Transfusion-transmitted hepatitis E in Germany, 2013

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The reported IgG seroprevalence against hepatitis E virus (HEV) in German blood donations is 6.8%, and HEV RNA detected in 0.08%, but documented evidence for HEV transmission is lacking. We identified two donations from a single donor containing 120 IU HEV RNA/mL plasma and 490 IU/mL. An infectious dose of 7,056 IU HEV RNA was transmitted via apheresis platelets to an immunosuppressed patient who developed chronic HEV. Further, transmission was probable in an immunocompetent child.

Hepatitis E virus (HEV) infection was diagnosed in December 2013 in Germany. Retrospective analysis identified the event as the first transfusion-associated hepatitis E virus (HEV) infection in the country. Here, we report baseline virological data on the case.

Case description

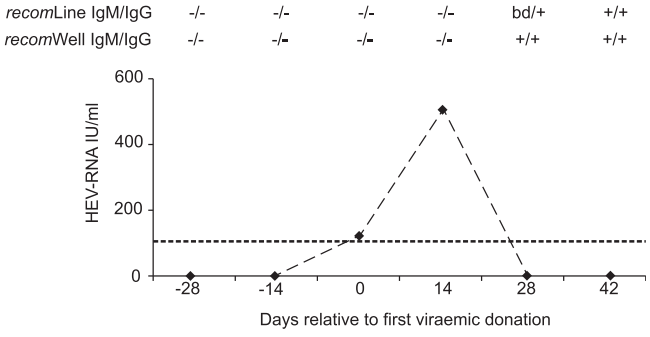
The patient (recipient 1), an immunocompromised man in his 40s, was positive for anti-HEV IgM and IgG using a recomLine HEV assay (Mikrogen, Munich, Germany), and HEV RNA was detected by real-time RT-PCR (Altona Diagnostics, Hamburg, Germany). Retrospective analysis showed that he had been chronically infected with HEV since 24 July 2013, when HEV RNA was detected for the first time. When reviewing the medical charts it was noticed that the patient had received apheresis platelets from a single donor on 4 July 2013.

A lookback procedure was initiated and two viraemic donations of this donor were identified. The donor was a man in his 40s and asymptomatic around the time of the blood donations. He donated blood regularly every 14 days. The first viraemic donation (donation 1, day 0) was from 1 July 2013 and contained 120 IU HEV RNA/mL plasma, and the second donation (donation 2, day 14) was from 15 July 2013 and contained 495 IU HEV RNA/mL plasma (Figure 1). This corresponds to an infectious dose of 7,056–8,892 IU HEV RNA in a total volume of 196–247 mL apheresis platelets transfused for donation 1 (assuming a residual plasma volume of 0.33 mL per 1 mL apheresis platelets). For donation 2, an infectious dose of 30,888–37,273 IU HEV RNA was calculated. Real-time RT-PCR results were confirmed using a nested RT-PCR protocol [1]. All other donations (n=4) of this donor before and after donations 1 and 2 tested negative by real-time RT-PCR and by nested RT-PCR (Figure 1).

The HEV nucleotide sequence of a 242 bp fragment of the ORF1 region was amplified and sequenced from donations 1 and 2 and from recipient 1 [1]. Phylogenetic analysis showed that the samples clustered together and were closely related to HEV genotype 3f, which is prevalent in Germany (Figure 2). The nearly complete nucleotide sequence (6,688 nt, GenBank accession number KJ873911) of the HEV isolate from recipient 1 was determined and compared to sequences from

Figure 1

Hepatitis E virus RNA concentration and serology results in an asymptomatic blood donor, Germany, 2013

