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REVIEW ARTICLE

Prevalence of hepatitis E viraemia among blood donors: a systematic review

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Background Hepatitis E virus (HEV) is usually transmitted by faecal–oral route. Recent reports have documented HEV viraemia in donated blood units and HEV transmission through blood transfusion. This systematic review summarizes the available data on prevalence of HEV viraemia in blood donors.

Methods Electronic databases were searched on 17 December 2018 to identify full-text English papers reporting original data on prevalence of HEV RNA in donated blood units. Two authors independently extracted the relevant data, which were pooled using simple aggregation as well as a random-effects meta-analysis; heterogeneity was assessed using the I^2 method.

Results In all, 59 data sets from 28 countries were identified. The available data showed marked heterogeneity. Of a total of 2 127 832 units studied, 561 (263·6 [95% confidence intervals = 242·7–286·4] per million units) tested positive for HEV RNA. On random-effects meta-analysis, the pooled prevalence was 60·9 [6·7–155·4] per million units. In the viraemic units, HEV RNA titre varied by nearly one million-fold, and most had genotype 3 HEV. The prevalence was higher in blood units with anti-HEV antibodies or elevated alanine aminotransferase. Only nearly one-fourth of viraemic units had anti-HEV antibodies.

Conclusions The prevalence of HEV viraemia among healthy blood donors is low, though the available data had limited geographical representation and marked heterogeneity. There is a need for further data on HEV viraemia in blood donors from areas with non-3 HEV genotype preponderance.

Key words: donor screening, hepatitis E virus, nucleic acid test, prevalence, ribonucleic acid, transfusion-transmitted infection, viraemia.

Introduction

Hepatitis E virus (HEV) consists of 27- to 34-nm-diameter virions that contain a single-stranded, RNA genome. Human HEV infections are caused mainly by four viral genotypes, named 1–4. Of these, genotypes 1 and 2 are known to infect only humans. These are highly endemic in Asia, Africa, the Mediterranean region and the Middle East, and cause acute hepatitis, either as outbreaks or as sporadic cases. The disease caused by these genotypes is particularly severe in pregnant women. Chronic infection with these genotypes is virtually unknown. By contrast, genotype 3 and 4 HEV circulate worldwide in several mammals, with occasional zoonotic transmission to humans resulting in sporadic human cases in the developed world. Infection with these genotypes has a propensity to persist, particularly in immunosuppressed people, leading to chronic hepatitis E, defined as HEV viraemia lasting longer than 6 months, which can progress to cirrhosis. Cases with genotype 3 HEV have been identified most often in Europe and North America; those with genotype 4, by contrast, have mostly been from South-East Asia and the Far East [1,2].

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Hepatitis E virus infection was initially considered as a disease of resource-constrained regions, with faecal–oral transmission mainly through contamination of water supply, and only occasional cases related to travel to endemic areas in high-resource countries. However, data collected in the last nearly 15 years show that locally acquired HEV infections are frequent in developed countries of Europe, North America and Japan. Initially, these genotype 3 or 4 HEV autochthonous infections were believed to be zoonotic, being related to ingestion of undercooked meat or contact with animals. However, later, several cases of HEV genotype 3 infection in developed countries were traced to transfusion of blood and blood products [3]. The occurrence of blood-borne transmission is supported by the demonstration of HEV viraemia among healthy blood donors in several countries [4]. Based on this, pre-transfusion screening of donated blood for HEV viraemia has been introduced in some jurisdictions [5], using nucleic acid tests (NAT) for HEV RNA. However, these tests are costly and need specialized equipment and manpower, making routine screening difficult in developing countries [6]. Further, even within Europe, countries with similar socio-economic and epidemiologic conditions and predominant HEV genotype, the prevalence of HEV viraemia has varied widely. An even greater variation in this prevalence may be expected in countries with differences in socio-economic conditions and predominant HEV genotypes.

In view of the above, there is a need to better estimate the prevalence of HEV viraemia among blood donors. We therefore undertook a systematic review of the available information on the prevalence of HEV viraemia among healthy blood donors.

Methods

Literature search and study selection

We searched electronic literature databases, namely PubMed/MEDLINE, Scopus and EMBASE (including EMBASE Classic), to identify English language papers published on or before 17 December 2018 that reported original data on prevalence of HEV RNA in blood donors or donated blood units. The search strategies used included various alternative terms related to HEV (such as HEV, hepatitis E or enterically transmitted hepatitis), viraemia (such as viremia, viraemia, HEV RNA or nucleic acid test) and healthy blood donors (such as donor, or blood donor) (Please see Supplementary information). Bibliographies of the retrieved articles were scanned to identify additional relevant publications.

