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Epidemiology of hepatitis E virus infection in a cohort of 4023 immunocompromised patients

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ABSTRACT

Objectives: The prevalence of active, chronic, and former hepatitis E virus (HEV) infections was investigated in a cohort of immunocompromised patients. The association with transfusion transmitted HEV was evaluated, and the HEV seroprevalence was compared with that in healthy blood donors.
Study design and methods: Serum samples from 4023 immunocompromised patients at Rigshospitalet, Denmark were retrospectively tested for HEV RNA and anti-HEV IgG. HEV RNA-positive patients were followed up by HEV testing, clinical symptoms, and transfusion history. Factors associated with anti-HEV were explored by multivariable logistic regression analysis. Samples from 1226 blood donors were retrospectively tested for anti-HEV IgG.
Results: HEV RNA was detected in six patients (0.15%) with no indications of chronic HEV infection. HEV RNA prevalence rates among recipients of allogeneic haematopoietic stem cell transplantation (allo-HSCT) and solid organ transplantation (SOT) were 0.58% and 0.21%, respectively. Transfusion transmitted infections were refuted, and transfusion history was not associated with anti-HEV positivity. The difference in HEV seroprevalence between patients (22.0%) and blood donors (10.9%) decreased when adjusting for age and sex (odds ratio 1.20, 95% confidence interval 0.97–1.48).
Conclusions: HEV viremia among allo-HSCT and SOT recipients suggests that clinicians should be aware of this diagnosis. The lack of association of blood transfusion with anti-HEV positivity supports food-borne transmission as the main transmission route of HEV common to both patients and blood donors.

Introduction

The zoonotic hepatitis E virus genotype 3 (HEV-3) causes human infections in Europe, with pigs serving as the predominant reservoirs. In Denmark, HEV-3 is widespread in pig herds and in pigs at the time of slaughter (Breum et al., 2010; Krog et al., 2019). The transmission of HEV-3 is mainly associated with the consumption of contaminated foodstuff, but transmission by blood transfusion and

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organ transplantation can also occur (Hewitt et al., 2014). Infection with HEV-3 usually leads to silent seroconversion. However, immunocompromised patients have been shown to develop chronic infections with the risk of cirrhosis (Kamar et al., 2008; Abravanel et al., 2014). In Denmark, the HEV RNA prevalence was 0.04% among healthy blood donors in 2015 (Harritshoj et al., 2016). However, the HEV epidemiology in immunocompromised patients is unknown. Immunocompromised patients may have a higher HEV RNA prevalence and HEV seroprevalence due to the possibility of chronic infection from environmental/food exposure and any exposure to transfusion-transmitted HEV (Tedder et al., 2017).

The primary aim of this cross-sectional study was to determine the HEV RNA prevalence in a wide spectrum of immunocompromised patients in Denmark and to describe the clinical course of HEV infection in this cohort. Secondary aims included the assessment of the association of transfusion history with active and former HEV infections among immunocompromised patients. Furthermore, we wanted to compare the prevalence of anti-HEV IgG between immunocompromised patients and healthy blood donors in Denmark, a country with a large pig industry.

Materials and methods

Study population, blood samples, and laboratory data—patients

PERSIMUNE is a centre of excellence in Rigshospitalet, Denmark, which serves a broad range of patient groups with impaired immune function, who are followed in the PERSIMUNE cohort (inclusion criteria are mainly solid organ transplantation (SOT), allogeneic haematopoietic stem cell transplantation (allo-HSCT), malignant or solid organ or haematological disease, rheumatological immunomodulation treatment, HIV infection). Enrolment in PERSIMUNE is independent of the time of inclusion diagnosis. The cohort is affiliated with the PERSIMUNE BioBank. Patients providing informed consent are enrolled in the BioBank, with blood samples drawn at enrolment and at follow-up time points. Frozen plasma samples obtained at enrolment from patients ≥18 years old enrolled in the PERSIMUNE BioBank between April 1, 2015 and May 15, 2017 were included in the study. Follow-up samples from patients with HEV RNA viraemia were samples from the PERSIMUNE BioBank or specific sampling after informed consent, according to ethical scientific approval H-16040755. Demographic data, affiliated department, routine laboratory data, status on treatment with transplantation (TX) with either SOT or allo-HSCT, and HIV diagnosis were available from the patient treatment databases. Patient transfusion histories were available from the laboratory information system in the Capital Region Blood Service. Transfusion histories from blood banks in other regions of Denmark were not available.

