Virology, serology, and demography of hepatitis E viremic blood donors in South East England

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BACKGROUND: Hepatitis E virus (HEV) Genotype 3 (G3) in England comprises two principal phylogenetic groups (Group 1 and Group 2) and can be transmitted by transfusion. Unselected screening identified 79 viremic donors; 76 participated in a follow-up study.

STUDY DESIGN AND METHODS: Viral RNA dynamics, phylogenetics, and seroconversion were characterized in the donors. Detailed demographic, travel, clinical, and lifestyle questionnaires were undertaken.

RESULTS: The majority of viremic individuals (57/79) were seronegative at time of donation but all seroconverted. Viremia was short-lived, with a median of 6.5 weeks to confirmed viral clearance. All infections were acquired in the United Kingdom and were G3, with Group 2 viruses predominating (43/54; 80%). Infection was associated with some clinical symptoms both at and after donation (8/77; 10%). Viral loads and symptoms were more pronounced in Group 1 infections. There was no serologic evidence of reinfection. Donors were more commonly male ($p = 0.002$); both male and female donors were older than comparator donors. Animal contact was unlikely to be the source of infection. Consumption of chicken and pig meat was common to all infected donors; processed pig meat was most commonly purchased from one particular retail chain.

CONCLUSION: Viremic donors represent primary infection in older members of the community and reflect a widespread zoonotic in the United Kingdom. The two phylogenetic groups of HEV G3 display different pathogenicity and the more common Group 2 appears less adapted to humans. There are no objective demographic criteria that can identify donors at enhanced HEV risk.

Hepatitis E virus (HEV) in England was previously considered as an imported infection. It is now established that HEV infections are common and probably acquired through the dietary route.[1] The increasing incidence of cases of hepatitis E in England and Wales is principally due to Genotype 3 (G3) infection, with the recent emergence of a dominant group (Group 2). This is now more common than the Group 1 G3 viruses that have circulated previously.[2] After recognition of the increasing frequency of G3 infections and the potential for HEV infection in donors,[3] a study was conducted to investigate the potential for human-to-human transmission of HEV through blood and component transfusion.[4] Donation screening during this study has provided an opportunity to identify donors with HEV infection at the time of donation, determine their

ABBREVIATIONS: G3 = Genotype 3; GP(s) = general practitioner(s); S/CO = signal to cutoff.

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demography, and examine the evolution of viral markers by acquiring archive and follow-up samples.

Studies investigating HEV RNA prevalence in blood donors have been conducted in a number of European countries, including Scotland,[5] the Netherlands,[6] Germany,[7] France,[8] Spain,[9] and Austria.[10] The prevalence of viremia in these investigations has ranged from 1 in 1200 in German donors to 1 in 15000 in Scottish donors. Observed differences may be related to local epidemiology as well as to the time of sampling of what, in humans, is likely to be a representation of an emerging European epizootic. Overall, the majority of donors gave blood in a seronegative window and have been reported to be asymptomatic at that time. Evidence of coincidence between asymptomatic elevated transaminases and viral RNA was noted first in Japanese studies.[11] A recent study conducted in the Netherlands describes "silent HEV infection" in blood donors.[12] A central plank of transfusion safety is excluding "at-risk" donors before donation by asking donors to disclose specific risk behaviors, and/or, as appropriate, applying additional screening to those who may be likely to be at increased risk of presenting with any specific transmissible infection. There are at present no objective data identifying supportable exclusion criteria to reduce the prevalence of zoonotic HEV viremia in donors except where infection is acquired overseas and donors are excluded on the basis of travel. Reflecting the observed English viremia prevalence[4] onto the UK population at large indicates a likely burden of between 100,000 and 150,000 HEV infections annually. The PHE Enhanced Surveillance Programme results for 2013 indicated that 479 of the 692 reported cases of hepatitis E were likely to be indigenously acquired confirming the relative lack of clinical symptoms in acute HEV G3 infections in England.

