical reports included demographic information, details on symptoms and severity of disease and the questioning of patients on potential risk factors, including zoonotic ones. Nevertheless, it was not always clear from these case reports how thorough and structured the questioning was.

We identified nine seroepidemiological surveys carried out on acute non-A–C hepatitis patients (Table 3). Within this group 4–39% were shown to have acute hepatitis E highlighting the probable under-detection of HEV since these patients are often classified as patients with 'unknown hepatitis'. Most studies contained information on demographics, symptoms, and risk factor questioning. A proportion of the identified HEV cases were confirmed by reverse-transcriptase–polymerase chain reaction (RT–PCR), gt3 being the most frequent genotype (Table 3).

In animals, studies assessing HEV RNA prevalence and RNA sequence analysis are of interest. We identified 24 studies in eight European countries showing HEV RNA in animal samples, most often in domestic pigs (Table 4). The proportion of pigs excreting HEV in faeces ranged between 5% and 55%. A small number of the studies ($n = 7$) have detected HEV RNA from food items ($n = 2$) [44, 45], sewage samples of human ($n = 4$) [19, 46–48] and animal ($n = 3$) origin (Table 4), and from water ($n = 3$) [45–47].

Evidence levels 2 and 3

A total of 31 seroepidemiological surveys in humans were identified as EL2. Two of these reports contained cases also reported under EL1 [31, 49]. These studies were carried out in different groups of people (Tables 5 and 6) and compared groups considered at higher risk of acquiring HEV infection either to a group considered not be at risk or to a baseline – most often healthy blood donors. The seroprevalence rates in the different groups were compared, either prospectively or retrospectively, to see if those considered at higher risk have a higher seroprevalence. Care should be taken in comparing results between sero-surveys as different methodologies and diagnostic tests were used, and sometimes different markers for infection.

It is only in recent years that nationwide epidemiological risk factor analyses and enhanced surveillance (Table 7) have been conducted. Three studies, in the UK [50], The Netherlands [19] and France [51]

conducted enhanced surveillance and a standardized hypothesis-generating questionnaire was applied to cases of autochthonous hepatitis E so that risk-factor analysis could be undertaken. Through enhanced surveillance it was verified that a significant proportion of hepatitis E cases are indeed not travel-associated (Table 7). Currently, only one analytical epidemiological study, a case-control study in Germany, has been carried out on hepatitis E in Europe [52]. However, no European study has reported identical sequences between potential source and human HEV in a logical time order.

Identification of risk factors and transmission routes

Age and sex as risk factors

There is a general trend for men and older people being reported with HEV gt3 infection as found in EL1 case series (Table 2), EL1 seroprevalence studies in non-A–C hepatitis patients (Table 3) and EL2 case series (Table 7). Acute hepatitis E patients are significantly more likely to be older than hepatitis A patients [53, 54]. Studies assigned to EL1–3 [32, 50, 52] have found that autochthonous hepatitis E cases in Europe are significantly older than those who have travelled to hyper-endemic areas. At present, only one HEV gt3 case has been reported in a child aged <15 years, and this was an immunocompromised boy who received infected blood by transfusion [55]. A number of EL2 comparative seroprevalence studies (Table 6) have also found an association between increasing age and anti-HEV IgG prevalence. In contrast to the case series in which about 70% of HEV cases are male (Tables 2 and 7), being male is rarely associated with seropositivity for anti-HEV IgG. An explanation for the higher proportion of men and older people among acute hepatitis E cases is not provided by any study. However, the lack of an association in the sero-prevalence studies conducted to date could suggest that the dominance of male cases is due to a higher risk for overt disease manifestations rather than for infection.

Comorbidity as a risk factor

Increasingly, cases of acute hepatitis E are being reported with chronic liver disease, liver cirrhosis, or history of high alcohol consumption (Table 2). Additionally, other comorbidities like diabetes mellitus, compromised immune status, hypertension, obesity, arthritis, ischaemic heart disease or previous HAV

Table 3. *Studies identifying autochthonous hepatitis E virus (HEV) cases among undiagnosed non A–C hepatitis patients (Evidence level 1)*

Country	Period when samples collected (duration)	Diagnostic marker	No. anti-HEV positive/total non-A–C (%)	No. RNA pos.	Genotype	Estimated no. autochthonous cases (gt3 detected *or* no travel reported)	Age and sex of autochthonous cases	Reported risk Factors for autochthonous cases (Y/N)*	Ref.
England & Wales	1996–2003	IgM, IgG, RNA	186/478 (38·9%)	11	gt3	17	Mean 68 yr (range 51–83), 13 (76%) male	Y	[32]
	1999–2007†	IgM, IgG, RNA	42/unknown	26	gt3	40	Median age 65 yr (35–86), 31 male	Y	[31]
	1998–2004†	IgM, IgG, RNA	23/333 (6·3%)	16	gt3	21	Median age 67 yr (35–83), 15 male	Y	[67]
France	2001–2002	IgG, RNA	46/431 (10·7%)	16	gt3	16	Mean age 52·2 (range 17–78), 13 male	Y	[125]
Italy	1994–1997	IgM, IgG, RNA	22/218 (10·1%)	6	1 × gt3	5	—	Y	[102] [126]
Spain	1989–1999	IgG, RNA	3/37 (8·1%)	3	2 × gt3	2	—	Y	[76]
	1999–2001	IgM (+WB), RNA	30/336 (8·9%)	1	gt3	1	—	N	[127]
The Netherlands	2002–2004	IgM, IgG (+WB), RNA	45/1027 (4·4%)	24	16 × gt3 8 × gt1	16	—	N	[6]
	2000–2002	IgM, IgG (+WB), RNA	11/209 (5·3%)‡	3	gt3	3–11	34 yr, male; 48 yr, male; 56 yr, male	N	[41]