Study population, blood samples, and demographic data—blood donors

Frozen HEV RNA-negative plasma samples chosen randomly (5%) from a blood donor study of HEV RNA prevalence in Denmark performed from January 12 to February 13, 2015 were included in the study (Harritshoj et al., 2016). Sex and age at donation were available demographic data.

HEV RNA detection and genotyping

All patient samples were retrospectively tested for HEV RNA using a CE-marked commercial qualitative nucleic acid amplification test (NAT) on automated analysers (Procleix HEV Assay on Procleix Panther Systems; Grifols Diagnostic Division, Emeryville, CA, USA). The assay detects HEV of genotypes 1–4 and uses transcription-mediated amplification to amplify highly conserved regions of HEV RNA targeted by captured oligonucleotides (Sauleda et al., 2015). Initially reactive HEV RNA samples were confirmed by Sanquin Blood Supply in the Netherlands for HEV RNA quantification and genotyping using methods described previously (Ijaz et al., 2014; Hogema et al., 2016). Due to limited sample material, a 1.25× dilution of samples was necessary to extract RNA from 0.4 ml of sample using the QIAamp MinElute Virus Spin Kit (Qiagen, Hilden, Germany). The PCR assay amplified a 74-bp fragment of open reading frame region 3 (ORF-3) of HEV with a 95% lower limit of detection (LLD) of 10.3 IU/ml in undiluted samples. HEV genotyping was performed by amplification and sequencing of 242-bp and 302-bp fragments of ORF-1 and ORF-2 and a 1390-bp fragment of ORF-2, as described elsewhere (Ijaz et al., 2014; Hogema et al., 2016). GenBank accession numbers are **MN602942**–**MN602947**.

Anti-HEV serology

HEV antibodies were detected using the Wantai Hepatitis E IgG and IgM ELISA (Nordic BioSite, Copenhagen, Denmark), performed according to the manufacturer's instructions, using a signal/cut-off (S/CO) ratio of ≥1.1 to define a positive result. Samples with a borderline S/CO ratio (0.9–1.1) were re-tested. All patient and donor samples were tested for anti-HEV IgG. Anti-HEV IgM testing was conducted for anti-HEV IgG or HEV RNA reactive samples.

Follow-up of HEV RNA-positive patients

A patient was considered HEV RNA reactive by enrolment in the PERSIMUNE cohort, if the patient's sample was confirmed HEV RNA reactive by confirmatory PCR test or if anti-HEV seroconversion was detected in a follow-up sample. Follow-up samples were tested for HEV RNA and HEV IgG and IgM antibodies. Plasma (p) liver enzyme alanine aminotransferase (p-ALT), p-bilirubin, p-creatinine, leukocyte count, and leukocyte subpopulations were reviewed from the treatment data obtained from enrolment (and nearest data within 1 year before enrolment) until the date of the latest follow-up sample. The persistence of HEV was defined by HEV viraemia for ≥6 months or more. Patient notes were investigated for the clinical symptoms of an HEV infection. Look-back procedures included HEV RNA and IgG testing of archived samples from blood transfusions received by HEV RNA-positive patients up to 6 months before enrolment in PERSIMUNE.

Phylogenetic analyses

Sequence alignment and construction of a neighbour-joining phylogenetic tree using the Tamura–Nei algorithm were performed using Mega software (version 10) and Figtree version 1.4. Genotype and tentative subtypes were assigned by comparison with sequences from the HEV reference set proposed by the International Committee on Taxonomy of Viruses (ICTV) *Herpesviridae* study group (Smith et al., 2016).

Statistical analysis

Nominal data were presented as percentages with 95% confidence intervals (CI), and continuous data were presented as medians with the interquartile ranges (IQR). Differences in frequency distributions were tested using the Chi-square test. Multivariable logistic regression was used to examine factors associated with positive anti-HEV IgG results and included age (linear), sex, previous blood transfusions (linear), allogeneic HSCT

or SOT, and HIV status; odds ratios (ORs) with 95% CI were calculated. For the comparison of HEV seropositivity between patients and donors, multivariable logistic regression analysis was used to adjust for age and sex. The statistical analysis was performed in R (R Core Team, 2017).

Ethical approval

The study of hepatitis E among PERSIMUNE patients was approved by the Regional Scientific Committee for the Capital Region of Denmark (H-16040755). The donor investigation was approved as a quality control project (H-2–2014FSP42).

