Hepatitis E virus blood donor NAT screening: as much as possible or as much as needed?

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BACKGROUND: The cost-benefit question of general screening of blood products for the hepatitis E virus (HEV) is currently being discussed. One central question is the need for individual nucleic acid amplification techniques (NAT) screening (ID-NAT) versus minipool NAT screening (MP-NAT) approaches to identify all relevant viremias in blood donors. Here, the findings of ID-NAT versus MP-NAT in pools of 96 samples were compared.

STUDY DESIGN AND METHODS: From November 2017 to January 2018, a total of 10,141 allogenic blood donations from 7650 individual German blood donors were screened for the presence of HEV RNA using MP-NAT (96 samples) (RealStar HEV RT-PCR Kit) compared to ID-NAT (cobas HEV assay) on the fully automated cobas 6800 platform.

RESULTS: Parallel screening of MP (n = 122, 96 samples/MP) using both methods detected seven reactive pools. After pool resolution, 8 HEV RNA–positive donations were identified by the in-house detection method, whereas 17 HEV RNA–positive donations were identified by ID-NAT with the cobas HEV assay. This resulted in an incidence of 1:1268 donations (0.079%) for MP-NAT screening and 1:597 donations (0.168%) for ID-NAT screening.

CONCLUSIONS: The detection frequency of HEV RNA was approximately 50% higher if ID-NAT was used compared to MP-NAT. However, viral loads of ID-NAT–only samples were below 25 IU/mL and will often not result in transfusion-transmitted HEV (TT-HEV) infection, taking into account the currently known infectious dose of 5.0E + 04 IU inevitably resulting in TT-HEV infection. The clinical relevance and need for identification of these low-level HEV-positive donors still require further investigation.

The emergence of the hepatitis E virus (HEV) as a potential new candidate pathogen of transfusion-transmitted HEV (TT-HEV) infections introduces procedural questions about the safety of blood products. The cost-benefit question of a general screening of blood products for HEV is being discussed extensively. An important factor influencing the discussion is the required minimum viral load to be detected in the donor blood, which is determined mainly by the currently known lowest infectious dose of a certain blood product triggering an infection in the recipient. Increased importance of TT-HEV has been recognized since 2004, although earlier reports pointed to the risk of infection.[1,2] Several European committees, local blood authorities, and a large number of blood transfusion facilities question the necessity of HEV-NAT screening of blood donors. In the "European Pharmacopeia," Chapter 8.3, the implementation of an HEV run control for screening of human plasma pools was recently demanded.[3] A summarized assessment of the current situation in 11 European countries by Domanovic et al. described the situation as "a shift to screening,"[4] but

ABBREVIATIONS: ALT = alanine aminotransferase; APC = apheresis-derived platelet concentrates; FFP = fresh frozen plasma; HEV = hepatitis E virus; ID-NAT = individual NAT; MP-NAT = minipool NAT; PPC = pooled platelet concentrate; RBC = red blood cell; SD = standard deviation; TT-HEV = transfusion-transmitted HEV.

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different countries have chosen different approaches. Nationwide HEV RNA screening of blood donations was introduced in Ireland, the United Kingdom, and The Netherlands. Selective screening intended for use in high-risk patients or universal screening for HEV RNA is performed, partially on a voluntary basis, in several blood establishments in Germany and France, and recently in Switzerland. Blood authorities in Greece, Portugal, Italy, and Spain are currently evaluating the situation.[4] However, at present, some authorities are reluctant to recommend a general HEV RNA screening of blood donors or plasma pools for fractionation but have recommended HEV monitoring of immunosuppressed patients.[5,6]

The practical implementation including the applied test system or screening strategy (MP-NAT versus ID-NAT) also varied among different countries. A large number of HEV NAT screening studies in Europe revealed HEV incidences ranging from 1:157 in central Italy to 1:14,520 in Scotland, depending on the donor cohort, test sensitivity, and pool size.[7] However, the optimal screening strategy is being discussed extensively, mainly driven by the question of the minimum required sensitivity in connection with the cost-benefit ratio. TT-HEV infection has been described for all types of blood products, including red blood cells (RBCs), platelet preparations, pooled granulocytes, and fresh frozen plasma (solvent-detergent treated, amotosalen-treated, secured by quarantine[8]). The likelihood of infection is strongly influenced by the viral load present in the donor and the residual plasma volume with respect to the infectious dose in the concerned blood product. The European Medicines Agency concluded that in-process testing of plasma pools for HEV RNA using an MP-NAT testing strategy might be helpful for screening out donations with high virus concentrations as an additional safety measure contributing to the safety margin of plasma-derived medicinal products, similar to screening for hepatitis A virus (HAV) RNA.[5] In the present study, we compare the findings of ID-NAT versus MP-NAT in pools of 96 samples. Based on our results and the amount of plasma in different blood products, we extrapolated the detection probability of HEV RNA–positive blood donors using different test strategies (NAT assay, ID vs. MP-NAT with different pool sizes). Furthermore, we systematically review published screening studies using different screening assays and screening strategies (ID-NAT versus MP-NAT) to evaluate the relation of MP size to the sensitivity of applied assay with the determined incidences.

