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INTERNATIONAL FORUM

Hepatitis E

J. Petrik, M. Lozano, C. R. Seed, H. M. Faddy, A. J. Keller, P. S. Prado Scuracchio, S. Wendel, A. Andonov, M. Fearon, G. Delage, J. Zhang, J. W. K. Shih, P. Gallian, R. Djoudi, P. Tiberghien, J. Izopet, J. Dreier, T. Vollmer, C. Knabbe, R. Aggarwal, A. Goel, A. R. Ciccaglione, K. Matsubayashi, M. Satake, K. Tadokoro, S.-H. Jeong, H. L. Zaaijer, E. Zhiburt, J. Chay, D. Teo, S. S. Chua, M. Piron, S. Sauleda, J.-M. Echevarría, H. R. Dalton & S. L. Stramer

Hepatitis E virus (HEV) is the aetiological agent of hepatitis E, discovered in 1980's during the military campaign in Afghanistan among Soviet soldiers with unexplained hepatitis. A faecal–oral route of transmission was directly experimentally established [1]. HEV was known to cause waterborne epidemics in developing countries, with human-infecting genotypes 1 and 2 responsible. In the last decade, however, HEV was established as a zoonotic infection in industrialized countries. Autochthonous infections are caused predominantly by genotype 3 and to a lesser extent by genotype 4, infecting humans, pigs and other mammals. Uncooked or undercooked pork, game and shellfish are considered main sources of zoonotic infections [2, 3].

Only a limited number of transfusion transmissions have been described to date, but more recent studies indicate a more frequent occurrence [4], although for various reasons, those transmissions are often underreported. Large proportions of blood and blood components are destined for recipients with natural, acquired or induced immunodeficiency. Chronic infections have been described in immunosuppressed individuals, mostly organ transplant recipients, but the scale of the problem is not clear and requires more detailed studies. The large majority of HEV infections in immunocompetent individuals are subclinical, and this fact combined with lack of hepatitis E awareness may lead to low level of reporting followed by testing or applying HEV tests at later stages, if at all [5].

The HEV seroprevalence figures and incidence of active infection in the general population and in blood donors are higher than expected in some jurisdictions and highly variable. They show as yet unexplained, significant differences between countries and regions, as well as within some countries [6]. New data raise a number of important questions about the measures which should be adopted to deal with a transfusion-transmissible agent, causing a mild self-contained infection in immunocompetent individuals, but a potentially fatal one for patients with underlying liver disease or transplant recipients. Such questions must be asked in the situation where a seemingly high level of transmission is not matched by the number of reported infections in the recipients, and at the same time, the zoonotic agent is being readily acquired through the food chain.

Due to the complexity of this topic and the lack of large studies, the expert views perhaps unsurprisingly differ on the necessity and/or the urgency to introduce HEV screening in blood donors [4, 6–8]. However, as HEV appears gradually becoming the dominant cause of new hepatitis cases, there is a recognized need for HEV testing at an early stage, alongside routine tests for other hepatic viruses, at least for high-risk individuals.

Pathogen inactivation technologies (PITs) represent another approach to improve blood safety, but their efficiency varies for different pathogen target groups. The PIT efficiency may not yet be sufficient for certain non-enveloped viruses such as HEV [9, 10] and needs further improvement.

First HEV prophylactic vaccine was approved by China's State of Food and Drug Administration (SFDA) in 2011. All clinical studies with Hecolin (hepatitis E vaccine produced in *E. coli*, Xiamen Innovax Biotech, Xiamen, China) were carried out in China and shown well tolerated and effective for genotypes 1 and 4 [11]. Data are not available on Hecolin efficiency in high-risk groups and also in relation to genotype 3, hampering a potential wider use outside China. WHO's Global Advisory Committee on Vaccine Safety recommended to carry out phase 4 postmarketing study to further assess the safety profile.

In this Forum, we have invited responses to nine questions related to different aspects of HEV epidemiology, pathology, diagnostics, perceived public health and blood safety importance, as well as potential measures to deal with this pathogen. The invitation was sent to establishments and experts in the field. Eighteen responses were received and are summarized below. Individual detailed participants' responses are available electronically on the Vox Sanguinis website (include link here).

Question 1

Are you aware of studies of HEV prevalence in the blood donors or general population in your region/country? What is the prevalence of HEV found in those studies?

Seroprevalence

Published data and Table 1 in this forum reveal wide variation in HEV seroprevalence figures. One of the reasons frequently cited for at least part of such variation is the performance of different assays used (see Q3 for more details). However, a closer inspection of Table 1 clearly shows significantly different prevalence figures for countries and/or regions established using the same assay. If we take a look at seroprevalence figures obtained only with the Wantai ELISA, largely accepted as the most sensitive current assay, the differences between countries and/or regions are significant. The lowest figures published until recently using Wantai were from Fiji (2·3%) [12] and New Zealand (4·0%) [13]. Several other countries and regions also show seroprevalence established with Wantai assay below 10% (Table 1), which could arbitrarily be labelled as 'low' seroprevalence: Australia (6%), Canada (5·9%), Latium region of Italy (9%), Scotland (4·7%). On the other side of the spectrum are countries and regions with seroprevalence above 20%, which could be considered 'high': South Korea (27%), China (30–42%), Netherlands (27%), France (24–52%), Abruzzo region of Italy (46%). 'Moderate' seroprevalence between 10% and 20% established with Wantai assay was reported from England (12–16%), USA (16%) and Spain (~20%). Such direct comparison of figures obtained with the same assay reveals significant differences even within the same country: UK's England and Scotland: 12–16 vs. 4·7%, north and south of France: 24% vs. 52%; Latium and Abruzzo regions of Italy: 9% vs. 46%. Potential reasons for such differences are discussed under Q4.

Studies which looked at the prevalence in various blood donor age groups confirmed increasing seroprevalence with age. In Canada, the over 50-year-old group had 9·9% seroprevalence against overall 5·9%, in Spain the over 61-year-old group had 39% vs. 17% overall figure, in Scotland 9·8% in over 55 group and 4·7% overall, in USA 22% (MP Biomedicals, Santa Ana, CA, USA) or 42% (Wantai, Beijing, China) in the over 65 group vs. average prevalence of 7·7% (MP) or 16% (Wantai). Several studies reported generally higher seroprevalence figures in male donors, but this was not confirmed in other studies.

RNA incidence

There seems to be some correlation between seroprevalence and RNA incidence, although these figures need to be interpreted with caution, due to the use of serological and NAT assays with differing parameters, as well as variable sample sizes for NAT assays. As in seroprevalence, there is a wide variation in RNA incidence figures among the blood donors from various countries and regions.

Highest RNA incidence (1 in 36 to 50; 2 to 2·7%) in Table 2 comes unsurprisingly from endemic India, although resulting from small studies. RNA prevalence in a range of 0·02–0·2% was reported among Chinese blood donors. The latest figures from the Netherlands are in a similar range of 1 in 658 (0·15%) and recent figures from some German trials (1 in 1240 or 0·08%; 1 in 1760 or 0·06%), as well as from France (1 in 2218 or 0·04%), England (1 in 2848 or 0·03%) and Spain (1 in 3333 or 0·03%) are not far behind. On the other side of the scale no RNA-positive donations were reported in Canadian and Australian studies, although numbers may not have been sufficiently high. Japan described 0·011% prevalence in endemic region of Hokkaido. USA with 1 in 9500 (0·01%) and Scotland with 1 in 14 520 (0·007%) recorded lower RNA incidence.

Question 2

What HEV assays have been used to establish seroprevalence and acute infection (RNA frequency) in blood donors and/or general populations in your country – commercial or in house? What was the sensitivity and specificity of these assays, if known?

Seroprevalence studies were performed predominantly using commercial assays. Only reports from India and Japan specified the use of in-house assays (Table 1). However, the sensitivity and specificity of various commercial assays varies significantly, a fact which is sometimes difficult to deduce from the sensitivity and specificity figures provided by the manufacturers. Meaningful direct comparison of data is therefore possible only for studies using the same assay, as shown above (Q1) for studies using the Wantai ELISA.

The number of laboratories using commercial and in-house NAT assays to establish HEV RNA incidence is more equally distributed than in the case of serological assays, reflecting the lack of available approved commercial tests until recently (Table 2). Seven establishments are using in-house PCR assays, although they may test individual samples or minipools up to 96–100 samples, which in some cases (Canada) are concentrated by ultracentrifugation. Among the establishments or laboratories using commercial kits, the Altona Diagnostics RT-PCR kit has been used most frequently (4×), closely followed by Hologic/Grifols TMA assay (3×) and DRK RT-PCR kit (3×).

Question 3

In your view, what are the dominant risk factors for HEV infection in your country? What are the prevalent genotypes and routes of HEV infection?

Table 1 Summary of HEV seroprevalence data

Country	Seroprevalence		Target group (number tested)	Region/year	Screening HEV assay	Confirmatory HEV assay	Sensitivity[a]	Specificity[a]	Ref[b]
	IgG %	IgM%							
1 Australia	0.4		BD		Genelabs indirect EIA				1
	2.2		Travellers						1
	7.7		Non AB hepatitis patients						1
	8		HIV infected patients						2
	6	0.02	BD (3237 IgG, 194 IgG positive tested for IgM)		Wantai ELISA		97.96–100 %		3
2 Brazil	6.1		Gold miners		Various assays used:		98.50%	99.50%	1
	3.3		GP		IgG: Abbott, Biokit ELISA	Microgen Immunoblot recomLine HEV IgM/IgG			
	2.0–7.5		BD		IgM: Biokit bioELISA				
	14.0–18.0		Prostitutes at risk of HIV		IgM: Microgen recomWell ELISA				
	12		IV drug users						
	1		Pregnant women						
	4.5		Children						
	3.3–6.1		Amazonian communities						
3 Canada	5.9		BD (4102)		Wantai IgG ELISA		<1.0 WHO U/ml (0.25 U/ml)	100% Based on in house panel	
4 China	30–42	0.4–1.7%	BD		Various commercial assays used		IgG: 93 %	98%	1, 2, 4
	25–66		GP				IgM: 95–99%	>99%	2, 3, 4
							IgM: 90%	99%	5
5 France I	24		BD (10 000)	France	Wantai ELISA				In prep
6 France II	23.6		BD	France	Wantai ELISA				
	52		BD	South West (Toulouse)	Wantai ELISA				
7 Germany	5.5		BD (109)	West (Hesse)	ELISA Mikrogen, MP Biomed	WB Mikrogen			[c]
	15.5		BD (116)	East (Thuringia)	WB Mikrogen				[d]
	16.8		Adults' exam. Survey (4422)	Germany	WB Mikrogen				2
	11		BD (301)	East (Berlin, Brandenburg)	WB Mikrogen				[e]

Table 1 (Continued)

Country	Seroprevalence		Target group (number tested)	Region/year	Screening HEV assay	Confirmatory HEV assay	Sensitivity[a]	Specificity[a]	Ref[b]
	IgG %	IgM%							
	18		Forestry workers (563)	East (Berlin, Brandenburg)	WB Mikrogen				[e]
	5·9		BD (336)	West (NRW, Lower Saxony, Hesse)	ELISA Mikrogen, MP Biomed	WB Mikrogen			5
	6·8		BD (1019)	North Schleswig- Holstein	ELISA Mikrogen				[f]
	50·7		Patients (1092)	Southeast (Bavaria)/1996	ELISA Axiom				3
	20·5		Patients (1092)	Southeast (Bavaria)/1996	WB Mikrogen				3
	34·3		Patients (1092)	Southeast (Bavaria)/2011	ELISA Axiom				3
	14·5		Patients (1092)	Southeast (Bavaria)/2011	WB Mikrogen				3
	1		Children (0–17 years)	Germany	ELISA Mikrogen, MP Blomed	WB Mikrogen			[h]
8 India	6.9 –14.0		Different soc.-econ. groups		In-house, orf 2-based assay				1
	17–55.2		Adults						
9 Italy	1–3		GP	North Central I, 1990s					1
	3–6		GP	Southern I, Islands, 1990s					2
	9		BD	Latium	Wantai ELISA		96.4% (IgM)		
	46		BD	Abruzzo			Based on 28 HEV RNA positive samples		Lucareli *et al.*
10 Japan	3.4		BD (12 600)		In-house or commercial		Not known	Not known	1, 2
	5.3		BD (22 027)		HEV ELISAs				3
11 Netherlands	27		BD		Wantai EIAs				
12 Russia	4.75	1.78	BD (505)		RPC Diagnostic Systems		IgG		2
	0.79 (both)	0.79 (both)					IgM: 98%	92.80%	
	2	1	HIV patients (500)						
	0.8 (both)	0.8 (both)							
	29.97		Afghanistan veterans (317)						3
	3.82		Other veterans (208)						
13 Singapore		0.0019	Confirmed acute hepatitis E patients		MP Biomedicals IgM ELISA		98.00%	97.80%	1, 2, 3 4
14 S Korea	11.9		GP (749)		GeneLab				
	23.1		GP (147)		Wantai				
	14.3		GP (147)		GeneLab				

Table 1 (Continued)

Country	Seroprevalence IgG %	Seroprevalence IgM%	Target group (number tested)	Region/year	Screening HEV assay	Confirmatory HEV assay	Sensitivity[a]	Specificity[a]	Ref[b]
15 Spain I	2.2		GP	Madrid	VariousAssays used:				1, 2, 3
	7.3		GP	Barcelona	Abbott, Biokit,				
	3.1		BD (492)	Granada	DiaPro,				
	2.8		BD (863)	Madrid	Microgen RecomBlot				
	18.6		Pig farm workers						
16 Spain II	7.3		GP (1280)	Catalonia /2006	Various Assays used:				1, 2, 3
	2.17		GP (2305)	Madrid /2012	Biokit bioelisa IgG;				
	4.6		Children (6–15 year)	Catalonia, 2008	Diagnostic Bioprobes				
	3.9		BD	Madrid /1995	Srl Ab; Abbott EIA				4
	20		BD (1082)	Catalonia /2013	Wantai IgG ELISA		99.08%	99.99%	5
	11		BD (1082)	Catalonia /2013	Microgen recomWell IgG		96.30%	98.20%	
17 USA	16		BD (1939)	Samples collected 2012	Wantai				1
	7.7 (total)	0.6	BD (18 829)	6 USA areas, samples 2013	MP BioMed double antigen and IgM		99.20%	99.20%	2
	6.0 (total)	0.5	GP aged 6 and over	USA, samples 2009–10	Diagnostic Systems		98%	95.20%	
18 UK	12		BD	England	Wantai				1
	16		BD	South West England	Wantai				2
	4.7		BD (1559)	Scotland	Wantai				3

BD, blood donors; GP, general population; WB, Westernblot.

[a]As stated by the manufacturers, unless specified otherwise.

[b]References numbers as in the corresponding country responses.

[c]Baylis *et al.*, Vox Sang 2010; 98:479.

[d]Krumbholz *et al.*, Med Microbiol Immunol 2012; 201:239–44.

[e]Dremsek *et al.*, Med Microbiol Immunol 2012; 201:189–200.

[f]Juhl et al., Transfusion 2013; 54:49–56.

[g]Krumbholz *et al.*, Pediatr Infect Dis J 2014; 33:258–62.