Results

PERSIMUNE patients

Among the 4062 adult patients (≥18 years old) enrolled within the first 2-year period of the PERSIMUNE cohort in Denmark, five patients had withdrawn their consent, 32 had a replacement social security number that was not compatible with access to treatment databases, and two patients lacked enough sample material. Thus, a total of 4023 patients were included in the study. Patient characteristics are summarized in Table 1. A total of 843 (21%) patients were treated with TX, of whom 344 were treated with allo-HSCT. Patients treated with SOT ($n = 499$) included 437 kidney, 27 liver, 22 heart, and 13 lung transplantation patients.

Table 1
Baseline characteristics of all patients; total $N = 4023$ (100%).

At time of Biobank inclusion of patients tested for HEV markers	Number of patients (%)
Sex	
Male	2793 (69.4)
Female	1230 (30.6)
Age, median (IQR) years	54 (42–67)
Age divided in age groups, years	
<30	351 (8.7)
30–39	528 (13.1)
40–49	802 (19.9)
50–59	801 (19.9)
60–69	889 (22.1)
≥70	652 (16.2)
HIV diagnosis	1162 (28.9)
Non-HIV diagnosis	2861 (71.1)
TX treatment (SOT or allo-HSCT)	843 (21.0)
Non-TX treatment	3180 (79.0)
Affiliation of patients with departments at Rigshospitalet	
Department of Infectious Diseases[a]	1469 (36.9)
HIV	1154 (28.7)
Non-HIV	315 (7.9)
Department of Haematology[b]	855 (21.3)
Allo-HSCT	344 (8.6)
Non-HSCT	511 (12.7)
Department of Oncology[c]	825 (20.5)
Department of Nephrology[d]	679 (16.9)
SOT	437 (10.9)
Non-SOT	242 (6.0)
Department of Hepatology or Liver Surgery[e]	100 (2.5)
SOT	27 (0.7)
Non-SOT	73 (1.8)
Department of Rheumatology[f]	50 (1.2)
Department of Cardiology or Thoraco-surgery[g]	45 (1.1)
SOT	35 (0.9)
Non-SOT	10 (0.2)

HEV, hepatitis E virus; IQR, interquartile range; TX, transplantation; SOT, solid organ transplant patients; allo-HSCT, allogeneic haematopoietic stem cell transplant patients. Inclusion criteria, diagnosis of patients: [a]HIV infection, common variable immunodeficiency, cystic fibrosis. [b]Malignant haematological disease, leukaemia, myelomatosis or lymphoma and allo-HSCT. [c]Patients before or on chemotherapeutic treatment, for solid organ malignancy: cancer in the lungs, pleura, bladder, prostate, testicles, head and throat. [d]Kidney transplantation, haemodialysis or peritoneal dialysis, vasculitis with kidney morbidity. [e]Autoimmune hepatitis, liver transplantation. [f]Rheumatological conditions treated with immunomodulating drugs. [g]Heart and lung transplantation, before heart surgery.

Among the 4023 patients, six were initially HEV RNA reactive and were confirmed to be HEV RNA reactive. Of these six patients, two had been treated with allogeneic HSCT and one patient had received a kidney transplantation (Table 2). The HEV RNA prevalence overall was 0.15% (95% CI 0.07–0.33%) and was 0.36% (95% CI 0.12–1.06%) among the TX subgroup; when broken down by allo-HSCT and SOT recipients, the prevalence was 0.58% (95% CI 0.16–2.1%) and 0.21% (95% CI 0.04–1.2%), respectively.

The HEV RNA prevalence rate of 0.15% (95% CI 0.07–0.33%) was slightly higher in this Danish PERSIMUNE cohort compared with an HEV RNA prevalence rate of 0.04% (95% CI 0.02–0.07%) determined among Danish blood donors ($p = 0.009$) using the same methodology (Harritshoj et al., 2016).

Among the five patients alive for follow-up, clearance of HEV viraemia and anti-HEV seroconversion were detected in the first follow-up sample at 12–26 months after enrolment. Persistent HEV infection during the first year after enrolment was not assessable due to the lack of sample material. Patient 5 had cleared the HEV infection in the first follow-up sample at 21 months after enrolment, but tested anti-HEV IgG-negative in the Wantai assay. However, anti-HEV IgG was detectable using another assay, recomWell (Mikrogen Diagnostik, Neuried, Germany). One patient died (breast cancer) before the possibility of follow-up.