IgM/G, immunoglobulin M/G; RNA, ribonucleic acid; WB, Western blot; yr, years.

All IgG and IgM by enzyme immunoassay unless WB also carried out which means confirmation also done by WB, all RNA detected through RT–PCR.

* Reported risk factors are summarized in Appendix Table 2.

† Overlapping patients.

‡ Comparison of two IgG diagnostic tests.

Table 4. *Studies on hepatitis E virus genotype 3 PCR-positivity in animal and animal sewage* (*Evidence level 1*)

Country	Sample	Source of virus detection	Positive/tested (where known)	Prevalence (%)	Ref.
England	Pig	Tissue and faeces	Faeces: 9/40 Faeces and tissue: 2/2	26–76	[62]
		Faeces	—	—	[79]
		Faeces and slurry	Faeces: 42/200 Slurry: 2/9	5–35 (mean 21·5) 22	[74]
France	Pig	Faeces (sewage)	5	—	[75]
Germany	Wild boar	Serum	10/189	5·3	[128]
Hungary	Pig	Faeces	—	—	[71]
		Serum (piglets only)	4/18	22·2	[129]
	Wild boar	Faeces	—	—	[71]
	Deer	Faeces	—	—	[71]
Italy	Pig	Faeces and serum	Faeces: 2/34 Serum: 0/22	5·9 0	[66]
		Faeces	115/274	42	[63]
	Wild boar	Bile	22/88	25	[130]
The Netherlands	Pig	Faeces	—	14–55	[131, 132] [45]
		Muscle	20/39	51·3	[45]
	Wild boar	Faeces	1/26	3·8	[45]
Spain	Pig	Serum, faeces, bile, liver, lymph nodes	—	15–50	[47, 59–61, 64, 65, 133]
		Sewage	4/4	100	[46]
		Faeces (slaughterhouse sewage)	1/12	8·3	[76]
	Wild boar	Serum	27/138	19·6	[68]
Sweden	Pig	Faeces	71/290	24	[134]

infection have been reported in acute hepatitis E cases. Even though some of these comorbidities were more likely associated with the comparatively older age of the patients, pre-existing diseases or behaviour affecting the liver do seem to have an impact on whether HEV infection results in clinical disease. Enhanced surveillance in France noted that 11/47 (23%) patients had high alcohol consumption [51], and the EL3 case-control study in Germany [52] found that a higher but non-significant percentage of cases than controls (6·7% *vs.* 1·5%) had chronic liver disease.

Although four EL2 seroepidemiological studies have also found that patients with comorbidities [5, 56–58] have a higher anti-HEV IgG positivity in comparison to the reference group a larger number of studies found no association (Table 6). This further supports the above-mentioned hypothesis that comorbidities affecting the liver are mainly associated with a higher risk of clinical disease but not infection.

Potential for zoonotic transmission by direct animal contact

The detection of widespread HEV gt3 RNA suggests that swine HEV is ubiquitous and epizootic in pigs in Europe (EL1) (Table 4). Infection in pigs can be symptomatic [59–61] but most often infections remain clinically undetected [47, 62–65]. Close relatedness has been found between HEV gt3 nucleotide sequences from pigs and wild boar and those from human cases [6, 32, 41, 51, 60, 61, 63, 64, 66–71]. HEV carried by a human case in the UK had 100% amino-acid sequence homology to a UK pig HEV sequence but no transmission link between them could be defined [72]. Such sequence comparison data suggest that in Europe, HEV carried by pigs and humans are genetically closely related and that cross-species transmission is occurring.

In a recent transmission study the R_0 (which defines the average total number of new infections caused by one typical infectious animal during its entire infectious period in a completely susceptible population) for transmission of HEV in pigs was estimated to be 8·8 under experimental conditions [73]. This indicates that HEV is able to spread efficiently between pigs and supports the hypothesis that pigs are a reservoir for HEV. During HEV infection, the virus is shed mostly via faeces. Consequently, HEV RNA has been found in the pig farm environment (Table 4) including

Table 5. *Comparative seroepidemiological studies assessing the risk of zoonotic hepatitis E virus (HEV) infection in occupational groups (Evidence level 2)*