The search results were entered into an EndNote X6 (Clarivate Analytics, Philadelphia, PA, USA) database, and duplicate entries were purged. Title, abstract and article type were reviewed by two reviewers to identify the relevant papers. In the next step, two authors reviewed full papers for the remaining records to identify studies that fulfilled the selection criteria. It was decided in advance to exclude any studies on special groups, such as stem cell donors, paid or illegal blood donors and pig farmers.

Data extraction

From each selected paper, two authors independently extracted the following information using a predesigned form: first author, year of publication, country, type of NAT (on individual donor units or pools of several units, and pool size, if the latter), donor characteristics, number of units tested for HEV RNA, number of units tested positive, HEV RNA concentration and HEV genotype in viraemic units. Any disagreements were resolved by the corresponding author (RA).

Several studies had used a 'pooled' NAT strategy, in which specimens from several blood units were pooled before testing for HEV RNA. In some such studies, the specimens included in a pool that tested positive were subjected to individual-unit NAT. In the studies where individual testing was not done, each positive pool was assumed to represent one HEV RNA-positive donor. If both individual-unit and pooled NAT were done, data from the former were used. Any equivocal test results were counted as negative.

In some studies, donated units had been first tested (pre-screened) for elevated alanine aminotransferase (ALT) or presence of HEV antigen or anti-HEV antibodies, followed by testing for HEV RNA only of screen-positive units. Data from such studies were also analysed separately.

Statistical analysis

For each eligible study, prevalence data on HEV viraemia were summarized as proportions. To combine results from several studies, a simple aggregation of data as well as pooling using a random-effects meta-analysis model was done. Inter-study heterogeneity was assessed using the I^2 and τ^2 methods. All analyses were done using Stata software, version 12 (StataCorp LLC, College Station, TX, USA).

As a form of subgroup analysis, separate meta-analyses were done for studies from data sets originating from similar geographical regions. In addition, in studies where blood units had also been tested for a biochemical or serological marker, the relationship of these markers with viraemia was assessed.

Results

Our electronic searches identified a total of 1199 unique citations. Of those, 1059 citations were deemed as not relevant during screening of titles and abstracts (Fig. 1). On review of full-text articles for the remaining 140 citations, 83 did not meet selection criteria. Of the 57 citations selected for final data synthesis [7–63], one provided three data sets [9]; hence, we had a total of 59 data sets from 28 countries (Fig. 2). These data sets included 109 to 620 140 blood units (median = 10 011) each (Table 1). A large majority of data sets were from Europe, high or high-middle income countries in Asia and North America (27, 12 and 4 data sets, respectively); by comparison, there were only eight studies from low/low-middle income countries of Asia and Africa; the remaining eight data sets were from other parts of the world. In 39 data sets, all the blood units had been tested for HEV RNA, whereas in 20 data sets, the blood units were first screened for the presence of anti-HEV antibodies (n = 17) [47–63] or for elevated serum ALT levels (n = 3) [44–46], with only the screen-positive units being tested for HEV RNA.

Of the 59 data sets, 29 were based on NAT on individual donor units and 28 on pooled NAT; of the remaining two studies, one had used individual-unit and pooled NAT for different subsets of specimens [27], and one had tested each blood unit with both individual-unit and pooled NAT [40]. In the studies that used pooled NAT, the number of blood units included in each pool varied from 2 to 500.

Viraemia was detected in one or more blood units in 42 of the 59 data sets, with prevalence of up to one every 65 units. Overall, of the 2 127 832 units studied in the 59 data sets, 561 units had tested positive for HEV RNA, with an aggregated HEV viraemia prevalence of 263·6 (95% CI = 242·7–286·4) per one million blood units, or one per 3792·2 (95% CI = 3491·6–4120·3) units.

A random-effects meta-analysis of all the 59 data sets revealed significant heterogeneity across studies (I^2 = 91·74%, $P < 0·001$; τ^2 = 0·00034), and a weighted pooled prevalence of HEV viraemia of 60·9 per one million donors (95% CI = 6·7–155·4) or one per 16 420 donors (one per 6436 to 149 416 units).

Separate analyses of data sets from different geographical regions showed that the pooled prevalence in Europe (n = 27 data sets), North America (n = 4), Asian countries in high or high-middle income category (n = 12), Asian or African countries in low or lower-middle income category (n = 8) was 21·6 (95% CI = 0·0–92·5), 10·9 (0·0–50·6), 658·1 (218·1–1291·4) and 3508·7 (406·7–8747·9) per one million donor units, respectively.

Subgroup analysis of studies by testing strategy

The available data sets were based on three different testing strategies, that is (i) testing for HEV RNA of all blood units studied (n = 39), (ii) screening of blood units using serological (anti-HEV antibody or HEV antigen) assay, followed by HEV RNA test in those that tested positive (n = 17) and (iii) screening for elevated serum ALT, followed by HEV RNA test in those that were positive (n = 3). The results for studies using these three strategies are compared in Table 2.