The immunological status at enrolment of the HEV viraemic patients (patients 1–5) included lymphocyte counts ranging between 0.7×10^9/l and 2.9×10^9/l and neutrophil counts ranging between 4.5×10^9/l and 8.1×10^9/l. In one 85-year-old patient with atypical chronic myeloid leukaemia, higher values included a lymphocyte count of 6.1×10^9/l and a neutrophil count of 20.7×10^9/l.

No symptoms of hepatitis or neurological disorders were described in five of the six patients available for follow-up. The patient notes revealed gastrointestinal symptoms including diarrhoea and/or abdominal pain in three patients. Two patients showed ALT elevation at the time of HEV diagnosis.

A 54-year-old female (patient 2) treated with sirolimus after allo-HSCT 45 months prior to the HEV diagnosis, had a high HEV viraemia level (590 059 IU/ml) and a peak ALT of 1100 IU/ml by the enrolment date. The normal ALT value of 27 IU/ml at 1 month before and the decline in value reaching 35 IU/ml at 5 weeks after enrolment were consistent with undiagnosed acute resolving hepatitis, with stomach ache as the only subjective symptom.

A minor ALT elevation (70 IU/ml) was found in a 47-year-old woman (patient 5) with anaemia and a diagnosis of coeliac disease, which had caused a continuous minor ALT elevation for years; this was also measured at follow-up after HEV RNA clearance.

A high HEV viraemia level (449 332 IU/ml) was also detected in patient 3, who was treated with the immunosuppressive drugs tacrolimus and mycophenolate mofetil due to a kidney transplantation 30 months prior to PERSIMUNE enrolment. Four months after HEV diagnosis, a 3-week period with diarrhoea was described. ALT values were registered at the enrolment date and 6 months after, with normal values (32 and 36 IU/ml).

A medium level of HEV viraemia (5714 IU/ml) was detected in a 41-year-old male (patient 4) no longer on any immunosuppressive drug after allo-HSCT 42 months prior to his HEV diagnosis. No infectious symptoms were described, and normal values of ALT were detected at 6 months before the HEV diagnosis and until 18 months after.

A total of 885 PERSIMUNE patients were anti-HEV IgG-positive with an HEV seroprevalence of 22% (95% CI 20.7–23.3%). Of these, 20 patients (0.5%; 95% CI 0.3–0.8%) were anti-HEV IgM-positive. The seroprevalence increased with age, whereas individuals living

Table 2
Characteristics and results from patients with an active HEV infection (HEV RNA-positive).

Characteristics and results					Laboratory data at time of enrolment									
UPN	Sex F/M	Age (years)	Diagnosis	SOT or allo-HSCT yes/no	HEV RNA NAT S/CO	Quantitative PCR IU/ml	Anti-HEV IgM/IgG	ALT IU/ml	Leukocytes × 10^9/l	Neutrophils × 10^9/l	Lymphocytes × 10^9/l	Symptoms	Number of follow-up months after enrolment	HEV RNA NAT pos/neg	Anti-HEV IgM/IgG, follow-up sample	Number of blood transfusions within 6 months
1/	F	60	Metastatic breast cancer	No	3.37	19	Neg/Neg	14	12.1	8.1	2.5	NA	NA	NA	NA	0
2/	F	54	MDS	Yes	51.11	590 059	Pos/Pos	1100	10.1	7.1	1.9	GI	24	Neg	Pos/Pos	0
3/	M	53	Uraemia	Yes	49.08	449 342	Pos/Pos	32	7.3	5.4	0.7	GI	26	Neg	Pos/Pos	0
4/	M	41	RAEB/MDS	Yes	56.43	5714	Neg/Neg	40	8.6	4.5	2.9	No	19	Neg	Neg/Pos	0
5/	F	47	Anaemia and coeliac disease	No	6.3	17	Neg/Inc.	71	6.5	5.5	1.4	GI	21	Neg	Neg/Pos[a]	0
6/	M	85	CML	No	2.41	34	Neg/Pos	19	28.7	20.7	6.1	NA	12	Neg	Neg/Pos	3

HEV, hepatitis E virus; UPN, unique patient number; F, female; M, male; SOT, solid organ transplantation; allo-HSCT, allogeneic haematopoietic stem cell transplantation; NAT, nucleic acid amplification test; S/CO, signal/cut-off ratio; Anti-HEV IgM, antibodies against hepatitis E virus immunoglobulin M; Anti-HEV IgG, antibodies against hepatitis E virus immunoglobulin G; ALT, alanine aminotransferase; pos, positive; neg, negative; Inc., inconclusive; NA, not available; MDS, myelodysplastic syndrome; GI, gastrointestinal symptoms; RAEB, refractory anaemia with excess blasts; CML, chronic myeloid leukaemia.
[a] Negative in Wantai assay but positive in recomWell (Mikrogen) assay.