MATERIAL AND METHODS

Blood donors and HEV RNA screening

From November 2017 to January 2018, a total of 10,141 allogenic blood donations from 7650 individual German blood donors were screened for the presence of HEV RNA using MP-NAT (96 samples, RealStar HEV RT-PCR Kit, Altona Diagnostic Technologies [ADT], Hamburg, Germany) compared to ID-NAT using the cobas HEV assay on the fully automated cobas 6800 platform (Roche Diagnostics, Mannheim, Germany). MPs of 96 samples were also screened in parallel with the cobas HEV assay. Testing algorithms including pool resolution are shown in Fig. 1. All donors underwent predonation medical examination and negated current diseases or any known risk factors for viral infection.

RealStar HEV RT-PCR Kit: MP-NAT for HEV RNA was performed as described previously.[9] In brief, master pools of 96 donations (200 µL/donor) and subpools of 10 donations (400 µL/donor) were extracted with the Chemagic viral DNA/RNA reagent kit (4.8 mL protocol, Viral 5 k, PerkinElmer chemagen Technologie GmbH, Baesweiler, Germany)

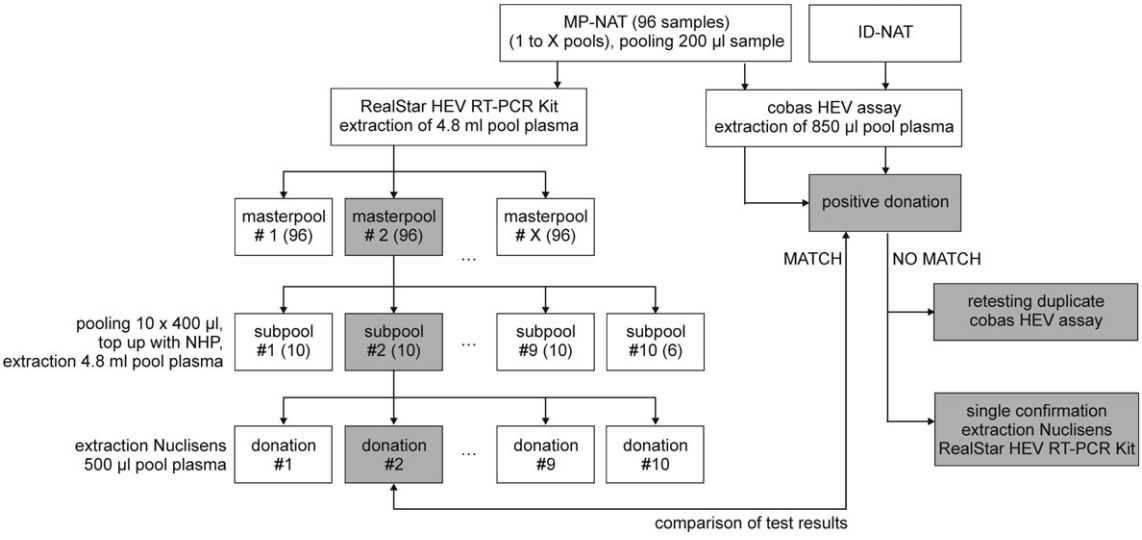

Fig. 1. Flow diagram of screening and confirmation procedure for HEV RNA.

combined with the automated Chemagic magnetic separation module MSMI (PerkinElmer chemagen Technologie GmbH). Donors of the reactive subpool were tested singularly and total RNA was extracted from 500 µL plasma using the NucliSens easyMAG (bioMerieux, Nürtingen, Germany) automated RNA/DNA extraction.

cobas HEV assay: Master pools of 96 donations and individual donor samples were analyzed according to the manufacturer's instructions. Reactive samples observed exclusively for ID-NAT were repeated twice.

The HEV virus titer in the plasma of HEV RNA–positive donors was quantified with the RealStar HEV RT-PCR Kit using the first World Health Organization (WHO) international standard for HEV RNA for NAT-based assays (Paul-Ehrlich institute, Langen, Germany). The linear range of quantification was from 25 to 10E + 07 IU/mL; therefore all positive results below this 25 IU/mL were represented as <25 IU/mL.

Analytical sensitivity and comparison of different amplification methods

The analytical sensitivity of the cobas HEV assay was reevaluated using a twofold dilution series of plasma inoculated with the first WHO international standard for HEV RNA for NAT-based assays (Paul-Ehrlich Institute) in 6 dilution steps and 24 replicates. The 95% detection limit was calculated by probit analysis using SPSS software (SPSS GmbH Software, version 14.0, SPSS, München, Germany).

Serological testing and measurement of liver-specific parameters

Plasma of HEV RNA–positive donors was screened for the presence of HEV-specific IgM and IgG antibodies using the Anti-HEV ELISA (IgM and IgG, Euroimmun, Lübeck, Germany). Samples were analyzed according to the manufacturer's instructions. Results of the immunoassay were classified into the following three categories: 1) no antibodies detectable (negative: IgM ratio < 0.8, IgG < 0.8 IU/mL), 2) evidence of the presence of antibodies (borderline: IgM ratio ≥ 0.8 to ≤1.1, IgG ≥ 0.8 to ≤1.1 IU/mL), and 3) antibodies detectable (positive: IgM ratio > 1.1, IgG > 1.1 IU/mL). Serum concentrations of glutamate dehydrogenase (GLDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin were measured in plasma samples using the respective enzymatic assays (Abbott Diagnostics Europe, Wiesbaden, Germany) on the Architect c8000 system (Abbott Diagnostics Europe).

