

Hepatitis E virus infection in the Irish blood donor population

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BACKGROUND: Hepatitis E virus (HEV) Genotype 3 (G3) infection is a zoonosis that may be transmitted during the acute phase by transfusion. The aim of this study was to determine the incidence of HEV and seroprevalence among Irish blood donors.

STUDY DESIGN AND METHODS: Anonymized samples from 1076 donations collected in 2012 were tested for HEV immunoglobulin (Ig)G using the Wantai enzyme-linked immunosorbent assay. A total of 24,985 anonymized donations collected between December 2013 and June 2014 were individually tested for HEV RNA using the Procleix HEV assay; reactive donations were confirmed by an in-house real-time polymerase chain reaction (PCR) test.

RESULTS: Seroprevalence for anti-IgG was 5.3% (95% confidence interval [CI], 4.0%-6.8%), ranging from 1.1% in the 18- to 29-years age group to 33.3% in males over 60 years. HEV RNA screening of 24,985 samples yielded five PCR-confirmed donations (1:4997, 0.02%; 95% CI, 0.0065%-0.0467%), only one of which was serologically reactive (HEV IgM reactive only). Viral loads ranged from 10 to 44,550 IU/mL. Genotype analysis on three samples identified HEV G3 virus. Four of the five viremic donations were from donors in the 18- to 29-years age group ($p = 0.01$).

CONCLUSION: Seroprevalence for anti-HEV IgG was low compared to some European countries, but 1 in 5000 donations was viremic. Viremia was predominantly in younger Irish donors. After Department of Health approval the Irish Blood Transfusion Service implemented individual blood donation HEV RNA screening initially for a 3-year period from January 2016.

Indigenously acquired hepatitis E virus (HEV) infections are mainly caused by Genotype 3 (G3) viruses in Europe. The close sequence homology between human HEV G3 strains and those circulating in pigs supports the concept of a zoonosis.¹ Most HEV G3 infections are asymptomatic in immunocompetent individuals or cause a minor self-limiting illness, but acute HEV has been linked to hepatic decompensation in individuals with existing, often unsuspected, chronic liver disease.^{2,3} Chronic HEV infection has been observed in 1% to 3% of immunosuppressed solid organ transplant recipients with rapidly progressive liver fibrosis and cirrhosis in some patients.⁴ Chronic HEV infection has also been reported in allogeneic hematopoietic stem cell recipients,⁵ as well as occasional immunosuppressed patients with human immunodeficiency virus (HIV).⁶

HEV seroprevalence and RNA detection rates among blood donors are highly variable across Europe and the

ABBREVIATIONS: G (when followed by a number) = Genotype; GDA = Greater Dublin Area; IBTS = Irish Blood Transfusion Service; ID = individual donation; IR = initial reactive; LOD(s) = limit(s) of detection; MP = minipool; RR = repeat reactive; S/CO = signal to cutoff; TMA = transcription-mediated amplification.

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developed world.⁷ A high incidence of HEV infection was reported from the Netherlands where HEV viremia was detected in 78 of 59,474 (0.13%, 1:762)⁸ Dutch blood donations while the seroprevalence for anti-HEV IgG was 27%.⁹ This compares with a seroprevalence of 4.7% in Scottish blood donors and a HEV RNA frequency of 1 in 14,520.¹⁰ HEV incidence and prevalence in Ireland have not been studied thus far.

In addition to several reports of isolated cases of transfusion-transmitted HEV from the United Kingdom, France, Germany, and Japan (see Dalton et al.¹¹), the largest series and the only donor/recipient linked transmission study, where 1 in 2848 donations were viremic, reported that 42% of such viremic donations transmitted infection; HEV persistence was considered to be related to the degree of immunosuppression in the patients.¹²

Not surprisingly for a nonenveloped virus, HEV has been transmitted by solvent/detergent (S/D)-treated plasma,¹³ such that from January 1, 2015, HEV RNA screening of S/D plasma pools is required by the European Pharmacopoeia.¹⁴ Amotosalen treatment of plasma has also failed to prevent the transmission of HEV in France.¹⁵