with HIV and TX patients were less likely to be anti-HEV IgG-positive on univariate logistic regression analysis (Table 3). Only age and HIV status remained statistically significant in the multivariable logistic regression analysis, with a 65% increased risk of anti-HEV positivity per 10 years (Table 3). The median (IQR) CD4+T cell counts among HIV-infected individuals did not differ between anti-HEV IgG-positive and anti-HEV IgG-negative patients, with 0.69 (IQR 0.36) × 10^9 cells/l and 0.67 (IQR 0.42) × 10^9 cells/l, respectively.

Of patients living in the Copenhagen capital region affiliated with the Capital Region Blood Service (n = 3128 patients), 27.7% had received blood transfusions with a median of 9 blood components (IQR 3–15, range 1–524 blood components). A multivariable logistic regression analysis in this subpopulation showed that the number of blood transfusions was not positively associated with anti-HEV positivity. Conversely, receiving more transfusions was associated with a slightly lower anti-HEV positivity (p = 0.04) (Supplementary Material, Table S1). Additionally, a non-significant result was obtained (p = 0.46) when restricting to a 5-year transfusion history in order to increase the possibility that patients had lived in the Capital Region for the entire observation period and thereby had not received blood transfusions from other blood services in Denmark (data not shown).

Comparison of patients and blood donors

Of 1226 blood donors from all five regions of Denmark with a median age of 42 years (IQR 30.5–53.5 years, range 17–67 years), 134 were anti-HEV IgG-positive, yielding an HEV seroprevalence of 10.9% (95% CI 9.3–12.8%). Of these, four donors were anti-HEV IgM-positive (0.3%; 95% CI 0.1–0.8%). Although the overall prevalence of anti-HEV IgG among the immunocompromised patients (22%) was higher than among blood donors in the same period of 2015–2017 ($p < 0.0001$), this was not significant when adjusting for age and sex: OR 1.20 (95% CI 0.97–1.48) (p = 0.1). An anti-HEV IgG frequency plot showed a similar increase in seroprevalence by age in both groups (Figure 1). Similarly, in a sub-analysis comparing blood donor anti-HEV positivity with individuals living with HIV or TX patients, we found no significant difference in anti-HEV positivity when adjusting for age and sex (p = 0.9 and p = 0.5, respectively); data not shown.

HEV ORF1 and ORF2 fragments were successfully sequenced for three patients who were all infected with HEV genotype 3 (HEV-3). One genotype had the highest homology to the 3c subtype, and two could not be assigned a subtype but were part of the 3efg clade (Figure 2).

Discussion

HEV-3 infections may persist and become chronic in immunodeficient patients (Kamar et al., 2008). In the current cross-sectional study of 4023 immunocompromised patients, we did not find this to be the case for any of the six patients who were determined to have HEV RNA viraemia.

The reported difference in HEV RNA prevalence rates in various studies is largely related to geographical region and to some extent to the sensitivity of the methods used. HEV RNA prevalence varies greatly among blood donor populations in European countries (Domanovic et al., 2017; Boland et al., 2019). The HEV RNA prevalence rate was slightly higher in our Danish PERSIMUNE cohort compared with the HEV RNA prevalence rate of 0.04% found among Danish blood donors (Harritshoj et al., 2016). In the Netherlands and the UK, HEV RNA-positive fractions among patients with allo-HSCT or haematological malignancies were 2.4% and 0.13%, respectively (Versluis et al., 2013; Ankcorn et al., 2019). Additionally, 1% and 1.16% HEV RNA reactivity were detected

Table 3
Risk factors for anti-HEV IgG seropositivity based on all patients tested.