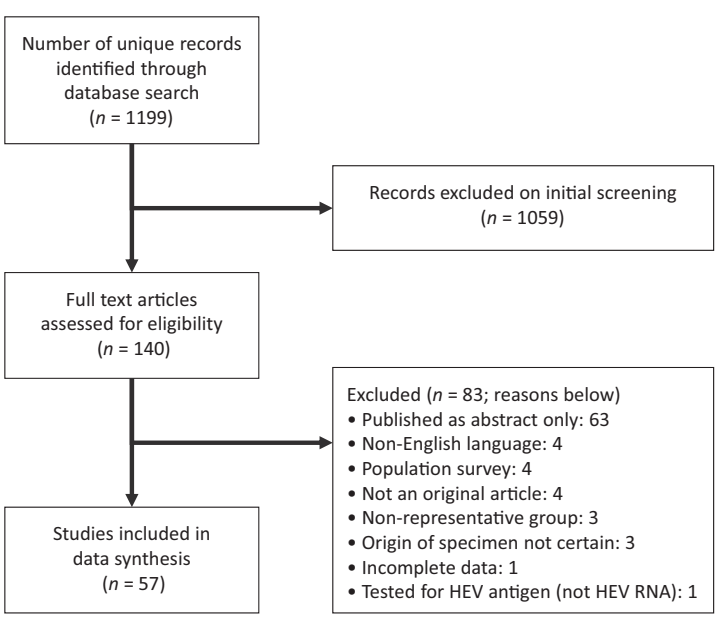

Fig. 1 PRISMA flow chart.

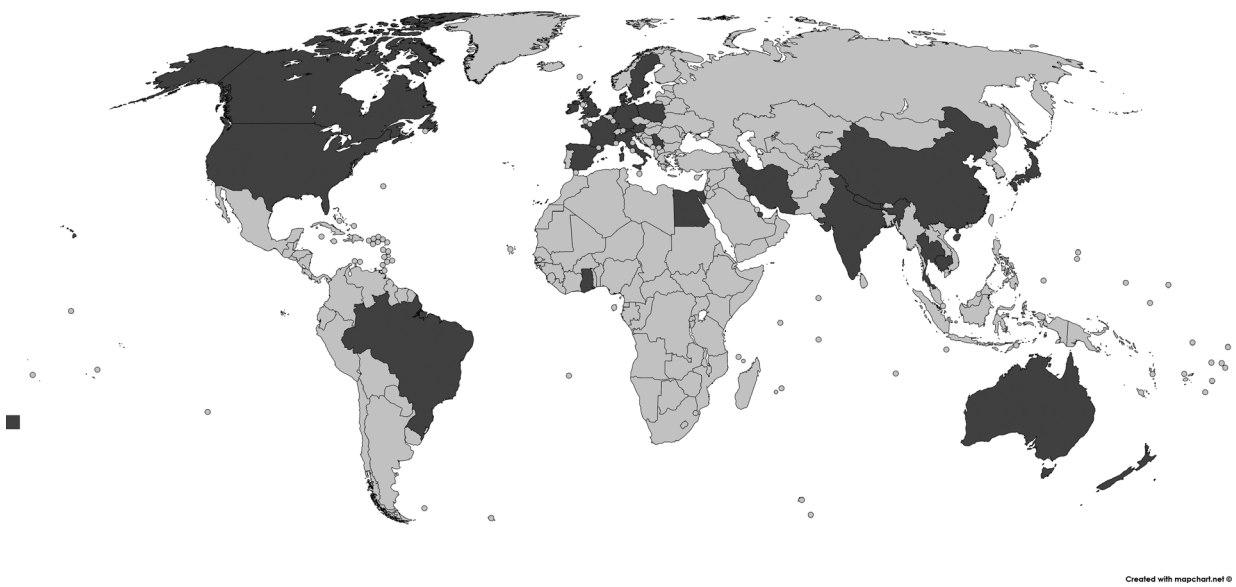

Fig. 2 World map showing the countries (in black) from which data on hepatitis E viraemia in blood donors were available.

Concentration of HEV RNA in viraemic units

Of the 42 data sets in which HEV RNA was detected, 27 reported data on concentration of HEV RNA in the viraemic units. In these studies, concentration of HEV RNA in viraemic units showed nearly one million-fold variation, being <10 to 11 200 000 IU/ml in the 21 studies that reported data as IU/ml, and <50 to 16 595 870 copies/ml in the four studies that reported these as copies/ml. Of the remaining two studies, one each reported data in genome equivalents per millilitre (<2000 genome equivalents/mL in each of the six specimens) and as semi-quantitative data (very low levels in each of the two viraemic specimens).