Country	Association with occupation	Risk group		Control group		Diagnostic marker	Ref.
		Occupation	No. anti-HEV IgG-positive/total (%)	Occupation	No anti-HEV IgG-positive/total (%)		
Austria	Yes	• Swine farmers • Swine slaughterhouse workers • Hunters • Poultry slaughterhouse workers	39/153 (25·5%) 27/95 (28·4%) 22/149 (14·8%) 4/46 (8·7%)	Townspeople (general population)	4/35 (11·4%)	IgG, IgM, RNA	[135]
Denmark	Yes	Farmers (sera from year 1983)*	144/286 (50·4%)	• Healthy blood donors (1983)* • Healthy blood donors (2003)*	55/167 (32·9%) 94/456 (20·6%)	IgM or IgG	[5]
France	Yes†	None	—	Healthy blood donors*	88/529 (16·6%)	IgG	[136]
Italy	No	• People professionally exposed to swine	3/92 (3·3%)	General population	101/3511 (2·9%)	IgG	[137]
Moldova	Yes	• Healthy swine farmers	135/264 (51·1)	General population	63/255 (24·7)	IgG	[92]
The Netherlands	Yes	• Healthy swine veterinarians • Healthy non-swine veterinarians	x‡/49 (11%) x‡/153 (6%)	General population	x‡/644 (2%)	Bayesian stochastical model combined IgG and IgM results	[22]
	No	• Healthy swine veterinarians • Healthy non-swine veterinarians	8·5% 2·3%	General population	6·4%	Diagnostic algorithm, IgG ELISA confirmed by WB	[22]
Spain	Yes	• People professionally exposed to swine	19/101 (18·8%)	General population unexposed to swine	4/97 (4·1%)	IgG: ELISA confirmed by WB	[94]
Sweden	No	• Healthy swine farmers	15/115 (13%)	Non-farmers (matched)	10/108 (9·3%)	IgG	[8]

ELISA, Enzyme-linked immunosorbent assay; IgM/G, immunoglobulin M/G; RNA, ribonucleic acid; WB, Western blot; yr, years.
* Risk analysis done retrospectively in each group after administering questionnaire to those tested.
† Hunting found to be an independent predictor in multivariate analysis ($P = 0.038$). Of those that did not travel, majority lived in rural areas ($P = 0.07$).
‡ Bayesian stochastical model combined results of different assays therefore exact numbers cannot be given.

Table 6. *Comparative seroepidemiological studies (IgG) assessing risk factors other than zoonotic exposure (Evidence level 2)*

Risk factor	Association found for: country [ref.]	No association found for: country [ref.]
Pre-existing conditions/comorbidity		
Chronic viral hepatitis patients, HBsAg-positive, chronic liver disease	HBsAg-positivity: Albania [58]*, Greece [57]*†; Chronic viral hepatitis patients: Greece [57]*†; Chronic liver disease: Albania [58]*	HBsAg positive: Greece [98]*, Italy [141]*; chronic liver disease: Albania [140]*, Portugal [142], England [31]*‡
Antibodies against other viral hepatic diseases (previous acute infection)	Anti-HAV-positivity: Denmark [56]*; Anti-HBc-positivity: Greece [57]*†	Anti-HAV: Italy [101]*†, Germany [99]*§, England [49]*‡; anti-HBc: Greece [98]*, Albania [58]*; anti-HBV: Denmark [56]*, Greece [98]*, Italy [141]*; HCV-infected children: Spain [97]†
Healthcare facility and parenteral exposures		
Hospitalization and surgery	Minor surgery and abdominal surgery: Spain [4]*	Hospitalization, surgical intervention, abortion, endoscopy, dental extraction, administration of gamma globulin: Spain [4]*; HCWs (general): Germany [99]*§, Spain [4]*
Healthcare workers (HCWs)	HCWs working in emergency admission and surgery: Germany [99]*§	HCWs (general): Germany [99]*§, Spain [4]*
Haemodialysis and transfused patients	Haemodialysis patients: Greece [98]*, [57]*†, Italy [96]†, Spain [97]†	Haemodialysis patients: Spain [4]*, Sweden [139]*; Haemodialysis for >33·5 (median) months: Greece [98]*; Transfused patients: Greece [57]*†, [98]*, Spain [4]*
Needle exposures	Tattoos: Denmark [56]*; injecting drug users with HBV exposure: Sweden [138]*	Tattoos: Denmark [56]*, Spain [4]*; injecting drug users: Denmark [56]*, Greece [57]*†, Italy [96]†, Spain [4]*; Piercing, acupuncture, injections: Spain [4]*
Demographics		
Age	Increasing prevalence with age: Denmark [56]*, Switzerland [93]*; Albania [58]*, Spain [4]*, Italy [101]*†, France [3]*, Sweden [139]*, England [31]*‡, Albania [140]*	Greece [57]*† [98]*, Germany [99]*§
Male	Spain [4]*, England [31]*‡	Denmark [56]*, Greece [98]*, Italy [141]*, Italy [101]*†, Spain [4]*, Germany [99]*§, Switzerland [93]*, France [3]*, Albania [58]*
Specific population categories		
Parity	Women being uni- or multiparous: Spain [4]*	No. of children: Germany [99]*§; pregnant women: Albania [140]*, Spain [143]
Migrant status	Immigrants: Italy [96]†; refugees: Greece [57]*†	Immigrants: Spain [144]†, Greece [98]*
Housing	>4 people in household: Italy [101]*†	
Education	<8 years schooling: Albania [58]*	>8 years schooling: Italy [101]*†; education: Switzerland [93]*
Occupation	Airline flying personnel: Norway [100]	Workers exposed to sewage: Switzerland [93]*; general occupation, years of occupation: Germany [99]*§
Other possible exposures		
Travel to endemic area	Airline flying personnel: Norway [100]	Travel to endemic area: Italy [141]*; travel to endemic area >5 years: Denmark [56]*, Switzerland [93]*