During the English blood donor study,[4] postdonation interviews, including a food questionnaire, were conducted along with repeat sampling to document viral clearance. Here we report data arising from these investigations that allowed detailed profiling of the time course of viremia and seroconversion in blood donors. In addition we describe unique data on the clinical features, lifestyle, and potential risks in HEV-infected donors.

MATERIALS AND METHODS

Ethical approval for study

This study and its protocols were reviewed and approved by the London Bridge Research Ethics Committee (Reference 12/LO/0987).

Detection, measurement, and characterization of donor samples

HEV-infected donors were identified as previously described.[4] Nucleic acid was extracted from plasma using a high-throughput system for preparing nucleic acid samples (MagNA Pure 96 platform, Roche Diagnostics Ltd, Sussex, UK). HEV RNA was quantified[13] and sequenced across part of ORF2[14] as previously described. HEV antibody was detected using the Wantai immunoglobulin IgM and IgG detection assays (Fortress Diagnostics, Antrim, Northern Ireland) in accordance with the manufacturer's instructions.

Notification and follow-up of HEV-infected donors

During the study period, all donors in the area selected for study (generally the east of England and the area immediately north of London) were informed that donations would be additionally tested for HEV infection and were given the opportunity to opt out of such testing, but very few did so. All 79 viremic donors were notified by letter of their test results, received an information leaflet about HEV infection, and were invited to telephone and discuss their test results with a member of the National Health Service Blood and Transplant (NHSBT) clinical team. This is standard practice for any donor found to be infected with a blood-borne virus. Their general practitioners (GPs) and the local health protection teams were also informed of the donors' HEV infection. Donors were told that they would not be able to donate again until further blood sampling confirmed viral clearance.

All donors were provided with explanatory information about the follow-up study, invited to enter into the study and consented for enrollment. Of the 79 donors, two did not provide consent to participate and an additional donor, having consented, failed to provide a further blood sample and was lost to follow-up. These three donors were deferred from donating for 1 year. Donors included in the follow-up study thus numbered 76, of whom 10 were apheresis platelet (PLT) donors, 66 were routine donors, and all but one were repeat donors.

Archive samples

The archived plasma sample from the most recent donation before the index HEV RNA–positive donation was retrieved for all 78 repeat donors and tested for HEV RNA and antibody. Where the most recent archive was found to contain HEV RNA the next most recent archive was retrieved and tested.

Questionnaires

The questionnaire had previously been developed specifically for investigation of confirmed cases of hepatitis E and included questions on travel, food consumption, food preparation, animal contact, and alcohol consumption. Seventy-three questionnaires were completed. Thirty-four (47%) of the 73 questionnaires were self-completed and returned by the donors, while 39 (53%) were completed over the phone with the NHSBT clinical team. The