Table 2 Summary of HEV RNA incidence data

Country	HEV RNA incidence	Target group (number tested)	Region and/or year	NAT Assay/sample size	Analytical sensitivity/Limit of detection (LOD)	Specificity	References
1 Australia	0	BD IgG positive (194)		TMA Procleic HEV, Hologic/individual	95% lower LOD 8-18 HEV RNA copies/ml		3[a]
2 Brazil	Genotyping purposes only						
3 Canada	0	BD (14 000)		RealStar HEV RT-PCR (Altona Diagnostics)/pools of 100 after ultracentrifugation.	Analytical sensitivity 15 IU/ml; 95% LOD is 25 IU per pool after ultracentrifugation corresponding to 250 IU/ml per individual sample before pooling (100 samples of 0.1 ml each).	100% (in-house RNA+ panel)	
4 China	0.02–0.2%	BD IgM positive		PCR, individual	Sensitivity 78 %	100%	1, 5
5 France I	1 in 2218	BD (53 234)		Real Star Reverse Transcription PCR (Altona Diagnostics) (pools of 96)	95% LOD 23 IU/ml; Confirmatory quantitative method: LOD 60 IU/ml		1
6 France II	1 in 2218	BD (53 234)		RealStar HEV RT-PCR kit 1.0" Altona Diagnostics	95% LOD: 23 IU/ml (WHO standard)		
7 Germany	1 in 1240 (0.08%)	BD (16 125)	2011	RealStar HEV RT-PCR kit (Altona)[c]/pools of 48	4.7 (ID)/226 (pool)		5
	1 in 4525 (0.02 %)	BD (18 100)	2012	In house RT-PCR /pools of 96	~250 ID/24 000 (pool)		[d]
	1 in 3050 (0.03%)	BD (12 200)	2012	HEV RT-PCR kit (DRK)[e]/pools of 100	12 ID/1200 pool		[f]
	1 in 1760 (0.06%)	BD (72 220)	2013	HEV RT-PCR kit (DRK)[e]/pools of 100	12 ID/1200 pool		[f]
	1 in 2027 (0.05%)	BD (91 216)	2014	HEV RT-PCR kit (DRK)[e]/pools of 100	12 ID/1200 pool		[f]
8 India	1 in 50 1 in ~36	BD (200) BD (107)		In-house			2, 3
9 Italy				RealStar HEV RT-PCR kit, version 1.0, (Altona Diagnostics)	95% LOD: 50 IU/ml		3, 4 [g]

Table 2 (Continued)

Country	HEV RNA incidence	Target group (number tested)	Region and/or year	NAT Assay/sample size	Analytical sensitivity/Limit of detection (LOD)	Specificity	References
10 Japan	1.1%	BD with ALT ≥ 200 IU/L (1389)	2003–2004	In house real time RT-PCR/pools of 20	95% LOD: 50 IU/mlz		2
	0.014%	Health check up individuals (22 027)	2002–2007	In house nested RT-PCR/indiviual samples or minipools of 50			3
	0.011%	BD (~2.5 million)	Hokkaido (endemic) 2005–2013	In house real time RT-PCR/pools of 20	95% LOD: 50 IU/mlz		
11 Netherlands	1 in 2436	BD (41 415)	2011	In-house			
	1 in 658	BD (11 191)	2014	PCR, pools of 96			
12 Russia							
13 Singapore							
14 S Korea	NA						
15 Spain I							
16 Spain II	0.03%; 1 in 3333	BD (~10 000)		Procleic HEV, Hologic/ Grifols	95% LOD: 7.9 IU/ml (WHO international standard)	99.99%	5
17 USA	0	BD (1939)	2014	Individual samples, TMA Panther platform (Hologic Grifols)	50% LOD: 2 IU/ml	99.96%	1 [h]
	1 in 9500	BD (18 829)					
18 UK	1 in 2848	BD	England				4
	1 in 14 520	BD (43 560)	Scotland	In-house, pools of 24	95% LOD: 201 IU/ml		3

BD, blood donors.

[a]Reference numbers as in the corresponding country responses.

[c]Altona, Diagnostics, Hamburg. Nucleic acid extraction carried out using Chemagen viral 5K (Perkin Elmer Chemagen).

[e]DRK Blutspendedienst, Frankfurt a.M. Nucleic acid extraction carried out using Zeleos x 100.

[d]Baylis *et al.* 2011, Vox Sang 103:89–90

[f]Hourfar *et al.* Transf Med Hemother 2014; 41 (suppl 1):70

[g]Lucarelli *et al.*, manuscript in preparation

[h]Unpublished.

The replies reveal that in a number of industrialized countries, the cases of imported HEV infections are still significantly higher than the number of autochthonous cases of zoonotic origin: >90% in Canada, similar proportion in Australia and Italy. In Singapore, the proportion of imported cases is around 55%. However, there is little doubt about ongoing zoonotic transmissions in industrialized countries where studies were carried out. There is a clear distinction of genotypes depending on route of transmission: mostly genotype 1 (some 2) for imported cases, mostly genotype 3, less frequently 4 in China and Hokkaido, Japan. Genotype 1 does not seem to cause chronic infection in immunosuppressed individuals, but causes significantly high mortality in pregnant women. The effects of the genotype 3 seem to be opposite. An interesting transition in dominant HEV genotype occurred in China in the last couple of decades – from genotype 1 being characteristic for faecal/oral route of transmission to genotype 4 suggesting there may have been shift to zoonotic route of transmission. Based on the Japanese data from Hokkaido, genotype 4 appears to cause more severe infections than genotype 3. While found in <10% of HEV RNA-positive blood donors, genotype 4 is responsible for about half of hepatitis E cases in Hokkaido. In Europe, 21 autochthonous genotype 4 cases were described in France between 2009 and 2012, all but two strains were related to Belgian swine-derived sequences. Five autochthonous cases were reported in Italy, not closely related to the majority of French cases, suggesting at least two different distinct sources [14].

In highly endemic countries with a dominant faecal/oral route of transmission, the public water supply and waste management systems are of primary importance, while zoonotic transmission via food and exposure/interaction with animal hosts plays a smaller role. Apart from imported infections, the dominant risk factor identified in majority of replies was zoonotic route via uncooked or undercooked pork, game and shellfish. However, these sources are unlikely to account for all autochthonous infections. Other potential sources of infection are discussed in next question.

Question 4

Significant HEV differences between countries as well as within some countries exist. Could you hypothesize reasons for such differences?

Different epidemiology and HEV genotypes involved can account for differences between highly endemic countries with primarily faecal–oral route of infections, and industrialized countries with primarily zoonotic route of infection for autochthonous cases. However, there are some striking regional differences within countries, such as in Latium and Abruzzo in Italy (9·0% vs. 46%), or south-west and north France (52% vs. 24%) (see Table 1 and references therein). The variation can be, in some cases, traced to the preparation/consuming habits of certain products, such as uncooked pig liver sausage figatellu in southern France [15] or raw-dried liver sausage consumption in Abruzzo region in Italy. In other instances, such as more than twofold higher seroprevalence in England compared with Scotland, it is harder to establish clear reasons for such a difference.

Dietary habits including cultural differences in cooking and consumption of food were the most frequently quoted reasons for differences (13/18 responses), followed by the issues related to the performance of assays used (9/18 responses). Other reasons cited in at least one-third of replies were exposure to animals, whether professional or during leisure activities, and issues related to water and environment waste management. The latter is obviously of primary importance in endemic countries with dominant faecal–oral route of transmission, but it is increasingly obvious that the pig manure management is important also in industrialized countries as contaminated water can be used for land applications and crop irrigation, in addition to contamination of waters close to shellfish farms [3, 16, 17]. Two European studies investigating possible contamination routes of enteric viruses in vegetables and some fruits where sampling included irrigation water, animal faeces and swabs at various points of production and points of sale. A percentage of 3·2 HEV lettuce contamination was identified at the point of sale [18] as well as in a sample from contaminated frozen raspberries [19].

Apart from the above-mentioned animal species most frequently associated with zoonotic HEV transmission, several recent papers reported on positive serology in other animal species, such as rabbits, rats, cattle, dogs and cats [17]. A paper by Liang *et al.* reported overall HEV seroprevalence determined by Wantai assay in pet dog and cat serum samples 21·12% and 6·28%, respectively in five big Chinese cities [20]. Omnivorous pet dogs and pet cats sharing kitchen residue food with the general population had higher HEV sero-positivity than pets fed the commercial food [20]. Due to a shared living space with some pets, a two-way HEV infection cannot be ruled out. It needs to be pointed out, however, that the sequences recovered from other animal sources are rather rare.

Question 5

In your country, do you see a relatively high HEV prevalence in blood donors/general population but infrequently

reported cases of clinical hepatitis E? If so, what is the basis of this discrepancy (misdiagnosis; insufficient testing; suboptimal assays; low HEV infectivity or low titre in immunocompetent individuals; lack of awareness among clinicians; subclinical infection, other)?

This scenario is not applicable to endemic countries such as India, where clinical hepatitis E due to predominantly genotype 1 infections is common.

Numbers of confirmed hepatitis E cases are generally low compared with numbers of projected cases. In Brazil, 967 cases were confirmed between 1999 and 2011. In the Netherlands, a country with one of the highest reported RNA prevalences (1 in 658 donors or 0·15%), 280 samples of 4067 requested by clinicians to be tested for HEV were IgM positive (6·8%), 144 of those IgM and RNA positive (3·5%) between 2009 and 2014 [5].

Most frequently quoted reason for the responders of this International Forum (13/18) was the fact that up to 98% of infections are asymptomatic/subclinical/mild with non-specific symptoms. Second most frequently quoted reason (10/18) was lack of awareness. Low rate of testing was specifically mentioned in several responses, but it seems a logical consequence of asymptomatic infections and lack of awareness. Misdiagnosis and assay limitations were also quoted in several replies. Other reasons included possible protective effect of ALT screening in Russia, and the fact that emerging clinical phenotypes (e.g. neurological) may be only mildly abnormal (UK reply). In relation to lack of awareness, only few countries indicated inclusion of hepatitis E among notifiable diseases which may be a factor.

It appears that low HEV awareness combined with mild infections in immunocompetent individuals and non-specific symptoms means that majority of community acquired cases are simply not referred for HEV testing. The situation seems to be improving in transplant setting where some laboratories started routine HEV tests alongside other hepatic viruses.

Question 6

Is hepatitis E infection perceived as a significant health problem in your country? Is hepatitis E virus infection in blood donors perceived as a problem for blood supply?

Hepatitis E perception as a (public) health problem appears to correlate with some extent with HEV prevalence and occurrence of hepatitis E. Three responses labelling hepatitis E infection a significant health problem came from endemic countries India and China, and from France with its hyperendemic region around Toulouse. Around one-third of respondents did not think it is considered a significant health problem in their own country, but indicated a gradual change – an increase in focus and awareness. But around a half of respondents noted hepatitis E is not considered a health problem in their countries.

Replies to the second part of the question indicated more awareness among blood bank establishments, labelling the HEV infection in blood donors as 'concern, possible threat, a (worrying) problem, or an issue' in about half of responses, including some which did not label hepatitis E as a health problem in the first part of the question. In India, while a significant health problem, the hepatitis E infection in blood donors is not considered a problem.

Question 7

Based on the current state of knowledge, in your opinion, do the data merit the introduction of HEV blood donation screening in your region? If yes, should it involve all donations or a subset (similar to CMV testing)? What should be the requirements for an efficient screening test?

High blood donor RNA incidence figures are sufficient reason for some experts to suggest the need for mass HEV RNA screening, in order to rid the blood supply of the potentially infectious donations. Others argue that transfusion transmissions represent a minority route of HEV acquisition since the majority cases arise via consumption of contaminated water or food. Yet another view was that while the HEV RNA incidence in blood supply is high, the actual clinical disease even in immunosuppressed is lower than expected. Predominant opinion seems to be that the data available are limited and further studies and cost-benefit analyses are needed for evidence-based decision making. However, if introduced, almost half of respondents would favour primarily the screening of products/components destined for at-risk/high-risk recipients such as immunosuppressed individuals. While some respondents suggested that speculating about the test parameters is currently a bit premature, where specified, the general agreement was that the test would have to be based on HEV RNA detection.

In a study on HEV transmission via blood components in England [4], 42% of recipients of HEV-contaminated components became infected with no morbidity or neurological complications. Viral clearance or length of seroconversion correlated largely with the level of immunosuppression. It was estimated 1200 HEV-containing components would be transfused each year. That is only a fraction of 80 000–100 000 acute HEV infections projected on the basis of 1 in 2848 HEV RNA-containing donations and 8-week-long viraemia

[4]. Infectious doses in transmitted cases were not determined in this study, although it was shown that donations associated with transmission, contained no or lower levels of anti-HEV antibodies and higher levels of plasma RNA. Huzly *et al.* described an infectious dose of 7056 IU HEV RNA genotype 3, transmitted via apheresis platelets to immunosuppressed patient who developed chronic hepatitis E [21]. It is probable that infectious dose will be affected by number of additional factors, such the HEV genotype and individual's immune status, age and gender.

Question 8

Have you implemented HEV screening of blood donations in your institution? What kind of HEV test are you using?

While no country has adopted the routine HEV screening of all blood donations yet, Japan has implemented a trial HEV RNA screening in 2005 in an endemic region, Hokkaido. The screening was carried out using an in-house RT-PCR on pooled samples until August 2014 when the Procleix HEV ID NAT assay was implemented.

A pilot study was conducted in 2011 at a contributing author's institution in Germany, and January 2015 was the start date for routine HEV RNA screening. In-house minipool (96 samples) testing assay has detection limit of 442 IU/ml per single donation.

EFS in France introduced HEV RNA screening for solvent–detergent (SD)-treated plasma in 2012 on minipools of 96 samples. Since November 2014 fractions of quarantined and Intercept-treated plasma are HEV RNA screened using a method described in reply to Q2. Similarly, in the Netherlands, a validated in-house minipool (96 samples) HEV RNA PCR was implemented for SD plasma production.

All other responders indicated no HEV screening at present, although pilot studies have been carried out or are ongoing.

Question 9

Should an effective and efficient HEV vaccine become widely available, are there plans or discussion to implement vaccination of the general public, selected groups such as blood or stem cell donors, or at-risk groups (organ and stem cell transplant recipients, cancer patients on immunosuppressive therapy, pregnant women, children)?

As mentioned earlier, Hecolin was approved in China in 2011 and is in use since 2012. Based on genotype 1 HEV, and effective and well tolerated also for genotype 4 infections, it can effectively deal with HEV circulating in China and potentially some neighbouring countries. Lack of data related to genotype 3 infections and unavailability of alternative approved vaccines is reflected in majority of replies. In most countries, there are no plans on a potential vaccine use, and in many cases, the issue has not yet even discussed.

Several respondents expressed the view supporting the vaccine use in high-risk groups, once the well-characterized effective vaccine is widely available.

Conclusion

Water and waste management seem the most important factors in endemic countries with faecal–oral route of HEV. In industrialized countries, the number of imported infections still outweighs autochthonous infections, but zoonotic transmission via contaminated food is increasing. Studies on seroprevalence and RNA incidence in blood donors revealed unexpectedly high figures although the numbers of confirmed hepatitis E cases are disproportionately low. Subclinical infection in immunocompetent individuals and low awareness of hepatitis E and HEV lead to small numbers reported for testing variation in assays available and other factors are all cited as potential explanation for such discrepancies.

The preferred solution would be identification and elimination of the environmental sources of HEV, but that appears a considerable task considering number of factors involved. These include regional, cultural, religious differences in food processing, preparation and consumption, the level of environmental contamination related to animal farm waste management and use of water for irrigation, general hygiene standards, the level of exposure/interaction with natural HEV hosts. In the meantime, we may have to focus on the improvements and standardization of HEV assays and their increased use. HEV awareness seems gradually increasing among clinicians and general public, at least in countries with high prevalence figures. Since hepatitis E is becoming a leading cause of new acute hepatitis cases, HEV testing should be carried out at early stages, alongside HBV, HCV and HAV, at least for high-risk groups. Blood screening is an option but according to majority of responders requires additional data and analyses. Widely available efficient vaccines and improved pathogen reduction technologies would significantly increase options available in the HEV-fighting armoury.

References

1 Balayan MS, Andjaparidze AG, Savinskaya SS, *et al.*: Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal-oral route. *Intervirology* 1983; **20**:23–31

2 Aggarwal R, Jameel S: Hepatitis E. *Hepatology* 2011; **54**:2218–2226

3 Kamar N, Bendall R, Legrand-Abravanel F, *et al.*: Hepatitis E. *Lancet* 2012; **379**:2477–2488
4 Hewitt PE, Ijaz S, Brailsford SR, *et al.*: Hepatitis E virus in blood components: a prevalence and transmission study in southeast England. *Lancet* 2014; **384**:1766–1773
5 Koot H, Hogema BM, Koot M, *et al.*: Frequent hepatitis E in the Netherlands without traveling or immunosuppression. *J Clin Virol* 2015; **62**:38–40
6 Dreier J, Juhl D: Autochthonous hepatitis e virus infections: a new transfusion-associated risk? *Transfus Med Hemother* 2014; **41**:29–39
7 Dalton HR: Hepatitis e: the 'new kid on the block' or an old friend? *Transfus Med Hemother* 2014; **41**:6–9
8 Pawlotsky JM: Hepatitis E screening for blood donations: an urgent need? *Lancet* 2014; **384**:1729–1730
9 Hauser L, Roque-Afonso AM, Beyloune A, *et al.*: Hepatitis E transmission by transfusion of Intercept blood system-treated plasma. *Blood* 2014; **123**:796–797
10 Owada T, Kaneko M, Matsumoto C, *et al.*: Establishment of culture systems for Genotypes 3 and 4 hepatitis E virus (HEV) obtained from human blood and application of HEV inactivation using a pathogen reduction technology system. *Transfusion* 2014; **54**:2820–2827
11 Wu T, Li SW, Zhang J, *et al.*: Hepatitis E vaccine development: a 14 year odyssey. *Hum Vaccin Immunother* 2012; **8**:823–827
12 Halliday JS, Harrison GL, Brown A, *et al.*: Hepatitis E virus infection, Papua New Guinea, Fiji, and Kiribati, 2003-2005. *Emerg Infect Dis* 2014; **20**:1057–1058
13 Dalton HR, Fellows HJ, Gane EJ, *et al.*: Hepatitis E in New Zealand. *J Gastroenterol Hepatol* 2007; **22**:1236–1240
14 Bouamra Y, Gerolami R, Arzouni JP, *et al.*: Emergence of autochthonous infections with hepatitis E virus of genotype 4 in Europe. *Intervirology* 2014; **57**:43–48
15 Colson P, Borentain P, Queyriaux B, *et al.*: Pig liver sausage as a source of hepatitis E virus transmission to humans. *J Infect Dis* 2010; **202**:825–834
16 Crossan C, Baker PJ, Craft J, *et al.*: Hepatitis E virus genotype 3 in shellfish, United Kingdom. *Emerg Infect Dis* 2012; **18**:2085–2087
17 Ruggeri FM, Di Bartolo I, Ponterio E, *et al.*: Zoonotic transmission of hepatitis E virus in industrialized countries. *New Microbiol* 2013; **36**:331–344
18 Kokkinos P, Kozyra I, Lazic S, *et al.*: Harmonised investigation of the occurrence of human enteric viruses in the leafy green vegetable supply chain in three European countries. *Food Environ Virol* 2012; **4**:179–191
19 Maunula L, Kaupke A, Vasickova P, *et al.*: Tracing enteric viruses in the European berry fruit supply chain. *Int J Food Microbiol* 2013; **167**:177–185
20 Liang H, Chen J, Xie J, *et al.*: Hepatitis E virus serosurvey among pet dogs and cats in several developed cities in China. *PLoS ONE* 2014; **9**:e98068
21 Huzly D, Umhau M, Bettinger D, *et al.*: Transfusion-transmitted hepatitis E in Germany, 2013. *Euro Surveill* 2014; **19**: pii=20812

Juraj Petrik
Guest Editor
Head, Microbiology & Components RDI
Scottish National Blood Transfusion Service
21 Ellen's Glen Road
Edinburgh
EH17 7QT UK
E-mail: juraj.petrik@nhs.net

Miguel Lozano
International Forum Editor
Department of Hemotherapy and Hemostasis
University Clinic Hospital
Villaroel 170
08036 Barcelona
Spain
E-mail: MLOZANO@clinic.ub.es

C. R. Seed, H. M. Faddy & A. J. Keller

Question 1

An early HEV seroprevalence study among selected Australian groups determined HEV IgG prevalence of 0·4% (blood donors), 2·2% (travellers) and 7·7% (non-A, non-B hepatitis patients and also refugees) [1]. A current but limited seroprevalence study among HIV-infected Australian patients determined a rate of 8% [2]. Our recent study among a cohort of 3237 Australian blood donors recorded an HEV IgG seroprevalence of 6%, while 4 of the 194 HEV IgG-positive donors had detectable HEV IgM, and none had HEV RNA [3].