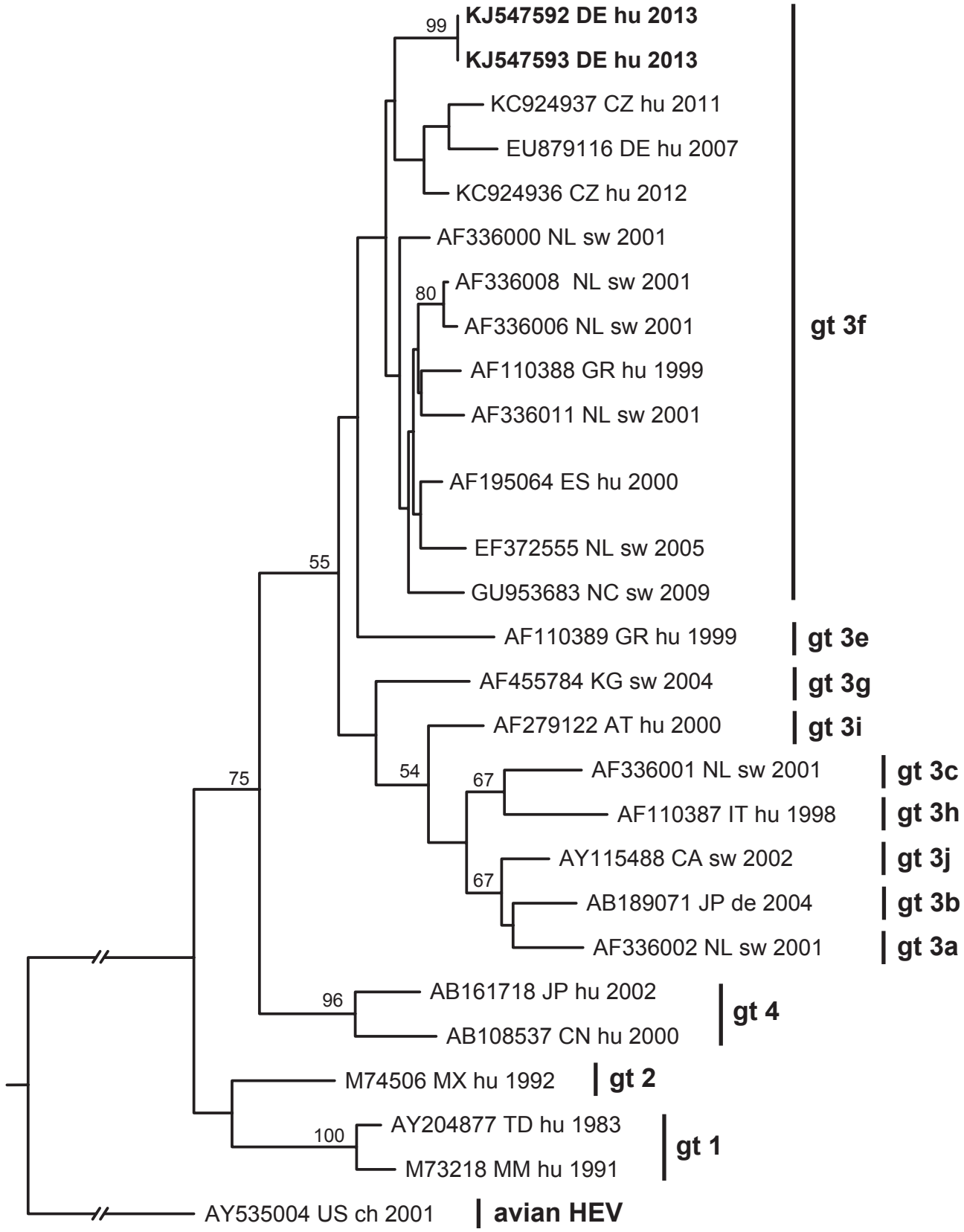


HEV: hepatitis E virus; IU: international units.

Viral RNA concentration is given on the y-axis in IU/mL as indicated by diamonds. The thin broken line indicates the limit of detection of real-time RT-PCR (Altona Diagnostics). Symbol – denotes a negative result as measured by recomLine or recomWell assay, symbol + denotes a positive result, bd indicates borderline result.

Figure 2

Rooted maximum likelihood phylogenetic consensus tree for ORF1 nucleotide sequences of selected hepatitis E virus isolates



AT: Austria; CA: Canada; ch: chicken isolate; CN: China; CZ: Czech Republic; DE: Germany; ES: Spain; GR: Greece; gt: genotype; hu: human; IT: Italy; JP: Japan; KG: Kyrgyzstan; MM: Myanmar; MX: Mexico; NC: New Caledonia; NL: Netherlands; sw: swine; TD: Chad; US: United States.

The sequences of the presented cases (KJ547592 and KJ547593, bold) cluster in subgenotype 3f. The selected sequences represent the nearest homologues in GenBank and typical members of genotype 1, 2 and 4 [15]. An avian hepatitis E virus sequence was used as an outgroup. Numbers at the nodes indicate bootstrap values of greater than 50%. Sequences are denoted by GenBank identification number, country, International Organization for Standardization country code, source, and year of isolation (or publication).

Table

Characteristics and outcome of recipients of hepatitis E virus-positive donations, Germany, 2013 (n=5)

	Transfusion recipients				HEV status		
	Transfused infectious dose HEV RNA	Recipient, sex and age	Immuno-compromised	Outcome	HEV status determined, time after transfusion	HEV PCR	Anti-HEV IgG status
Blood products from donation 1							
Apheresis platelets (196 mL)	7,056 IU	#1, male 47 years	Yes	Chronic HEV infection	6 months	Positive	Positive
Apheresis platelets (247 mL)	8,892 IU	#2, male 6 years	No	Probable HEV infection	8 months	Negative	Positive
Apheresis platelets (243 mL)	8,748 IU	#3, female 70 years	Yes	Died, sepsis	NA	NA	NA
Blood products from donation 2							
Apheresis platelets (208 mL)	30,888 IU	#4, male 71 years	Yes	No HEV infection	5 months	Negative	Negative
Apheresis platelets (251 mL)	37,273 IU	#5, male 71 years	No	Died, arrhythmia	NA	NA	NA
Apheresis platelets (249 mL)	36,976 IU	#5, male 71 years	No	Died, arrhythmia	NA	NA	NA

HEV: hepatitis E virus; NA= Not applicable.

donation 2 (4,251 nt, KJ873912). The nucleotide sequences were 100% identical proving transfusion-associated transmission.