Searching criteria

For the systematic review of publications on HEV RNA incidence in blood donors, the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed/) was searched. PubMed is a public search engine maintained by the United States National Library of Medicine (NLM) at the National Institutes of Health (NIH) that provides access to over 24 million citations in all fields of the life sciences, mostly from MEDLINE (Medical Literature Analysis and retrieval System Online). The database was searched for publications between 2010 and 2018 (publications dates) using specific search strings including "hepatitis E/HEV infection", "transfusion transmitted hepatitis E/HEV infection", and hepatitis E/HEV blood donor screening."

RESULTS

Analytical sensitivity

To reevaluate the analytical sensitivity of the cobas HEV assay, a twofold dilution series of HEV RNA–positive plasma was used. The 95% detection limit was calculated to 11.71 IU/mL (confidence interval: 9.31–17.12 IU/mL), and the 50% detection limit was calculated to 4.19 IU/mL (confidence interval: 2.68–5.57 IU/mL). The analytical sensitivity of the RealStar HEV RT-PCR assay in combination with the 4.8 mL nucleic acid extraction protocol was determined previously for the 95% detection limit as 4.66 IU/mL (confidence interval: 3.60–7.55 IU/mL)[9] resulting in a detection limit of 447.4 IU/mL for a single donation in a master pool of 96 sample. The 50% detection limit was calculated as 1.44 IU/mL (confidence interval: 0.60–2.03).

Screening of blood donors

A total of 10,141 individual donations were screened for the presence of HEV RNA. Characteristics of HEV RNA–positive donations are summarized in Table 1. Parallel screening of MPs (n = 122, 96 samples) with the in-house detection method and the cobas HEV assay detected seven reactive pools with both methods (concordant positive MP- and ID-NAT). After pool resolution, a total of 8 HEV RNA–positive donations were identified by the in-house detection method, whereas 17 HEV RNA–positive donations (from 16 different donors) were identified by ID-NAT using the cobas HEV assay. This resulted in an incidence of 1:1.268 donations (0.078%) for the pool screening procedure and 1:597 donations (0.168%) for ID screening. Remarkably, in one case, MP-NAT with the in-house detection method also detected an HEV RNA–positive donation with a viral load below the linear range of quantification of 25 IU/mL per single donation, which is far below the specified 95% detection limit of the MP-NAT assay. All ID-NAT–only positive donations had viral loads <25 IU/mL. The detected fluorescence signals were very weak, with CT values >40 cycles. The two samples donated by one donor (D4) within an interval of 7 days were identified by ID-NAT–only twice, showing very high CT values >42 without an increase in the viral load. At the time of initial identification, all the 17 HEV RNA–positive donations were NAT-only positive (negative for anti-HEV IgM and anti-HEV IgG).

TABLE 1. HEV RNA concentration, HEV genotype, HEV antibody status, concentration of liver-specific enzymes of HEV-positive donors

	HEV RNA			Anti-HEV				HEV RNA		Anti-HEV		
Donor (age/sex)	ID-NAT initial c6800 (CT)	ID-NAT retest c6800 (CT)	ID IH c_{RNA} (IU/ml)	IgM (SCO)	IgG (IU/ml)	ALT (U/l)	Follow-up (d)	ID c6800 (CT)	ID IH (c_{RNA} IU/ml)	IgM (SCO)	IgG (IU/ml)	Completion of seroconversion (IgM-, IgG+, PCR-)
ID-NAT positive only												
D1 (24, M)	P (42.61)	N/N	N	N (0.08)	N (<0.20)	22	70	N	N	P (1.38)	P (1.45)	147
D2 (30, M)	P (41.97)	N/N	<25	N (0.19)	N (0.64)	13	72	N	N	N (0.66)	P (8.90)	72
D3 (31, F)	P (42.96)	P (40.93) P (40.78)	N	N (0.10)	N (0.30)	8	NA	NA	NA	NA	NA	NA
D4 (22, M)	P (42.57)	P (46.61) N	N	N (0.14)	N (0.28)	11	7	P	N	N (0.07)	N (0.23)	NA
D5 (37, M)	P (41.04)	P (43.12) N	N	N (0.94)	N (<0.20)	19	67/130	N	N	N (0.22)	N (<0.20)	no seroconversion†
D6 (19, M)	P (40.57)	P (38.94) P (39.38)	<25	N (0.08)	N (0.24)	41	22	P	2.09E + 03	N (0.11)	N (0.32)	56
D7 (44, M)	P (42.66)	N/N	N	N (0.04)	N (<0.20)	23	62	P	8.52E + 02	N (0.70)	P (4.96)	62
D8 (60, M)	P (42.32)	N/N	<25	N (0.04)	N (0.23)	24	8	N	N	N (0.04)	N (<0.20)	102
Concordant positive MP- and ID-NAT												
D9 (25, M)	P (30.15)	NT	1.25E + 04	N (0.06)	N (<0.20)	27	164	N	N	N (0.36)	P (3.70)	164
D10 (25, M)	P (35.98)	NT	2.12E + 03	N (0.04)	N (<0.20)	114	94	N	N	N (0.37)	P (>25)	94
D11 (62, M)	P (26.67)	NT	1.98E + 06	N (0.06)	N (<0.20)	27	NA	NA	NA	NA	NA	NA
D12 (22, M)*	P (37.48)	NT	<25	N (0.17)	N (0.68)	49	104	N	N	N (0.20)	P (4.78)	104
D13 (25, M)	P (31.16)	NT	4.47E + 04	N (0.07)	N (<0.20)	61	NA	NA	NA	NA	NA	NA
D14 (50, F)	P (36.43)	NT	4.47E + 02	N (0.13)	N (<0.20)	20	124	N	N	N (0.58)	P (18.61)	124
D15 (49, M)*	P (35.15)	NT	1.50E + 03	N (0.40)	N (<0.20)	23	NA	NA	NA	NA	NA	NA
D16 (26, M)	P (31.11)	NT	1.09E + 05	N (0.08)	N (<0.20)	45	106	N	N	N (0.45)	P (6.44)	106