* Result is from retrospective study done in a certain population group (e.g. haemodialysis patients, blood donors, prisoners, healthcare workers) rather than comparing a previously identified risk group to a control group [either as this was (i) original study design, or (ii) in studies with risk and control groups that then looked at subgroups within these, (iii) studies which looked at risk factors within whole HEV positive population, i.e. risk and control group merged]. Study in Denmark [56] included two different population groups: prisoners and drug-treatment-centre patients.

† Confirmation by immunoblot/Western blot.

‡ Report includes other study type of EL1 – [31] is also reported in Table 3 and [49] in Table 2.

§ Significant result from logistic regression.

Table 7. *Analytical studies (Evidence level 3) and case series with risk factor analysis (Evidence level 2) for hepatitis E virus (HEV) infection or hepatitis E*

Country	Study period	Study design	Source of cases	Case definition	No. interviewed/ total no. with autochthonous hepatitis E	No. genotype 3/no. genotyped	Demographics	Ref.
Germany	2006–2007 (16 months)	Case-control study (45 cases, 135 controls), enhanced surveillance	State and local health offices in Germany	Clinical manifestation and IgM or PCR positive	45/66	14/15	Median age 46 yr (33% ⩾ 50 yr) 76% male	[52]
England & Wales	2005 (12 month)	Case series, Enhanced surveillance	Diagnostic laboratories in England and Wales	IgM or rising IgG or PCR positive	28/33	14/33	Median age 65 yr (70% > 55 yr) 82% male	[50]
France	2004–2007 (48 months)	Case series, enhanced surveillance	French general hospitals (affiliated to the ANGH) gastro-enterological departments	ALT >10× higher than normal value plus rising IgG, or IgM, or PCR	47/53	14/14	Mean age 56·1 ± 15·6 yr (for 53 cases) 68% male	[51]
The Netherlands	2004–2006 (24 months)	Case series, enhanced surveillance	99 diagnostic laboratories in The Netherlands	Patient with acute viral hepatitis – HEV IgM and/or PCR positive, without travel to endemic country 3 mos. prior to onset of illness	19/19	13/13	Median age 50 yr (range 25–84) 90% male	[19]

ALT, Alanine aminotransferase; ANGH, Association Nationale des Hépato-Gastroentérologues des Hôpitaux Généaux (a national network of 96 Hepato-gastroenterology centres in France); IgM/G, immunoglobulin M/G; PCR, polymerase chain reaction; RNA, ribonucleic acid; WB, Western blot.

in pig sewage [46, 74, 75], slaughterhouse sewage [76], ditch manure [65] and a pig water trough [64].

Several EL1 case reports (Table 2) and seroprevalance studies in non-A–C hepatitis patients (Table 3) have alluded to pig contact (i.e. from working with pigs to living in a rural area) as being potential risk factors for the acquisition of HEV infection [32, 35, 39, 53, 67, 77–81]. Anti-HEV IgG seroprevalence studies have reported that those working with pigs are at increased risk of infection in some countries in Europe (Austria, Denmark, Moldova, Spain), but not in others (France, Italy, Sweden) (Table 5). In The Netherlands, an increased risk has been found for veterinary surgeons working with pigs when Bayesian statistics were applied to a set of five tests, but no increased risk was found when a validated diagnostic protocol was followed [22]. Apart from one patient in the case series in France being given a pet pig 8 weeks before onset of fatigue [51, 82], remarkably, there seems to be no correlation between pig contact and being a hepatitis E case in the EL2 enhanced surveillance studies and the German EL3 case-control study [52]. Despite the ubiquity of HEV in a considerable proportion of pig herds in Europe, there have been no studies proving that contact with pigs is a risk factor for developing hepatitis E.

HEV gt3 RNA has been isolated from wild boar in a number of European countries (Table 4) and deer have also been found positive for HEV gt3 in Hungary [71]. In The Netherlands, HEV RNA was not detected in faeces of a pet pig, horses, cows [19], musk rats [45] or in liver tissues in dogs with hepatitis [83], but only a few samples were analysed. Having pets (dogs and cats) [19, 50], contact with horses [5, 19], hunting or living in rural areas (Table 5) was reported to be associated with HEV infection in some EL2 studies. The German EL3 case-control study did not find an association, indicating that further analytical studies are needed.

Potential for foodborne zoonotic transmission

Recent studies in Spain [59, 64, 65] and Italy [63] have shown that RNA is present in faeces, serum and liver of pigs at the age of going to slaughter, which implies that infected meat and organs are potentially on the market. Two studies in Europe (EL1) have attempted to isolate HEV from pig livers. In The Netherlands, 4/62 (6·5%) pig livers on retail sale were found positive for HEV RNA and sequencing analysis found HEV gt3 to be closely related to HEV gt3 of pigs (97%) and humans (93%) in The Netherlands [44].