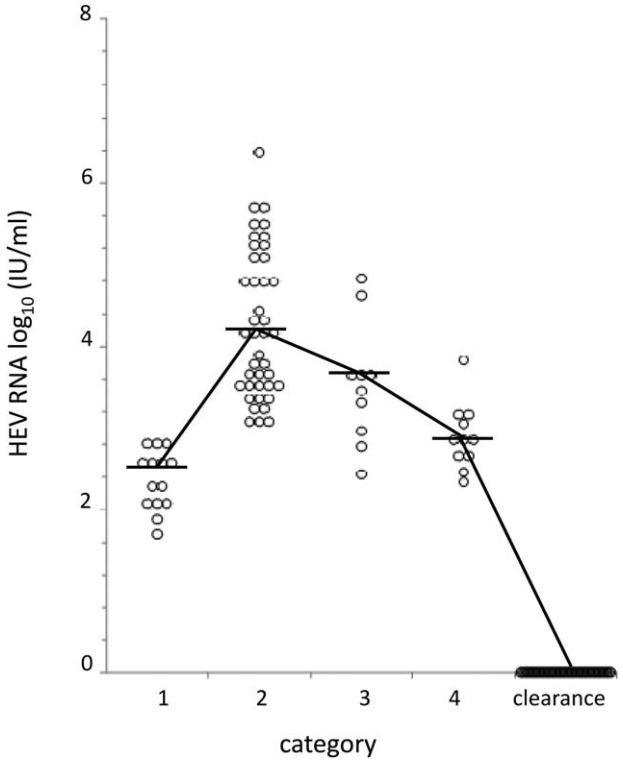


Fig. 1. Inferred time course of viremia in donors. Viral load present in 79 index samples shown individually with the donor characterized in the following categories: 1 = very early acute (seronegative, followed by a second seronegative sample); 2 = early acute (seronegative, followed by seroconversion in the next sample); 3 = seroconversion (seropositive and remaining viremic at next sampling); and 4 = late (seropositive, decreasing IgM, and RNA negative at next sampling). Data at viral clearance are also indicated. Line represents median value for RNA IU/mL.

completeness of questionnaires overall was excellent with the majority answering all questions.

Donor demography

The comparison donor data set included 199,172 donors drawn from a population consisting of all first-time and repeat volunteer, nonremunerated blood and component donors and their associated donations given to NHSBT in England during 2012. These were selected by identifying donors in the South East of England mapped using postcode of residence and taking those resident in London, north of the Thames with a latitude greater than the South London Tooting Blood Centre (N51.458217 degrees), and resident to the East of the major A1 arterial road (E0.213974 degrees). New and repeat donor status was assigned using the first donation during the calendar year. Demographic data were extracted from the NHSBT donor database and included a unique donor ID, full UK postcode, date of birth, ethnicity, donation date, donation type, new or repeat donor flag, and most recent previous donation date.

Statistical analysis

Categorical variables were compared using chi-squared tests. Given the skewed nature of the age distribution, ages were compared using Wilcox-Mann-Whitney non-parametric tests using the R statistical environment on a Linux system.

RESULTS

Viral dynamics

To assemble a time course for the 76 enrolled viremic donors, index donation samples were categorized into four groups depending on the dynamics of HEV RNA and antibody in all samples from each donor:

1. Very early (n = 14)—viremic and seronegative at donation; seronegative at next test.
2. Early (n = 41)—viremic and seronegative at donation; seroconversion at next test.
3. Seroconversion (n = 10)—viremic (high) and seropositive (+IgM) at donation; remaining viremic at next test.
4. Late (n = 11)—viremic (low) and seropositive at donation; RNA negative and decreasing IgM at next test.

This procedure allowed the assembly of the time course for viral load in donors (Fig. 1).

The median elapsed time for donors presenting in Groups 1 to 4 to have cleared viremia was 7.0, 7.1, 5.7, and 5.4 weeks, respectively. There was considerable variation in the duration of viremia inferred by elapsed time to demonstration of clearance, compounded by differing time intervals to the follow-up blood sample (Fig. 2). Overall, 11 donors presenting with early (three very early) infections remained viremic at the first follow-up sample (range, 3-5 weeks); four of these donors had provided their initial follow-up sample less than 4 weeks after the index donation.

In 25 of the viremic donors, the RNA level was insufficient at any time point to allow sequencing. All of the remaining 54 donors were harboring G3 infections; 11 (20%) were representative of Group 1 and the remaining 43 (80%) of Group 2. There was no difference in the proportion of each group that was seropositive in the index donation (three and five, respectively). The viral RNA level in the index seronegative donation was significantly different between the individuals in Group 1 (n = 8; median, 104,758 IU/mL; range, 2240-616,000 IU/mL) and Group 2 (n = 38; median, 5810 IU/mL; range, 75-2,370,000 IU/mL; Table 1).