In the absence of any data concerning HEV infection in Ireland, this study was initiated by the Irish Blood Transfusion Service (IBTS), to determine the seroprevalence rates for HEV IgG as a measure of exposure to the virus as well as to investigate the current incidence of infection in Irish blood donations using a sensitive HEV RNA assay in individual-donation (ID) nucleic acid testing (NAT) on a fully integrated and automated NAT system for blood and plasma screening (Panther, Grifols Diagnostic Solutions, Inc.). This format is already in use for routine NAT for HIV-1 and -2, hepatitis C virus, and hepatitis B virus at the IBTS. The IBTS is a noncommercial state agency set up under statute to organize and administer a blood transfusion service.

MATERIALS AND METHODS

Samples for seroprevalence

A total of 1076 anonymized, unlinked samples in plasma preparation tubes (PPT, Becton Dickinson) from blood donations collected by the IBTS from September 1 through to December 31, 2012, were frozen at less than -25°C pending testing for anti-HEV. Samples were unselected apart from the exclusion of those reactive for infectious disease markers. Seroprevalence was assessed using the anti-HEV IgG (Wantai, Fortress Diagnostics) assay, which is based on detection of recombinant peptides, according to the manufacturer's instructions. This assay has previously been shown to efficiently detect G3 HEV infection.¹⁶ Samples found to be HEV IgG reactive were repeat tested in duplicate and considered antibody positive (repeat reactive [RR]) if found reactive in two or

three replicates. Samples reactive for IgG were further tested with the Wantai anti-HEV IgM assay in accordance with manufacturer's instructions.

Samples for HEV RNA screening

Plasma preparation tube samples from 25,000 IBTS whole blood donations collected between December 2013 and June 2014 were rendered unlinked and anonymized before HEV RNA testing. Retained donor information was sex, age bracket, and clinic source to provide the geographical location of the clinic. Samples were retrieved on the day after completion of the routine mandatory NAT and frozen at less than -25°C pending HEV RNA testing. Ethical approval for the HEV RNA testing was obtained from the Research Ethics Committee of the Royal College of Physicians of Ireland on March 26, 2014 (Reference ID RECSAF 22).

The 1076 archived plasma samples stored at less than -25°C from the seroprevalence study were also tested for HEV RNA in ID-NAT. The stability of HEV RNA has been assessed by the manufacturer where positive HEV clinical specimens obtained from two blood bank organizations were stored in frozen condition (-15 to -35°C) for at least 1 year and subjected to two freeze-thaw cycles. Testing in the Procleix HEV assay was done upon receipt of the samples and again approximately 1 year later. All specimens showed 100% reactivity at both time points suggesting that no HEV RNA degradation occurred during storage (J. Linnen, Hologic, Inc., personal communication, 2016).

Procleix HEV assay

The Procleix HEV assay (Grifols Diagnostic Solutions, Inc.; developed in collaboration with Hologic, Inc.) is a transcription-mediated amplification (TMA) assay as previously described¹⁷ and performed on the fully automated Procleix Panther system. The analytical sensitivity of the Procleix HEV assay was determined by testing serial dilutions of the first WHO International Standard (IS) for HEV RNA G3a (PEI Code 6329/10). The dilution series concentrations were 90, 30, 10, 3, 1, and 0 IU/mL. All dilutions were prepared in normal human plasma qualified negative for HEV RNA. Eight replicates from each of the three dilution series were tested with the HEV assay on three different days giving a total of at least 24 replicates per dilution. The 95 and 50% limits of detection (LODs) were determined by probit analysis.

The robustness of the Procleix HEV assay was further assessed by checking the ability to consistently detect low levels of the target by testing 20 replicates of low concentration controls (30 IU/mL dilution of WHO HEV International Standard) for HEV RNA. Two HEV seroconversion panels obtained from Biomex GmbH (Heidelberg, Germany) were tested in duplicate. Reactivity was determined

from the date the first panel member tested positive where all subsequent panel members were consistently positive. Where only one of two replicates was reactive, three additional replicates were tested.