ID-NAT = individual NAT; MP-NAT = minipool NAT; ID IH cRNA = quantification of individual samples by in house PCR; CT = crossing threshold; P = positive; N = negative; NA = not available; NT = not tested.

* Confirmation of HEV viremia in previous retention sample (D12: 3.48E + 02 IU/mL).

† No seroconversion detectable after either 67 or 130 days.

Confirmation of HEV infection by follow-up analysis

To evaluate the accuracy of obtained results, follow-up samples were analyzed to confirm HEV infection. Follow-up samples were available for 12 donors (ID-NAT-only: n = 7, MP-NAT: n = 5). Three donors identified concordant positive by MP- and ID-NAT have not yet returned, thereby preventing confirmation of HEV infection by evidence of HEV seroconversion. However, these samples had viral loads >1000 IU/mL and have been detected repeatedly as positive; therefore false-positive screening results can be excluded with reasonable certainty. For the remaining five donors identified as concordant positive by MP- and ID-NAT, as well as for five ID-NAT–only positive donors, HEV infection was confirmed by determination of the serostatus in subsequent samples (range 7–164 days, Table 1) showing complete seroconversion with IgM-negative and IgG-positive status.

Unfortunately, the accuracy of results for two of the eight ID-NAT–only positive samples cannot be assessed definitively due to the absence of subsequent samples. One donor (D3) did not return for donation. For a second ID-NAT–only positive donor (D4), only one follow-up sample with a small temporal interval of seven days was available. Samples from this donor were repeatedly reactive with ID-NAT, either on Day 0 or Day 7; two of three replicates were positive. No anti-HEV antibodies were detectable at this time, and the in-house detection method remained negative at both time points. Most likely, this donor is in a very early phase of acute primary HEV infection with very low viremia.

Surprisingly, one of the ID-NAT–only positive donors (D5) did not show seroconversion after 67 or 130 days. The serostatus was reconfirmed using the Wantai IgG ELISA (Sanbio B.V., Uden, The Netherlands), and the recomWell HEV IgG ELISA (Mikrogen GmbH, Neuried, Germany). Reconsideration of results raises suspicion of a repeat false-positive screening result, since only two of three replicates were positive with the cobas HEV assay. However, the assay had a very high specificity and the occurrence of contamination is likewise conceivable. Unfortunately, we had no chance to ultimately solve this issue due to the absence of sufficient sample material to repeat analysis with a greater number of replicates.

DISCUSSION

There is an ongoing discussion regarding the need for HEV blood donor screening, with divergent opinions and regulatory requirements in different countries.[4] One important aspect is the currently unknown clinical relevance and burden of TT-HEV infection. The second major aspect is determination of the most appropriate and effective strategy to minimize the risk of TT-HEV infection, balancing necessity and practicability. On the one hand, screening may constitute a universal approach to include all blood products, or selective screening can be performed for only the products that would be used in patients at risk. This issue is influenced primarily by hospital-wise distinct definition of at-risk patients and logistic implications for the order of blood products and their supply, as well as the logistics of testing influencing availability on the part of the blood establishment.[8] Certainly, authorities in the United Kingdom have already recommended a general screening approach, driven principally by the technical complexity and double inventory costs needed for selective screening.[4] On the other hand, the required minimum viral load to be detected in the blood donor, which is influenced by the currently known lowest infectious dose of a blood product triggering an infection in the recipient, is currently unknown. In this context, the necessity of ID-NAT compared to MP-NAT is often called into question, also dependent on logistics and costs.

In this study, attention is focused on the limit of detection of NAT, depending on the applied screening strategy (ID-NAT vs. MP-NAT). This is the first study to comparatively assess the performance of MP-NAT (96 samples) versus ID-NAT in a consistent cohort of German blood donors. Our MP screening setting is, to the best of our knowledge, the most sensitive HEV NAT. The fully automated nucleic acid extraction method chemagic Viral DNA/RNA Kit allows the processing of large plasma volumes (4.8 mL), thereby increasing the processed sample volume compared to other commercial HEV NAT screening methods, resulting in a higher number of HEV plasma-equivalents per polymerase chain reaction (PCR) assay.[8] It is nevertheless not surprising that the detection frequency of HEV RNA–positive donations was approximately 50% higher using ID screening. The ID-NAT applied in our study had an approximately 50× greater sensitivity related to the viral load in the single donation. Gallian et al. compared the incidence of HEV RNA in French blood donors obtained by MP-NAT (6 samples and 96 samples) and ID-NAT with similar results, although individual blood donor cohorts were used for each screening strategy.[10] Hogema et al. estimated how many donations were missed by pool screening by testing all donations made up to 60 days before or after HEV RNA-positive donations. In total, 37% of donations with viral loads in the range of 20–750 IU/mL were positive when tested individually, whereas they had not been detected with MP-NAT screening.[11]