Question 2

The 1995 study by Moaven *et al.* [1] used a commercial, indirect enzyme immunoassay (Genelabs Inc., Redwood City, CA, USA) to determine the presence/absence of anti-HEV IgG antibodies. This assay and subsequent versions have been shown to underestimate the IgG seroprevalence by as much as 4·5 times [4] compared with the Wantai HEV-IgG ELISA (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd., Beijing, China) used in our more recent study [3]. The manufacturer of the Wantai assay claims a sensitivity of 97·96–100%, which is consistent with the study by Bendall *et al.* [4] who derived a sensitivity of 98% with 99·6% specificity. All HEV IgG-positive samples in our study were also tested for HEV RNA with a prototype transcription-mediated amplification (TMA) assay (Procleix HEV, Hologic Inc, San Diego, CA, USA). The assay manufacturer claims a provisional 95% lower limit of detection of 8–18 HEV RNA copies/ml.

Question 3

HEV is a notifiable disease in Australia, and the surveillance data indicate between 20 and 40 notifications annually since 2010. The vast majority of notified cases are individuals with a history of recent travel to countries endemic for HEV. Reports of domestically acquired acute HEV in Australia are uncommon, but are increasing in frequency. In a recent case involving a solid organ recipient, several possible routes of infection were postulated, including the donor liver, transfused blood products or contaminated food or water (D. Speers, personal communication). In our recent seroprevalence study [3], 14 HEV IgG-positive donors had no previous history of overseas travel. Demonstration of HEV serological markers in such donors suggests the possibility of their infection being acquired within Australia. HEV seroprevalence in Australian pig herds [5] suggests that zoonotic transmission by ingestion of contaminated pork products may be possible. A recent report of an increased prevalence of HEV among Australian HIV-infected men suggests that male-to-male sex might also contribute to HEV transmission in Australia [2].

Question 4

Some of this variation is undoubtedly a true reflection of differing HEV epidemiology (for example, the large waterborne outbreaks of human-only infecting genotypes 1 and 2 in developing countries vs. sporadic cases of human- and swine-infecting genotypes 3 and 4 in industrialized countries). However, other variation is a result of the differing performance characteristics of applied tests – particularly related to test sensitivity [4]. The situation in France is illustrative of both factors. Ingestion of contaminated pork products has resulted in highly endemic regions within France, where regional seroprevalence can exceed 50%. However, in one study, the seroprevalence in Toulouse (a highly endemic region) that was initially assessed as 16% increased to 52% using an assay with superior sensitivity [6]. This highlights that comparison is only valid between studies using the same HEV test.

Question 5

Our seroprevalence of 6% among donors is mid-range when compared to figures from other non-endemic countries where the same test was used. Should the donor seroprevalence be representative of the general population, then, in the context of 6% previous exposure, the small number of notifications is surprising and indicates substantial underreporting. Possible causes include the low rate of HEV testing, the high rate of asymptomatic infection and a possible lack of awareness among clinicians. In Australia, the testing algorithm for laboratory diagnosis of the viral causes of acute hepatitis includes testing for the more common HAV, HBV and HCV only; HEV testing is only considered for patients with recent overseas travel history. This may also contribute to underreporting and possible misdiagnosis.

Question 6

No, hepatitis E is not currently perceived as a significant health problem in Australia, although this is changing. Recent locally acquired HEV cases in New South Wales (Australia's most populous state) in which pork liver consumption was implicated prompted a media statement from the state health authority. The general public and food handlers in particular were advised to ensure thorough cooking of pork products and good food hygiene of all raw meats (http://www.health.nsw.gov.au/news/Pages/20140911_01.aspx). In terms of blood safety, HEV has been a concern for some time – prompting our seroprevalence study [3]. The magnitude of the risk is unclear pending an accurate estimate of the rate of HEV viraemia in Australian blood donors, which is currently in progress.

Question 7

The accumulated data are insufficient to make a final determination on the need for any additional HEV blood safety measures, including donation testing. While we failed to find any RNA-positive (presumed viraemic) donations among the 194 HEV IgG-positive samples in our seroprevalence study [3], a larger study targeting HEV RNA (pending) is required to accurately assess the risk within the donor population. It is premature to speculate on the optimal donation testing strategy except, perhaps to say that it should be based on HEV RNA detection as the proxy for infectivity. The clinical significance of chronic hepatitis E infection, for example in immunosuppressed patients, has not yet been formally studied in Australia.

Question 8

No, HEV blood donation testing is not currently performed in Australia.

Question 9

We are not aware of any planned general HEV vaccination programmes. From a blood safety perspective, vaccination of high-risk recipients (e.g. organ and stem cell transplant recipients) is certainly worthy of consideration.

References

1 Moaven L, Van Asten M, Crofts N, *et al.*: Seroepidemiology of hepatitis E in selected Australian populations. *J Med Virol* 1995; **45**:326–330

2 Yong MK, Paige EK, Anderson D, *et al.*: Hepatitis E in Australian HIV-infected patients: an under-recognised pathogen? *Sex Health* 2014; **11**:375–378

3 Shrestha AC, Seed CR, Flower RLP, *et al.*: Hepatitis E virus and implications for blood supply safety, Australia [letter]. Emerg Infect Dis 2014; **20**:1940–2, Nov [date cited]. http://dx.doi.org/10.3201/eid2011.140412

4 Bendall R, Ellis V, Ijaz S, *et al.*: A comparison of two commercially available anti-HEV IgG kits and a re-evaluation of anti-HEV IgG seroprevalence data in developed countries. *J Med Virol* 2010; **82**:799–805

5 Chandler JD, Riddell MA, Li F, *et al.*: Serological evidence for swine hepatitis E virus infection in Australian pig herds. *Vet Microbiol* 1999; **68**:95–105

6 Mansuy JM, Bendall R, Legrand-Abravanel F, *et al.*: Hepatitis E virus antibodies in blood donors, France. *Emerg Infect Dis* 2011; **17**:2309–2312

Clive R. Seed and Anthony J. Keller
Australian Red Cross Blood Service
Level 1- 69 Walters Dr.
Osborne Park
WA 6017, Australia
E-mails: cseed@redcrossblood.org.au; akeller@redcrossblood.org.au

Helen M. Faddy
Australian Red Cross Blood Service
44 Musk Ave (PO Box 145)
Kelvin Grove
Qld
4059 Australia
E-mail: hfaddy@redcrossblood.org.au

P. S. P. Scuracchio & S. Wendel

Question 1

In Brazil, there are few studies of HEV prevalence in different population groups, as depicted in Table 3, with no differences according to the geographical regions [1]. The Epidemiological Bulletin of Viral Hepatitis published by Brazilian Ministry of Health in 2012 described that in the period from 1999 to 2011, 967 confirmed cases of hepatitis E were reported, most of them in south-east (470 cases: 48·6%) and north-east (173 cases: 17·9%) regions. This document also described that between 2000 and 2011, there were 86 deaths by hepatitis E: in 51 cases, HEV was the main cause and in 35 cases, it was an associated cause, most of them (58·1%) occurred in the south-east region [2].

Table 3 Prevalence of anti-HEV IgG in different population groups [1]

Population groups	Prevalence (%)
Gold miners	6·1
General population	3·3
Blood donors	2·0–7·5
Prostitutes in risk of HIV	14–18
Intravenous drug users	12·0
Pregnant women	1·0
Children	4·5
Amazonian communities	3·3–6·1

Question 2

Blood donors or general population in Brazil were tested for specific anti-HEV IgG antibodies by enzyme-linked immunosorbent assay (ELISA), using commercially available reagents (ABBOTT and Biokit). According to the manufacturer, these assays show 98·5% sensitivity and 99·5% specificity. Acute infections were established by testing anti-HEV IgM using two immunoassays: bioELISA HEV IgM (Biokit) and recomWell HEV IgM (Mikrogen). The reactivity of samples in HEV ELISAs was confirmed by immunoblot assay (IB) recomLine HEV IgM/IgG (Mikrogen). Both Mikrogen assays, ELISA and IB, are based on genotypes 1 and 3, while bioELISA HEV IgM/IgG detects genotypes 1 and 2 viruses [1]. All reported studies using RNA assays were performed only for genotyping and not for population prevalence or incidence studies.

Question 3

It seems that the risk factors for HEV infection in Brazil can be related to the consumption of contaminated shellfish, undercooked pork, wild game and direct exposure to pigs. The prevalent genotype detected is HEV 3, either in patients with acute hepatitis or in pigs. Outbreaks of HEV infection have not been described yet, but it can exist, considering that our country is very big and we have great social differences and sanitary conditions. Furthermore, it is important to highlight the zoonotic origin of these infections in Brazil, especially because HEV infection is common among Brazilian swine livestock responding to viral strains from genotype 3. A first case report described the identification of human autochthonous hepatitis E virus (genotype 3b) acute infection in Brazil, suggesting a likely zoonotic origin for the infection [3]. Also, we had had case reports of chronic HEV infection in adult renal transplant recipients and paediatric liver transplant recipient, demonstrating that chronic HEV infection can occur in immunocompromised patients, as described by others

studies in developed countries. In these cases, chronic hepatitis E was diagnosed on the basis of positive results for anti-HEV IgG, IgM antibody tests, by the detection of HEV-RNA genotype 3 in the serum and/or stool and the presence of elevated liver enzymes levels for at least 6 months [4].

Question 4

In terms of epidemiology of HEV infection, it is observed that in Brazil and Argentina, they are identical to the characteristics of most countries from North America and Europe, and therefore, it is a potential zoonotic disease with evidences of HEV circulation among humans and swine livestock, but it seems to behave similarly to other regions considered of low endemicity. Maybe, the infections are subclinical or asymptomatic or underreported. In Brazil, studies performed in other animals species found anti-HEV IgG also among pigs, dogs, cattle, chicken and wild rodents, with prevalence rates ranging from 1·4% to 50% [5]. However, in the Caribbean and Mexico, the HEV involves the waterborne, non-zoonotic viral genotypes responsible for epidemics in Asia and Africa [5]. Another fact that could explain some differences between countries is the inconsistency of the assays for HEV-specific immunoglobulin M and G antibody detection due to the diversity of the HEV recombinant antigens used by the different assays and to the genetic variations between the different HEV strains. Although all of them were performed by well-established immunoassays, technical performance may vary from assay to assay as well as the sequence diversity of the antigens and the differences in sampling criteria. Similarly, in some cases, anti-HEV IgG is undetectable or disappears rapidly in some assays, which makes them unsuitable for detecting previous infection.

Question 5

In Brazil, the prevalence of HEV in blood donors is not so high comparing to other countries in America (16·2% in Bolivia and 8% in Chile) and in Europe (9·3% in Sweden, 13·5% in England and 14% in Belgium). Few case reports of clinical hepatitis E were described. However, it is very important to remember that the commercial assays currently used have some limitations in terms of sensitivity and specificity, and we also believe that many clinicians are still not aware of this kind of infection in immunocompetence of immunocomprised patients, besides the significant extra-hepatic complications, for example neurological symptoms, kidney injury, pancreatitis, thombocytopenia and aplastic anaemia [6].

Question 6

As we began to have published some case reports of chronic HEV infection in renal and liver transplant recipients in Brazil, it is critical that hepatitis E infection must be perceived as a significant public health problem by the authorities in our country, besides other types of hepatitis that are considered a concern for the general population (hepatitis A, B, C and D). Although it is a reportable disease like others viral hepatitis, we do not have any case of transfusion transmission of hepatitis E reported yet, but we believe that hepatitis E should be studied regarding a possible threat to the blood supply.

Question 7

Currently, we do not consider the introduction of HEV blood donation screening in our blood service, but further studies are necessary to investigate the real situation of this virus in our population of blood donors. Studying the epidemiology implies the application in properly standardized serological and molecular assays that should be adopted in order to accurately identify current and past infections. It is also important that the clinicians consider the hepatitis E infection as a differential diagnosis, especially in immunocompromised patients, and also recommend that this group of patients avoid the ingestion of raw or undercooked porcine meat.

Question 8

In our country, we have not implemented HEV screening of blood donation yet.

Question 9

According to some HEV vaccine trials published, vaccination might be useful in high-risk groups such as immunocomprised patients, that is organ and stem cell transplant recipients, cancer patients on immunosupressive therapy, those with chronic liver disease, in addition to individuals intending to travel to endemic areas [6]. However, there are no plans or discussions about vaccination of general or selected groups, for example blood donors or immunocomprised patients.

References

1 Vitral CL, Silva-Nunes M, Pinto MA, *et al.*: Hepatitis A and E seroprevalence and associated risk factors: a community-based cross-sectional survey in rural Amazonia. *BMC Infect Dis* 2014; **14**:458

2 Brasil. Ministério da Saúde. Boletim Epidemiológico Hepatites Virais. Ano III - N°I, July 25, 2012. Available at: http://www.aids.

gov.br/sites/default/files/anexos/publicacao/2012/51820/boletim_epidemiol_gico_hepatites_virais_2012_ve_12026.pdf
3 Santos DRL, Lewis-Ximenez LL, Silva MFM, *et al.*: First report of a human autochthonous hepatitis E virus infection in Brazil. *J Clin Virol* 2010; **47**:276–279
4 Passos-Castilho AMP, Porta G, Miura IK, *et al.*: Chronic hepatitis E infection in a pediatric female liver transplant recipient. *J Clin Microbiol* 2014; **52**:3944–3951
5 Echevarria JM, Gonzalez JE, Lewis-Ximenes LL, *et al.*: Hepatitis E virus infection in Latin America: a review. *J Med Virol* 2013; **85**:1037–1045
6 Dreier J, Juhl D: Autochthonous hepatitis E virus infection: a new transfusion-associated risk? *Transfus Med Hemother* 2014; **41**:29–39

Patricia Santos Prado Scuracchio
Attending Physician
Blood bank of Hospital Sírio Libanês
Rua Adma Jafet, 91
São Paulo
01308-050 Brazil
E-mail: scuracchiop@ihsl.com.br

Silvano Wendel
Medical Director
Blood bank of Hospital Sírio Libanês
Rua Adma Jafet, 91
São Paulo
01308-050 Brazil
E-mail: snwendel@terra.com.br

Dr. A. Andonov, Dr. M. Fearon & Dr. G. Delage

Question 1

In 2013–14, Canadian Blood Services, Hema-Quebec and the National Microbiology Laboratories conducted an HEV seroprevalence survey based on testing of 4102 Canadian blood donors and estimated that the prevalence of anti-HEV IgG was 5·9% (1). None of 14 000 donors tested in 100 member pools followed by ultracentrifugation in order to reduce the dilution effect was positive for HEV RNA (threshold of detection 250 IU/ml). Prevalence for HEV antibody increased with age, with donors in the 50+ age group having a prevalence of 9·9%.

