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Hepatitis E virus is the leading cause of acute viral hepatitis in Lothian, Scotland

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Abstract

Acute viral hepatitis affects all ages worldwide. Hepatitis E virus (HEV) is increasingly recognized as a major cause of acute hepatitis in Europe. Because knowledge of its characteristics is limited, we conducted a retrospective study to outline demographic and clinical features of acute HEV in comparison to hepatitis A, B and C in Lothian over 28 months (January 2012 to April 2014). A total of 3204 blood samples from patients with suspected acute hepatitis were screened for hepatitis A, B and C virus; 913 of these samples were also screened for HEV. Demographic and clinical information on patients with positive samples was gathered from electronic patient records. Confirmed HEV samples were genotyped. Of 82 patients with confirmed viral hepatitis, 48 (59%) had acute HEV. These patients were older than those infected by hepatitis A, B or C viruses, were more often male and typically presented with jaundice, nausea, vomiting and/or malaise. Most HEV cases (70%) had eaten pork or game meat in the few months before infection, and 14 HEV patients (29%) had a recent history of foreign travel. The majority of samples were HEV genotype 3 (27/30, 90%); three were genotype 1. Acute HEV infection is currently the predominant cause of acute viral hepatitis in Lothian and presents clinically in older men. Most of these infections are autochthonous, and further studies confirming the sources of infection (i.e. food or blood transfusion) are required.

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Keywords: Clinical virology, Edinburgh, public health, RNA virus, virus diagnostic

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Introduction

Viral hepatitis affects millions of people worldwide, with over 20 million people diagnosed with acute infections annually and over 150 million with chronic viral hepatitis (<http://www.who.int/mediacentre/factsheets/fs328/en/>, <http://www.who.int/mediacentre/factsheets/fs204/en/>, <http://www.who.int/mediacentre/factsheets/fs164/en/>, <http://www.who.int/mediacentre/factsheets/fs280/en/>). Viral hepatitis is the world's eighth leading cause of death, responsible for 1.4 million deaths every year ([\[worldhepatitisday.org/\]\(http://worldhepatitisday.org/\)\). The main causes of viral hepatitis are the hepatitis viruses A, B, C, and E \(HAV, HBV, HCV and HEV, respectively\). These diverse viruses are members of different virus families, with HAV belonging to the *Picornaviridae* family, HBV to the *Hepadnaviridae* family, HCV to the *Flaviviridae* family and HEV to the *Hepeviridae* family. HAV, HCV and HEV are all single-stranded positive-sense RNA viruses, whereas HBV is a circular DNA virus. Generally HAV and HEV cause self-limiting acute hepatitis, although HEV infection can become chronic in immunocompromised individuals \[1\]. In contrast, HBV and HCV infections are mostly asymptomatic in the acute phase but frequently give rise to chronic infections \(<http://www.who.int/mediacentre/factsheets/fs204/en/>, <http://www.who.int/mediacentre/factsheets/fs164/en/>\).](http://www.</p>
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Although the burden of viral hepatitis has been underappreciated for a long time, global data on viral hepatitis is collected by the World Health Organization and Europe-wide

surveillance is collated by the European Centre for Disease Prevention and Control. In 2011, there were over 55 000 reported cases of HAV, HBV and HCV in the European Union member states, although these figures may be an underestimate because diagnosis, case definition and reporting vary among countries (<http://ecdc.europa.eu/en/publications/Publications/annual-epidemiological-report-2013.pdf>, <http://ecdc.europa.eu/en/publications/Publications/hepatitis-b-c-surveillance-europe-2012-july-2014.pdf>). In the case of HEV, global and European data are still mostly lacking because these infections are neither consistently diagnosed nor notified in most countries. The presentation of acute viral hepatitis ranges from asymptomatic to fulminant hepatic failure. The viruses cause very similar presentations and are clinically difficult to tell apart, but they can be distinguished through laboratory testing (<http://www.who.int/mediacentre/factsheets/fs328/en/>, <http://www.who.int/mediacentre/factsheets/fs204/en/>, <http://www.who.int/mediacentre/factsheets/fs164/en/>, <http://www.who.int/mediacentre/factsheets/fs280/en/>).

HEV infection in Europe used to be considered only in patients travelling from endemic areas such as central and Southeast Asia, northern Africa and Central America. Recently, increased importance has been placed on autochthonous infection [2]. HEV infection in Europe is often related to zoonotic transmission via the consumption of undercooked food, but transfusion of blood products has recently been recognized as an additional risk factor [3]. Molecular characterization of HEV strains has shown that infections acquired from endemic areas are mostly associated with virus of genotypes 1 (and rarely 2), whereas in industrialized countries genotype 3 (and rarely 4) is the main cause of sporadic cases.