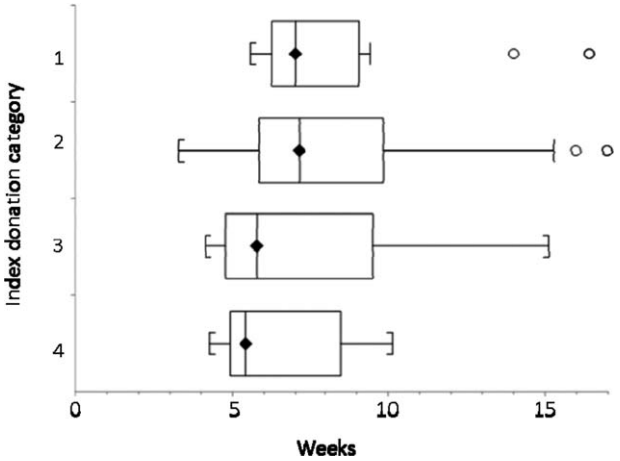


Fig. 2. Time elapse from index donation to observed clearance. Box and whisker plots (median, middle quartiles, and range in weeks) for time elapsed from index sample identification to confirmed viral clearance.

TABLE 1. Comparison of index viral load and symptomatology between donors infected by Group 1 or Group 2 HEVs

Donor characteristic	Group 1 (n = 11)	Group 2 (n = 43)
Sex split, male:female	2.7:1 (8:3)	1.3:1 (25:19)
Median viral load index donation (IU/mL)	7.70×10^{4}*	5.63×10^{3}
Seropositive index donation	3 (27%)	5 (12%)
Illness reported	6 (55%)†	10 (24%)

* Wilcox p 0.007.
† Chi-square $p < 0.02$.

Clinical illness among HEV-infected donors

None of the donors reported an illness shortly before or at the time of donation in their donor health check questionnaire. Telephone discussions with 77 of the 79 donors took place approximately 4 weeks after donation when 69 (90%) recalled no illness at the time of donation. Eight donors, in retrospect, considered that they had been "below par" at the time they donated. Four were fatigued and had dark urine, three had dark urine in the absence of fatigue with coincident pale stools in two, and one had diarrhea. Fifty-three of the 69 also reported no illness over the time period between donation and interview. However, 16 donors who were well at donation, in addition to the eight retrospectively identified to be unwell at donation, recalled some symptoms in the postdonation period, bringing to 24 (31%) the total number of donors with some symptoms of illness. In only 20 were the declared symptoms temporally compatible with their HEV infection. The most commonly recalled symptoms among these 20 donors were dark urine and/or pale stools in 12, with one noticing a "slight yellowness in the eyes." General gastrointestinal symptoms (nausea, loss of appetite, diarrhea, and vomiting), joint pains, and lethargy were reported in the remaining eight. Symptoms in these 20 donors occurred a median of 11 days (range, 3-28 days) after donation; however, none of the affected donors had reported their postdonation illness to NHSBT. Nine donors went to see their GP because of illness; two of the GPs requested liver function tests, which in both donors indicated a transaminitis. Neither was further investigated for hepatitis. No neurologic symptoms were reported in any donor. Symptoms were significantly more likely in donors infected by G3 Group 1 than in those infected by G3 Group 2 (6 of 11 Group 1 compared with 10 of 43 Group 2; $p \leq 0.02$; Table 1).