Question 2

Anti-HEV IgG ELISA (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd., Beijing, China); sensitivity <1·0 WHO units/ml. Specificity based on an in-house panel of HEV RNA (+) samples was 100%. Based on published reports, the sensitivity of the assay is 0·25 WHO units/ml. HEV RNA was detected by RealStar HEV RT-PCR kit (Altona Diagnostic Technologies [ADT], Hamburg, Germany). The analytical sensitivity of the test in our laboratory was 15 IU/ml.

Question 3

Majority of laboratory-confirmed HEV acute infections in Canada are imported (>90%) from India, Pakistan and Bangladesh. Almost all of the imported cases belong to genotype 1 (we have found only two imported genotype 4 cases from China during the last 6 years). The risk of acquiring the infection is significantly higher for immigrants visiting their home countries compared with Caucasian Canadians travelling to the same countries as tourists. During the period 2006–2013, there were only 14 locally acquired laboratory-confirmed cases belonging to genotype 3. Eight of these were solid organ transplantation patients (seven liver transplants and one kidney transplant). Two were HIV-infected patients, and only four were immunocompetent; in one of the latter, deer hunting was the only risk factor found.

The incidence of HEV infection in Canada may be underestimated as the disease is not on the list of the Nationally Notifiable Diseases (NND), and not all health jurisdictions collect proper epidemiological information. HEV infection is common in Canadian swine herds, and in a recent survey, 5·7% of retail pork liver was found to be positive for HEV RNA (2,3). Most probably, HEV infection in Canada may be considered as a food zoonosis.

Question 4

Often, the disparity is clearly due to the performance of the assays used. In some cases, the difference could be explained with the dietary habits of the population in different regions/countries where foodborne route of transmission is assumed to be the main one. The level of exposure to farm animals, particularly pigs may contribute to differences in prevalence between countries or different geographic regions within countries.

Question 5

HEV seroprevalence in Canadian blood donors is not high; it may be somewhat higher in the general population, especially in immigrants from endemic areas whose representation among the general population should be taken into account. Regardless, the extremely small number of laboratory-confirmed autochthonous HEV cases among immunocompetent individuals is difficult to

associate even with this modest seroprevalence observed in Canadian blood donors (5·9%). A possible explanation is that locally acquired HEV infection is asymptomatic or subclinical in nature in the great majority of cases. There is increased awareness during the last few years among clinicians especially regarding at-risk patients. In fact, we have observed significant increase on a yearly basis for number of HEV tests requested. However, the disease is likely to be underdiagnosed and underreported as it is not on the NND list and may not undergo the same rigorous epidemiological investigation as HAV, HBV and HCV acute infections. Basically, we have limited data on HEV seroprevalence in Canada.

Question 6

HEV infection is not a significant health problem in Canada. There are very few clinically manifested cases, most of which are imported. There are no documented cases of HEV transmission through blood products in Canada. The seroprevalence is relatively low compared with other developed countries. The fact that none of 14 000 blood donors had detectable viraemia is very encouraging and indicates that asymptomatic HEV infection is probably very rare and not a significant threat to the blood supply.

Question 7

At the present time, the data on HEV infection in Canada and Canadian blood donors do not support the introduction of blood donation screening for HEV.

Question 8

No.

Question 9

Currently, there are no discussions to implement HEV immunization for the general population or at-risk groups.

References

1 Fearon M, Scalia V, O'Brien S, *et al.*: Babesia and hepatitis E seroprevalence in Canadian blood donors. *Can J Infect Dis Med Microbiol* 2014; **25** No 2:e38
2 Yoo D, Willson P, Pei Y, *et al.*: Prevalence of hepatitis E virus antibodies in Canadian swine herds and identification of a novel variant of swine hepatitis E virus. *Clin Diagn Lab Immunol* 2001; **8**:1213–1219
3 Wilhelm B, Leblanc D, Houde A, *et al.*: Survey of Canadian retail pork chops and pork livers for detection of hepatitis E virus, norovirus, and rotavirus using real time RT-PCR. *Int J Food Microbiol* 2014; **185**:33–40

Anton Andonov
Head
Molecular and Immunodiagnostics Bloodborne Pathogens & Hepatitis
Public Health Agency of Canada
National Microbiology Laboratories
Winnipeg
MB
Canada R3E 3R2

Department of Medical Microbiology
University of Manitoba
1015 Arlington St., Suite 4800
Winnipeg
MB
Canada R3E 3R2
E-mail: anton_andonov@phac-aspc.gc.ca

Margaret Fearon
Executive Medical Director
Medical Microbiology
Canadian Blood Services
67 College St.
Toronto
ON
Canada M5G2M1
E-mail: margaret.fearon@blood.ca

Gilles Delage
Vice-Président aux affaires médicales
Microbiologie
Héma-Québec
4045 Côte-Vertu
Ville Saint-Laurent
QC
Canada H4R 2W7
E-mail: gilles.delage@hema-quebec.qc.ca

J. Zhang & J. W. K. Shih

Question 1

There are several studies in China to disclose the seroprevalence of anti-HEV IgG in blood donors and general population. The results showed that the prevalence of anti-HEV IgG is 30–42% and 25–66% in blood donors [1] and in general population [2], respectively. The prevalence of anti-HEV IgM among blood donors is 0·4–1·7% [1]. A study of blood donors from Beijing Blood Bank showed that the proportion of abnormal alanine aminotransferase (ALT) in anti-HEV IgM-positive donors (17%) is 7·7 (95% CI 3·9–15·5) times higher than that of IgM-negative donors (2%). The prevalence of HEV RNA in

blood donors was 0·02–0·20% when a screening strategy of RT-PCR following a positive finding of anti-HEV IgM was used [1].

Question 2

In China, most HEV serological testing is performed using commercial assays, but RNA testing is performed by in house assays. The sensitivity and specificity of the assays were varied with the 'true positive' and 'true negative' criteria of the evaluation panel. In most cases when HEV RNA-positive samples were set as 'true positive' and samples from blood donors or healthy population were set as 'true negative', the sensitivity and specificity of IgM assay are usually 95–99% and >99%, respectively [3, 4]. In a recent evaluation, the sensitivity and specificity of the generally used IgG assay are 93% and 98%, respectively [4]. In a longitudinal study in which acute hepatitis cases were sequentially followed up and several HEV infection markers were tested in parallel to establish unbiased diagnosis of HE or non-HE, the sensitivity of IgM assay and RNA assay is 90% and 78%, respectively, and the specificity of either assays is 99% and 100%, respectively [5].

Question 3

The exact dominant risk factors for HEV infection remained unclear. It is believed that the close contacts with uncooked swine products (pork, blood and internal, which are all widely consumed in China), swine farming and processing play important role. The predominant HEV genotype in China had changed from genotype 1 to genotype 4 in recent ten to twenty years. Food contaminant is the most common route of HEV transmission.

Question 4

The differences might be correlated with various reasons, that is (1) basic public water supply and sewage management system for human genotypes; (2) natural hosts and their interaction with human beings for zoonosis genotypes; (3) swine consumption habits, etc.

Question 5

Yes. The reasons might be the following: (1) the relative low virus load but wide distribution in the environment, which leads to lots of asymptomatic infection but rarely clinical hepatitis E (the ratio of subclinical and clinical infections is about 50:1 in general population) [6]; (2) insufficient testing; (3) lack of awareness among clinicians; (4) misdiagnosis.

Question 6

Yes. Yes.

Question 7

Yes. It should involve blood products for a subset of high-risk blood recipients, such as immunocompromised patients, patients with liver diseases and pregnant women.

Question 8

No.

Question 9

Yes.

References

1 Guo QS, Yan Q, Xiong JH, *et al.*: Prevalence of hepatitis E virus in Chinese blood donors. *J Clin Microbiol* 2010; **48**:317–318
2 Zheng Y, Ge S, Zhang J, *et al.*: Swine as a principal reservoir of hepatitis E virus that infects humans in eastern China. *J Infect Dis* 2006; **193**:1643–1649
3 Khan A, Tanaka Y, Kurbanov F, *et al.*: Investigating an outbreak of acute viral hepatitis caused by hepatitis E virus variants in Karachi, South Pakistan. *J Med Virol* 2011; **83**:622–629
4 Abravanel F, Chapuy-Regaud S, Lhomme S, *et al.*: Performance of anti-HEV assays for diagnosing acute hepatitis E in immunocompromised patients. *J Clin Virol* 2013; **58**:624–628
5 Huang S, Zhang X, Jiang H, *et al.*: Profile of acute infectious markers in sporadic hepatitis E. *PLoS ONE* 2010; **5**:e13560
6 Zhang J, Zhang XF, Zhou C, *et al.*: Protection against hepatitis E virus infection by naturally acquired and vaccine-induced immunity. *Clin Microbiol Infect* 2014; **20**:0397–405

Jun Zhang
Xiamen University
Xiang'An Campus
Xiang'An
Xiamen
Fujian
361102 China
E-mail: zhangj@xmu.edu.cn

James Wai Kuo Shih
National Institute of Diagnostics and Vaccine Development
School of Public Health
Xiamen University
Xiang'An Campus
Xiang'An
Xiamen
Fujian
361102 China
E-mail: jwshih@xmu.edu.cn

P. Gallian, R. Djoudi & P.Tiberghien

Question 1

Estimation of HEV RNA prevalence among blood donors has been performed by screening plasma samples (minipools of 96 samples, corresponding to 53 234 blood donations) from blood donations (apheresis) contributing to SD-plasma production for the presence of HEV RNA. The detection rate was 1 HEV-positive sample/2218 blood donations. Most samples (22/24) from viremic donors were negative for IgG and IgM against HEV [1]. Regarding IgG anti-HEV seroprevalence, testing individual (nationwide) donations included in 9 RNA HEV-positive pools for the presence of anti-IgG resulted in a 23·6% prevalence [1]. In some parts of the country, a higher rate has been observed. A study conducted in blood donors collected in the south-western of France (Toulouse area) found an IgG prevalence of 52% [2].

Question 2

HEV RNA screening included nucleic acid extraction [Nuclisens easyMAG (bioMerieux, Marcy l'Etoile, France) followed by RT-PCR (RealStar HEV RT-PCR kit 1.0" Altona Diagnostics, Hamburg, Germany (CE marked)]. Sensitivity (95% detection rate) was of 23 UI/ml (WHO standard). Anti-HEV antibody screening was performed by ELISA (Wantai Biologic Pharmacy, Beijing China).

Question 3

To date, only genotype 3 strains have been detected in French blood donors supporting zoonotic infection via mainly uncooked meal, sausages products, etc.

Question 4

In addition to variability in zoonotic HEV exposure which will need additional studies, comparison of seroprevalence studies according to the ELISA kit manufacturer or to the sensitivity of the test (using WHO standard for IgG) is necessary to fully understand the differences in seroprevalence. Similarly, interpretation of HEV RNA prevalence rates needs to be taken into consideration both the sample pool size and NAT test sensitivity.

Question 5

Reports of clinical hepatitis are infrequent because 98% of HEV-3 infections are asymptomatic. Diagnosis of acute infection in patients is increasing because HEV RT-PCR is available in most hospital laboratories and clinicians are increasingly aware of HEV infection, particularly for transplanted immunocompromised patients [3].

The first documented case of HEV transfusion-transmitted infection (TTI) was recorded in France in 2006 [4]. Since then till end of 2013, 16 cases of patients infected through transfusion of a blood product from an HEV viremic donor have been reported (Djoudi *et al*., manuscript in preparation), most of these cases having occurred in 2012 and 2013. Blood products involved included red blood concentrates, platelets (pooled whole blood-derived and apheresis) and plasma, including SD-plasma (prior to detection implementation) and plasma treated by amotosalen-HCL + UV-A illumination (Intercept plasma) [5], thus establishing resistance of HEV to such pathogen reduction technology. Patients were in a majority of cases kidney or liver transplants, often having undergone plasma exchange. All but one were immunocompromised. At least five patients developed chronic hepatitis [6]. Viral strains identified were all of genotype 3. In most cases, phylogenetic analysis of involved viral strains firmly established transfusion imputability. The most important region (Paris region), which uses 19% of all blood products reported a majority of these 16 cases. Variable awareness by clinicians probably contributes to such a finding.

Question 6

Hepatitis E infection is perceived as a significant health problem and NAT screening for blood donations is under consideration.

Question 7

Considering the reported cases of TTI hepatitis E, the current uncertainties as to the scope (i.e. neurological and renal diseases in addition to hepatitis) and potential severity of HEV-mediated pathology, as well as the present frequency of RNA HEV-positive blood donors, we believe HEV screening of blood donations should be considered, at least for high-risk patients such as immunosuppressed patients and chronic hepatopathies. Similar safety measures should be considered for pregnant patients depending on HEV genotypes present among blood donors.

Question 8

HEV testing was introduced for EFS-produced plasma-SD in December 2012. EFS has stopped producing and delivering SD-plasma in January 2015. Until then, SD-plasma constituted 30% of delivered plasma by the EFS, the remaining two-thirds being quarantined plasma and Intercept plasma. To insure continuous availability of plasma verified for the absence of HEV RNA for high-risk patients as defined above, a fraction of quarantine and

Intercept plasma produced by the EFS is now tested for the presence HEV RNA. The test used for HEV detection is described in response to Question 2.

Question 9

We are not aware of such plans in France. However, such vaccination approaches should obviously be considered if available.

References

1 Gallian P, Lhomme S, Piquet Y, *et al.*: Hepatitis e virus infections in blood donors, France. *Emerg Infect Dis* 2014; **20**:1914–1917
2 Mansuy JM, Bendall R, Legrand-Abravanel F, *et al.*: Hepatitis E virus antibodies in blood donors, France. *Emerg Infect Dis* 2011; **17**:2309–2312
3 Abravanel F, Lhomme S, Chapuy-Regaud S, *et al.*: Hepatitis E virus reinfections in solid-organ-transplant recipients can evolve into chronic infections. *J Infect Dis* 2014; **209**:1900–1906
4 Colson P, Coze C, Gallian P, *et al.*: Transfusion-associated hepatitis E, France. *Emerg Infect Dis* 2007; **13**:648–649
5 Hauser L, Roque-Afonso AM, Beylouné A, *et al.*: Hepatitis E transmission by transfusion of Intercept blood system-treated plasma. *Blood* 2014; **123**:796–797
6 Féray C, Pawlotsky JM, Roque-Afonso AM, *et al.*: Should we screen blood products for hepatitis E virus RNA ? *Lancet* 2014; **383**:218

Pierre Gallian, Rachid Djoudi and Pierre Tiberghien
Etablissement Français du Sang
20 Avenue du Stade de France
93218 La Plaine St Denis
France
E-mails: pierre.gallian@efs.sante.fr; rachid.djoudi@efs.sante.fr; pierre.tiberghien@efs.sante.fr

J. Izopet

Question 1

The prevalence of anti-HEV IgG antibodies in the French blood donors has been determined in a collaborative study involving the French Establishment of Blood and the National Reference Center for Hepatitis E (Toulouse University Hospital). A total of 10 000 donors were tested. The detection rate of anti-HEV IgG was 24% with variations between regions (manuscript in preparation). In addition, the prevalence of HEV RNA in French plasma donors (minipools of 96 samples, corresponding to 53 234 blood donations) was 1 HEV-positive samples/2218 blood donations (Gallian, *Emerg Infect Dis* 2014).

Question 2

IgG against HEV were detected using the Wantaï test. HEV RNA was detected by using the RealStar Reverse Transcription PCR (Altona Diagnostics, Eurobio, Courtaboeuf, France). The 95% detection limit was 23 IU/ml. In addition, all HEV-positive samples were confirmed by a validated quantitative method (limit of detection 60 IU/ml).

Question 3

The dominant risk factor for HEV infection in France is consumption of undercooked pork, game meat and shellfish. The prevalent genotypes 3f, 3c and 3e are also found in pig populations (Abravanel, *Emerg Infect Dis* 2009; Bouquet, *Emerg Infect Dis* 2011). The main route of HEV infection is enteric transmission via contaminated food or water.

Question 4

Differences between countries and within countries could be linked to food habits.

Question 5

The high HEV prevalence in blood donors and the infrequently reported cases of clinical hepatitis E seen in France is primarily due to the fact that most HEV infections are asymptomatic. The number of cases reported by the National Reference Center for Hepatitis E is increasing due to improvements in diagnostic tests and diagnostic algorithms.

Question 6

The health problem raised by HEV infection is probably underestimated in France. However, HEV infection in blood donors is perceived as a problem for blood supply.

Question 7

HEV blood donation screening must rely or nucleic acid testing (NAT) because most of viraemic donors are negative for anti-HEV IgG and IgM. Implementation of NAT relies on several parameters:

(1) Risk of transmission to recipients (high, 42% in a UK study).
(2) Clinical outcome of transfusion-transmitted HEV: diagnosis of HEV infection is crucial in immunocom-

promised recipients because the risk of chronicity is 60% and ribavirin therapy is effective.
(3) cost-effectiveness of the different strategies.