The main aim of this study was to investigate the current viral causes of acute hepatitis in Lothian, and in particular to assess the role and epidemiology of HEV infection.

Methods

Between January 2012 and April 2014, a total of 3204 blood samples from patients living in the NHS Lothian area with suspected acute hepatitis were sent to the specialist virology centre at the Royal Infirmary of Edinburgh. All samples were screened for HAV, HBV and HCV; a subset of samples ($n = 913$) was also screened for HEV. Until the inclusion of HEV testing as a part of initial testing for acute hepatitis for all individuals with alanine aminotransferase (ALT) levels over 100 IU/ml in 2013, only samples submitted from the Department of Hepatology or from individuals with a history of travel were tested for HEV. Previously published virologic results from samples collected in the year 2012 [4] were also included in this study, together with

additional detailed clinical information and virus sequence analysis. The positive samples were placed into a data set with name of individual, date of birth, specimen number, virology results and specimen collection date.

Clinical information including age at the time of diagnosis, ethnicity, peak ALT within 2 weeks from sampling, presenting symptoms, antiviral treatment, fulminant liver failure status, known risk factors and presence of chronic infection was collected from patients' electronic records. In addition, because all confirmed cases of acute HEV (i.e. RNA-positive individuals) were also notified to the local public health team with a view to identifying possible sources of infection and limiting further transmission of HEV, the public health records on these cases were also reviewed. Acute hepatitis was defined as an acute illness with an ALT >100 IU/L or the presence of jaundice, or in the case of HCV, ALT >400 IU/L (Centers for Disease Control and Prevention case definition; <http://www.cdc.gov/nndss/script/casedef.aspx?CondYrID=723&DatePub=1/1/2012%2012:00:00%20AM>).

Laboratory screening included testing for HAV immunoglobulin (Ig M and IgG antibodies, hepatitis B surface antigen (HBsAg) and HCV IgG antibody and antigen (Abbott, USA), whereas HEV IgM and IgG antibodies were tested with RecombiWell assay (Mikrogen, Germany). All HAV and HEV IgM-positive samples were also tested by HAV- and HEV-specific polymerase chain reaction (PCR), respectively [5,6]. HBsAg-positive samples were further tested for HBV core IgG and IgM antibodies and e antigen and antibodies. Virus detection and genotyping on confirmed HEV RNA positive samples was done in nested PCR using the primers outersense (6390) 5'-CARGGYTGGCGYTCNGTYGAGAC-3', innersense (6486) 5'-TAYACYAAAYACRCCYTAYACYGG-3', innerantisense (6850) 5'-GTCGGCTCGCCATT GGCYGAGACRAC-3' and outerantisense (6895) 5'-CCCTTRTCYTGTGNGCRTTCTC-3'. Virus RNA was extracted from samples and amplified using the Access RT-PCR system (Promega), and nucleotide sequences were obtained of PCR products with the BigDye Terminator Cycle Sequencing kit (Applied Biosystems). All sequences were submitted to the GenBank database (KP835485–511, JX486781, JX270855 and KT893458).

The statistical analysis used to compare independent groups of sampled data (ALT levels and age) was a nonparametric Kruskal-Wallis test, whereas Fisher's exact was used for statistical analysis of proportions. p values of <0.05 were considered statistically significant. The study was performed under an NHS Lothian BioResource license (10/SI402/33).

Results

From January 2012 to April 2014, 82 patients with a laboratory confirmed diagnosis of acute viral hepatitis were identified