HEV genotype in viraemic donor blood units

Of the 42 data sets in which viraemia was identified, 33 reported data on HEV genotype; in the remaining studies, genotyping was either not attempted (n = 7) or failed (n = 2). Only one genotype each was identified in 31 studies, including GT3 in 26 studies, and GT4 in two studies (both from China) and GT1 in three studies (one each from India, Iran and Nepal). Two studies identified multiple HEV genotypes, namely GT1 and GT4 from China [50] and GT3 and GT4 from Japan [45].

Relation of serum ALT level with HEV viraemia

Four studies (China 3, Japan 1) had assessed HEV viraemia as well as ALT levels in the blood units studied [25,49,59,63] (Table 3). The studies from China and Japan used an ALT cut-off of 40 IU/l and 60 IU/l, respectively, to define normal or elevated ALT. In these studies, HEV viraemia was more frequent in the units with elevated serum ALT (10/7357; 1359 viraemic units per one million units screened) than in those with normal ALT (2/19 409; 103 per million units), with a pooled relative risk of 13·07 (95% CI = 2·51–68·00; P = 0·002). The genotypes identified in the donor blood with elevated ALT were either 3 or 4.

Relation of anti-HEV antibodies with HEV viraemia

In 10 data sets, all blood units had been tested for HEV viraemia as well as for a serological marker of recent (IgM anti-HEV or HEV antigen) or recent/remote (IgG) HEV infection. Of the 14 817 blood units analysed in these studies (Table 4), two (0·11%) of the 1,781 units with a detectable marker and five (0·04% of the 13 036) without these had detectable HEV RNA, with a pooled relative risk of 2.90 (95% CI: 0.57–15.08; P = ns).

In addition, 25 data sets provided data on anti-HEV antibody positivity in 372 HEV viraemic units (Table 5). Of these, 69/293 (23·5%) and 43/248 (17·3%) had tested positive for anti-HEV IgM and anti-HEV IgG, respectively. Overall, only 26·6% of the viraemic units had one or both of these antibodies.

Follow-up data on viraemic blood donors

Ten studies [8,13,16,17,20,24,35,41,42,49] described the temporal course of viraemia in the viraemic blood donors. Of the 144 such donors included in these studies, 111 (genotype 3 in 92, genotype 4 in 17 and unknown in 2)

Table 1 Summary of data from the studies identified for inclusion in the review

Continent/ region	Country	Author, year	Number of blood units screened	Number of units tested +ve for HEV RNA	HEV RNA concentration, median (range)	HEV genotype identified
a. Studies with testing of all blood units for HEV RNA without any prior screening						
Europe	Austria	Fischer, 2015 [13]	58 915	7	22 000–2 900 000 IU/ml	3
	Denmark	Harritshoj, 2016 [17]	25 637	11	<10–920 IU/ml	3
	France	Gallian, 2014 [15]	53 234	24	468–5 155 800 IU/ml	3
		Gallian, 2017 [14]	68 768	53	Data not reported	3
	Germany	Vollmer, 2012 [39]	41 325	13	12·8–68 100 IU/ml	3
		Baylis, 2012 [9]	18 100	4	1819–223 872 IU/ml	3
		Corman, 2013 [11]	93 955	14	1259–6 309 610 IU/ml	3
		Westholter, 2018 [42]	18 737	23	120–11 200 000 IU/ml	3
		Vollmer, 2018 [40]	10 141	17	<25–1 980 000 IU/ml	Not attempted
	Ireland	O'Riordan, 2016 [30]	26 061	5	10–44 550 IU/ml	3
	Italy	Lucarell, 2016 [24]	313	2	100–10 000 IU/ml	3
		Spada, 2018 [36]	10 011	0		
		Marcantonio, 2018 [26]	198	1	Not attempted	3
	Netherlands	Slot, 2013 [35]	45 415	17	<25–470 000 IU/ml	3
		Hogema, 2016 [20]	59 474	45	80–2 320 000 IU/ml	3
	Poland	Grabarczyk, 2018 [16]	12 664	6	16–6586 IU/ml	3
	Serbia	Petrovic, 2014 [31]	200	0		
	Spain	Sauleda, 2015 [33]	9998	3	250–2755 IU/ml	3
	Sweden	Baylis, 2012 [9]	95 835	12	1 585–89 125 IU/ml	3
	United Kingdom	Ijaz, 2011 [21]	42 000	6	<2000 GEq/ml	Not attempted
		Cleland, 2013 [10]	43 560	3	Data not reported	3
		Hewitt, 2014 [18]	225 000	79	50–2 370 000 IU/ml	3
		Thom, 2018 [38]	94 302	38	Data not reported	3
North America	USA	Baylis, 2012 [9]	51 075	0		
		Stramer, 2016 [37]	18 829	2	14 IU/ml*	Not attempted
		Roth, 2017 [32]	128 021	3	1000–6310 IU/ml	3
	Canada	Fearon, 2017 [12]	13 993	0		
Asia, high/ high-middle income	China	Xu, 2013 [43]	1939	0		
		Ma, 2015 [25]	816	0		
		Wen, 2018 [41]	11 747	28	10 900–715 000 copies/ml	4
	Japan	Minangi, 2016 [28]	620 140	36	<50–16 595 870 copies/ml	3
	Thailand	Intharasongkroh, 2018 [22]	30 115	26	Not reported	3
Asia/Africa, low/low-middle income	Cambodia	Nouhin, 2016 [29]	301	1	956 IU/ml	3
	India	Arankalle, 1999 [8]	200	3	Data not reported	Not attempted
		Katiyar, 2018 [23]	1799	0		
	Ghana	Meldal, 2013 [27]	239	0		
Others	Australia	Shrestha, 2016 [34]	14 799	1	15 000 IU/ml	3
		Hoad, 2017 [19]	74 131	1	180 IU/ml	Unsuccessful
	New Zealand	Hewitt, 2018 [7]	5000	0		
b. Studies with screening for HEV antigen or anti-HEV antibody, followed by testing for HEV RNA of screen-positive units						
Europe	France	Mansuy, 2015 (IgM) [54]	3353	1	630 copies/ml	Unsuccessful
	Italy	De Sabato, 2016 (IgG followed by IgM) [48]	170	0		
	United Kingdom	Beale, 2011 (IgG followed by IgM) [47]	595	0		
	China	Guo, 2010 (IgM) [50]	44 816	30	Data not reported	1 (n = 17), 4 (n = 13)