HEV RNA screening of anonymized, unlinked donation samples and confirmatory testing

In total, 24,985 anonymized, unlinked donation samples in ID-NAT format were tested with the Procleix HEV assay (research use only reagents) according to the manufacturer's instruction. HEV RNA initial reactive (IR) samples were repeated in singleton and were regarded as RR if RNA positive on both occasions. All IR samples (regardless of repeat result) underwent confirmatory testing at Sanguin using a HEV polymerase chain reaction (PCR) assay as previously described.^{9,18} The lower LOD (95% cutoff) of the confirmatory assay was previously established to be 10.3 IU/mL.⁸ Where possible, HEV genotyping was performed by nested PCR amplification and sequencing of 285- and 304-bp fragments of the ORF1 and ORF2 region as previously described.^{9,18-20} Sequences were submitted to GenBank with Accession Numbers KT873489-873493. A neighbor-joining phylogenetic tree was computed using the Tamura-Nei algorithm for the ORF1 fragment sequenced using HEV reference sequences from Smith and colleagues²¹(G3), Baylis and colleagues²² (G1, G2, and G4), and sequences from 37 Dutch blood donors and 126 Dutch patients obtained from samples from 2010 to 2014 (Genbank Accession Number KR362607-362769). Additional phylogenetic comparison across the ORF2 region was performed using ORF2 sequences from the same reference set and Dutch isolates and with HEV G3 sequences from donors and patients from England and Wales. All NAT IR samples were also tested for anti-HEV IgG and IgM using the Wantai assays.

Statistical analysis

Probit analysis was performed using computer software (Minitab, Version 17.1.0, Minitab, Inc.). Fisher's exact test or the chi-squared test was used to compare proportions of positive samples in different groups using Minitab software. A p value of less than 0.05 was considered significant.

RESULTS

HEV seroprevalence

A total of 57 of 1076 donation samples (5.3%; 95% CI, 4.0%-6.8%) tested RR for the presence of HEV IgG antibodies. The mean enzyme-linked immunosorbent assay (ELISA) signal-to-cutoff (S/CO) ratios for positive samples was 7.38 ± 6.6 . Anti-HEV IgG seroprevalence increased from 1.1% in the 18- to 29-year age group to 21.9% in those more than 60 years of age (Table 1). There was no significant difference in the seropositive rate between males and females except in the over 60 age cohort where 33.3% of donations from males were anti HEV IgG positive in comparison to just 6.5% of females ($p = 0.004$). Two of the 57 (3.5%) also tested repeat positive for anti-HEV IgM: one male in the 18- to 29-years age group, IgM S/CO 1.42, anti-HEV IgG S/CO 19.47, and a female (50- to 59-years age group) IgM S/CO 1.69, IgG S/CO 6.77.

HEV RNA assay

The 95 and 50% LOD values for the HEV RNA assay were determined to be 5.5 IU/mL (95% CI, 3.8-19.4 IU/mL) and 1.7 IU/mL (95% CI, 0.2-2.8 IU/mL), respectively. All 20 replicates of the WHO standard were positive at 30 IU/mL. Seroconversion panel SCP-HEV-001b (Table 2A) from a 27-year-old individual with HEV, G3e, showed consistent HEV RNA reactivity 33 days before first IgM positivity. For the second seroconversion panel SCP-HEV-006b, this 23-member panel from an HEV G3c-infected individual showed consistent HEV RNA reactivity 47 days earlier than the first positive result for HEV IgM (Table 2B). The TMA assay was reactive in two of five replicates of Panel Member 2 where the reference PCR test was negative.