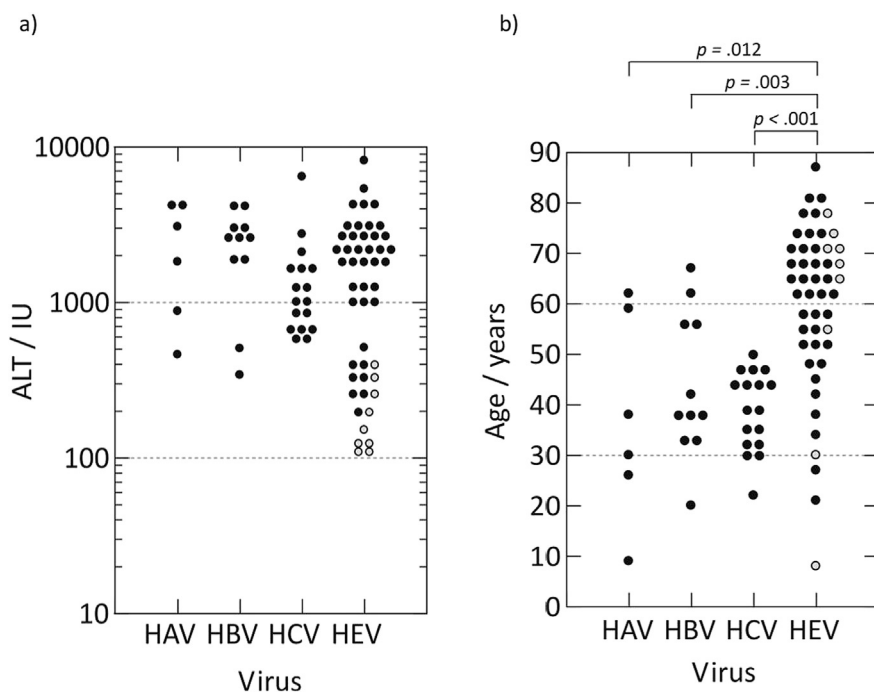


FIG. 1. Peak ALT levels (a) and age (b) of individuals infected with hepatitis A (HAV), B (HBV), C (HCV) and E (HEV) virus. Forty cases were RNA positive (solid circles), and the remaining eight were RNA negative (open circles).

(2.5% of all acute hepatitis samples tested). Six were infected with HAV, 11 with HBV and 17 with HCV. However, the majority of patients ($n = 48$, 59%), were shown to be infected with HEV. The number of cases of HEV infection has increased in recent years, with 13 diagnosed in 2012, 20 in 2013 and 15 in the first 4 months of 2013. Despite the introduction of routine HEV laboratory testing for all patients with suspected acute viral hepatitis at the beginning of 2013, only 56% of samples fulfilling diagnostic criteria for acute hepatitis (all tested for HAV) were screened for HEV.

Among patients with acute viral hepatitis, peak ALT values ranged from 142 to 8144 IU/L, with median values highest for HBV infection (2529 IU/L), followed by HAV (2438.5 IU/L), HEV (1820.5 IU/L) and HCV (1035 IU/L) (Fig. 1a). Interestingly, ALT levels of individuals who tested as HEV RNA negative had lower ALT levels observed than in HEV RNA-positive individuals. The demographics of patients differed; HAV infection was only seen in women (M:F 0:6), while the other viruses displayed a male dominance (HEV M:F 31:17, HBV M:F 9:2, HCV 14:3). Of patients with ethnicity documented (65/82, 79%), 60 were white British (including Scottish or Welsh), two were of Pakistani/Pakistani British origin, one was Indian/Indian British, one was Brazilian, and one was from Australasia. Acute viral hepatitis was diagnosed in individuals aged 8 to 87 years (Fig. 1b). HEV infection was significantly more common in older age groups (median age 64 years) compared to acute HAV, HBV and HCV infections (median ages 34, 39 and 39 years, respectively, $p < 0.05$).

Information on presenting symptoms was available for 75 of the 82 patients (Table 1). Four patients had no clinical symptoms. The most common presentations were jaundice (55%), nausea and/or vomiting (35%) and dark urine with or without pale stools (35%). Jaundice was seen in most patients with HAV, HBV and HCV (83%, 73% and 77%, respectively) but in only 40% of HEV patients ($p < 0.05$).

Potential risk factors associated with transmission of viral hepatitis remained unknown for 37 of the 82 patients (Table 2). The most common risk factors included foreign travel for HAV (3/6, 50%), unprotected sex for HBV (7/11, 64%) and intravenous drug use for HCV (11/17, 65%). For transmission of HEV, foreign travel was a commonly documented risk factor (14/48, 29%), and the majority had also eaten pork (31/48, 65%).

Whereas none of the HEV infected individuals developed chronic infection, chronic HBV infection was seen in one patient (1/11, 9%) and chronic HCV infection eight (8/13, 62%; four patients lost to follow-up). Three patients developed fulminant hepatitis, one with acute HCV infection and two with acute HEV infection, one of whom had coexisting chronic HCV while the other had a history of alcohol misuse. All three fulminant hepatitis cases died in hospital. No acute HAV or HCV patients received antiviral treatment, but one patient with acute HBV infection was treated with tenofovir, and one patient with acute HEV received ribavirin.