Table 1 (Continued)

Continent/ region	Country	Author, year	Number of blood units screened	Number of units tested +ve for HEV RNA	HEV RNA concentration, median (range)	HEV genotype identified
Asia, high/ high-middle income		Ren, 2014 (IgM and/or HEV antigen) [59]	11 538	4	Data not reported	4
		Wang, 2017(IgM and/or HEV antigen) [63]	9069	6	Data not reported	Not attempted
	Qatar	Nasrallah, 2017 (IgM) [55]	5809	4	Data not reported	Not attempted
	Japan	Fukuda, 2004 (IgG) [49]	5343	2	Data not reported	3
Asia/Africa, low/low-middle income	Egypt	Ibrahim, 2011 (IgM) [53]	760	2	Very low level	Not attempted
	India	Tripathy, 2018 (IgM) [62]	2447	2	35 000–4 60 000 copies/ml	1
	Iran	Parsa, 2016 (IgG and/or IgM) [56]	700	7	Data not reported	1
	Nepal	Gupta, 2016 (IgM) [51]	581	9	Data not reported	1
Others	Australia	Shrestha, 2014 (IgG) [61]	3237	0		
	Brazil	Passos-Castilho, 2016 (IgG) [57]	300	0		
		Passos-Castilho, 2017 (IgG) [58]	500	0		
		Hardtke, 2018 (IgG) [52]	199	0		
	South Caribbean	Schreuder, 2016 (IgG) [60]	600	0		
c. Studies with screening for high ALT, followed by testing for HEV RNA of screen-positive units						
Europe	Germany	Baylis, 2010 [44]	109	0		
Asia, high/ high-middle income	Japan	Gotanda, 2007 [46]	6700	9	Data not reported	3
		Fukuda, 2007 [45]	4019	11	Data not reported	3 (n = 8), 4 (n = 3)

IU, international units; GEq, genome equivalents.

*RNA concentration could be measured in only one of the two HEV RNA-positive units.

had been followed up, and all showed clearance of HEV RNA.

Follow-up of recipients of HEV viraemic units

Four studies [17,18,24,42] provided follow-up data on the recipients of HEV viraemic blood (Table 6). All these recipients had received blood or blood components that contained genotype 3 HEV, with HEV RNA ranging between <10 and 11 200 000 IU/ml. Of the 74 recipients, only 2 had clinical hepatitis.

In three of these studies [17,18,42], recipients had follow-up biochemical, serological or virological testing. Of the 61 recipients tested, five had biochemical hepatitis (elevated ALT) and seven had anti-HEV seroconversion. HEV viraemia was looked for in 54 recipients and was detected in 14 (26%) individuals, including 3 immunocompetent and 11 with an immunocompromised state.

Discussion

In the current systematic review, we retrieved published data on the prevalence of HEV viraemia in blood donors. In all, 59 data sets from 28 countries, with data on more than two million units, were identified. The prevalence in these studies varied widely. Aggregated data from these data sets showed an overall prevalence of HEV viraemia of 263·7 per million units, and a formal random-effects meta-analysis revealed a pooled prevalence of 60·9 per one million units. Separate meta-analyses for different geographical areas showed pooled prevalence varying from 21·6 to 3508·7 per one million units. Blood units with elevated ALT had a nearly 13-fold higher prevalence of HEV viraemia than those with normal ALT. Prevalence of viraemia was no different in the units with and without anti-HEV antibodies, and only about one-quarter of viraemic units were sero-positive for anti-HEV. The concentration of HEV RNA in the viraemic units varied widely. The HEV genotype most commonly identified was genotype 3, with minor contributions from genotypes 1 and 4.