However, is it necessary to identify those low-level viremic donations from the current point of knowledge regarding TT-HEV infection? All HEV RNA positive donors exclusively identified by ID screening had a corresponding viral load of <25 IU/mL. This means, in fact, that the resulting blood products will contain the following maximum infectious doses depending on the respective mean plasma content (Table 2): RBCs: <250 IU, pooled platelet concentrates (PPCs) with additive solution: <3565 IU, plateletpheresis concentrates (APCs) in plasma: <5000 IU, fresh frozen

TABLE 2. Overview of different blood products and their plasma content; detection probability of different screening strategies

	RBC	PPC (in additive solution)	APC (in plasma)	FFP
	Volume of blood product			
	310 mL (220–400 mL)	310 mL (260–360 mL)	250 mL (205–295 mL)	290 mL (200–380 mL)
	Plasma proportion			
	10 mL/RBC	0.46 mL/mL (0.40–0.52 mL/mL)	0.8 mL/mL	0.78 mL/mL (0.75–0.82 mL/mL)
Blood product specification	Plasma volumes*			
	10 mL	35.65 ml†	200 mL	226 mL
	Infectious dose inevitably resulting in TT-HEV infection (5.0E + 04 IU), corresponding viral load to be identified in donor			
	5000 IU/mL	1403 IU/mL	250 IU/mL	221 IU/mL

Detection probability‡ for different screening strategies based on the 95% or 50% LOD of NAT assays

		95/50% LOD (IU/ml)			95/50% LOD (IU/ml)			95/50% LOD (IU/ml)			95/50% LOD (IU/ml)	
Pool size	viral load (IU/ml)	RealStar	cobas	viral load (IU/ml)	RealStar	cobas	viral load (IU/ml)	RealStar	cobas	viral load (IU/ml)	RealStar	cobas
		4.7/1.4	18.6/3.9		4.7/1.4	18.6/3.9		4.7/1.4	18.6/3.9		4.7/1.4	18.6/3.9
ID	5000	+	+	1403	+	+	250	+	+	221	+	+
8	625.0	+	+	175.4	+	+	31.3	+	+	27.6	+	+
24	208.3	+	+	58.5	+	+	10.4	+	(+)	9.2	+	(+)
48	104.2	+	+	29.2	+	+	5.2	+	(+)	4.6	(+)	(+)
96	52.1	+	+	14.6	+	+	2.6	(+)	–	2.3	(+)	–
	Lowest infectious dose resulting in TT-HEV infection (7.1E + 03 IU), corresponding viral load to be identified in donor											
	710 IU/mL			199 IU/mL			36 IU/mL			31 IU/mL		

Detection probability‡ for different screening strategies based on the 95% or 50% LOD of NAT assays

		95/50% LOD (IU/ml)			95/50% LOD (IU/ml)			95/50% LOD (IU/ml)			95/50% LOD (IU/ml)	
Pool size	Viral load (IU/ml)	RealStar	Cobas	Viral load (IU/ml)	RealStar	Cobas	Viral load (IU/ml)	RealStar	Cobas	Viral load (IU/ml)	RealStar	Cobas
		4.7/1.4	18.6/3.9		4.7/1.4	18.6/3.9		4.7/1.4	18.6/3.9		4.7/1.4	18.6/3.9
ID	710	+	+	199	+	+	36	+	+	31	+	+
8	88.8	+	+	24.9	+	+	4.5	(+)	(+)	3.9	(+)	(+)
24	29.6	+	+	8.3	+	(+)	1.5	(+)	–	1.3	–	–
48	14.8	+	(+)	4.1	(+)	(+)	0.8	–	–	0.6	–	–
96	7.4	+	(+)	2.1	(+)	–	0.4	–	–	0.3	–	–

* Residual plasma volumes were obtained from blood product specifications in our transfusion facility or from the literature (RBC,[13,18] PPC.[18]

† Total 142 mL from 4 buffy coats, assuming only one positive donation resulting in 35.65 mL HEV RNA positive plasma.

‡ Assessment detection probability, +: 95% LOD < viral load, (+): 95% LOD > viral load but 50% LOD < viral load, –: 95% and 50% LOD > viral load.