In contrast, no pig livers were found positive in a study conducted in 80 retail outlets in Cornwall, UK [84]. Moreover, in The Netherlands, 20/39 (51%) muscle samples from experimentally infected pigs, used as proxies for pork at retail, were found positive [45]. Positive livers contained infectious HEV as revealed by the virus extracted from them being used to successfully infect pigs. However, this success appears to be dose-dependent as it only occurred when the inoculum contained a high dose of virus [44].

EL1 case reports (Table 2) and seroprevalence studies in non-A–C hepatitis patients (Table 3) have suggested that the following food items are risk factors for the acquisition of HEV infection: pork pies [85], liver paté [39], wild boar [77, 86], under-cooked or raw pork [67, 72, 87, 88], home-made sausages [70], meat (in general) [31, 67], unpasteurized milk [78], shellfish [32, 39, 67, 85, 89, 90] and ethnic foods [91]. Handling raw pig liver is also suggested [67]. Unpasteurized milk was associated with HEV infection in an EL2 seroepidemiological study [92]. In a risk factor EL2 analysis using a structured questionnaire, dried sausages and uncooked shellfish (mussels, oysters, or both) were considered risk factors associated with cases in The Netherlands and France, respectively, but these studies did not use controls [19, 51]. In the EL3 case-control study in Germany, Wichmann et al. found that eating any offal or wild boar was independently associated with autochthonous HEV infection [52]. No significant association was found for other food products from other animals including shellfish.

Potential for transmission through human sewage

HEV RNA has been detected in raw human sewage in Barcelona, Spain [19, 46–48] and in France [47]. An EL2 study among sewage workers in the canton of Zurich did not find a higher seroprevalence in those exposed to waste water [93]. In the EL3 case-control study, one of the 45 autochthonous cases had contact with sewage, but there was no statistical difference compared to the control group [52].

Potential for water-borne transmission

A number of EL1 case reports have described water exposure as a possible risk factor for acquiring HEV infection: swimming in a lake [90], through exposure to water from a high- pressure hose, and water sourced from a stream which ran adjacent to a free-range pig farm [79]. In The Netherlands 2/12 (17%) of

monthly samples of river water used for drinking water production and recreation were found positive for HEV RNA in two separate years [45]. By contrast, river water samples in Spain were all found to be negative [46].

During EL2 enhanced surveillance in The Netherlands, HEV RNA was found in a water sample taken from outside a patient's home and it had an identical sequence to that of the patient but it was thought more likely that the patient contaminated the water [19]. An EL2 risk factor study conducted in France found that 15 cases (32%) drank from their own water supply (spring or well) [51] and 1/35 samples taken from the spring was positive for HEV RNA. An EL2 seroprevalence study found that untreated water consumption was a risk factor (OR 5·6, $P = 0.01$) for anti-HEV IgG seroprevalence confirmed by immunoblot [94]. Conversely, the EL3 case-control study found that drinking well water and contact with surface was not related to HEV infection [52]. No waterborne outbreaks have been reported in Europe to date.

Potential for parenteral transmission

As HEV does not usually produce a chronic carrier state, non-enteric (faecal–oral) sources were not considered as an important cause of HEV transmission. However, cases linked to blood transfusion have now been reported in England [95] and France [55]. In The Netherlands, enhanced surveillance using a structured questionnaire detected two cases of hepatitis E who had previously received blood transfusions [19]. The German EL3 case-control study did not identify blood transfusion as a risk factor, which shows that parenteral transmission does not play a significant role in Germany (none of 46 included autochthonous cases had received a recent blood transfusion) but does not exclude that parenteral transmission in single cases is possible [52]. In France, a student acquired HEV 7 weeks after practising surgical procedures on a pig [77].

The majority of EL2 seroepidemiological studies looking into risk factors involving needle exposure and many types of hospital exposure did not find a significant difference between groups tested (Table 6). However, haemodialysis patients in four different studies [57, 96–98], those who had undergone minor surgery and abdominal surgery in Spain [4], health-care workers in emergency admission and surgery in Germany [99] and male prisoners and drug treatment patients in Denmark [56] were found to have a

higher anti-HEV IgG prevalence than the reference group.

Potential for other transmission routes and risk factors

Some seroepidemiological studies have found other risk groups to have higher anti-HEV antibody prevalence in comparison to a reference group (Table 6). For immigrants [96], refugees [57] and probably airline flying personnel [100], the HEV antibodies probably did not result from HEV gt3 infection but from gt1 infection when staying in an hyper-endemic country. In contrast, three seroepidemiological studies did not find an association with travel to endemic areas (Table 6) and in one case-control study no association was found with travel within Europe or close contact with returning travellers [52]. The finding of an association with a larger family size supports the possibility of person-to-person transmission [101] as has been reported for gt1 and gt2 HEV infection and is further suggested in a case series from France [90] and a case in Italy [102]. However, efficient person-to-person transmission is unlikely since very few reports mention outbreaks or family clusters of cases. In the EL2 enhanced surveillance conducted by Borgen et al. in The Netherlands it was specifically asked if any family members or close contacts had signs of hepatitis in the months preceding or following the disease period of the cases, but no positive responses were elicited and a limited contact investigation found no evidence of HEV infection [19]. During the enhanced surveillance conducted for the case-control study in Germany, unstructured household investigations identified five additional IgM-positive HEV cases in three households of autochthonous cases [52], one of whom was PCR-positive. All were asymptomatic or complained only of mild abdominal pain or fatigue. Due to possibility of asymptomatic infection and the wide range of the incubation period, it was not possible to say if these were cases due to person-to-person transmission or if they were infected through the same source (in one household the whole family ate wild boar, in another they had all eaten offal).