HEV RNA screening of blood donations

Of the 24,985 donation samples tested for HEV RNA, 10 (0.04%) were IR; five of the 10 were RR on the TMA HEV assay. All five RR samples were confirmed positive by PCR (Table 3). All five of the RR samples were serologically negative with the exception of one IgM only reactive sample. These data give a prevalence of HEV viremia in current Irish blood donations of 0.02% (95% CI, 0.0065%-0.0467%) or 20 HEV RNA-positive samples per 100,000

TABLE 1. Anti-HEV IgG seroprevalence

Age group (years)	Overall		Males		Females		Chi-square test p value
	Samples	Positives (%)	Samples	Positives (%)	Samples	Positives (%)	
18-29	271	3 (1.1)	93	2 (2.2)	178	1 (0.6)	0.251
30-39	262	8 (3.1)	113	4 (3.5)	149	4 (2.7)	0.692
40-49	240	10 (4.2)	114	7 (6.1)	126	3 (2.4)	0.142
50-59	230	20 (8.7)	116	9 (7.8)	114	11 (9.6)	0.611
>60	73	16 (21.9)	42	14 (33.3)	31	2 (6.5)	0.004
Total	1076	57 (5.3)	478	36 (7.5)	598	21 (3.5)	0.004

TABLE 2. Seroconversion panel

Date	Days	Viral load (IU/mL)*	Valuation IgM†	Valuation IgG†	Procleix HEV assay‡	
					Results	Mean S/CO
A. SCP-HEV-001b (Biomex)						
06/10/2011	0	Negative	Negative	Negative	Nonreactive	0.00
07/13/2011	+33	2.63E+04	Negative	Negative	Reactive	49.62
07/18/2011	+38	5.13E+03	Negative	Negative	Reactive	48.11
07/21/2011	+41	2.10E+03	Borderline	Negative	Reactive	38.22
07/26/2011	+46	1.51E+02	Positive	Positive	Reactive	28.98
07/29/2011	+49	9.99E+01	Positive	Positive	Reactive	21.94
08/02/2011	+53	3.01E+01	Positive	Positive	Reactive	18.05
08/26/2011	+77	Negative	Negative	Positive	Nonreactive	0.00
B. SCP-HEV-006b (Biomex)						
07/25/2011	0	Negative	Negative	Negative	Nonreactive	0.00
07/28/2011	+3	Negative	Negative	Negative	Reactive§	2.315§
08/04/2011	+10	4.94E+01	Negative	Negative	Reactive	5.78
08/12/2011	+18	6.77E+01	Negative	Negative	Reactive	10.15
08/22/2011	+28	3.15E+02	Negative	Negative	Reactive	29.25
08/25/2011	+31	6.05E+02	Negative	Negative	Reactive	42.8
09/06/2011	+43	2.13E+04	Negative	Negative	Reactive	48.35
09/09/2011	+46	3.42E+03	Negative	Negative	Reactive	39.99
09/13/2011	+50	9.02E+01	Positive	Negative	Reactive	30.16
09/19/2011	+56	4.49E+01	Positive	Borderline	Reactive	15.13
11/07/2011	+105	Negative	Negative	Positive	Nonreactive	0.035¶

* RealStar HEV RT-PCR kit: Altona Diagnostic Technologies.
† ReomWell HEV IgM and IgG by Mikrogen.
‡ Tested in replicates of two.
§ Two of five replicates reactive with Procleix HEV assay.
|| Four of five replicates reactive with Procleix HEV assay.
¶ Panel Members 12 to 23 not shown due to results similar to those of Panel Member 11.

TABLE 3. HEV RNA-reactive donation samples

Sample	M/F	Age bracket (years)	Procleix HEV assay		HEV PCR assay			ELISA Wantai	
			IR S/CO	RR S/CO	Result	Viral load (IU/mL)	Genotype	HEV IgM S/CO	HEV IgG S/CO
1	F	18-29	6.12	3.32	Pos/Neg Pos/Pos	10	N/A	Neg	Neg
2	M	18-29	54.36	44.99	Pos/Pos	44,550	3efg	Neg	Neg
3	M	18-29	19.4	15.7	Pos/Pos	340	N/A	Neg	Neg
4	M	40-49	45.49	41.39	Pos/Pos	5,660	3abchij	Neg	Neg
5	M	18-29	33.63	21.44	Pos/Pos	2,140	3efg	1.5	Neg
6	F	30-39	1.55	Neg	Neg/Neg			Neg	Neg
7	M	60	15.6	Neg	Neg/Neg			Neg	Neg
8	M	40-49	6.51	Neg	Neg/Neg			2.27	6.81
9	M	18-29	15.05	Neg	Neg/Neg			Neg	Neg
10	F	18-29	2.12	Neg	Neg/Neg			Neg	Neg