The overall prevalence of HEV viraemia among unselected blood donors was relatively low. Introduction of routine screening of donated blood for HEV RNA is thus likely to prevent only about a small minority of HEV infections. The number of clinical cases prevented may be

Table 2 Comparison of results of three different strategies for hepatitis E viraemia detection in donated blood units

Parameter	Testing strategy used		
	Testing of all units without prior screening	Testing of units which tested positive for serological tests	Testing of units which had high levels of serum alanine aminotransferase
Number of data sets using the particular strategy	39	17	3
Countries represented in the data sets using the particular strategy	22	13	2
Number of data sets from			
a. Europe	23	3	1
b. North America	4	0	0
c. Asia: high/high-middle income	5	5	2
d. Asia/Africa: low/low-middle income	4	4	0
e. Others	3	5	0
Total number of blood units studied	2 026 987	90 017	10 828
Number of units studied from			
a. Europe	1 053 843 (52·0%)	4118 (4·6%)	109 (1·0%)
b. North America	211 918 (10·5%)	0 (0·0%)	0 (0·0%)
c. Asia: high/high-middle income	664 757 (32·8%)	76 575 (85·1%)	10719 (99·0%)
d. Asia/Africa: low/low-middle income	2539 (0·1%)	4488 (5·0%)	0 (0·0%)
e. Others	93 930 (4·6%)	4836 (5·4%)	0 (0·0%)
Number of blood units tested positive for HEV RNA	484	67	20
Aggregate viraemia prevalence, number per one million (95% confidence interval)	238·8 (218·2–261·0)	744·3 (58·6–94·5)	1847 (1160–2907)
Pooled prevalence using a random-effects model, number per one million (95% confidence interval)	49·6 (2·3–140·4)	325·5 (26·7–837·4)	696 (66–1759)
Heterogeneity			Not applicable*
I^2 value	93·16	73·94	
P value	<0·001	<0·001	

*The number of studies was too few to calculate I^2.

Table 3 Relationship of elevated serum alanine aminotransferase levels with prevalence of HEV RNA in healthy blood donors

Country	Author, year	Donated units with normal ALT		Donated units with high ALT	
		Number of units screened	Number of units that tested HEV RNA-positive	Number of units screened	Number of units that tested HEV RNA-positive
China	Ren, 2014 [59]	10 741	2	797	2
	Ma, 2015 [25]	366	0	450	0
	Wang, 2017 [63]	4046	0	5023	6
Japan	Fukuda, 2004 [49]	4256	0	1087	2
Total		19 409	2	7357	10

even smaller, since most of the transfusion-related HEV infections are asymptomatic, with only a small proportion developing icteric hepatitis, and even fewer progressing to severe liver injury or death [64]. However, such screening may still be important for blood units destined for administration to recipients in whom HEV infection may carry serious consequences. This includes persons with an inherited or acquired immune deficiency state, including those with solid organ transplantation, who are at a risk of developing chronic HEV infection and consequently chronic liver disease, and those with an underlying chronic liver disease, who are at risk of acute-on-chronic liver failure.

Importantly, the available data were limited by marked heterogeneity between studies. Also, most of the studies were from developed countries in Europe and North

Table 4 Relationship of presence of anti-HEV antibodies with that of HEV RNA in blood units from healthy blood donors in studies which tested such units for both anti-HEV antibodies and HEV RNA

Country	Author, year	Number of blood units screened	Anti-HEV antibody isotype tested	Number of units that tested positive for antibody	Number of serologically reactive units with detectable HEV RNA	Number of units that tested negative for antibody	Number of serologically non-reactive units with detectable HEV RNA
Cambodia	Nouhin, 2016 [29]	301	IgG, IgM	86	1	215	0
China	Xu, 2013 [43]	1939	IgG	364	0	1575	0
	Ma, 2015 [25]	816	IgG, IgM	175	0	641	0
Ghana	Meldal, 2013 [27]	239	IgG, IgM	32	0	207	0
India	Arankalle, 1999 [8]	200	IgG	37	0	163	3
Italy	Lucarell, 2016 [24]	313	IgG, IgM	153	1	160	1
	Spada, 2018 [36]	10 011	IgG	869	0	9142	0
Serbia	Petrovic, 2014 [31]	200	IgG	30	0	170	0
South Caribbean	Schreuder, 2016 [60]	600	IgG, IgM	26	0	574	0
Italy	Marcantonio, 2018 [26]	198	IgG, IgM	9	0	189	1