In donation 1 and 2, anti-HEV IgG and IgM were not detected using two different serological HEV assays (recomLine HEV and recomWell HEV, Mikrogen, Munich, Germany). Seroconversion of the donor was observed 14 days after donation 2 (Figure 1). Levels of alanine aminotransferase, aspartate aminotransferase, bilirubin and gamma-glutamyl transferase were within normal range from days –28 to 42 relative to the first HEV RNA-positive donation. Detailed anamnestic exploration of possible risk factors for HEV infection (e.g. occupational exposure to pigs) remained inconclusive and the travel history was negative.

Another four recipients were identified, who had received apheresis platelets from donations 1 or 2 (Table). An immunocompetent child with a history of congenital heart disease tested positive for anti-HEV IgG and borderline for anti-HEV IgM (recomLine HEV and recomWell HEV) in a single sample eight months after receiving apheresis platelets from donation 1. Real-time RT-PCR from this sample was negative (Table). Clinical symptoms suggestive of acute HEV infection were not reported. The available samples from the remaining recipients were all negative for HEV markers (Table). Two patients died for reasons other than HEV infection.

Discussion

HEV recently emerged as a transfusion-transmissible pathogen, with reports from France, the United Kingdom, and Japan [2-4]. In Europe, the vast majority of autochthonous HEV infections are caused by HEV genotype 3 (gt-3) and are linked to the consumption of contaminated food. In general, HEV gt-3 infection remains asymptomatic or presents as mild self-limited acute hepatitis [5]. HEV IgG seroprevalence in Europe ranges from 17% in Germany to 26% in France among the general population, indicating widespread contact with HEV [6,7]. A HEV IgG seroprevalence of 6.8% was determined among German blood donors in 2011, and HEV RNA was detected in 0.08% of donations [8,9]. Juhl et al. reported an HEV IgG incidence in donors of 0.35% per year [9]. A total of 7.4 million blood products were administered in Germany in 2013, and between 1,600 and 5,900 HEV RNA-positive blood donations could be occurring in Germany per year [8,10]. In the Netherlands, one HEV-positive donation per day was reported, which implies that transmission by transfusion could be a likely event in both countries [11].

An estimated 30–40% of blood products in Germany were transfused to immunocompromised patients and these patients are at risk of developing chronic HEV gt-3 infection with increased mortality [5]. Sequence analysis of HEV strains from the Czech Republic, Germany and the Netherlands showed close homology indicating a geographically confined circulation [8]. This is supported by the high degree of sequence identity of our and recent Czech and Dutch sequences. Zoonotic transmission from pigs to humans seems to be the major mode of infection, but occupational exposure to pigs was not reported in our case [6].

Two important observations were made in this study. Firstly, we could show that the infectious dose required for HEV infection seems to be low, i.e. HEV RNA

concentrations close to the limit of detection of the real-time RT-PCR. Low levels of HEV RNA in asymptomatic donors have already been reported but without evidence for transmission [8,9]. Interestingly, Juhl et al. speculated that viraemia of around 125 IU/mL in the presence of anti-HEV IgM was not sufficient for transfusion-associated infection [9]. However, it is not clear if HEV antibodies can prevent infection. A recent study showed that infectious HEV could be propagated in cell culture in the presence of HEV-specific antibodies, suggesting that they do not efficiently reduce virus infectivity [12]. In addition, a clinical study demonstrated that anti-HEV IgG did not uniformly protect against reinfection [13].

Secondly, the duration of viraemia in our asymptomatic donor did not exceed 45 days, based on the time interval between the last and the first HEV RNA-negative donation. The interval of 14 days between first and last HEV RNA-positive donation was even shorter than the 27 to 58 days reported by Slot et al., but could be due to the shorter sampling interval in our study [11]. From our and previously published data it is obvious that highly sensitive methods would be required if screening for HEV RNA were to be considered for blood products.

The second HEV transfusion-associated transmission possibly occurred in a child. However, we were not able to definitely prove transmission since only one sample was available. In light of the very low HEV seroprevalence among children in Germany it seems probable that this child was infected by donation 1 [14]. It remains unclear why transfusion of donation 2 with a fourfold higher HEV RNA concentration did not result in infection, but this could be related to host factors.

To conclude, we could demonstrate that transmission of HEV by asymptomatic donors with low-level viraemia is possible. Current German guidelines in transfusion medicine do not recommend testing for HEV. Importantly, with regard to the possible severe consequences of transfusion-associated transmission of HEV, especially in immunocompromised patients, the necessity of screening for HEV RNA needs to be discussed in countries with a high HEV prevalence. However, more data regarding the HEV disease burden due to blood transfusions are needed before recommendations can be made.

Conflict of interest

None declared

Authors' contributions

DH, HH, MP wrote the manuscript. DB, PH, ES, RT took part in the clinical management of the patient. MU, TC, FE, RH, CSH took part in the look-back procedure. JJW, MP collaborated in molecular biology techniques. OK, SM, RU collaborated on the public health investigation. All authors participated in the investigation. All authors read and approved the final manuscript.

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