F = female; M = male; Neg = negative; Pos = positive.

donations (95% CI, 6.5-46.7) or 1:5000. The HEV viral loads in the five viremic donations ranged from near the detection limit of the confirmatory assay of less than 10 to 44,550 IU/mL (Table 3). HEV sequencing of ORF1 and ORF2 fragments was successful for three confirmed samples, yielding three ORF1 fragments and two ORF2 fragments. All sequences were G3, with two sequences clustering in GT3 Group 1 (3efg) and one in GT3 Group 2 (abchij)²⁰ (Fig. 1). Phylogenetic analysis of the two ORF2 sequences yielded highly similar results (data not shown).

The five samples that were IR but non-RR on the TMA assay were PCR negative on the confirmatory assay and are considered false-positive samples (Table 3). Three of these nonconfirmed IR samples occurred in one test run of 487 samples, suggesting a possible contamination event. However, one of these nonconfirmed TMA IR samples was positive for anti-HEV IgG and IgM, although the S/CO ratio of the IgG-reactive sample at 6.8 was lower than expected for acute seroconversion. This may indicate a false-positive anti-HEV IgM in a previously infected

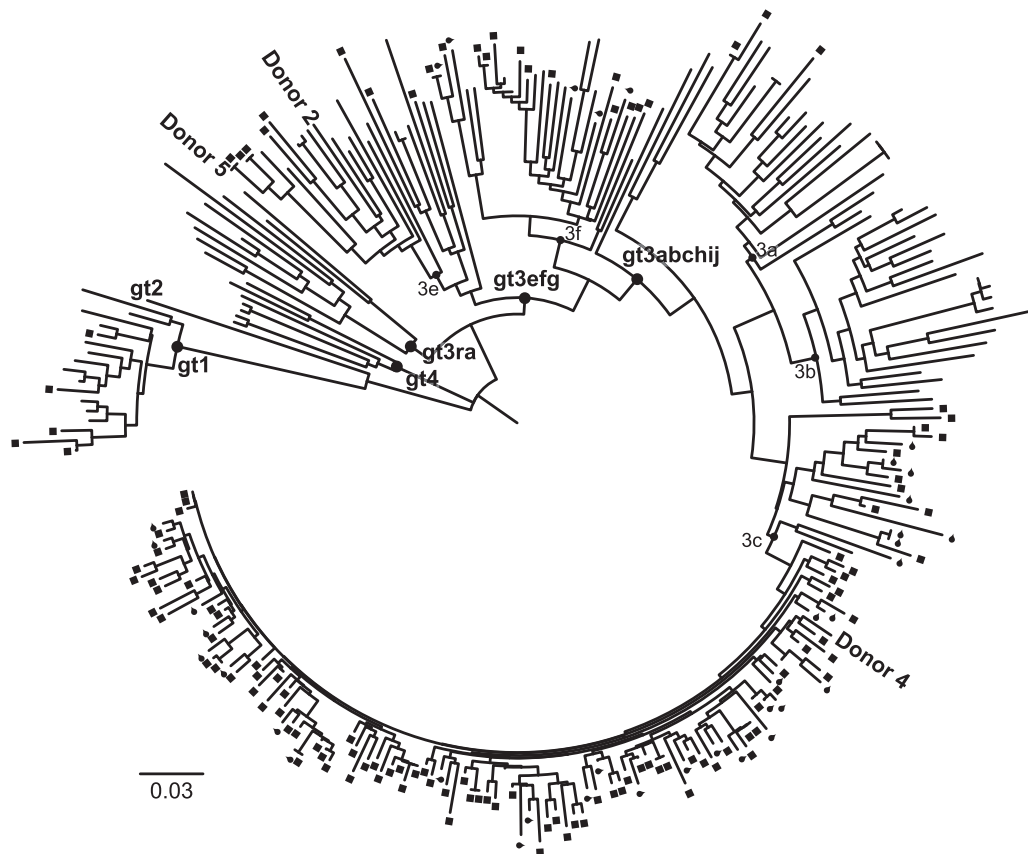


Fig. 1. Phylogenetic analysis of the 242-bp ORF1 sequences. Reference sequences from Smith et al.²¹ (G3) and Baylis et al.²² (G1, G2, and G4) are unmarked. Dutch blood donors and patients are marked by “drops” and squares, respectively, and the three Irish blood donors are marked as Donors 2, 4, and 5 (see Table 1). Genotypes and subtypes are assigned based on the full genome reference sequences. The three major gt3 clades 3ra (rabbit), 3efg, and 3abchij are indicated. Subtypes are indicated by letters and circles at the nodes where 100% of the reference sequences are from the relevant subtype (based on full genome sequences). The scale bar denotes the 0.03 substitutions per site.

donor, but without a follow-up sample we cannot exclude the possibility that this NAT IR sample or the other four non-RR samples represent low-level viremia cases.