Table 5 Prevalence of anti-HEV antibodies in HEV viraemic blood units

Author, year	Number of HEV RNA–positive blood units studied	Number of HEV RNA-positive blood units that tested positive for anti-HEV antibodies		
		IgG isotype	IgM isotype	Either IgG or IgM isotype
Arankalle, 1999 [8]	3	0	1 (33%)	1 (33%)
Ijaz, 2011 [21]	6	6 (100%)	1 (17%)	6 (100%)
Baylis, 2012 [44]	8	1 (13%)	0	1 (13%)
Baylis, 2012 [9]	4	0	1 (25%)	1 (25%)
Vollmer, 2012 [39]	13	1 (8%)	3 (23%)	3 (23%)
Slot, 2013 [35]	17	8 (47%)	6 (35%)	8 (47%)
Gallian, 2014 [15]	24	2 (8%)	2 (8%)	2 (8%)
Hewitt, 2014 [18]	79			23 (29%)*
Fischer, 2015 [13]	7	0	0	0
Sauleda, 2015 [33]	3	3 (100%)	3 (100%)	3 (100%)
Harritshoj, 2016 [17]	11	5 (45%)	4 (36%)	5 (45%)
Hogema, 2016 [20]	45	11 (24%)	Not tested	11 (24%)
Lucarell, 2016 [24]	2	1 (50%)	1 (50%)	1 (50%)
Minangi, 2016 [28]	36	7 (19%)	2 (5%)	7 (19%)
Nouhin, 2016 [29]	1	1 (100%)	1 (100%)	1 (100%)
O'Riordan, 2016 [30]	5	0	1 (20%)	1 (20%)
Shrestha, 2016 [34]	1	0	0	0
Stramer, 2016 [37]	2	1 (50%)	1 (50%)	1 (50%)
Hoad, 2017 [19]	1	1 (100%)	1 (100%)	1 (100%)
Roth, 2017 [32]	3	1 (33%)	0	1 (33%)
Intharasongkroh, 2018 [22]	26	9 (35%)	2 8%)	9 (35%)
Marcantonio, 2018 [26]	1	0	0	0
Thom, 2018 [38]	38	8 (21%)	9 (24%)	9 (24%)
Vollmer, 2018 [40]	17	0	0	0
Westholter, 2018 [42]	19	3 (16%)	4 (21%)	4 (21%)
Total	372	69/293 (23·5%)	43/248 (17·3%)	99/372 (26·6%)

*Data for IgG and IgM isotype were not reported separately.

Table 6 Follow-up of persons who received HEV RNA-positive blood units

Author, year	Number of recipients of HEV RNA-positive blood followed up	Number of recipients who developed clinical hepatitis	Number of recipients tested for HEV infection during follow-up	Number of recipients who had ALT elevation	Number of recipients who had seroconversion to anti-HEV antibodies	Number of recipients who developed HEV viraemia	Comment
Hewitt, 2014 [18]	43	1	43	4	6	12	10 of 12 recipients who developed HEV viraemia were immunosuppressed
Harritshoj, 2016 [17]	15	0	7	0	0	0	
Lucarelli, 2016 [24]	2	0	0	Not done	Not done	Not done	
Westholter, 2018 [42]	14	1	11	1	1	2	1 of 2 recipients who developed HEV viraemia was immunosuppressed

America where the burden of HEV disease may be lower than in the countries in Asia and Africa. Even within these European countries with relatively homogenous socio-economic and cultural practices, the prevalence of viraemia showed marked variation. The reasons for this heterogeneity even within the same geographical region are unclear. Since HEV infection in Europe is believed to arise from animal-to-human transmission, it is possible that this variation arises from differences in practices for rearing pigs or in the food-eating habits, that is propensity to eat under- or uncooked meat. The studies were also heterogeneous in terms of the sensitivity of the NAT test used and the testing approach (i.e. pooled versus individual specimen testing with a dilution effect leading to reduced sensitivity in the former; pre-NAT testing screening or no screening). This could have influenced the estimates of viraemia prevalence in individual studies, and hence in our pooled estimates.

Only a few studies were available from developing countries in Asia, but these showed higher prevalence. Hence, the pooled prevalence of HEV viraemia for all the geographical regions taken together that we calculated may be an underestimate. Thus, additional large studies, from developing countries where HEV disease is endemic, to obtain more accurate estimates of prevalence of HEV viraemia and burden of transfusion-related HEV infection are needed.

Interestingly, the prevalence of HEV viraemia was higher in studies that first screened the donated blood units for a serological marker of HEV infection or elevated ALT and then tested the screen-positive units for HEV RNA than in the studies that tested all the units directly for HEV RNA. This is somewhat counterintuitive since the initial screening would be expected to fail to detect HEV viraemia in the units that lacked the marker used for screening. The observed differences between the various strategies thus possibly represent the inherent variability across studies and not the effect of a particular strategy. This is also supported by the observation that the studies in which such screening was employed were more often from high-income countries in Asia, whereas the studies that did not employ such screening were more often than from Europe and North America.