TABLE 3. Detection frequency of HEV RNA in unselected blood donations (e.g., preselection according to antibody status) and a minimal number of 1000 donors screened

Country	Donations [donors] (n)	RNA positive (n)	Pool size	Incidence donations (%)	Test system (LOD)	LOD screening (IU/ml)*	Study period (duration)	Ref
Europe								
Austria	58,915 [NS]	7	96	1:8416 (0.011)	RealStar HEV† (11,6 IU/mL)	1114	02/2013–04/2014 (14 mo)	23
UK	225,000 [NS]	79	24	1:2848 (0.035%)	in-house (22 IU/mL)	528	10/2012–11/2013 (14 mo)	12
UK	42,000 [NS]	6	48	1:7040 (0.014)	NS	NS	2007 (1 y)	24
Denmark	25,637 [25,637]	11	ID	1:2331 (0.043)	Procleix TMA+ (7.9 IU/mL)	7.9	01–02/2015 (2 mo)	16
France	53,234 [NS]	24	96	1:2218 (0.045)	RealStar HEV (23 IU/mL)	2208	11/2012–12/2013 (14 mo)	25
France	1560 [NS]	1	6	1:1560	RealStar HEV (23 IU/mL)	138	12/2014–09/2015 (10 mo)	10
	17,305 [NS]	1	96	1:1442		2208	12/2012–10/2014 (11 mo)	
	5211 [NS]	7	ID	1:744	Procleix TMA+ (7.9 IU/mL) cobas HEV‡ (18.6 IU/mL)	7.9–18.6	06–07/2015 (2 mo)	
Germany	10,141 [7650]	8	96	1:1268 (0.079)	RealStar HEV (4.66 IU/mL)	447	11/2017–01/2018 (3 mo)	this study
		17	ID	1:597 (0.168)	cobas HEV (18.6 IU/mL)	18.6		
Germany	41,325 [16,125]	13	48	1:3179 (0.031)	RealStar HEV (4.66 IU/mL)	224	07–09/2011 (3 mo)	9
Germany	18,100 [NS]	4	96	1:4525 (0.022)	in-house (250 IU/mL)	24.000	NS	26
Germany	18,737 [NS]	23	24	1:815 (0.123%)	cobas HEV (18.6 IU/mL)	447	10/2016–05/2017 (8 mo)	15
Ireland	24,985 [NS]	5	ID	1:4997 (0.020)	Procleix TMA+ (7.9 IU/mL)	7.9	12/2013–06/2014 (6 mo)	27
Scotland	43,560 [NS]	3	24	1:14,520 (0.006)	in-house (201 IU/mL)	4824	NS	28
Scotland	51,388** [NS]	23	24	1:2234 (0.045)	cobas HEV (18.6 IU/mL)	447	02/2016–02/2017 (12 mo)	29
	42,914** [NS]	15		1: 2860 (0.035)			03–05/2017 (3 mo)	
	T: 94,302 [NS]	38		1:3929 (0.040)			02/2016–05/2017 (15 mo)	
Spain	9998 [9998]	3	ID	1:3333 (0.030%)	Procleix TMA+ (7.9 IU/mL)	7.9	06–12/2013 (6 mo.)	30
Sweden	95,835 [NS]	12	96	1:7986 (0.012)	in-house (250 IU/mL)	24.000	NS	26
The Nether-lands	59,474 [NS]	40	96	1:1487 (0.067)	in-house (38.4 IU/mL)	7373	01/2013–12/2014 (2 y)	11
		44	192	1:1351 (0.074)	in-house (10.3 IU/mL)	4800		
		45, 41 donor	96/ 192	1:1321 (0.069)	-	4800–7373		
	59,474 [NS]	78	various	1:762 (0.130)	in-house (10.3–38.4 IU/mL, mean 25 IU/mL)	4800–7373	01/2013–12/2014 (2 y)	
The Nether-lands	20,016 [NS]	7	48	1:2859 (0.035)	in-house (25 IU/mL)	1.200	11/2011–01/2012 (3 mo)	20
	20,160 [NS]	6	480	1:3360 (0.030)		12.000	04–05/2012 (2 mo)	
	5239§ [NS]	4	ID	1:1310 (0.076)		25	03/2011 (2 d)	
	T: 45,415 [NS]	17	-	1:2671 (0.037)		-	-	
North America								
Canada	13,993¶ [NS]	0	48/ 100	1:13,993	RealStar HEV (15 IU/mL) in-house (10 IU/mL)	480–1500	07/2013–12/2015 (2.5 y)	31
USA	51,075 [NS]	0	96	1:51,075	in-house (250 IU/mL)	24,000	NS	26
USA	128,021 [NS]	4, 3 donors	96	1: 32,005 (0.003%)	cobas HEV (18.6 IU/mL)	1785	06–08/2015 (~3 mo)	32
USA	1939 [1939]	0	8	< 1: 2000	in-house (200 IU/mL)	1600	2006/2012	33
USA	18,829 [NS]	2	ID	1:9500 (0.011)	Procleix TMA+ (7.9 IU/mL)	7.9	02–07/2013 (6 mo)	34
Others								
Australia	14,799 [NS]	1	ID	1:14,799	Procleix TMA+ (7.9 IU/mL)	7.9	09–10/2014 (2 mo)	35
Australia	74,131 [NS]	1	6	1: 74,131 (0.001)	Procleix TMA+ (7.9 IU/mL)	47.3	05–11/2016 (11 mo)	36
China	44,816 [NS]	30	NS	1:1494 (0.067)	in-house (NS)	NS	12/2002–10/2008 (6 y)	37
Japan	107,756 [NS]	2	500	1: 53,788 (0.002)	in-house (152 IU/mL)	76,000	2004–2014 (2y)	38
Japan	512,564 [NS]	34	50	1: 15,075 (0.226)		7600		