With the exception of the more than 60 years age group, there were approximately equal numbers of samples tested within each of the respective age groups. Four of the five PCR confirmed-positive samples were from the 18- to 29-year age group indicating a significantly higher HEV RNA detection rate in this group when compared to those aged more than 30 years (Fisher’s exact test, $p = 0.010$; Table 4). The age profile of the anonymized, unlinked donation samples screened for HEV RNA in this study was exactly the same as all whole blood donations given during the 7-month study period for sample accrual (data not shown).

Two of the HEV-positive donations were collected in a Dublin city clinic ($n = 3216$) with the remaining three collected by a mobile clinic largely servicing the Greater Dublin Area (GDA) 83.2%; ($n = 2887$). However, one cannot exclude the possibility of a HEV-positive donation outside the GDA as 584 samples were collected from the

North West of Ireland (16.8% of mobile clinic samples). The GDA is urban with a population of 1.2 million (26% of total population of the country). The five possible false-positive samples were donated outside of the GDA.

The clinical specificity for the TMA HEV assay on screening 24,985 donation samples was 99.98% (95% CI, 99.95%-99.99%). The overall assay performance on the Panther system demonstrated satisfactory robustness with no run failures in 111 worklists. The invalid sample rate of 0.25% was comparable with that of the Ultrio Elite assay for routine screening of IBTS donations.

HEV RNA screening of samples used in the seroprevalence study

One of the 1076 samples was IR ($S/CO = 2.04$) on the Procleix HEV assay but was nonreactive on repeat and negative by PCR. The sample was anti-HEV IgG positive, IgM negative. All other samples including the two IgM-reactive samples were negative for HEV RNA.

TABLE 4. HEV RNA screened donation sample cohort*

Age (years)	Males		Females		Total	
	Samples	HEV RNA positive	Samples	HEV RNA positive	Samples (%)	HEV RNA positive
18-29	2,635	3	2,890	1	5,525 (22.1)	4†
30-39	3,298	0	1,980	0	5,278 (21.1)	0
40-49	4,044	1	2,155	0	6,199 (24.8)	1
50-59	3,724	0	1,923	0	5,647 (22.6)	0
>60	1,622	0	714	0	2,336 (9.4)	0
Total	15,323	4	9,662	1	24,985	5

* Male 4:15,323 vs. female 1:9662, $p = 0.655$.

† Four of 5525 vs. 1 of 19,460, $p = 0.010$ (Fisher's exact test).