In my opinion, screening pools of 10 samples (viraemia >230 IU/ml detected) for donors whose blood will be used for patients with high risk of severe form of hepatitis E could be efficient. Systematic testing of recipients with high risk of severe form of hepatitis E seems very important.

Question 8

HEV RNA screening for plasma processed with solvent–detergent started in November 2012 in France. The Altona test was used on minipools of 96 samples. Therefore, viraemia >2300 IU/ml were detected, and the blood was discarded.

Question 9

I am not aware of plans or discussions to implement vaccination of the general public, selected groups or at-risk groups in Europe.

Jacques Izopet
Laboratoire de Virologie
Institut Fédératif de Biologie de Purpan
330 Avenue de Grande Bretagne
31059 Toulouse Cedex 9
France
E-mail: izopet.j@chu-toulouse.fr

J. Dreier, T. Vollmer & C. Knabbe

Question 1

The HEV seroprevalence in different German studies is summarized in Table 4. The anti-HEV prevalence in blood donors ranged from 5·5% to 15·5% [1]. Determination of anti-HEV seroprevalence in an unselected cohort revealed an overall HEV seroprevalence of 16·8% with an increasing seroprevalence by increasing age, but no dependence on sex or location of residence [2]. Interestingly, Wenzel and co-workers demonstrated a significant decrease in the anti-HEV prevalence in south-eastern Germany in the past decades, concluding that the phenomenon of HEV being an emerging pathogen is most probably due to an increasing awareness of the disease [3]. However, the comparison of seroprevalences determined with different serological assays is inadequate since it has been shown that test performance characteristics of anti-HEV IgG assays strongly influence the estimation of hepatitis E seroprevalence [4]. For example, comparison of the seroprevalence in south-western France showed significantly different results using the Wantai HEV ELISA (53%) compared with the Mikrogen ELISA (17%) [2]. We have already evaluated different immunological assays by testing serially diluted HEV samples (genotype three-infected individual and the WHO Reference Reagent for hepatitis E virus antibody (WHO-Standard, NIBSC) and seroconversion panel of ten HEV genotype three-infected individuals. We have also seen differences in the sensitivities and specificities of different anti-HEV assays, with anti-HEV IgM assays being more divergent than anti-HEV IgG assays (Vollmer *et al*., unpublished data).

Question 2

HEV assays for the determination of seroprevalences are summarized in Table 4. Assays for the detection of HEV RNA used for HEV blood donor screening are shown in Table 5. The sensitivity of PCR assays ranged from 4·7 to 250 IU/ml.

Question 3

We suppose that the dominant risk factors for HEV infection in our country primarily include foodborne zoonosis since consumption of pork meat is very common. In a case–control study by Wichmann and co-workers in 2008, the consumption of offal and wild boar meat was independently associated with autochthonous HEV infection. The German central institution for health protection [Robert-Koch Institute (RKI)] reported genotype 3 as the most prevalent genotype.

Question 4

In our opinion, the main reasons for regional differences of HEV infection are due to the differences in hygienic standards and different lifetime exposures (foods that may serve as transmission vehicles or animals serving as zoonotic reservoirs). In Germany and other industrialized countries, HEV infection is most likely to be transmitted by the zoonotic or foodborne route (uncooked or undercooked pork, wild boar). The HEV seroprevalence is high in domestic pig herds, providing increasing evidence for pigs as a reservoir for foodborne transmission [2]. Studies by Krumbholz and co-workers further described a higher HEV seroprevalence in persons with occupational exposure to pigs than in control groups [1]. Seasonal differences are also conceivable; for example, the occurrence of undercooked pork meat at summer barbecues is not uncommon.

Table 4 Anti-HEV prevalence in Germany

German region (country)	Population	*n*	Seroprevalence (%)	Method	Year [References]
West (Hesse)	Blood donors (ALT > 68 IU/ml)	109	5·5	ELISA[b] (Mikrogen, MP Biomedicals), Westernblot[c] (Mikrogen)	2010 [Baylis *et al.*, Vox sang 98:479]
East (Thuringia)	Blood donors	116	15·5	Westernblot[b] (Mikrogen)	2011 [Krumbholz *et al.*, Med Microbiol Immunol 201:239–44]
Germany	German Health Examination Survey for Adults	4422	16·8	Westernblot[b] (Mikrogen)	[2]
East (Berlin, Brandenburg)	Blood donors Forestry workers	301 563	11 18	Westernblot[b] (Mikrogen)	2012 [Dremsek *et al.*, Med. Microbiol Immunol 201:189–200]
West (NRW[a]/Lower Saxony/ Hesse)	Blood donors	336	5·9	ELISA[b] (Mikrogen, MP Biomedicals) Westernblot[c] (Mikrogen)	[5]
North (Schleswig-Holstein)	Blood donors	1019	6·8	ELISA[b] (Mikrogen), Westernblot[c] (Mikrogen)	2013 [Juhl *et al.*, Transfusion 54:49–56]
South-east (Bavaria)	Patients (with no preselection)	1092	*1996:* 50·7 (EIA) 20·5 (Blot) *2011:* 34·3 (EIA) 14·5 (Blot)	ELISA[b] (Axiom) Westernblot[b] (Mikrogen)	[3]
Germany	Children (0–17a)	1646	1·0	ELISA[b] (Mikrogen, MP Biomedicals Axiom) Westernblot[c] (Mikrogen)	2008–2010 [Krumbholz *et al.*, Pediatr Infect Dis J. 33:258–62]

[a]North-Rhine Westphalia.
[b]Screening assay.
[c]Supplemental assay for confirmation.

Question 5

The reporting frequency of hepatitis E has significantly increased during the last years (e.g. 2006: $n = 51$, 2008: $n = 104$, 2010: $n = 221$, 2012: $n = 386$, 2013: $n = 532$), which might be due to enhanced diagnostic methods (e.g. availability of commercial HEV RT-PCR detection assays), but also due to an increasing sensibility regarding HEV infection. However, there is still a major discrepancy between reported cases and the expected number of clinical hepatitis E cases. The RKI estimated a mean annual incidence of HEV seroconversion of 3·9 (95% CI 3·6–4·2%) per 1000 population using a catalytic model [2]. Based on this incidence and a German population of 80·62 m, the annual report rate is supposed to be 300 000 cases. In our opinion, this discrepancy has three major causes: (a) occurrence of subclinical infections, (b) misdiagnosis, in combination with (c) a lack of awareness among clinicians. The combination of (b) and (c) results in diagnostic confirmation or exclusion of hepatitis A/B/C in cases of acute hepatitis without subsequent systematic follow-up of other viral hepatitis sources in cases of non-A/B/C hepatitis.

Question 6

Currently, HEV infection is not realized as a significant health problem since most infections tend to present with subclinical or asymptomatic courses. However, serious complications have occurred. To evaluate the significance of HEV infection as a significant health problem, the progression of severe HEV infection is of major interest, but studies regarding the outcome of these patients are rare. Furthermore, one assumes that the estimated number of unreported severe cases due to misdiagnosed or lack of awareness of HEV infection is potentially high.

Table 5 Incidence of HEV RNA detection in German blood donors

Year	*n*	Incidence (%)	Nucleic acid extraction /NAT method	Analytical Sensitivity) ID/pool (IU/ml)	References
2011	16 125	1: 1240 (0·08)	Chemagic viral 5K (Perkin Elmer Chemagen)/RealStar HEV RT-PCR Kit (Altona Diagnostics, Hamburg)	4·7/226	[5]
2012	18 100	1: 4525 (0·02)	In-house RT-PCR	~250/24 000	Baylis *et al.* 2011, Vox Sang 103: 89–90
2012	12 200	1:3050 (0·03)	Zeleos x100/HEV RT-PCR Kit (DRK Blutspendedienst,	12/1200	Hourfar *et al.*, 2014, Transf Med
2013	72 220	1:1760 (0·06)	Frankfurt a.M.)		Hemother 41 (suppl 1): 70
2014	91 216	1:2027 (0·05)			

Question 7

In our opinion, the introduction of HEV RNA screening is currently the only option to avoid transfusion-transmitted HEV infection and should be implemented in regions where HEV is endemic. At the end of September this year, members of the Blood Working Party (German Advisory Board of the Federal Ministry of Health) met to discuss special topics on HEV infection risks in Germany. Possibly, recommendations regarding the limitation of screening to a special subset of blood products comparable to CMV might be given, but the results of this meeting are currently pending. In our opinion, this approach is not an option up to now, because the infective dose of the different blood products is undetermined. At present, only one comprehensive study by Hewitt and co-workers has provided data regarding the context of involved blood products and infection of the respective recipient [6]. Results of this study revealed that packed red blood cells did not seem to be particularly important for HEV transmission. The residual plasma volume of the transfused blood product appears to play an important role, raising the question of the correct estimation of the infective dose, which should be calculated in reference to the volume of transfused IU per blood product rather than as the viral titre measured in the donor (IU/ml). Another possible approach could be classification into tested and untested blood products followed by provision of HEV RNA tested blood products for high-risk transfusion recipients since the immune status of the recipient has a major impact on the actual risk of infection caused by contaminated blood products.

The most rapid and easy solution for implementation of HEV RNA screening is the simple addition of HEV screening to current routine screening procedures. Most of the German blood donation services perform minipool screening of up to 96 samples. However, concerns about the most useful screening sensitivity remain. Taking the average demonstrated progression of HEV viraemia in blood donors into account (Vollmer *et al.*, unpublished data), a sensitivity of 500 IU/ml will cover at least the majority of viraemic phases. Donors with low viraemic phases were not identified with this strategy, but it remains to be seen in the future what infective doses are relevant to transfusion transmission of HEV infection (also refer to Question 6).

Question 8

We conducted a pilot study with routine HEV blood donor screening in minipools of 48 samples for 3 months (July – September 2011), revealing 13 of 16 125 individual HEV RNA-positive blood donors (0·08%, Table 5, [5]). The introduction of routine HEV blood donor screening is planned in our institution for January 2015. We are using an in-house detection method performing a high-volume RNA extraction (4·8 ml plasma sample, Chemagic Viral 5k, Perkin Elmer) combined with the RealStar HEV RT-PCR amplification kit. The 95% detection limit of this method was determined to 4·7 IU/ml [5]. Minipools will consist of 96 samples; therefore, the detection limit is 442 IU/ml per single donation.

Question 9

To our knowledge, implementation of vaccination in the case of an available efficient vaccine has so far not been discussed in detail in our country. However, this topic is potentially also discussed by members of the Blood Working Party in the September meeting.

References

1 Dreier J, Juhl D: Autochthonous Hepatitis E Virus Infections: A New Transfusion-Associated Risk? *Transfus Med Hemother* 2014; **41**:29–39

2 Faber MS, Wenzel JJ, Jilg W, *et al.*: Hepatitis E virus seroprevalence among adults, Germany. *Emerg Infect Dis* 2012; **18**:1654–1657

3 Wenzel JJ, Sichler M, Schemmerer M, *et al.*: Decline in hepatitis E virus antibody prevalence in southeastern Germany, 1996–2011. *Hepatology* 2014; **60**:1180–1186

4 Wenzel JJ, Preiss J, Schemmerer M, *et al.*: Test performance characteristics of anti-HEV IgG assays strongly influence hepatitis E seroprevalence estimates. *J Infect Dis* 2013; **207**:497–500
5 Vollmer T, Diekmann J, Johne R, *et al.*: Novel approach for the detection of Hepatitis E virus infection in German blood donors. *J Clin Microbiol* 2012; **50**:2708–2713
6 Hewitt PE, Ijaz S, Brailsford SR, *et al.*: Hepatitis E virus in blood components: a prevalence and transmission study in southeast England. Lancet 2014; **384**:1766–73;. doi:10.1016/S0140-6736(14)61034-5

Jens Dreier and Tanja Vollmer
Department of Gastroenterology
Sanjay Gandhi Postgraduate Institute of Medical Sciences
Lucknow 226014
India
E-mails: jdreier@hdz-nrw.de; tvollmer@hdz-nrw.de

Cornelius Knabbe
Director, Department of Gastroenterology
Sanjay Gandhi Postgraduate Institute of Medical Sciences
Lucknow 226014
India
E-mail: cknabbe@hdz-nrw.de

R. Aggarwal & A. Goel

Question 1

Most of the seroprevalence studies for anti-HEV antibodies in India have been small and have included either school children or selected groups of adults. Seroprevalence rates in these studies have varied widely from 4% to around 55% in different populations. This variation may represent true differences in seroprevalence across different geographic regions or socio-economic groups, or confounding due to differences in age and gender group studied and in performance characteristics of the anti-HEV antibody assays used, or both. In general, seroprevalence rates were higher in older age groups, men and among persons with lower socio-economic status.

In a population-based study, anti-HEV antibody was detected in 6·9% of 884 healthy persons belonging to urban higher socio-economic group, 10·6% of those belonging to urban lower socio-economic group and 14·0% of those belonging to rural lower socio-economic group; positivity rates among adult subsets in the three population groups were 35/206 (17·0%), 38/210 (18·9%) and 197/357 (55·2%), respectively [1].

Data on prevalence of HEV RNA in general population are limited to two small studies [2, 3]; in these studies, HEV RNA was detected in 4 of 200 and 3 of 107 blood donors, respectively. However, larger studies are needed to obtain reliable estimates of HEV RNA in healthy persons.

Question 2

For testing seroprevalence in blood donors and general population, a wide variety of assays with variable sensitivity and specificity have been used. The large population-based study referred to above [1] was based on an in-house assay based on a recombinant open reading frame 2 protein expressed in insect cells. For RNA testing in the two studies in blood donors too, in-house assays were used.

Question 3

The dominant mode of transmission of HEV infection in India is through faecal contamination of water supplies and environment. All human cases have been related to genotype 1 HEV, which is transmitted by faecal–oral route [4].

Question 4

These differences in HEV prevalence in different countries (and between different regions in the same country) are most likely related to differences in water quality, and in frequencies of ingestion of uncooked meat and contact with animals [4].

Question 5

This does not apply to India. Clinical hepatitis E is common in India.

Question 6

Hepatitis E is a significant health problem in India. However, HEV infection in blood donors is not considered a problem.

Question 7

As of now, screening of blood units for HEV RNA is not indicated in India. We need further data before we can think of this. Also, the prevalent HEV genotype in India is genotype 1 and that does not seem to lead to chronic HEV infection, which is the primary reason for screening of blood products.

Question 8

Blood donations in my institution are not screened for presence of HEV.

Question 9

Not yet. The cost-effectiveness of such approaches needs to be worked out. In fact, no data are yet available even on immunogenicity of the currently available vaccine (licensed in China) in high-risk groups listed above (such as organ and stem cell transplant recipients, cancer patients on immunosuppressive therapy, pregnant women and children).

References

1 Arankalle VA, Chadha MS, Chitambar SD, *et al.*: Changing epidemiology of hepatitis A and hepatitis E in urban and rural India (1982–98). *J Viral Hepat* 2001; **8**:293–303
2 Arankalle VA, Chobe LP: Hepatitis E virus: can it be transmitted parenterally? *J Viral Hepat* 1999; **6**:161–164
3 Khuroo MS, Kamili S, Yattoo GN: Hepatitis E virus infection may be transmitted through blood transfusions in an endemic area. *J Gastroenterol Hepatol* 2004; **19**:778–784
4 Aggarwal R, Jameel S: Hepatitis E. *Hepatology* 2011; **54**:2218–2226

Rakesh Aggarwal and Amit Goel
Department of Gastroenterology
Sanjay Gandhi Postgraduate Institute of Medical Sciences
Lucknow 226014
India
E-mails: aggarwal.ra@gmail.com; agoel.ag@gmail.com

A. R. Ciccaglione

Question 1

In Italy, several studies conducted in 1990s reported a moderate prevalence of anti-HEV IgG antibodies in general population (1–3% in north–central Italy and 3–6% in southern Italy and the Islands) [1, 2]. In the last years, two surveys conducted among blood donors in Latium and Abruzzi regions (central Italy) have revealed an anti-HEV IgG prevalence of 9% and 48%, respectively. This difference in prevalence seems to be linked to particular local food habits (Lucarelli, manuscript in preparation).

Question 2

In the last years, the presence of anti-HEV IgM and IgG antibodies in acute infections was evaluated by recently developed commercial assays (Wantai, Biologic Pharmacy Enterprise, Beijing, People's Republic of China). The sensitivity of the IgM assay, evaluated on 28 HEV RNA-positive samples, was 96·4%. Detection of HEV RNA was carried out by the RealStar HEV RT-PCR kit, version 1.0, (Altona Diagnostics, Hamburg, Germany). on Rotor-Gene Q 5/6 plex Platform (Qiagen, Hilden, Germany). This kit includes primers and a probe-targeting ORF3 region. It sensitivity, reported as 95% limit of detection, was assessed to be 50 IU/ml of HEV RNA. In order to have an estimation of viral load in HEV-positive samples, an external standard curve, made of log dilution series of HEV RNA WHO International Standard code 6329/10 (Paul Ehrlich Institute, Langen Germany) from 5×10^4 to 5×10^1 IU/ml was used (3, 4 and Lucarelli, manuscript in preparation).