In contrast to hyper-endemic countries [11], there have been few studies and no reports of sexual transmission or vertical transmission during pregnancy in Europe. Seroepidemiological studies have found no association with sexual contact [4] or pregnancy (Table 6). One HEV infection during pregnancy occurred in Germany but without complications [52].

DISCUSSION

This review found that there is no evidence for one main transmission route or risk factor for HEV infection or disease in Europe. Rather, multiple routes of transmission are likely to exist. Autochthonous HEV gt3-associated hepatitis E cases are on average older than the general population and predominantly male, which could be due to higher likelihood of pre-existing comorbidity, different dietary preferences and differences in other behaviours. Our results do indicate that zoonotic transmission of HEV, either by direct contact or food, is likely due to the following observations: (1) the virus is highly prevalent in domestic pigs, wild boar and to a lesser extent in deer; (2) animal stool and tissues may contain sufficient viral loads to be infectious; (3) high sequence relatedness has been found between animal and human HEV gt3 isolates; (4) several EL2–3 studies have identified zoonotic risk factors. It should be noted that not all autochthonous infections were attributable to these factors. Other routes of transmission reported in hyper-endemic countries do not appear to be applicable to gt3 HEV infection, and our results therefore provide further evidence for a unique clinical and epidemiological identity of disease conferred by this genotype [12]. We also found evidence suggesting that cases of hepatitis E with underlying diseases are at increased risk of developing clinical manifestations or severe disease.

Current epidemiology suggests that HEV gt3 is the predominant strain circulating in pigs in Europe and that pigs are the natural host [20, 103]. However, the virus can also infect a considerable proportion of the human population, but only a minority appear to develop symptoms [3–8]. The key to understanding HEV transmission in Europe may lie in determining how the virus that circulates among pigs is transmitted to humans in sufficiently high doses to cause infection or overt disease. Direct animal contact could lead to infection associated with low-dose exposure, thus one could argue that infection does not occur or that the resulting infection is more likely to be asymptomatic. Larger doses through, for example, the consumption of heavily contaminated and insufficiently cooked meat could lead to infections that are more likely to result in clinical symptoms. Occasional instances of HEV transmission through sewage or consumption of contaminated water seem possible, if a sufficiently high dose of HEV is ingested. The failure of the virus to generate an infecting dose when excreted by humans could be a plausible explanation for the low person-to-person transmission rates.

Evidence for foodborne zoonotic transmission of HEV in Europe is generally weak but this is mainly because of an insufficient number of good analytical epidemiological studies. The only case-control study conducted to date has suggested that, at least in Germany, foodborne zoonotic transmission is the main route [52]. Additional strong evidence for foodborne transmission is available from Japan where sequence identity between deer meat [104, 105] and hepatitis E patients who consumed that meat, and HEV genetic similarity between boar meat and patient samples [106], have been found. HEV has been shown to be viable and infectious in pigs' liver in the USA [107] and resistant to temperatures up to 60 °C [108]. Consequently, the role of foodborne transmission in European countries other than Germany should be assessed through further analytical studies. Interestingly, consumption of bivalve molluscs [32, 39, 51, 67, 85, 89, 90] and contact with horses [5, 19] are mentioned as possible sources of infection in studies we reviewed. Even though anti-HEV antibodies have been detected in many other animal species including rats, cats, monkeys, dogs, cattle, sheep, goats and mongooses [17, 109, 110] it is not clear if these animals can be infected with the HEV strains known to date, and thus their role as possible reservoir is doubtful. Nonetheless, the possibility of another reservoir than swine transmitting HEV to swine and humans cannot be excluded.

It is of interest that questioning of HEV patients for enhanced surveillance studies and the case-control study (Table 7) often found evidence for food exposures whereas a number of seroepidemiological studies conducted in healthy populations (Table 5) found other risk factors such as animal contact. This could be due to confounding factors such as different dietary behaviour in rural areas where there is also more direct animal contact than in urban settings. Since case series and case-control studies involve interviewing patients with a known date of onset of hepatitis E symptoms it is also possible to enquire about the consumption of specific food items during the incubation period, but one should be aware of recall problems due to length and variation of incubation periods. This kind of assessment is not possible for seroepidemiological studies since the date of HEV infection cannot be estimated. Moreover, seroepidemiological studies assess risk factors for infection

whereas case series and case-control studies assess risk factors for clinically overt disease. Risk factors for asymptomatic HEV infection might be different from risk factors for hepatitis E disease if, as suggested above, the ingested dose of HEV determines whether infection subsequently leads to overt clinical disease. A clear limitation especially of less recent sero-epidemiological studies is the use of poor diagnostic kits and the results of these studies should be interpreted with caution.