DISCUSSION

Seroprevalence for anti-HEV IgG was 5.3% among Irish blood donations, which is on the lower end of a range from seroprevalence studies of blood donors in developed countries using the same HEV IgG detection assay: Scotland, 4.7%;¹⁰ Australia, 5.9%;²³ England and North Wales 12%;²⁴ Netherlands, 27%;⁹ Spain, 20%;¹⁷ Denmark, 19.8%;²⁵ United States, 18.8%;²⁶ and France, overall 23.6%²⁷ and 52% in southwest France.²⁸ In these studies, the presence of anti-HEV IgG marker reactivity in the Wantai assay was used to provide the population seroprevalence for this virus. The Wantai assay has been shown to be a sensitive assay in clinical samples from HEV-infected individuals,^{16,29} but without an equally sensitive and more specific confirmatory assay, it may suffer from some level of nonspecific reactivity as an indirect antibody test.³⁰ Nonetheless, at the epidemiologic level, HEV IgG seroprevalence is a useful marker for the burden of exposure to HEV in different geographical areas. As in all these studies seroprevalence increased with age in our study but with a dramatic increase in the more than 60-year-old male donors, which may represent an age cohort effect due to cumulative exposure over time and to higher exposure of older generations in the past which has been described in studies from Denmark,³¹ England,³² and the Netherlands.³³ Detection of IgM in two of the 57 IgG-reactive samples in the serologic study indicated that infection was occurring among Irish blood donors at present and thus the larger molecular study was performed to assess rates of active infection.

The 95% LOD of the Procleix HEV assay used in this study was determined to be 5.5 IU/mL (95% CI, 3.8-19.4 IU/mL), similar to the LOD of 7.9 IU/mL recently reported by the manufacturer.¹⁷ With five of the 10 TMA IR samples not confirmed in this study, the assay specificity on screening 24,985 donations was 99.98% similar to the 99.99 and 99.96% specificities reported respectively from Spanish¹⁷ and US³⁰ donor ID-NAT studies using the same assay. All five RR samples on the HEV TMA assay were confirmed positive by real-time PCR assay resulting in a prevalence of viremia of 0.02% (95% CI, 0.0065%-0.0467%)

or 1:5000 donations. With a similar seroprevalence to Scottish blood donors (4.7%), we had expected perhaps a similar frequency of HEV RNA detection of approximately 1:14,000.¹⁰ However, differences in assay sensitivity with ID testing versus 24 minipool (MP)-NAT are likely to have contributed to the observed difference.

Overall our HEV RNA prevalence of 1 in 5000 is within the range reported in blood donors in developed countries, albeit in MP NAT studies, of 1:2218 from France;²⁷ 1:760 from the Netherlands in 2013 to 2014;⁸ and 1:2848 in England.¹² For ID-NAT, frequencies were reported of 1 in 3333 from Spain¹⁷ and 1 in 9500 from the United States.³⁰

Of concern is that four of the five viremic donations in our study were from the 18- to 29-years donor age bracket, where the IgG seroprevalence is just 1.1%. This significant association suggests an emerging zoonosis in young Irish donors. A recent Dutch study showed that donors aged 18 to 21 had a decreasing HEV seroprevalence from 19.8% in 1988 to 4.3% in 2000, followed by an increase to 12.7% in 2011, but there are no indications that the incidence of HEV infection in the Netherlands is age dependent.³³

We were able to genotype three of the five HEV RNA-positive samples by sequencing. All three were HEV G3 viruses; two sequences clustered with G3 efg (Group 1) and one sequence clustered with 3abchij (Group 2). The latter sequence cluster within the dominant group of viruses that have emerged in England and Wales coinciding with an increase in case numbers.²⁰ In contrast, sequences from UK pigs cluster with the 3efg clade.³⁴ The source(s) of HEV in the food chain in Ireland has not been studied. In the United Kingdom, an investigation of the pork food chain found that 10% of pork sausages at point of sale had detectable HEV RNA but it is worth noting that five of six of these sausages came from one batch and the origin of the sausages was not known.³⁵ A case-controlled study in England and Wales indicated consumption of ham and sausages from a major UK supermarket chain was significantly associated with indigenous HEV infection.³⁶ Some major UK food supermarket chains also supply the Irish market. The only Irish data come from a

seroprevalence study of HEV infection in the Irish pig population, which showed that 89 pigs (27%) in 13 of 18 herds (81%) were seropositive in 2010 and 2011.³⁷ The HEV strains circulating among Irish pigs have not been reported.