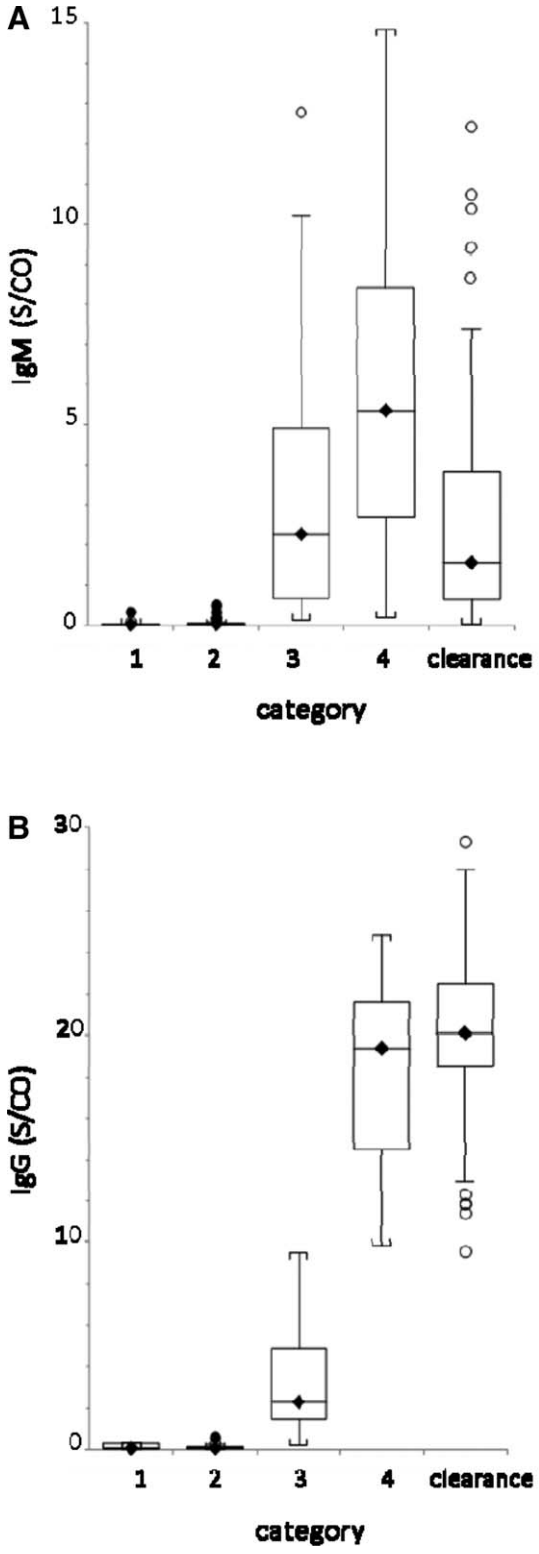
Seroconversion patterns

Fifty-six donors were seronegative at the time of the index sample. The remaining 23 of 79 (29%) were serologically reactive in their index donations. Seventeen of these samples were concordantly IgM and IgG anti-HEV reactive. Six samples were discordantly reactive (Table 2): two index samples were reactive for IgM alone and four for IgG alone. The archive samples of these six donors as well as the remaining 72 repeat donors were unreactive (Table 2). All of the donors developed a strong serologic response, IgM reactivity peaked in the late stage of infection, and IgG reactivity continued to increase through to recovery with viral clearance (Figs. 3). Twenty-two donors failed to display a detectable IgM response in either the acute or the recovery phase. The recovery phase samples were on average taken 49 days after the index in those 54 of 76 donors who mounted a detectable IgM response compared to 59 days in the 22 donors who did not. The signal-to-cutoff (S/CO) ratios (mean, 0.089; median, 0.067; range, 0.364-0.013) for the archive samples from the 22 repeat donors who failed to mount a detectable IgM response were indistinguishable from the distribution of seronegative archive samples from the 54 donors who did (mean, 0.119; median, 0.058; range, 0.721-0.00).