When the opportunity presents, routes of transmission other than zoonotic ones may exist. The parenteral route of transmission has been documented in people who received HEV-contaminated blood transfusions [55, 95] and in a student practising surgical procedures on a pig [77]. It is plausible that patients develop disease in these instances due to the high infectious dose inoculated. Person-to-person spread of HEV is an uncommon event but this is not surprising if, as proposed earlier, infection and disease are dose-dependent [44] and require a higher infective dose than, for example, for HAV transmission.

This is the first systematic review attempting to define sources and transmission routes of HEV infection and disease. In conducting this review we found that studies of higher grade evidence are lacking and further case-control and cohort studies are required, preferably population-based and using sensitive diagnostic techniques such as Western blot and RT–PCR for HEV confirmation. Standardization of questionnaires and studies across a number of European countries would also assist evaluation and comparison of data. Where possible, water and food samples of HEV patients should be analysed and HEV strains should be genotyped to attempt to link their infection to a source [104, 106]. Further studies are also required to test for HEV RNA in potential commercial food sources in Europe (e.g. pork and pig offal, and wild boar meat) and in potential animal reservoirs other than pigs.

The lack of evidence for one main transmission route or risk factor, and the high possibility for multiple transmission routes, for HEV gt3 infection and disease in Europe limits the possibility to propose effective intervention strategies. However, proper cooking of all pork products and prevention of cross-contamination in the kitchen are generally accepted guidelines that should help to prevent HEV infections. Although the role of direct zoonotic or environmental transmission is still unclear, consideration should still be given to improve education of occupationally HEV-exposed people such as pig farmers and sewage workers. Although evidence for parenteral transmission is limited, it is recommended that a risk assessment, including determination of the frequency of HEV contamination, be conducted to determine whether general screening of organs for transplantation and blood products should be undertaken.

Several HEV vaccine candidates based on HEV gt1 recombinant capsid protein have been developed. To date, two have progressed to human clinical trials with promising preliminary results [111, 112]. Whether these vaccines provide long-term immunity to hepatitis E still needs to be established. Even though HEV gt1–4 are considered to belong to one serotype, whether they have similar efficacy to protect from HEV gt3 infections requires further study. If licensed, the main target of the vaccine would be those living in or travelling to HEV hyper-endemic countries but consideration should also be given to people living in industrialized countries with low HEV endemicity who are at risk of a severe outcome (e.g. patients with chronic liver disease or transplant organ recipients).

In summary, this first systematic review of HEV in Europe found that there is no evidence for one main transmission route or risk factor for HEV although evidence points towards zoonotic transmission. Multiple routes of transmission are likely to exist, and these need to be further defined so that specific and appropriate preventive measures can be implemented.

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DECLARATION OF INTEREST

None.

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Appendix Table 1. *Risk factors in case reports and case series on autochthonous hepatitis E infections in Europe, published 1998–2008 (Evidence level 1)*

Country	Estimated no. autochthonous cases (gt3 or non-travel)	Risk factors implicated for autochthonous cases	Chronic comorbidity/pre-disposing factors	Ref.
Austria	1	None identified.	• Reported HAV in 1960s	[113, 114]
England	1	Work involved being heavily splashed with water from using a high pressure hose. Water extracted from a stream which ran adjacent to a free-range pig farm. Pigs on this farm were HEV positive but of non-identical sequence homology. Stream and pond samples were negative for HEV	• Previously consumed 40 units alcohol/week • Type 2 diabetes mellitus • Previously undiagnosed cirrhosis on biopsy	[79]
	13	2 ate shellfish, 3 ate liver paté of unspecified animal origin. 38% dog owners	• 4 × type 2 diabetes mellitus • 3 × hypertension, • 1 × high alcohol intake	[39]
	28	5 travelled to non-endemic areas	• 3 × alcohol intake >28 U/week • 3 × diagnosed with cirrhosis (2 × alcoholic, 1 × idiopathic)	[49, 54]
	3	All consumed meat products including pork	• None identified	[115]
	8	gt1 patient was Asian and had contact with jaundiced individual (person to person transmission likely). Of the other 7: 1 daily consumption of pork pies, 1 frequently consumed shellfish	• None identified	[85]
	2	Blood transfusion for 1 recipient. No risk factors identified for donor	• Donor: none • Recipient: B-cell lymphoma	[95]
	1	Had eaten raw sausage and bacon but >3 months prior to illness	• None identified	[72]
	1	None identified	• None identified	[116, 117]
	4	None identified	• 1 previously reported viral-type illness with jaundice and was HAV IgG-positive	[118]
France	14	2 reported contact to animals (1 with chickens and rabbits, 1 with wild birds)	• All with organ disease and immuno-suppressive therapy: 3 × liver, 9 × kidney, 2 × combined kidney and pancreas transplants	[29]
	1	Consumed pork (no comment on cooking)	• Kidney transplant recipient 2 years previously	[28]
	1	Occasional consumption of medium-cooked pig meat including in incubation period	• Hypertension • Obesity	[87]
	1	Blood transfusion	• Had received chemotherapy for kidney rhabdoid tumour	[55]
	1	Consumed wild boar but 4 days before onset, surgical training with pigs 7 weeks before onset thought most likely source	• None identified	[77]
	1	Consumed uncooked shellfish during travel in Greece 1 month before onset, Contamination in France can not be ruled out	• None identified	[90]