To investigate the epidemiology of HEV, virus ORF2 nucleotide sequences were obtained. Of 39 HEV RNA-positive samples, five were unavailable for analysis (not found or sample finished),

TABLE 1. Presenting signs and symptoms in study patients

Sign or symptom	HAV (n = 6)		HBV (n = 11)		HCV (n = 17)		HEV (n = 48)	
	n	%	n	%	n	%	n	%
Jaundice	5	83	8	73	13	76	19	40
Vomiting/nausea	3	50	4	36	8	47	14	29
Dark urine	3	50	4	36	5	29	17	35
Malaise	4	67	1	9	2	12	15	31
Abdominal pain	0		5	45	4	24	10	21
Lethargy/fatigue	0		2	18	5	29	10	21
Anorexia/loss of appetite	1	17	2	18	2	12	11	23
Sweating/fever	1	17	2	18	4	24	9	19
Aches ^a	0		2	18	1	6	6	13
Weight loss	0		2	18	3	18	3	6
Myalgia	2	33	2	18	0		4	8
Itch	1	17	0		2	12	4	8
Headache	0		2	18	1	6	2	4
Altered bowel habit	0		0		0		4	8
No symptoms	0		1	9	1	6	2	4
Unknown	0		1	9	1	6	5	10

HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus.
^aAches include joint, neck or back pain, and loin discomfort.

and four were PCR negative with the sequencing primers. Of the remaining 30 samples, three contained genotype 1 sequences and the remainder (27/30, 90%) genotype 3 sequences (Fig. 2). Two of the genotype 1-infected patients had a recent history of travel to India, and the third was of South Asian ancestry. The majority of the genotype 3 sequences (n = 22) grouped together with the subtype 3c reference sequence FJ705359, while two additional clusters grouped with the reference sequences 3a AF082843 (2/27) and 3e AB248521 (3/27). Subtypes 3a and 3c are both included in the clade described as group 2 by Ijaz et al. [7] that includes the majority of genotype 3 virus sequences obtained in England and Wales since 2011 among patients with acute hepatitis and also among blood donors [8].

Discussion

Laboratory testing is required to establish the viral cause of acute hepatitis. Clinical presentation is similar between the

TABLE 2. Factors potentially associated with disease transmission in study patients

Factor	HAV (n = 6)	HBV (n = 11)	HCV (n = 17)	HEV (n = 48)
Travel	3	4	0	14 ^a
Family member	1	0	0	0
Sexual partner	0	7	4	0
Tattoo	0	2	2	0
IVDU	0	0	11	0
Prison	0	0	1	0
Fights/altercations	0	0	1	0
Food	0	0	0	34 ^b
Unknown	2	3	2	5

HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; IVDU, intravenous drug user.

^aOf the 14 HEV patients who had travel as their risk factor, eight had travelled to Europe, three to Asia, one to South America, one to southern Africa, and one to Europe, northern and western Africa and Asia.

^bOf the 34 HEV patients who had eaten pork (n = 31), wild boar (n = 1) or venison (n = 2), five had also travelled to an HEV-endemic country.

different viral hepatitis and can vary from asymptomatic to fulminant (<http://www.who.int/mediacentre/factsheets/fs328/en/>, <http://www.who.int/mediacentre/factsheets/fs204/en/>, <http://www.who.int/mediacentre/factsheets/fs164/en/>, <http://www.who.int/mediacentre/factsheets/fs280/en/>) [4]. Identifying the cause of infection is important because it influences the management of patients and protection of others, whether or not this includes medical treatment.

Over the 28-month study period, 82 laboratory-confirmed cases of acute HAV, HBV, HCV or HEV infection were identified. The major cause of acute virus hepatitis was HEV (59% of infections), in keeping with our previous report [4]. The incidence of acute HEV appears to be increasing in Lothian since 75% of the 2013 total number of cases was observed in first 4 months of 2014. HEV was also narrowly the most frequent cause of viral hepatitis in Lothian between July 2010 and December 2012 [4]. In Scotland as a whole, the number of reported HEV infections has increased 10-fold over the last 5 years (<https://www.food.gov.uk/sites/default/files/HPS-FSA-IID-report-2013.pdf>), although some of this change may reflect increased clinical awareness and improved diagnosis. A previous study has documented a change in HEV genotype 3 variants over time [7]; the present study is consistent with this finding, with genotype 3 “group 2” viruses predominating. We also detected genotype 1 viruses (3/30, 10%), although these were at a lower frequency than described in a previous study (3/10, 30%) [9].