Since HEV RNA testing is costly and requires specialized equipment and manpower, it would be useful to identify surrogate markers for HEV viraemia. In various studies, elevated serum ALT levels and anti-HEV antibodies have been investigated as potential markers for HEV viraemia. The available studies show a higher prevalence of HEV viraemia among donor units with elevated ALT than among those with normal ALT [44–46]. However, the use of serum ALT as a surrogate marker for HEV viraemia has some inherent limitations. First, the data on

yield of ALT screening were quite limited in terms of the number of units screened and geographical distribution. Second, these data did not allow estimation of the proportion of HEV viraemic units that have elevated ALT. Third, the optimum ALT cut-off for such screening remains unclear and may be influenced by several factors, such as age, gender and country of residence of the blood donor. Furthermore, the available data on ALT levels among healthy persons are based on specimens collected after an overnight fast; these may not be applicable for blood donor screening since serum ALT levels rise after food intake and blood donation is often not done in a fasting state. Thus, it appears that serum ALT is unlikely to be a useful screening tool to identify the HEV viraemic donors.

Anti-HEV antibodies can also be expected to serve as a surrogate marker for HEV viraemia. However, in the available studies, viraemia was equally common in the sero-positive and sero-negative units. Furthermore, only a minority of HEV viraemic blood units were sero-positive. Hence, tests for these antibodies are unlikely to be useful for screening donated blood.

During any viral infection, viral proteins are released in the host and it may be possible to detect these in body fluids. A test for the detection of HEV antigen in various body fluids has been developed, and it provides an alternative method for the detection of HEV viraemia. The test appears to have a good concordance with the detection of HEV RNA. Detection of HEV antigen may also have the advantage of a lower likelihood of being affected by genomic variations in the virus. However, the available data are mostly from patients with HEV disease [65]. There is thus a need for further studies on the potential use of this test as a screening tool among blood donors in whom the viral concentration can be low, though arguably the risk of transmission of HEV may be associated only with blood with high viral load.

Genotype 3 was the most commonly identified HEV genotype in the viraemic donors which could be because of two reasons. First, this could be either because the available studies included in this meta-analysis were mostly from areas with predominant circulation of genotype 3 HEV. This is supported by data from a recent meta-analysis of Chinese studies, which also included studies published in the Chinese language; in that analysis, genotype 4 and 1 were the predominant HEV genotypes in viraemic blood donors, and genotype 3 was not identified [66]. Second, genotype 3 has a propensity to cause asymptomatic prolonged infection in healthy persons. This genotype differs from the more common genotype 1 in having a large animal reservoir, zoonotic spread to humans, potential to persist in immunosuppressed persons [67], and to produce non-hepatic manifestations [68]. In contrast, chronic infection is virtually unknown with genotype 1 HEV [69,70].

On follow-up, all the HEV viraemic donors cleared the virus spontaneously and without developing any disease. However, data on the duration of viraemia were very sketchy. It would be useful, in future studies, to follow such persons more closely to understand the natural history of asymptomatic HEV viraemia. Since the titre of HEV RNA in viraemic donors varied as much as a million-fold, it would also be useful to study the temporal profile of HEV RNA titre in asymptomatic HEV infection, and whether the outcomes vary with the level of HEV viraemia.

Our review showed that a proportion of recipients of HEV viraemic blood developed biochemical hepatitis and detectable HEV viraemia, and clinical hepatitis was infrequent. However, a large proportion of persons who developed HEV infection had an underlying immune-compromised state.

In conclusion, the prevalence of asymptomatic HEV viraemia among healthy blood donors is highly variable across studies both within and across similar geographical settings, though relatively low overall. Thus, the impact of implementation of NAT testing for HEV RNA in blood transfusion services on the overall burden of HEV disease is difficult to assess. However, such testing may still be important for persons who are at a high risk of serious disease following HEV infection, such as those with immunosuppression. Thus, screening may be considered primarily for blood destined to recipients who are immunocompromised such as organ transplant recipients and those with limited liver reserve, in particular in countries where genotype 3 HEV is predominant. In addition, our review points to a need for further data on prevalence of HEV viraemia, as also on the time-course of such viraemia, in healthy persons, particularly from developing countries where non-3 HEV genotypes are prevalent, and consequences of transfusion of HEV viraemic blood with different HEV RNA concentrations.

Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contributions

AG and RA conceived the study. AG involved in literature search and prepared the first draft. VHJ and HK screened the study and extracted the data. RA involved in literature search, analysed the data and edited the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

File S1 Search strategy used to search various databases for initial identification of potential studies for inclusion in this systematic review.