Donor demography

All but one of the 79 HEV-infected donors were Caucasian and white British (98.7% compared with 93% in the comparator donor population). All were UK-born, apart from one White Canadian and one Iranian. There was a significantly higher proportion of males in infected compared to the control donors (64.1% in infected and 46.8% in 2012 comparator donors, $p = 0.002$). The median age of infected donors was significantly higher than the comparators (median, 51.5 and 45.0 years, respectively; Wilcoxon Mann-Whitney $p < 0.001$). This held true for both males (median, 52.0 and 47.0 years respectively; $p < 0.001$) and females (median, 49.5 and 44.0 years, respectively; $p < 0.01$). Age distribution density plots show the

concentration of the infected population of both sexes within the 40- to 60-year-old age group (Fig. 4); this was seen in both males and females (data not shown). Only four (5%) were taking proton pump inhibitors. A significantly ($p = 0.004$) higher proportion of the HEV-infected donors were repeat donors (96.2%) compared to the 2012 comparator donor population (83.6%). This may reflect the fact that HEV infections tend to occur in the older age group, and older blood donors tend to be repeat and apheresis donors.



Travel history

Twenty-one donors traveled outside the United Kingdom before their donation. Further details were available in 17. Only four had traveled in the 8 weeks preceding the donation. One had traveled to North America in the 2 preceding weeks; the remaining three travelers had been abroad as follows:

- 3-4½ weeks earlier in North Africa (seronegative, HEV G3, Group 2);
- 4½-5 ½ weeks earlier in Cyprus (seropositive, too low to sequence);
- 4 weeks earlier for a month in the Caribbean (seronegative, HEV G3, Group 2).

Both sequences were embedded in the G3 dominant Group 2 seen in England.

Lifestyle and risks

Eleven donors (15%) were retired and five (7%) were unemployed. Two had occupational links to animals: one farmer (contact with pigs, poultry, sheep, and cattle) and one poultry worker (poultry contact only). Fifty-three of 73 (72%) had contact with pet animals, commonly dogs (37; 47%) and cats (30; 38%). Those that had animal contact often had contact with more than one animal. In addition, seven had contact with farm animals, including pigs in three cases, and poultry, sheep, cows, and horses.

Food preferences

None of the donors were vegetarians. Of the 18 food exposures listed in the questionnaire five items were consumed by more than 80% of donors. These were chicken (100%), bacon (93%), ham (90%), sausages containing pork (88%), and pork (84%). Pate was consumed by 73% and shellfish by 56%. All ate pig products (sausages and ham). Sausages were purchased from a number of outlets, with the majority (36/73, 46%) from a single supermarket

Fig. 3. Distribution of antibody reactivity in grouped donor samples. (A) Reactivity for IgM anti-HEV. (B) Reactivity for IgG anti-HEV. Box and whisker (median, middle quartiles, and range) distribution of S/CO ratios for IgM anti-HEV (A) and IgG anti-HEV (B) for all samples provided by each donor over the course of their infection where each sample has been classified according to the stage of infection in the donor is at the sampling point: very early acute (1), early acute (2), seroconversion (3), late (4), and the first sample negative for viremia (5).

TABLE 2. Evolution of serologic markers in six donors whose index sample was discrepantly reactive solely for either IgM or IgG antibody to HEV

	Archive S/CO	Index sample S/CO		Rebleed sample S/CO		
ID	IgG	IgM	IgG	Elapse*	IgM	IgG
E06/12	0.107	2.27	0.21	23	0.81	23.31
E12/12	0.085	0.68	4.86	41	0.90	29.32
E04/13	0.190	0.59	9.48	39	0.33	18.15
E17/13	0.073	0.18	7.91	39	1.79	19.56
E49/13	0.055	12.78	0.23	47	10.74	19.16
E59/13	0.045	0.14	1.46	30	0.18	19.62

* Days elapsed between index and rebleed.