* Calculated 95% LOD of the screening assay depending on pool size, minimal viral load detected in the donor.
† RealStar HEV RT-PCR-Kit.
‡ Cobas HEV assay (either on the cobas 6800 or cobas 8800 platform).
§ Preselected donor cohort anti-HEV IgG positive, excluded from Fig. 2.
¶ Preselected donor cohort, prevalence study *Babesia microti,* excluded from Fig. 2.
** Regionally selected donor cohort (n = 51,388) and nationwide screening (n = 42,914).

plasma (FFP): <5655 IU. Some studies characterized the infectious dose[12–14] and, furthermore, we recently reviewed published single-case reports of TT-HEV infection.[8] Not surprisingly, components causing TT-HEV infection had a considerably higher median infectious dose than components not causing TT-HEV infection.[8,12,13] We determined that the lowest infectious dose currently known to result in TT-HEV infection was 7.05E ± 03 IU.[8] Taking into account the type of blood product, the lowest infectious dose transfused was 7.05E + 03 IU for platelet concentrates (no subdivision of APCs and PPCs), 3.16E + 04 IU for RBCs, and 3.60E + 04 IU for FFPs. Our systematic case review analysis showed that all components with an infectious dose >5.00E ± 04 IU caused TT-HEV infection. All nontransmitting components with the exception of one single case were contaminated with infectious doses below this value.[8] Correspondingly, the infectious doses reported in additional, recently published case reports did not exceed these values.[15] However, one of the reported cases further lowered the infectious dose known to result in TT-HEV infection for RBCs to 7.60E ± 03 IU. Based on current knowledge of the lowest infectious dose known to result in TT-HEV infection, transfusion of blood products derived from HEV RNA–positive blood donors exclusively identified by ID-NAT most likely will often not result in TT-HEV infection. However, this dose has a certain chance of causing HEV transmission in particular cases, but the likelihood is very low. A certain dose of HEV has a certain chance of transmission, just as certain levels of HEV have certain chances of being detected. Taking APCs as an example, a level of 25 IU/mL will result in 5000 IU (mean residual plasma content) or 5900 IU (maximum residual plasma content, according to Table 2). Both values were only barely below the lowest infectious dose known to result in TT-HEV infection and the likelihood of being transmitted is much than higher than for an HEV contaminated RBC, with a maximum dose of 250 IU). No evidence of TT-HEV infection was found after transfusion of HEV RNA–positive blood products (mainly RBCs) obtained from Danish blood donations carrying low viral loads (median HEV RNA level, 13 IU/mL[16]).

In fact, the discussion of blood donor NAT screening is about the dose received versus the probability of infection and about the level of safety that is desired. Taking into account the current knowledge regarding the lowest infectious dose currently known to result in TT-HEV infection, the infectious dose inevitably resulting in TT-HEV infection, and the analytical sensitivities of the two screening methods applied in this study, we further extrapolated the detection probability of HEV RNA–positive blood donors. In addition, we considered the amount of plasma in the different blood products and calculated the infectious doses needed to be found depending on the screening strategy (ID-NAT vs. MP-NAT with different MP sizes, Table 2). Strategies are based on the most common strategies currently used in published HEV RNA screening studies with ID screening (7 studies), MP screening in 24 or 48 samples (7 studies), or MP screening with 96 samples (9 studies, Table 3). For the cobas HEV assay, we included the analytical sensitivities stated in the package insert. If the initially stated NAT sensitivity of 100 IU HEV RNA/ml per single donation is taken into account,[17] not even ID testing is sufficient to detect the minimum viral load in the donor to avoid TT-HEV infections based on the lowest infectious dose currently known to result in TT-HEV infection of 7.1E ± 03 IU. Contaminated APCs (≥36 IU/mL) or FFPs (≥31 IU/mL, Table 2) could still result in TT-HEV infection. Considering the currently known infectious dose of 5.0E + 04 IU inevitably resulting in TT-HEV infection,[8] this sensitivity is only achievable for all potential blood products if ID screening is applied. Unrestricted consideration regardless of a defined minimum sensitivity revealed that even pool screening in MP of 96 samples with the 2 assays compared in this study will detect most donations inevitably resulting in TT-HEV infections, independently of the risk of the blood product transfused. Likewise for the lowest infectious dose currently known to result in TT-HEV infection, all donations resulting in blood products with a lower infectious risk due to the lower plasma content were identified using MPs of 96 samples.

Unfortunately, the discussion about the desired level of safety extends the discussion to the dietary risk versus transfusion risk of HEV. The blood safety discussion is often confronted with the argument that most HEV infections were transmitted via the zoonotic or food-borne route, which may be an explanation for the diversity of HEV transfusion policies in Europe. The risk of HEV infection in vulnerable transfusion recipients may be less determined by contaminated blood products than by dietary and environmental exposures. Taking model data from The Netherlands, a total of 133,000 HEV infections are expected annually; taken together with an estimated number of 187 TT-HEV infections, only 1 in 700 HEV transmissions are due to contaminated blood products.[18] Another model comparing blood transfusion and dietary risks estimated that approximately 13 donor exposures are required to provide the same risk of infection as dietary exposure for 1 year, based on the annual seroconversion rate of 0.2% in the UK population.[13] However, the authors further calculated that at-risk patients including stem cell or solid organ recipients may be exposed to more than 60 blood components, increasing the transfusion risk close to 1%, equivalent with 5 years of dietary exposure. Again taking the model data from The Netherlands, the authors conclude that of estimated chronic cases due to TT-HEV infection, 1 of 3.5 chronic cases in The Netherlands would be via blood transfusion.[18]