HEV-infected individuals in Lothian were mostly older (median age 64 years; Fig. 1), male (65%) and of white British ethnicity. This is comparable to findings seen elsewhere [1,10–13], although these reports found an even higher proportions of cases in men (70–90%). The reason for these biases is still unclear and remains an area for future research.

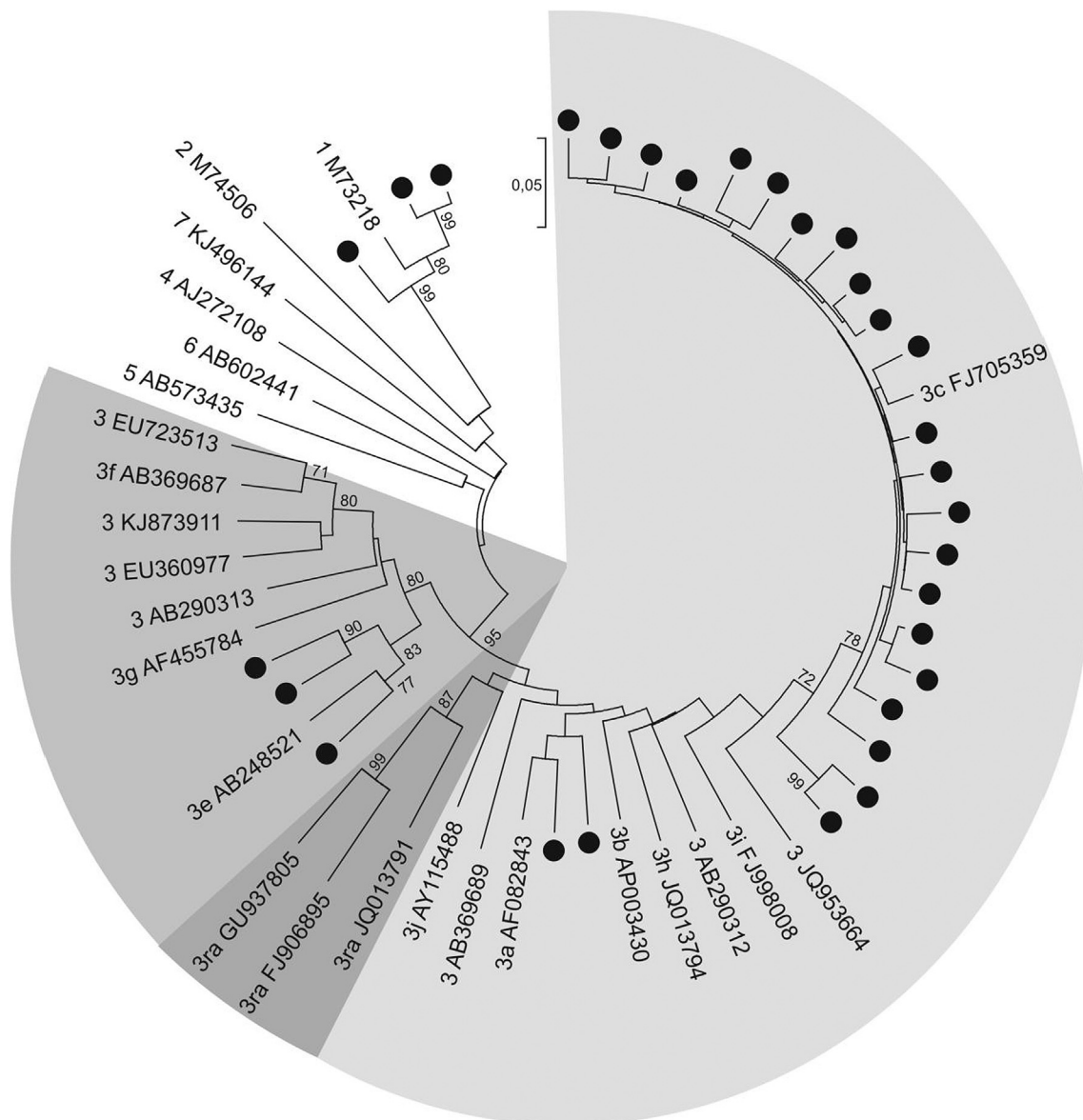


FIG. 2. Phylogenetic analysis of hepatitis E virus open reading frame 2 sequences. Nucleotide sequences (positions 6057–6370, numbered relative to AF082843) from patients (solid circles) or reference sequences (labelled with genotype and for genotype 3, subtype where assigned) were aligned and used to produce neighbour-joining tree using MEGA 6. Branches supported by >70% of bootstrap replicates are indicated. Three clades per group within genotype 3 are shaded.