Question 3

In a long-term prospective Italian study conducted over 15 years, 134 of 651 (20·6%) non-A-C patients had acute HEV infection. All of them were anti-HEV IgM and IgG positive, and 96 (71·6%) were also positive for HEV RNA by a nested-PCR assay. Moreover, 39 (6%) patients were anti-HEV IgG positive but negative for both anti-HEV IgM and HEV RNA. Among the acute hepatitis E cases, most were imported and caused by genotype 1, while some autochthonous cases were caused by genotype 3 [5]. Similar results were described in other Italian studies in which most infections, due to genotype 1, were associated with travel to endemic areas (Bangladesh, India and Pakistan), while the remaining infections, due to genotype 3, were autochthonous presumably linked to consumption of raw seafood, pork liver sausages and wild boar (3, 4 and Lucarelli, manuscript in preparation).

Question 4

Several reasons contribute to the differences such as studies performed in different periods, variable composition of the studied populations (age, sex, occupational exposure, etc.), different sensitivity and specificity of the diagnostic tests as well as higher exposure to infection of inhabitants from specific areas compared to other.

Question 5

During the period 2007–2010, 60 cases of acute HEV infection were reported to the Italian Surveillance System for Acute Viral Hepatitis (SEIEVA). Most cases occurred among persons aged 25–34 years and 35–54 years (35%

and 33% of cases, respectively). Most of the infected persons (88%) were males, and 57% were from central Italy. The actual number of cases of acute infection reported to SEIEVA is relatively low. This can be explained by the high occurrence of subclinical infections and to a lesser extent by misdiagnosis, insufficient resting and lack of awareness among clinicians.

Question 6

The perception that hepatitis E was a public health problem began when it was described as a zoonotic infection. Before then, it was not recognized in most cases and it was relegated only to travel-associated infections.

Question 7

The transfusion medicine community is currently aware of the problem but realizes that more data are needed to propose recommendations on the management of this infection with possible implication for blood safety [6]. Perhaps, the screening of blood components intended for immunosuppressed patients seems to be the most reasonable choice at the moment.

Question 8

Recently, we found a very high HEV IgG prevalence in blood donors from a region in central Italy suggesting that HEV infection is highly diffused in this area. The seroprevalence increases with age, and it is associated with consumption of raw-dried pork liver sausages. Moreover, 1·3% of HEV IgG positive were positive for HEV IgM, and two blood donors were positive for HEV RNA, genotype 3. This high seroprevalence, in contrast with low recorded incidence of hepatitis E, confirmed that HEV infection is underestimated, suggesting that most of infection are subclinical or undiagnosed. The presence of antibodies to HEV in this population was evaluated by the anti-HEV IgM and IgG ELISA (Wantai). Detection of HEV RNA was carried out by the RealStar HEV RT-PCR kit, version 1.0, (Altona Diagnostics) on Rotor-Gene Q 5/6 plex Platform (Qiagen) (Lucarelli, manuscript in preparation).

Question 9

It seems more reasonable to recommend the vaccine to risk groups than to blood donors. The severity of the infection, the chances of prevent it, the cost-benefit are important considerations for the implementation of the vaccine on a large scale.

References

1 Zanetti AR, Dawson GJ: Hepatitis type E in Italy: a seroepidemiological survey. Study Group of Hepatitis E. *J Med Virol* 1994; **42**:318–320
2 Pavia M, Iiritano E, Veratti MA: Prevalence of hepatitis E antibodies in healthy persons in southern Italy. *Infection* 1998; **26**:32–35
3 Candido A, Taffon S, Chionne P: Diagnosis of HEV infection by serological and real-time PCR assays: a study on acute non-A-C hepatitis collected from 2004 to 2010 in Italy. *BMC Res Notes* 2012; **5**:297
4 La Rosa G, Fratini M, Muscillo M: Molecular characterisation of human hepatitis E virus from Italy: comparative analysis of five reverse transcription-PCR assays. *Virol J* 2014; **11**:72
5 Romano L, Paladini S, Tagliacarne C: Hepatitis E in Italy: a long-term prospective study. *J Hepatol* 2011; **54**:34–40
6 Marano G, Vaglio S, Pupella S: Hepatitis E: an old infection with new implications. *Blood Transfus* 2014; **12**:1–12

Anna Rita Ciccaglione
Director
Viral Hepatitis Unit
Department of Infectious, Parasitic and Immunomediated Diseases
Istituto Superiore di Sanita'
Viale Regina Elena 299
00161 Rome
Italy
E-mail: annarita.ciccaglione@iss.it

K. Matsubayashi, M. Satake & K. Tadokoro

Question 1

Some nationwide surveys on HEV infection were conducted in Japan. We showed that the prevalence of anti-HEV IgG in qualified blood donors and anti-HEV IgG and HEV RNA in blood donors with elevated ALT (≥200 IU/l) in Japan was 3·4% (431/12 600), 3·2% (45/1389) and 1·1% (15/1389), respectively [1, 2]. Another study conducted in individuals who had health check-ups showed that the prevalence of anti-HEV IgG and HEV RNA was 5·3% (1167/22 027) and 0·014% (3/22 027), respectively [3]. To understand the status of HEV infection in blood donors, trial screening for HEV RNA has been conducted since 2005 in Hokkaido, an island in the north of Japan, where HEV infection is endemic. The overall prevalence of HEV RNA during 2005 and 2013 was 0·011% (279/~2.5 million).

Question 2

For the seroprevalence studies, in-house or commercial HEV ELISAs have been used, but the sensitivity and

specificity are unknown. For the HEV RNA prevalence studies, in-house nested RT-PCR or real-time RT-PCR using individual or minipooled samples has been used [2, 3]. The 95% limit of detection of our in-house real-time RT-PCR assay for HEV RNA was approx. 50 IU/ml.

Question 3

Besides imported cases, accumulating evidence demonstrates that zoonotic foodborne transmission plays the most important role in the autochthonous HEV infection in Japan. Consumption of uncooked or undercooked meat or viscera of animals acting as reservoirs of HEV, such as pigs, wild boar and deer, seems to be a major cause of HEV infection, which may account for higher seroprevalence and incidence of HEV infection in older males because they may be at greater risk of exposure. Transfusion transmission is another established route of HEV transmission but rarely occurs. We have experienced more than ten cases of transfusion-transmitted HEV infection during the past 15 years. The risk of HEV transmission by haemodialysis is low, and no evidence of vertical transmission has been obtained. However, the source or transmission route of the HEV infection is not fully specified in many domestic cases, and other causes may exist in this country.

HEV has been recovered from humans, animals, bivalves and environmental samples in Japan and is classified into genotype 3 or 4. Many of these strains are closely related and are indigenous to Japan. Recently, in addition, two novel strains were isolated from wild boar in Japan. Genotype 3 HEV is dominant and is widespread throughout Japan, whereas genotype 4 HEV is rarely found and primarily localized in Hokkaido [1, 3, 4]. Genotype 1 is detected only in patients with imported hepatitis E.

Question 4

The seroprevalence data are directly affected by the sensitivity and specificity of the assay used in the studies and size, sex and age distribution of the target population, which leads to the observed differences. The epidemic of waterborne disease is commonly associated with inadequate sanitary conditions. The seroprevalence of HEV among the general population is high in developing countries and low in developed countries. The major route of HEV transmission in developed countries is zoonotic foodborne; therefore, eating habits or cultural as well as religious practices could affect the seroprevalence of HEV. The seroprevalence of HEV was higher in eastern Japan than in western Japan, and individuals in eastern Japan are more likely to consume pork than beef [1]. Among pregnant women in Bali, Indonesia, the seroprevalence of HEV was significantly less frequent in Muslims, who were strictly prohibited from eating pork, than Hindus, who have no such restrictions. The genotype of HEV could be another reason for the differences. As mentioned above, two HEV genotypes, namely genotypes 3 and 4, are circulating in Japan and genotype 4 HEV causes more severe hepatitis than genotype 3 HEV [4]. Genotype 4 HEV is responsible for about half of the cases of HEV infection in Hokkaido; however, genotype 4 is found in <10% of the HEV RNA-positive blood donors in Hokkaido.

Question 5

Yes. The reason for the discrepancy may include all the points listed above. Reported cases of clinical hepatitis E has increased dramatically after the first licensed diagnostic assay for HEV (anti-HEV IgA assay) came into the market in Japan in 2011.

Question 6

Autochthonous HEV infection seems to be much more common than previously thought and has been identified throughout the country. Although most cases are subclinical, acute or fulminant cases reportedly account for 15% of all cases [4]. Fatal cases were also reported. Two cases with liver fibrosis in immunosuppressed patients and two cases with sustained viremia in liver transplant recipients were attributed to transfusion-related HEV infection. Although there is a growing awareness of hepatitis E among physicians, the issue of HEV has not yet been regarded as a significant health problem.

Having data on high HEV RNA prevalence among blood donors through trial NAT screening, blood professionals have perceived the HEV issue as a noteworthy problem for blood supply.

Question 7

Several critical issues have to be carefully discussed before deciding whether or not HEV RNA screening should be implemented. We will continue HEV ID-NAT in the Hokkaido area which will provide us with novel prevalence data. Our look back study, although insufficient in number, suggests the low infectivity of HEV-contaminated blood components. We need more data on chronic hepatic dysfunction among transplant recipients or heavily immunosuppressed patients or patients with underlying liver disease. It is also necessary to

monitor the incidence of fulminant hepatitis caused by genotype 4 HEV in areas other than Hokkaido.

Question 8

Trial screening for HEV RNA was implemented in 2005 in the Hokkaido area where hepatitis E infection is endemic and several cases of transfusion transmission of HEV infection have been observed. Since 2005, in-house real-time RT-PCR for HEV RNA, using pools of samples, has been used for annual screening of ~280 000 blood donors. The in-house assay was replaced in August 2014, with the implementation of ID-NAT using the Procleix HEV Assay (Grifols).

Question 9

Vaccination against HEV in not being discussed in Japan.

References

1 Takeda H, Matsubayashi K, Sakata H, *et al.*: A nationwide survey for prevalence of hepatitis E virus antibody in qualified blood donors in Japan. *Vox Sang* 2010; **99**:307–313
2 Sakata H, Matsubayashi K, Takeda H, *et al.*: A nationwide survey for hepatitis E virus prevalence in Japanese blood donors with elevated alanine aminotransferase. *Transfusion* 2008; **48**:2568–2576
3 Takahashi M, Tamura K, Hoshino Y, *et al.*: A nationwide survey of hepatitis E virus infection in the general population of Japan. *J Med Virol* 2012; **82**:271–281
4 Takahashi M, Okamoto H: Features of hepatitis E virus infection in humans and animals in Japan. *Hepatol Res* 2014; **44**:43–58

Keiji Matsubayashi
Japanese Red Cross Hokkaido Block Blood Center
2-1, Nijuyonken
Nishi-ku
Sapporo
Japan
E-mail: kmatsu@hokkaido.bc.jrc.or.jp

Masahiro Satake and Kenji Tadokoro
Central Blood Institute
Blood Service Headquarters
Japanese Red Cross Society
2-1-67, Tatsumi
Koto-ku
Tokyo
Japan
E-mails: m-satake@jrc.or.jp; k-tadokoro@jrc.or.jp

S.-H. Jeong

Question 1

I am aware of two studies of HEV prevalence in adult population, one is using 749 blood samples collected in a central laboratory (seroprevalence, 11·9% by GeneLabs ELISA for anti-HEV IgG) [2] and the other is using 147 blood samples from health-check examinee in a general hospital which was age-, and sex-matched to general population of South Korea (seroprevalence, 23·1% by Wantai anti-HEV IgG assay, and 14·3% by Genelab assay) [1]. I do not know any study in blood donors in Korea.

Question 2

Genelabs anti-HEV IgG assay is the most popular assay in Korea, which is commercial. Recently, Wantai ELISA is also available in limited laboratories, I heard.

HEV RNA detection is not available except in research laboratory.

Question 3

Zoonotic infection through eating raw meat or liver/bile juice of pig, wild boar or deer [4].

The reported genotypes are genotypes 3 and 4.

Question 4

It may be related to eating behaviours of raw meat or to the sanitary condition of cooking.

Question 5

I think that underrecognition of the disease and suboptimal assays of ELISA and subclinical infection are the reasons [3].

Question 6

HEV is not perceived as a significant health problem in South Korea. It is not perceived a s a problem for blood supply.

Question 7

I think HEV blood donation screening has merit in a subset, such as immunocompromised host transfusion, because HEV infection in such situation resulted in chronic infection and rapid progression.

The efficient screening test should be HEV RNA test.

Question 8

Not at al.

Question 9

I do not think the vaccination is considered for general population. Because of low awareness and low disease burden in Korea, plans for implementing HEV vaccine in immunosuppressed patients are difficult to discuss [3].

References

1 Park HK, Jeong SH, Kim JW, *et al.*: Seroprevalence of anti-hepatitis E in a Korean population: comparison of two commercial anti-HEV assays. *BMC Infect Dis* 2013; **12**:142
2 Ahn JM, Kang SG, Lee DY, *et al.*: Identification of novel human hepatitis E virus isolates and determination of the seroprevalence of HEV in Korea. *J Clin Microbiol* 2005; **43**:3042–3048
3 Jeong SH: Current Status of Hepatitis E Virus Infection in Korea. *Gut Liver* 2011; **5**:427–431
4 Kim YM, Jeong SH, Kim JY, *et al.*: The first cast of genotype 4 hepatitis E related to wild boar in South Korea. *J Clin Virol* 2011; **50**:253–256

Sook-Hyang Jeong
Department of Internal Medicine
Seoul National University Bundang Hospital
College of Medicine
Seoul National University
Seoul
Korea
E-mail: jsh@snubh.org

H. L. Zaaijer

Question 1

The Dutch anti-HEV seroprevalence and HEV RNA incidence have been described in our publications:

Silent hepatitis E virus infection in Dutch blood donors, 2011 to 2012.
Slot E, ea. Euro Surveillance 2013 Aug 1;18(31).
and
Past and present of hepatitis E in the Netherlands.
Hogema BM, ea. Transfusion. 2014 May 29. doi: 10.1111/trf.12733

In 2011, countrywide 27% of Dutch donors tested positive for HEV IgG; and 17 of 45·415 donors tested (confirmed) positive for HEV RNA. The incidence seems to be increasing: in the last 6 months (April – September 2014), the routine screening of plasma pools (96 donations/pool) shows that 17/11·191 (or 1:658) Dutch donors is (confirmed) HEV RNA positive.

Question 2

For HEV antibody testing of Dutch donors, Wantai EIAs are being used; for HEV RNA testing, we employ an in-house developed PCR assay.

Question 3

The prevalent genotype of endemic HEV in the Netherlands is genotype 3. Risk factors and routes of infection are unknown. Among vegetarian blood donors, the anti-HEV seroprevalence is half that of the general donor population.

Question 4

Differences could arise by the use of EIAs with insufficient sensitivity for HEV gt3 antibodies and of course by differences of HEV infection pressure, possibly caused by differences in food production and/or handling.

Question 5

In the Netherlands, we see many cases of clinical, endemic hepatitis E, especially among middle-aged men. Because hepatitis E is not a notifiable disease in the Netherlands, the clinical impact of endemic hepatitis E probably is underestimated.

Question 6

Hepatitis E is perceived as a problem by physicians caring for immunosuppressed patients, such as transplant patients and haematological patients. Regarding the safety of the blood supply, again these physicians, plus the Dutch blood bank, are worried. There seems to be no concern among the general public, nor among governmental officials.

Question 7

In my opinion, not the blood supply should be made HEV safe, but the cause of the 'outbreak' should be eliminated. Daily practice shows that blood is only a minor source of HEV infection in Dutch vulnerable patients.

By the way, considering the very high infection pressure of HEV gt3 in the Netherlands (see item 1), and the age and gender distribution of Dutch endemic hepatitis E

patients, HEV genotype 3 seems not to be a problem for newborns, children, adolescents and pregnant women.

Question 8

Routine HEV screening of 96 pools, using a validated in-house HEV RNA PCR, has been implemented for SD plasma production in the Netherlands.

Question 9

An HEV vaccine would be nice, but primarily the source of HEV infections should be eliminated.

Hans L. Zaaijer
Sanquin Blood Supply Foundation – Blood-borne Infections
Plesmanlaan 125
1066 CX Amsterdam
The Netherlands
E-mail: h.zaaijer@sanquin.nl

E. Zhiburt

Question 1

Hepatitis E virus (HEV) was first described in 1983 in Russia following the investigation of a hepatitis outbreak in a Soviet military camp in Afghanistan [1].

Hepatitis E virus seroprevalence was defined in blood donors and patients with HIV in Nizhny Novgorod, Russia. A total of 505 donor sera provided by regional blood centre and 500 sera from HIV-infected patients provided by Regional Center for prevention and control of AIDS and infection diseases were studied for the presence of antibodies to hepatitis E virus. The levels of IgM or IgG antibodies against HEV were determined with the CE-marked EIA kits (RPC 'Diagnostic Systems', Russia).