Country	N			Ref
	2	On holiday within France. Ate undercooked pig meat 4 weeks before onset of jaundice	• None identified	[88]
	1	Had travelled to HEV endemic areas (Egypt & Nepal) but gt3 isolated. Rarely ate undercooked meat	• None identified	[119]
	7	2 travelled in Europe (Spain & Portugal), 1 lived on a barge, 5 lived in rural areas, 1 had pet cat	• 5 × active drinkers (>40 g/day), 6 × chronic liver disease	[35]
	11	1 travelled to Portugal month before, 1 lived in La Reunion, 1 owned dog, 1 owned cat, 1 owned dog and cat	• 45% active drinkers	[120]
	20	7 travelled within Europe, 6 lived in rural areas, 12 had household pets incl. cats, dogs and canaries. 1 had poultry	• 7 × excessive alcohol consumption, 3 × chronic liver disease, 1 liver cirrhosis, 1 type 2 diabetes mellitus	[53]
	1	Lives in rural community and hunts partridge	• T-cell lymphoma with chemotherapy and stem-cell transplant	[81]
	3	Man and wife consumed shellfish. Family swam in lake. Intrafamilial transmission possible	• None identified	[89]
	1	Lived in rural region, visited farm from time to time, drank unpasteurized milk	• Renal transplant 14 years ago, on immunosuppressive therapy	[78]
	1	Hobby gardening. Poultry near house	• None identified	[121]
Germany	1	None identified	• None identified	[69]
	1	Patient Turkish – had eaten ethnic foods (maize flour, dried nettle, sheep cheese, mushrooms in salt water) brought as a gift by Turkish visitor	• Serological markers for past HAV, HBV, CMV, EBV, HSV infection. Eggs for *Ascaris lumbricoides* in stools	[91]
Hungary	1	Ate pork sausages pre-prepared from house slaughtered meat 1 month previously	• None identified	[70]
Italy	2	None identified	• None identified	[122]
Spain	7	One presumptive case (IgG-positive only) ate boar meat	• 1 × IgM anti-HBc • 1 chronic HCV infection	[86]
	1	Slaughterhouse worker	• Type 2 diabetes mellitus • Weekly consumption of 7 l beer and 1·5 l cognac	[80]
The Netherlands	2	Suggest that immunosuppressed long-term carriers of HEV may be a source	• Both liver transplant recipients	[26]
	2	None identified (speculate about pig meat)	• 1 × used bisphosphonate and a proton pump inhibitor daily. • Other history of epilepsy, hypertension, diabetes, hypercholesterolaemia, and ischaemic heart disease	[123]
	3	Had contact with domestic animals – 1 with cat, 1 with dog and 1 with goat. All drank tap water	• None identified	[124]

CMV, Cytomegalovirus; EBV, Epstein–Barr virus; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; IgG, immunoglobulin G; RNA, ribonucleic acid.

Appendix Table 2. *Risk factors in studies identifying autochthonous hepatitis E virus (HEV) cases among undiagnosed non-A–C hepatitis patients (Evidence level 1)*

Country	Estimated no. of autochthonous cases (gt3 detected *or* no travel reported)	Risk factors for autochthonous cases	Ref.
England & Wales	17	Comparing non-travel- to travel-associated cases: significantly more non-travel patients lived in dense pig-holding areas or in coastal/estuarine areas. Two cases habitual shellfish consumption	[32]
	40	All were pork eaters. 21 retired, 2 unemployed. One worked as a butcher. Seasonal variation of HEV case numbers with peaks in spring and summer. 3 patients with previously undiagnosed cirrhosis. HEV IgG increased with age (also did seroprevalence study comparing risk groups)	[31]
	21	One worked as butcher. One fed cat raw pigs' liver regularly. One had contact with pigs. All ate pork (2 uncooked), 7 shellfish. 9 kept pets. 14 lived in urban, 16 within 10 km of coast or estuarine settlements. 1 became anti-HAV + 1 week after hospitalized.	[67]
France	16	2 had travelled to Tunisia, 3 to Spain. Not questioned for other risk factors.	[125]
Italy	5	1 case person-to-person transmission.	[102, 126]
Spain	2	1 was an injecting drug user (RNA also detected in slaughterhouse sewage with 92–94% nucleotide homology to human strains)	[76]
	1	No questioning.	[127]
The Netherlands	16	No information on questioning but 'did not find formal proof of zoonotic link',1 patient anti-HAV antibody positive	[6]
	3–11	Did not find clustering of cases with anti-HEV antibodies in rural areas. Significant differences between hepatitis patients and matched control group were found for age groups 41–60 years	[41]

HAV, Hepatitis A virus; IgG, immunoglobulinG; RNA, ribonucleic acid.