HEV-infected individuals in this study presented with a variety of symptoms and signs, the most common being jaundice, dark urine, malaise, and nausea and vomiting (Table 1). Although jaundice was the most common sign, more than half of the patients presented without this. This frequency of symptomatic hepatitis is lower than previously described for patients with autochthonous HEV infection in Europe [10,12] and in developed countries [14]. On the other hand, some reviews claim that HEV infection in high-income countries does not tend to present with classical symptoms such as jaundice, dark urine and

gastrointestinal upset [15,16]. Although data on rash and arthralgia were not systematically collected, one individual with HEV presented with rash and arthralgia [17]. Because our study focused on patients with clinically apparent hepatitis (jaundice or ALT >100 IU/L), we might have overestimated the number of symptomatic individuals with acute HEV infection and have likely missed most asymptomatic presentations due to this. This could be addressed by investigating the close contacts of those with HEV infection. Furthermore, it is known that HEV RNA is usually detectable in blood only up to 3 weeks after the onset of

symptoms [18], and thus a negative HEV RNA result does not necessarily exclude recent infection if individuals are sampled late in the symptomatic phase of their illness. This was also demonstrated in our study where individuals known to have been sampled late presented without HEV RNA in blood.

Two of the 48 patients with HEV infection died during the study period, one who had HCV coinfection and the other with a history of high alcohol consumption. Both patients died during their stay in hospital as a result of liver failure, one despite ribavirin treatment. This strengthens the association between increased mortality of HEV infection in patients with chronic liver disease, and increased decompensation of preexisting liver disease [12,19,20]. Ribavirin has been shown to be an effective treatment in chronic HEV infection [21], and it has also been successfully used in a few cases of severe acute HEV infection [22,23]. However, no systematic studies on its role in treating acute HEV infections have been published.

The epidemiology of acute HEV in Europe has clearly changed. The risk factor previously thought to be the most common in HEV, travel to endemic areas, was not shown to be a key factor in our study. Only 14 patients with HEV in our study had a travel history, with the majority of these patients travelling to areas of low endemicity (9/14, Table 2). Infection in patients with no significant travel history has been seen elsewhere in Europe and the United States, increasing the awareness of autochthonous HEV infection in developed countries [10–12]. Most infections seen in these countries are caused by HEV genotype 3, which is known to infect both humans and animals, making zoonotic transmission a possibility [1]. Of the samples genotyped in the present study, 27 were genotype 3 HEV.

Potential zoonotic sources of infection are contact with pigs and consumption of poorly cooked pork or game meat [1,10,24]. Interestingly, 70% of HEV patients in our study had eaten pork or game meat in the few months before infection. Although detailed information was collected by the local public health team, further studies trying to confirm the sources of HEV (e.g. a specific pork product or pig farm) are required.

An emerging important risk factor for acute HEV in developed countries is blood transfusion [3,8,13,25]. This has been recognized in other European countries, such as the Netherlands [13] and Germany [3], and has recently gained more attention in the United Kingdom as well [8,25]. We were not able to obtain blood transfusion history for the patients, as this is not documented on electronic patient records and it was not mentioned in patient notes as a risk factor for any of the patients. In the future it would be beneficial to include transfusion history as a part of public health investigations for all acute HEV cases for the months preceding infection, as this could provide further clues to the source of infection in many of the patients with unclear risk factors.

Some aspects that we did not focus on in this study but that we noted when analysing the data are points to improve on in the future. Firstly, HEV has a long incubation period, ranging from 14 to 63 days [10,12], so identifying sources of infection can be difficult. Patients may not recall associated risk factors because the time between exposure and clinical symptoms is long. Secondly, as awareness of other sources of viral transmission increases, risk factors like previous blood transfusion may go unreported and risk factors like consumption of meat (not limited to pork), contact with animals and environmental exposure may require source identification studies [13,15]. Lastly, awareness of acute HEV should be increased; diagnosis should be recommended for all cases of acute hepatitis, and it should be made a notifiable infection throughout Europe. This would enable an estimation of its real impact in Europe.

Conflict of Interest

None declared.

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