A limitation of our study is the anonymized, unlinked nature with no donor follow-up and a sample accrual over a 7-month period with the possibility of serial donations from an infected donor. We consider it unlikely that the five yield cases are an overestimation of the prevalence of viremia in current Irish donations, as the duration of viremia in asymptomatic blood donors is less than the standard 3-month deferral period for whole blood donations.^{8,9}

A further limitation is that transfused recipients could not be followed up for evidence of transfusion transmission. Four of the five HEV viremic donations in this study were in the early seronegative phase of infection, which is associated with a higher likelihood of transfusion transmission.¹² In our study, the HEV RNA titers were low, ranging from 10 to 44,550 IU/mL, and some donations may not have been capable of transmission. In the transmission study from England, the median viral load for donations associated with transmission was 4.53 log IU/mL compared to 2.57 log IU/mL for donations not associated with transmission¹² and there was a trend for components with larger plasma volumes to transmit. A low-level viremia of 120 IU HEV RNA/mL in a dose of apheresis platelets (PLTs), equaling to a product infectious dose of 7056 IU HEV RNA G3, resulted in transmission to an immunosuppressed patient who developed chronic HEV in Germany.³⁸

The IBTS issues 140,000 labile blood components per annum and we estimate that per year approximately 30 HEV viremic donations are currently being issued for clinical use. The Procleix HEV assay in terms of sensitivity, specificity, and robustness on the Panther platform as demonstrated in this study is a suitable assay for blood donation screening whether individually or in small MP format. Hokkaido, an island in the north of Japan, is the only region to have implemented universal HEV RNA screening of their blood supply since 2005 with a prevalence of HEV RNA of approximately 1:8960 with MP-NAT.⁷ The only prospective donor/recipient transmission study from England showed little acute morbidity in recipients with only one patient developing mild clinical hepatitis, but the authors do raise the concern about the beginning of persistence in 55% of patients which correlated with the level of immunosuppression.¹² A high proportion of transfusion recipients are immunocompromised; approximately 60% of PLTs and 30% of red blood cells are transfused to patients with hematologic and nonhematologic malignancies in the United Kingdom.^{39,40} A selective screening strategy for categories of patients in whom chronic hepatitis E has been described could be adopted similar to cytomegalovirus (CMV) screening. However, the

at-risk transfusion population probably exceeds those selected to prevent transfusion-transmitted CMV as acute HEV infection has been linked to hepatic decompensation with a high mortality in patients with existing chronic liver disease.^{2,3} How commonly HEV causes decompensation in such patients in developed countries has not been systematically studied. Hepatitis E appears to be a rare cause of acute liver failure in the United States⁴¹ and does not appear to be a significant cause of hepatic decompensation among persons with advanced chronic hepatitis C in the United States.⁴² However, in a recent retrospective study from Germany approximately 10% of patients with acute liver failure had evidence for acute HEV infection.⁴³ The 2014 Serious Hazards of Transfusion Report documents the occurrence of transfusion-transmitted acute HEV precipitating hepatic encephalopathy in a patient with existing ethanol-related liver disease.⁴⁴

We conclude that HEV IgG seroprevalence at 5.3% among Irish blood donations is on the lower range for developed countries, suggesting relatively lower exposure to HEV in the past. However, the finding that approximately 1 in 5000 current donations are viremic predominantly from young donors is a concern particularly as there is little clinical awareness about HEV in Ireland. HEV was not a notifiable disease in Ireland at the time of this study and there is little if any examination of the food chain to determine food sources. The IBTS requested funding from the Department of Health for HEV ID-NAT screening of all donations for 3 years to measure and track the incidence of the virus in blood donors and thereby provide epidemiologic surveillance for the public health authorities. We felt that we would be unable to consistently supply HEV tested blood for all patients with severe liver disease across all hospitals in Ireland and concluded that for the time being universal HEV NAT screening would be preferable in particular since it is clear in EU law that the IBTS would carry consumer liability for any harm attributable to HEV infection from blood transfusion. In light of this, the IBTS implemented universal ID HEV RNA testing in January 2016 for a period of at least 3 years along with recommending that hepatology and transplant units also test susceptible patients for HEV in a regular program of surveillance. The identification of persistently infected patients is crucial as viral clearance can be achieved by modifying immunosuppressive regimen or by ribavirin treatment.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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