A total of 19 (3·8%) of 500 sera from HIV-infected patients examined for markers of viral hepatitis E were sero-positive. Four patients of 500 had both anti-IgM and anti-IgG (0·80%), five patients (1·0%) had only anti-IgM, and 10 patients had only anti-IgG (2·0%). Detection rate of hepatitis E antibodies in healthy population of NizhnyNovgorod was 7·3% (37 individuals). Frequency only of IgM marker occurrence was 1·78% (nine individuals); only IgG marker occurred with the frequency 4·75% (24 individuals). Simultaneous presence of IgM and IgG marker in donor sera was 0·79% (4 of 505 donors).

According to the results of the study described above, frequency of occurrence of hepatitis E markers in control group of blood donors was higher than in HIV-infected patients. Lower percentage of detection of hepatitis E markers in HIV-positive patients may be caused by interactive effect of viruses in case of HIV/HEV coinfection [2].

In 1979–1989, a limited contingent of Soviet Army took part in war actions in Afghanistan. Veterans of this war have had a higher risk of exposure to HEV during their presence in Afghanistan. The aim of this study was to investigate the current prevalence of anti-HEV in veterans who have been at war in Afghanistan in 1979–1989 and in those who have had their military service in the same years in regions of former USSR, non-endemic for hepatitis E.

Two groups of veterans were studied: group A – veterans who have had their military service in Afghanistan (n = 317), and group B – veterans who have had their military service in non-endemic for HEV regions of former USSR (n = 208). All individuals currently living in non-endemic for HEV area (Sverdlovsky Region, Russia) are of the same age and have had their military service at the same years (1979–1989). Individuals who have not been in Afghanistan denied any visits in south regions of former USSR. Anti-HEV testing was performed in ELISA with commercially available assay ('Diagnostic Systems', Russia).

At the time of investigation (2004–2005), the prevalence of anti-HEV in veterans who have had their military service in Afghanistan was 29·97% (95/317) and was significantly higher than that observed in veterans who have had their military service in non-endemic for HEV regions of former USSR (3·8% [8/208], PR [95% Cl] = 7·8 [3·9–15·7]. $P < 0·0001$). These results suggest that at least approximately 30% of veterans who have been at war in Afghanistan have been exposed to HEV. In none of cases, anti-HEV IgM were detected.

So, the military service in endemic for hepatitis E regions is consistent to a higher risk of HEV infection and demands preventive measures, including vaccination against hepatitis E [3].

Question 2

Russian company 'Diagnostic Systems' produces serologic assay for IgM anti-HEV. Its diagnostic sensitivity and specificity were 98% and 92·8%, respectively, and its analytic sensitivity was 9 Walter Reed Units/ml [4]. They also produce serologic assay for IgG anti-HEV. The both kits are CE-marked. Another company 'Vector-Best' is waiting to receive CE-mark for own serologic assays for IgG and IgM anti-HEV in nearest months.

Question 3

The detection frequency of antibodies to hepatitis E virus (HEV) was studied in residents of the South and of the Middle European Part of the Russia Federation as well as of Siberia. Antibodies to HEV were most often found both in patients with hepatic pathologies and in subjects with diseases unrelated with a primary hepatic lesion, in particular, in patients with skin and venereal diseases and with HEV. A higher concentration of antibodies to HEV was noted also in blood donors, medical personnel and isolated communities, such as prisons or psychiatric clinics. A correlation was established between the rate antibodies to HEV are registered and such risk factor as contacting with blood or a gross violation of the hygienic rules [5].

Question 4

The detection of antibodies to HE virus among different groups of the population of Russia (i.e. a hepatitis E non-endemic region of the world) suggests that they are frequently detected in the absence of disease notification. The fact that viral HE RNA has been detected in the pigs and wild boars inhabiting in the country is the evidence in favour of an assumption that the virus is spread among these animals [6].

Question 5

Lack of awareness among clinicians and subclinical infection. Possibly blood donor screening for ALT activity protect blood recipients against HEV transmission [7–9].

Question 6

No. No.

Question 7

No.

Question 8

No.

Question 9

No.

References

1 Balayan MS, Andjaparidze AG, Savinskaya SS, *et al.*: Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal-oral route. *Intervirology* 1983; **20**:23–31

2 Sharipova IN, Berezhnaya AV, Puzyrev VF, *et al.*: Hepatitis E virus seroprevalence in patients with HIV in Nizhny Novgorod, Russia. *BMC Infect Dis* 2014; **14**(Suppl 2):P100

3 Michailov M.I., Morsi I.A., Popova O.E., *et al.*: Prevalence of anti-HEV in veterans who have been at war or have not been at war in Afghanistan in 1979–1989. 12th International Symposium on Viral Hepatitis and Liver Disease, Paris, 2006.- P.465

4 Drobeniuc J, Meng J, Reuter G, *et al.*: Serologic assays specific to immunoglobulin M antibodies against hepatitis E virus: pangenotypic evaluation of performances. *Clin Infect Dis* 2010; **51**:e24–e27

5 Fedorova OE, Alatortseva GI, Pavlov NN, *et al.*: Prevalence of antibodies to hepatitis E virus among residents of different climate and geographic zones of Russia Federation. *Vopr Virusol* 2004; **49**:4–7

6 Mikhaĭlov MI, Zamiatina NA, Poleshchuk VF: Viral hepatitis E. The problems of its study. *Vopr Virusol* 2005; **50**:20–22

7 Zhiburt EB, Kodenev AT: Prescreening of the activity of alanine aminotransferase enhances the cost-effectiveness of blood storage. *Klin Lab Diagn* 2009; **(11)**:14–16

8 Zhiburt EB: Alanine aminotransferase is a substitute marker of viral hepatitis. *Vopr Virusol* 2005; **50**:18–20

9 Zhiburt EB, Bel'gesov NV, Vashchenko TN, *et al.*: Alanine aminotransferase is a surrogate marker of viral hepatitis. *Vopr Virusol* 1995; **40**:25–27

Eugene Zhiburt MD, PhD
Head, Department of Blood Transfusion
Pirogov Russian National Medical Surgical Center
70, Nizhnyaya Pervomaiskaya ul.
Moscow
105203 Russia
E-mail: ezhiburt@yandex.ru

J. Chay, D.Teo & S. S. Chua

Question 1

There are currently no studies of HEV prevalence among blood donors in Singapore.

The prevalence of acute HEV infection in the Singapore population was 1·9 per 100 000 in year 2012 [1–3]. The population prevalence of acute HEV infection has steadily increased from 0·05 per 100 000 in year 2000 to 1·9 per 100 000 in year 2012. There were no recorded deaths from acute HEV infection during this period.

HEV infection is a notifiable disease in Singapore under the Infectious Diseases Act. All cases of laboratory-confirmed HEV infection must be reported to the Ministry of Health (MOH) within 72 h of diagnosis. In this respect, acute HEV infection is defined as a clinically compatible disease that has been serologically confirmed by the presence of anti-HEV IgM antibody.

Following notification, investigations are routinely conducted by the MOH which include relevant demographic and epidemiological information such as the date of onset of illness, food items consumed and travel history within 8 weeks prior to the onset of illness, and contact with a known clinical case of acute hepatitis within the same period. Contact tracing is initiated where there is a cluster involving two or more cases with epidemiological linkage.

Question 2

The published population prevalence for acute HEV infection in Singapore is based on the presence of clinical illness coupled with the detection of anti-HEV IgM antibody. HEV testing is conducted in a centralized laboratory at the Singapore General Hospital which performs testing of all notifiable cases.

The current assay in use is the anti-HEV IgM ELISA 3.0 (MP, Biomedicals, Singapore) assay. Samples are tested once and if positive, are then retested in duplicate as per test kit manufacturer's instructions. Data provided by the manufacturer indicate that the test has a sensitivity of 98·0%, specificity of 97·8%, positive predictive value (PPV) of 94·9% and negative predictive value (NPV) of 98·7% [4].

HEV testing by NAT is not routinely performed and limited to a few specialized reference or research centres, which only perform qualitative HEV PCR assays. HEV genotyping is not performed.

Question 3

Epidemiological studies of HEV infection in Singapore suggest that overseas travel and possibly the consumption of porcine products (zoonotic transmission from undercooked pork) are the main risk factors for acquiring infection [1].

The majority of acute HEV infections in Singapore are imported; imported cases are defined as those with a recent travel history outside Singapore within 8 weeks prior to onset of symptoms. These accounted for 54·5% of all cases (262 cases of a total of 481 cases) reported from year 2000 to year 2011, and 57 imported cases (of a total of 104 cases, or 54·8%) in Year 2012. Most of the imported cases originated from South-East Asia and the Indian subcontinent.

The exact mode of transmission within Singapore (indigenous cases) could not be determined despite careful epidemiological enquiries although indirect evidence suggests that consumption of porcine products may play a role [1]. The predominance of males in the 25–34 years age group mirrors those reported in other developed countries such as the United States, the United Kingdom, Japan, Hong Kong, New Zealand and Australia.

No information is available on the genotypes of HEV infection in Singapore.

Question 4

Possible reasons for differences in HEV prevalence within and between countries include the following:

(1) Differences in the sensitivity and specificity of the assays used to detect hepatitis E [5];
(2) Differences in detection of cases, for example subclinical/asymptomatic cases;
(3) Differences in socio-economic conditions which influence public health, for example public sanitation and infrastructure, universal access to a safe, clean water supply influence frequency of HEV infections transmitted via the waterborne route (Genotype 1 & 2);
(4) Dietary and food preparation habits of the population that influence the frequency of HEV infection transmitted via the zoonotic route (Genotype 3 & 4).

Question 5

HEV seroprevalence among blood donors in Singapore is currently not established.

Question 6

The incidence of acute HEV infection in Singapore is relatively low, and it is not perceived as a significant health problem although it is a notifiable disease in the country. However, it is noted that acute HEV infection is on a rising trend in Singapore and is now more common than acute hepatitis A (1·8 per 100 000 in 2012). As the current available population prevalence data is limited to acute HEV infection with clinical illness, the actual prevalence may be higher if asymptomatic and subclinical cases are included.

There are also no studies of the prevalence of HEV infection in blood donors although this has been planned. The risk of HEV transmission through the blood supply is therefore unknown at present.

Question 7

There is no data available on the prevalence of HEV infection in blood donors. The efficiency of transmission is also unknown as there are similarly no seroprevalence studies in the population. The factors that would need to be taken into consideration in a decision whether to implement HEV testing are as follows:

(1) Donor prevalence
(2) Efficiency of transmission
(3) Severity of transfusion-transmitted HEV

(4) Any public health implications (e.g. any persistent chronic carrier state)
(5) Performance of available tests (sensitivity, specificity, impact on unit discards)
(6) Suitability of available tests for blood supply screening (high volume, high throughput)
(7) Cost-effectiveness of the test.

The requirements for an efficient screening test for the blood supply include the following:

(1) Test sensitivity and specificity for optimal detection of HEV-infected donations with minimum false positives resulting in unnecessary discard;
(2) Testing performed on automated platforms with high-volume throughput, fast turnaround time and no crossover contamination;
(3) Minimum cross-reactivity with other virus infections;
(4) Stability and robustness of test reagents and consumables during storage and transport;
(5) Accompanied with good service support from the manufacturer;
(6) Reasonably priced.

The decision whether to test all donations (universal testing) or a subset (selective testing) depends on either the ability to identify donor subgroups at risk (i.e. testing only at-risk donors) or transfusion recipients at risk (providing HEV-negative units to only patients at risk of serious complications [6]). As risk factors are not clearly defined in either groups within the donor and recipient population, it would not be feasible at this time to consider testing only a subset if HEV testing is to be implemented.

Question 8

HEV screening of blood donations has not been implemented in Singapore.

Question 9

As far as we know, there are no plans at the moment for hepatitis E vaccination in Singapore.

References

1 Tan LTC, Tan J, Ang LW, *et al.*: Epidemiology of acute hepatitis E in Singapore. *J Infect* 2013; **66**:453–459
2 Communicable Diseases Division, Ministry of Health, Singapore. Communicable Diseases Surveillance in Singapore 2011 Report. Available at: http://www.moh.gov.sg/content/moh_web/home/Publications/Reports/2012/_communicable_diseases surveillanceinsingapore2011.html [Last accessed 6 October 2014]
3 Communicable Diseases Division, Ministry of Health, Singapore. Communicable Diseases Surveillance in Singapore 2012 Report. Available at: http://www.moh.gov.sg/content/moh_web/home/Publications/Reports/2013/Communicable_Diseases_Surveillance_in_Singapore_2012.html [Last accessed 6 October 2014]
4 MP Anti-HEV IgM ELISA 3.0 ® [package insert]. Singapore; MP Biomedicals, Singapore. Aug 2009
5 Khudyakov Y, Kamili S: Serological diagnostics of hepatitis E virus infection. *Virus Res* 2011; **161**:84–92
6 Aggarwal R: Clinical presentation of hepatitis E. *Virus Res* 2011; **161**:15–22

Jason Chay, Diana Teo and Sze Sze Chua
Blood Services Group
Health Sciences Authority
11 Outram Road
169078 Singapore
Singapore
E-mails: Jason_CHAY@hsa.gov.sg; Diana_TEO@hsa.gov.sg; CHUA_Sze_Sze@hsa.gov.sg

M. Piron & S. Sauleda

Question 1

Few studies present data of HEV seroprevalence in Spain. In general adult population, data were published in 2006 in Catalonia (1280 samples) and in 2012 in Madrid region (2305 samples) describing seroprevalence of 7·3% and 2·17%, respectively. In paediatric population, a 4·6% HEV seroprevalence rate was described in 2008 in a cohort of 1249 healthy Catalan children (6–15 years) [1–3].

In blood donors, a study performed in 1995 in Madrid region described a prevalence rate of 3·9% (HEV IgG initially reactive samples) [4]. In 2013, our blood bank performed a study including nearly 10 000 Catalan blood donors. Our objective was to determine the RNA prevalence as well as the HEV IgG seroprevalence. We observed a prevalence of HEV IgG of 20% or 11% in the cohort of 1082 donors, depending on the commercial assay used. Significantly higher sero-positivity rates were observed in male donors vs. female donors, and the rate of exposure increased proportionally to the donors' age (39% or 17% in donors older than 61 years, depending on the HEV IgG test used). As for the RNA prevalence, the HEV RNA positivity rate was 1 per 3333 donations (0·03%, 95% CI: 0·01–0·09%) [5].

Question 2

All the seroprevalence results previously published in the Spanish population were obtained using different commercial enzyme-linked immunoabsorbent assays (bioelisa

HEV IgG Biokit, Diagnostic Bioprobes Srl HEV Ab, Abbott HEV EIA).

As for our study in Catalan blood donors, two different commercial ELISA tests were used for HEV IgG detection. The HEV-IgG ELISA test (Beijing Wantai Biological Pharmacy Enterprise CO., LTD., Beijing, China) showed the highest seroprevalence rate (20%). The sensitivity and specificity claimed by the manufacturer are 99·08% (in acute HEV phase) and 99·99%, respectively. The other assay used was recomWell HEV IgG test (Mikrogen GmbH, Neuried, Germany) which presented a seroprevalence of 11% in the same donors' population. The sensitivity and specificity claimed by the manufacturer are 96·3% (in acute HEV phase) and 98·2%, respectively. As for the RNA frequency, we used the Procleix HEV assay on the Procleix Panther system (Grifols Diagnostic Solutions Inc, Emeryville, CA, USA, developed in collaboration with Hologic Inc., San Diego, CA, USA). The assay showed a 95% limit of detection of 7·9 IU/ml using the HEV WHO International Standard (PEI code 6329/10) and a specificity of 99·99%.

Question 3

Zoonotic and foodborne transmissions must be considered the most probable sources of infection in our country. Genotype 3 accounts for hepatitis E autochthonous cases in our region.

Question 4

There is a strong need for a standardized test that would allow a correct comparison between countries or regions. In our study, we could indeed observe a nearly double rate of HEV IgG prevalence in the same blood donors, depending on the test used. Additionally, geographical differences can be explained by different food habits and different degrees of HEV environmental contamination.

Question 5

We could actually observe a fairly high HEV IgG seroprevalence in our blood donors (almost 40% in donors over 61 years with Wantai test, and 17% with Mikrogen test), but this does not correlate with the reported clinical hepatitis E cases. This leads us to suggest that most of HEV infections remain subclinical in immunocompetent individuals. However, Catalan hepatologists are increasingly aware of the potential HEV clinical impact, especially among immunosuppressed patients. Therefore, we will probably observe a higher number of reported acute and persistent HEV infections in our region.

Question 6

Hepatitis E infection is certainly not perceived as a significant health problem in Spain, and, for the moment, it is not considered a problem for blood supply.