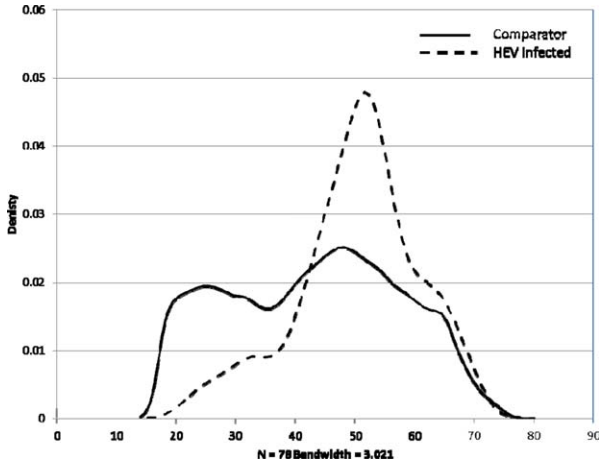


Fig. 4. Age density plot for HEV-infected donors compared with the comparator donor panel. Age density plots for comparator and HEV-infected donors that show the marked concentration of the infected donor population within the 40- to 60-year-old age group.

chain. Fifty-eight percent of the donors (42/73) purchasing pig products (sausages and ham) did so from the same chain, which holds a market share of approximately 28%.

Loss of donors

Donors were very supportive of the study. One donor questioned the need to enroll but nevertheless consented. Two donors declined and along with a third donor did not provide follow-up samples and could only be returned to the active donor panel after 1 year.

DISCUSSION

This study lays out a reconstructed time course which appears plausible and is based on the evolution of the plasma markers at the time of pick-up (Figs. 1 and 2). Similar data have been described recently by the Dutch.[6] The duration of viremia remains difficult to determine with precision; this study was managed as part of routine donor practice and follow-up samples depended on when it was convenient for a donor to have a blood sample taken at their general practice. We intended to requalify donors for return to the donor panel through retesting for HEV RNA at 28 days. Eleven donors with early infections remained viremic on the first follow-up at a median of 4.3 weeks later, (range, 3-5 weeks), four returning slightly earlier than the intended deferral period. Those donors providing a follow-up sample not containing detectable HEV RNA did so at a median of 6 weeks (range, 3.3-17 weeks). Overall, the elapsed time between the index donation and the first RNA-negative donation was between 6 and 7 weeks. We believe that donors should not donate again within 8 weeks unless shown to be HEV RNA free; however, deferral for 6 months would avoid a need for further RNA testing.

The postulated time course is likely to represent the normal for primary G3 HEV infection in humans. No serologic reactivity was detected in the RNA-negative archive samples of the 79 donors, even in those whose IgM anti-HEV response was blunted. Failure to develop a detectable IgM antibody response does not appear a likely marker of reinfection as suggested by others.[15] If reinfection was a common feature of infection in the immunologically intact human,[16,17] we would expect to have found supporting evidence among the archive samples in some of the 79 viremic donors.

The majority of the infected donors (57/79) were identified as expected in the serologic window phase and subsequent seroconversion was seen in all 76 donors available for follow-up. As anticipated anti-HEV IgM was detected in the acute or recovery phases of the majority of donors; however, those in whom it was not detected were characterized by an IgM seroreactivity (S/CO median, 0.65; range, 0.43-0.90), which though below the manufacturer's cutoff was clearly removed from the negative population and was associated with a concomitant high IgG binding ratio. Although the IgM levels decrease relatively quickly as the IgG levels increase, in a small number of donors this reactivity has persisted for months in the absence of detectable plasma viremia.

All infections sequenced were caused by HEV G3 viruses as previously reported[4] and showed the current dominance of HEV G3 Group 2 (43/54; 80%).[2] The contemporary infection by two different groups of HEV G3 provided an unique opportunity to compare the clinical outcome of infection by Group 1 and Group 2 infections. The viremia level in the index donations (Table 1) points toward a replicative difference between the two virus groups with the Group 2 viruses displaying a lower viremia. Soft indicators of illness in donors infected with G3 Group 1 were significantly less common in donors infected with Group 2 viruses (Table 1). This phenotypic difference between the two groups is certainly less

pronounced than the clinical differences between different genotypes but may be a reflection of the degree to which the two groups of porcine viruses are able to replicate in humans. These diffuse and generally mild symptoms at the time of infection could lead to donors postponing attendance for donation, leading to a degree of self-exclusion. We hypothesize that this response might be more likely in the potential first-time donor, partly explaining the higher proportion of repeat donors in the viremic group. The high number of repeat donors may also be accounted for in part by the age of the infected donors and by the inclusion of several PLT component donor clinics in the study area.