In recent years, a large number of reports of screening studies using different screening assays and screening strategies (ID-NAT vs. MP-NAT) have been published (Table 3), showing considerable differences regarding the observed rates of HEV RNA positivity. These differences may be explained by the following reasons, considered individually

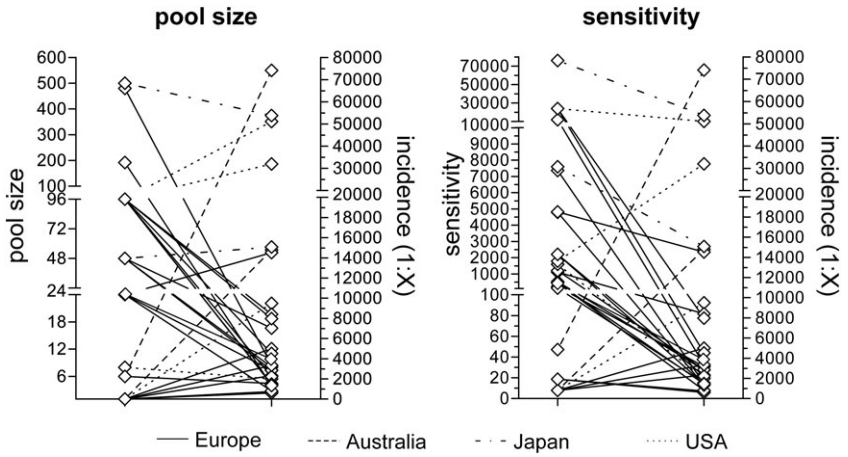

Fig. 2. Incidence of HEV infection among blood donors around the world. Incidence specified in different studies according to Table 2 analyzed by pool size and sensitivity of applied screening method.

or together: 1) a higher sensitivity of the NAT assay used for screening, which is related to 2) the MP size, 3) variations in the incidence of HEV infection between countries as an effect of the particular donor population, especially within the context of the zoonotic potential and transmission of HEV, or 4) variations of the incidence of HEV infection over time (short-term: seasonal differences, long-term: varying disease burden). In this context, we evaluated the relation of MP size versus the sensitivity of applied assay with the determined incidences (Fig. 2). First of all, studies represent a high heterogeneity regarding the reference point donors or donations. Depending on the study period, the study design or potential postponing of positive donors, the type of blood donor (plasma and/or whole blood) significantly influence the calculated incidences. Most often, the number of donations is given, whereas the number of screened donors is repeatedly lacking. For our subsequent analysis, we refer to the number of screened donations. In general, Australia, the United States, and Japan showed a significantly lower HEV incidence compared to Europe, independently of the MP size or the sensitivity of the assay used; therefore this is an effect of the particular donor population (Fig. 2, Table 3). Hence, Kupferschmidt is right with his assessment that HEV is "Europe's new hepatitis problem."[19] Taking a closer look at the determined incidences in Europe, the MP size is not inversely proportional to the detected incidences. Greater MP sizes did not inevitably result in lower incidences, and the observed effects were influenced mainly by the combination of MP size and sensitivity of the assay used. With some exceptions, it is clearly visible that a higher assay sensitivity resulted in a higher incidence, concordantly to the results obtained in this study. Of interest, despite a switch from MP consisting of 48 samples to 480 samples without changing the NAT assay, the incidence in equivalent donor cohorts remained constant, even though the sensitivity was reduced by factor 10.[20] This further supports the explanation of variations in the incidence of HEV infection over time. Therefore, a reasonable comparison of HEV incidences is challenging. Further data from individual countries is required, ideally using assays with a constant sensitivity over a greater time frame, not least for optimization of risk assessment and cost-effectiveness analyses, since the determined incidences are a principal factor for the calculation basis. Other major uncertainties of risk assessment and cost-effectiveness analyses are the probability of transmission by viral dose. The viral loads present in the donors cause a third uncertainty because a higher viral load in the donor enhances transmission probability, on one hand, but also increases the detection probability, on the other.[18]

Just recently, the German regulatory authorities (Paul-Ehrlich Institute) announced the probable introduction of mandatory HEV NAT screening from September 2019. They recommend a minimum sensitivity of 2000 IU HEV RNA/mL (per single donation[21]), independently of the adopted screening strategy (MP- or ID-NAT). This required sensitivity is achieved with both assays used in this study. The cobas HEV assay can be used for both testing strategies, ID- and MP-NAT, whereas the in-house detection method using the RealStar HEV RT-PCR assay is applicable only for a MP-testing strategy due to the lower degree of automation and throughput.

Based on the current knowledge and potential future regulatory requirements in Germany, we will continue with our routinely implemented in-house MP screening strategy (96 samples) to identify highly viremic donors, although some other European countries have implemented HEV ID-NAT screening.[4] Taking into account the observed progression of viremia in seven Genotype 3 infected blood donors, our implemented in-house MP-NAT method will detect 51.3% (95% limit of detection (LOD)) or 71.8% (50% LOD) of viremic donations.[22] This procedure is also in line with the

current statement of the European Medicines Agency, recommending minipool screening in their reflection paper on hepatitis E.[5]

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CONFLICTS OF INTEREST

The authors have disclosed no conflicts of interest.

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