Question 7

In England, Hewitt *et al.* [6] recently described an RNA prevalence of one in 2848 donations that is very close to the HEV RNA prevalence observed in our blood donations (1 in 3333). Although the authors observed a transmission rate of 42% through HEV RNA-positive blood components, they showed that infectivity was affected by the degree of immunosuppression in the recipient, and by the viral load and the presence of HEV IgG in the donor. We think that the factors that affect infectivity need to be established in order to evaluate the residual transmission risk. Then, the relevance and the cost-effectiveness of the HEV screening could be discussed. In any case, an optimal screening would be an HEV RNA test that should possess enough sensitivity and a sufficiently high throughput to allow validation of donations in parallel with the other routine screening tests.

From a logistical point of view, we do not think that a selective HEV RNA screening would be recommendable because as much as 60% of blood recipients, for their immunological status, can be susceptible to HEV infection.

Question 8

We are not currently testing for HEV.

Question 9

Our health authorities are not currently considering the possibility of HEV vaccination.

References

1 Buti M, Domínguez A, Plans P, *et al.*: Community-based seroepidemiological survey of hepatitis E virus infection in Catalonia, Spain. *Clin Vaccine Immunol* 2006; **13**:1328–1332

2 Fogeda M, Avellón A, Echevarría JM: Prevalence of specific antibody to hepatitis E virus in the general population of the community of Madrid, Spain. *J Med Virol* 2012; **84**:71–74

3 Buti M, Plans P, Domínguez A, *et al.*: Prevalence of hepatitis E virus infection in children in the northeast of Spain. *Clin Vaccine Immunol* 2008; **15**:732–734

4 Mateos ML, Camarero C, Lasa E, *et al.*: Hepatitis E virus: relevance in blood donors and risk groups. *Vox Sang* 1999; **76**:78–80

5 Sauleda S, Ong E, Bes M, *et al.*: Seroprevalence of hepatitis E virus (HEV) and detection of HEV RNA with a transcription-

mediated amplification assay in blood donors from Catalonia (Spain). Transfusion 2015 May; 55(5):972–9
6 Hewitt PE, Ijaz S, Brailsford SR, *et al.*: Hepatitis E virus in blood components: a prevalence and transmission study in southeast England. *Lancet* 2014 Nov 15; 384(9956):1766–73

Maria Piron PhD and Silvia Sauleda PhD
Transfusion Safety Laboratory
Banc de Sang i Teixits
Doctor Frederic Duran i Jordà building
Passeig Taulat, 116
08005 Barcelona
Spain
E-mails: mpiron@bst.cat; ssauleda@bst.cat

J.-M. Echevarría

Question 1

The prevalence of the HEV infection among the general population of Spain has been studied in Barcelona and Madrid on representative samples stratified by age. The overall rate of anti-HEV reported ranged from 1·1% in Madrid to 6·0% in Barcelona and increased with age (0·5–4·6% among children and 2·1–7·3% among adults, respectively). Data from Madrid corresponded to immunoblot-confirmed samples (confirmation rate on screening-reactive samples: 50%), and data from Barcelona to samples reactive in screening. They are not, therefore, fully comparable, but HEV would perhaps be more prevalent in Barcelona than in Madrid. Data from blood donors have been reported from Granada (492 samples) and Madrid (863 samples), and the anti-HEV rate found was not significantly different (3·1% and 2·8%, respectively). The highest anti-HEV rate reported from Spain was found among the workers of a swine farm (18·6%) [1–3].

Question 2

The studies mentioned above for the general population and for blood donors were performed using four different commercial tests (Abbott, Biokit, DiaPro, and Mikrogen RecomBlot). Other studies performed on particular populations were done with other assays. Diagnosis of acute HEV infection by RNA testing is mainly performed by in-house, conventional PCR methods. A few cases diagnosed by commercial, real-time PCR tests have been also reported. At present, many hospitals perform IgM testing, but very few have incorporated RNA testing.

Question 3

No risk factors for HEV infection have been yet identified in Spain among indigenous cases. One proven case of transmission by pork meat has been, however, described recently [4], and HEV RNA has been found among sausage samples [5] and in mussels [6].

Question 4

Some results suggest that acute hepatitis E might be more frequent in the Northern regions of Spain than in the rest of the country [3], but this remains to be confirmed by prospective studies. Consumption of pork sausage elaborated by particular procedures (i.e. smoke treatment instead of long-term drying of meat in conditions of low temperature and humidity) might account, at least in part, for such apparent difference, though this is just hypothetical.

Question 5

I do, but the issue is difficult to assess because the present lack of consensus criteria for measuring anti-HEV prevalence. Our recent, yet unpublished experience does not confirm the large differences in sensitivity between assays for anti-HEV IgG testing reported by some other research groups, and would support immunoblot confirmation as adequate to find the real rates in settings of low prevalence dominated by genotype 3 strains. In regard to diagnosis of acute infection by anti-HEV IgM detection, our results suggest also that most commercial tests perform properly, but in the case of sampling during the window period of the infection, which seems not very frequent in practice. Once, most Spanish clinicians became aware of the disease, as they are at present, my thought is that many acute HEV infections are not detected because they are symptomless or produce mild, non-specific clinical symptoms that do not suggest acute liver disease. False-negative results in PCR testing because low level viraemia is not a problem if anti-HEV IgM testing is also performed, which is the case in most hospitals.

Question 6

Not at present among the public health authorities, but this is in train to change quickly since testing of blood units for HEV RNA is being considered. The Spanish Society for Blood Transfusion included for the first time a round table on HEV in its recent national congress (July, 2014), which shows that our transfusion setting is awakening in regard to the problem. This is mainly due to the several reports of serious infections among the immunocompromised lacking

identifiable risk factors other than transfusion of blood components or industrial plasma derivatives, which might, theoretically, be involved as the source of the infection.

Question 7

I think that protection of Spanish immunocompromised patients requires HEV-free blood components and plasma derivatives. Therefore, what I would see right is mandatory RNA testing for plasma units destined to industrial fractionation and for a subset of blood units reserved to the immunocompromised. If testing is performed on individual units rather than on pools, I would not think, initially, that exquisite sensitivity was a major issue, but much more data on viral loads among symptomless people experiencing the acute infection are needed before getting any conclusion. Let us, therefore, begin as soon as possible and learn from the experience in order to improve.

Question 8

My institution is involved in the transfusion setting just as a national reference laboratory in the field of human virology that provides confirmation of cases and genotyping of viral strains. Anyway, I am not aware that any Spanish transfusion centre is yet performing HEV RNA testing, though this might be already changing.

Question 9

A proper cost-benefit analysis in regard to universal vaccination against HEV would require much more information about the incidence and the prevalence of the infection among the Spanish population than the one available at present. Public health authorities, both at the national and EU levels, must pay better attention to the issue to make that possible through proper financial support. This is different, of course, in regions where waterborne, epidemic genotypes circulate, which is not the case of Spain or of any other EU country. In the meantime, vaccination of the immunocompromised patients, including all cases listed in the question, would be recommendable.

References

1 Echevarría JM: Light and darkness: prevalence of hepatitis E virus infection among the general population. *Scientifica* 2014; **2014**:481016
2 Echevarría JM: Autochthonous hepatitis E virus infection in Europe: a matter of concern for public health? *J Clin Transl Hepatol* 2014; **2**:7–14
3 Echevarría JM, Fogeda M, Avellón A: Epidemiology of hepatitis E virus infection in Spain. *Enferm Infecc Microbiol Clin* 2015; **33**:281–286, in press
4 Riveiro M, Mínguez B, Gironés R, *et al.*: Phylogenetic demonstration of hepatitis E infection transmitted by pork meat ingestion. *J Clin Gatroenterol* 2015; **49**:165–168, in press
5 Di Bartolo I, Díez-Valcarce M, Vasickova P, *et al.*: Hepatitis E virus in pork production chain in Czech Republic, Italy, and Spain, 2010. *Emerg Infect Dis* 2012; **18**:1282–1289
6 Díez-Valcarce M, Kokkinos P, Söderberg K, *et al.*: Occurrence of human enteric viruses in commercial mussels at retail level in three European countries. *Food Environ Virol* 2012; **4**:73–80

José-Manuel Echevarría
Department of Virology
National Centre of Microbiology
Instituto de Salud Carlos III
Majadahonda
Madrid
Spain
E-mail: jmecheva@isciii.es

H. R. Dalton

Question 1

Yes. The IgG seroprevalence in England is 12% [1]. In SW England, it is 16% [2]. In Scotland, it is 4·7% [3]. In England, 1 in 2848 donors were found to be contaminated with HEV RNA [4]. In Scotland, the figure is 1 in 14 500 [3].

Question 2

The above studies were all performed using the same commercially available IgG assay. The sensitivity is 98%. The specificity for distant infection has not been fully established. The PCR assays conformed to recently established WHO standards.

Question 3

The prevalent genotype in the UK is HEV genotype 3. Clinically apparent infection is most common in middle-aged and elderly men. Excessive alcohol consumption may be a risk factor for developing overt signs of infection [5]. Consumption of contaminated pork products is probably the most important route of infection, but other foodstuffs, for example shellfish and strawberries, have found to be contaminated with HEV. There is also widespread contamination of the environment. The role of these other factors in human infection deserves further exploration.

Question 4

In the developed world, the differences in the amounts of circulating HEV in human populations probably relates to differences in pig husbandry (including safe disposal of pig effluent), cultural differences in attitudes to cooking and consumption of food and differences in hunting practices.

Question 5

The number of infections in England is estimated at 100 000 per year [4]. About 650 were reported by Public Health England last year. The reasons for this discrepancy, in descending order of importance, are as follows:

(1) Most infections are asymptomatic,
(2) Lack of awareness among clinicians,
(3) Insufficient testing,
(4) Misdiagnosis,
(5) Emerging clinical phenotype of HEV, for example HEV can cause a range of neurological injury; for example, in these cases the LFTs may be only mildly abnormal [6, 7].

Question 6

The profile of HEV in England is starting to rise, but has some way to go. Most patients have never heard of HEV. Many clinicians are also unaware that HEV is such a common infection in the UK. HEV is considered an issue for the UK blood supply.

Question 7

The blood supply should be HEV-free. This can only be achieved by screening with an accurate assay such as highly sensitive PCR.

Question 8

Blood donations are currently not screened for HEV.

Question 9

We have considered studying the HEV vaccine's safety and efficacy in patients with end-stage liver and kidney disease awaiting transplantation.

References

1 Beale MA, Tettmar K, Szypulska R, *et al.*: Is there evidence of recent hepatitis E virus infection in English and North Welsh blood donors? *Vox Sang* 2011; **100**:340–342
2 Dalton HR, Stableforth W, Hamad N, *et al.*: HEV in SW England; clinical features, natural history and complications and the HEV IgG seroprevalence in blood donors, patients with chronic liver disease and individuals over the age of 60 years. *EuroJGastrohepatol* 2008; **20**:784–790
3 Cleland A, Smith L, Crossan C, *et al.*: HEV seroprevalence and HEV RNA presence in plasma minipools of Scottish blood donors. *Vox Sang* 2013; **105**:283–289
4 Hewitt PE, Ijaz S, Brailsford SR, *et al.*: Hepatitis E virus in blood components: a prevalence and transmission study in southeast England. Lancet 2014 Nov 15; **384**(9956):1766–73, doi:10.1016/S0140-6736(14)61034-5
5 Dalton HR, Bendall RP, Rashid M, *et al.*: Host risk factors and autochthonous hepatitis E infection. *Eur J Gastroenterol Hepatol* 2011; **23**:1200–1205
6 van den Berg B, van der Eijk AA, Pas SD, *et al.*: Guillain-Barre syndrome associated with preceding hepatitis E virus infection. *Neurology* 2014; **82**:491–497
7 van Eijk JJ, Madden RG, van der Eijk AA, *et al.*: Neuralgic amyotrophy and hepatitis E virus infection. *Neurology* 2014; **82**:498–503

Harry Dalton
Royal Cornwall Hospital
2 Penventinnie Lane
Treliske
Truro
Cornwall
TR1 3LQ UK
E-mail: Harry.Dalton@rcht.cornwall.nhs.uk

S. L. Stramer

Question 1

Xu *et al.* [1] published finding no RNA-positive US blood donors of 1939 tested. Testing included pools of 7–8 donors by both real-time and nested PCR assays (50% LOD 200 and 50 IU/mL, respectively). In contrast, HEV IgG seroprevalence was 16% for donors collected in 2012 (Wantai HEV IgM and IgG ELISAs). Similar to many other countrys' findings, Xu *et al.* observed a stepwise increase in anti-HEV seroprevalence with age; donors in the oldest age group (65 years or greater) had a 42% seroprevalence. No transfusion transmissions were documented in 362 prospectively followed recipients. In a subsequent study by the American Red Cross (unpublished), 18 829 donors from six geographic regions in the USA collected in 2013 were tested individually for RNA using transcription-mediated amplification (TMA) on the Panther platform (Hologic/Grifols). The 50% LOD of the assay is 2 IU/ml with 2 TMA repeat reactives

identified for a rate of 1:9500 and assay specificity of 99·96% (95% CI: 99·92–99·98%). The two reactives both had low-level RNA confirmed by real-time PCR performed at Sanquin in the Netherlands. One of the two RNA confirmed-positive samples was antibody negative having an estimated 14 IU/ml of RNA, and the second had RNA that was too low to quantify but the sample was both total Ig and IgM positive (MP Biomed 4.0 HEV double-antigen ELISA and MP BioMed HEV IgM ELISA) confirming early infection. Total antibody prevalence was 7·7%, IgM seroprevalence was 0·6%, and similar to Xu *et al.*, there was a stepwise increase in anti-HEV seroprevalence with age. Donors 65 years or greater had a 22% seroprevalence. Lastly, the HEV seroprevalence in 2009–2010 for a sampling of the US population aged 6 and older was estimated by NHANES (National Health and Nutritional Evaluation Survey). This survey found a 6% (95% CI: 5·1–6·9%) total HEV antibody seroprevalence and 0·5% IgM seroprevalence, both very similar to the Red Cross findings but using different antibody tests for total antibody and IgM (Diagnostics System). The sensitivity and specificity of the MP Biomed assays are both 99·2% as reported by the manufacturer and those for the Diagnostics System's assays were reported as 98% and 95·2%, respectively (Ditah *et al.* [2]). NHANES found increasing age as highly significant ($P < 0·001$) by both univariate and multivariate analyses. Univariate analyses also found birth outside of the USA, Hispanic ethnicity and meat consumption (>10 times per month) as significant.

Question 2

The HEV assays used to establish seroprevalence and acute infection in blood donors and the general population in the USA are described in Response 1.

Question 3

The dominant risk factors for HEV infection in the USA are travel to an endemic country (waterborne or foodborne; genotypes 1 and 2) consistent with epidemic hepatitis E. Smaller numbers of cases are reported in immunosuppressed individuals and usually genotype 3. The US CDC reported on clinical cases of HEV in the USA from 2005 to 2012 in persons with acute hepatitis whose samples were submitted for diagnostic workups but were seronegative for hepatitis A and B viral markers. Of 154 persons, 26 (17%) were anti-HEV positive including 11 who had travelled to an endemic area and 15 non-travellers assumed to have acquired infection in the USA. Travellers were younger (mean age 32 years) vs. non-travellers (61 years). Acute hepatitis was present in 92% of travellers, and none was genotype 3. In contrast, genotype 3 was common in nontravellers of who nearly half were organ transplant recipients (Drobeniuc *et al.* [3]).

Question 4

Differences in HEV incidence and prevalence rates reported between various countries would be explained by different dietary habits and agricultural practices including the management of water, as well as differences in assays used in various studies.

Question 5

Reporting of HEV infection in patients including those who are immunosuppressed including organ transplant recipients is fairly infrequent; see Drobeniuc *et al.*

Question 6

HEV infection is not perceived as a significant health problem at this time in the USA.

Question 7

Additional studies are needed in the USA prior to making a decision on the need for blood donation screening; one option that may be the best use of public health resources would be to test donors who products will be transfused to recipients at greatest risk (e.g. those who are immunosuppressed especially organ transplant recipients or those severely immunocompromised).

Question 8

No HEV screening using RNA or antibody tests has been implemented in the USA; only studies have been done at the present time.

Question 9

Discussions regarding the use of an HEV vaccine of the general public or selected risk groups in the USA have not commonly occurred.

References

1 Xu C, Wang R, Schecherly C, *et al.*: An assessment of hepatitis E virus in US blood donors and recipients: no detectable HEV RNA in 1939 donors tested and no evidence of HEV transmission to 362 prospectively followed recipients. *Transfusion* 2013; 53:2505–2511

2 Ditah I, Ditah F, Devaki P, *et al.*: Current epidemiology of Hepatitis E virus infection in the United States: low seroprevalence

in the National Health and Nutrition Evaluation Survey. *Hepatology* 2014; **60**:815–822
3 Drobeniuc J, Greene-Montfort T, Le NT, *et al.*: Laboratory-based surveillance for hepatitis E virus infection, United States, 2005–2012. *EID* 2013; **19**:218–222

Susan L. Stramer
American Red Cross
Scientific Support Office
Gaithersburg
MD, USA
E-mail: Susan.stramer@redcross.org