We show that males have a higher risk of acquisition of infection than women and that age in both sexes is also a risk factor (Fig. 4). Only four donors were taking proton pump inhibitors and this is unlikely to have influenced our findings. The male age dominance also seen in cases of hepatitis E[2] is explained by susceptibility to infection rather than an increased susceptibility to develop clinical hepatitis.[18] While it is true that males on average consume some 15% more pig derived foodstuffs than women,[19] this is unlikely to explain the different attack rate.

A proportion (21/79, 27%) of the donors had traveled outside the United Kingdom before their index donation but only four had done so in the relevant time frame of 8 weeks before the index donation. The phylogeny of the viruses in two donors that could be sequenced clearly embedded these viruses in the emergent Group 2 viruses. Their infection will either have been acquired from the same continental source that is causing the increased HEV activity in Europe or been acquired in the United Kingdom either before or after their travel. Similar findings are described in the Netherlands.[20] No HEV G1 or G4 infections were identified, reflecting perhaps the temporary exclusion applied to those who travel to malaria-endemic or other countries where G1 and/or G4 infections are common. This donor selection policy will remove HEV G1 from the active donor panel but have negligible effect on G3 infections.

Turning finally to the question "Is it possible to exclude HEV risk donors from the active donor panel?" it is necessary to define the current view on the acquisition of HEV in the United Kingdom. Extensive literature from outside Europe serve to confirm that HEV G3 and G4 are globally widespread infections in swine, which lead to zoonotic infections in man.[21] Within Europe G3 infections are also common in swine and the viral phylogeny in pigs is reflected in the human viruses, confirming the probable human acquisition of G3 from pigs. Two consecutive case-control studies and data from this study indicate that dietary exposure to pork-derived foodstuffs[1] is the most likely route of infection for the majority of all indigenous hepatitis E cases and HEV infections in England. None of the HEV-infected donors were vegetarian. All donors consumed pork products and the majority (58% of respondents) purchased these from a single retail chain. Since the current dominant group of human viruses are not seen in UK pigs at slaughter,[22] the implication is that the retailer in question must be importing meat from abroad. This fact does not, however, provide a workable deferral criterion for blood donors. Control of HEV infection in swine herds seems far off and animal contact and husbandry are unlikely to have contributed to HEV acquisition in these donors.

An alternative approach to HEV risk reduction in the blood supply takes account of the absence of seropositivity in archived samples from those donors who subsequently became viremic. This suggests that any level of naturally acquired antibody in the plasma of a donor may prevent subsequent viremia contrasting with what has been described in the clinical trial of Hecolin vaccine in China.[16] Where the prevalence of anti-HEV in the donor panel was high enough it would be possible to select seropositive donors who would not carry the potential of developing viremia, even if the natural immunity is not sterilizing,[23] for provision of blood to vulnerable recipients. Clearly with a current prevalence of 10% (J. Newham, NHBST, personal communication, 2014) this approach would not be feasible in the United Kingdom.

The enthusiastic interest and support which the investigators received from volunteer blood donors in this study was unexpected but extremely welcome. Ultimately, all but three donors were returned to the donor panel on the basis of documented clearance of HEV RNA and seroconversion on follow-up blood samples, and only one of the nonreturnees actively elected not to donate in the future. We believe that the information from this study is informative when addressing the possibility of HEV screening of donors and express our thanks to all who participated.

DEDICATION

To the memory of our coauthor Dr Sam Lattimore who died in November 2015. His wit, wisdom, and intellect will be greatly missed by all who knew and worked with him. He leaves an indelible fingerprint on this article, which we dedicate to him.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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