Variable	Univariable logistic regression analysis			Multivariable logistic regression analysis	
	Anti-HEV IgG positivity *n*/*N* (%) Total *n*/*N* 885/4023 (22.0)	OR (95% CI) Anti-HEV IgG positivity	*p*-Value	OR (95% CI) Anti-HEV IgG positivity	*p*-Value
Sex		1.01 (0.86–1.19)	0.90	1.15 (0.96–1.38)	0.13
Male	616/2793 (22.1)				
Female	269/1230 (21.9)				
Effect of age, per 10 years[a]		1.67 (1.58–1.77)	<0.001	1.65 (1.55–1.75)	<0.001
Age (years)					
<30	15/351 (4.3)				
30–39	57/528 (10.8)				
40–49	100/802 (12.5)				
50–59	149/801 (18.6)				
60–69	289/889 (32.5)				
≥70	275/652 (42.2)				
HIV diagnosis	183/1162 (15.7)	0.57 (0.48–0.69)	<0.001	0.59 (0.42–0.85)	0.003
Non-HIV	702/2861 (24.5)				
TX treatment (SOT or allo-HSCT)	154/843 (18.3)	0.75 (0.62–0.91)	0.003	0.84 (0.65–1.08)	0.17
Non-TX treatment	731/3180 (23.0)				
Affiliation with department					
Department of Infectious Disease	235/1469 (16.0)	1.24 (0.56–3.28) Inf vs. Car	0.63	1.84 (0.75–5.24)	0.21
Department of Haematology	220/855 (25.7)	2.25 (1.01–5.99) Hae vs. Car	0.07	1.43 (0.61–3.91)	0.44
Department of Oncology	239/825 (28.0)	2.65 (1.19–7.04) Onc vs. Car	0.03	1.48 (0.63–4.10)	0.40
Department of Nephrology	157/679 (23.1)	1.95 (0.87–5.22) Nep vs. Car	0.13	1.70 (0.73–4.67)	0.25
Department of Hepatology or Liver Surgery	15/100 (15.0)	1.15 (0.43–3.42) Hep vs. Car	0.79	1.34 (0.48–4.15)	0.59
Department of Rheumatology	13/50 (26.0)	2.28 (0.81–7.08) Rhe vs. Car	0.13	2.28 (0.76–7.49)	0.15
Department of Cardiology or Thoraco-surgery	6/45 (13.3)	1		1	

Anti-HEV IgG, antibodies against hepatitis E virus immunoglobulin G; OR, odds ratio; CI, confidence interval; SOT, solid organ transplantation; allo-HSCT, allogeneic haematopoietic stem cell transplantation; TX, transplantation; Inf, Department of Infectious Disease; Car, Department of Cardiology or Thoraco-surgery; Hae, Department of Haematology; Onc, Department of Oncology; Nep, Department of Nephrology; Hep, Department of Hepatology or Liver surgery; Rhe, Department of Rheumatology.
[a] Linear variable.

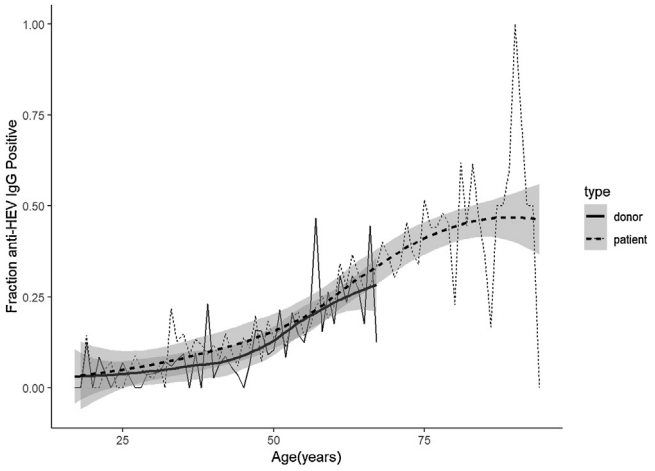

Figure 1. Anti-HEV frequency by age.

among SOT patients (Pas et al., 2012; Ankcorn et al., 2018) compared with 0.13% and 0.04% HEV RNA reactivity among blood donors (Hogema et al., 2016; Hewitt et al., 2014).

However, a higher fraction of HEV RNA positivity among immunocompromised patients compared with blood donors may indicate longer periods of HEV viraemia among immunocompromised patients due to less effective clearance of the infection.

The courses of the HEV infections in this study were either acute and self-limiting or subclinical. Among three HEV RNA viraemic TX patients, two were treated with immunosuppressive drugs. In general, the HEV RNA viraemic patients in this study were not profoundly immunosuppressed according to their lymphocyte cell counts at the time of HEV diagnosis, reducing the risk of developing chronic HEV infections. Among SOT patients, the calcineurin inhibitor tacrolimus, which blocks T cell activation, has been shown to be an independent risk factor associated with chronic HEV infections (Kamar et al., 2011). Additionally, chronic HEV infections among SOT patients are more likely to develop in profoundly immunosuppressed patients. In particular, the CD2, CD3, and CD4 T cell subpopulations are significantly lower in patients with chronic HEV infections than in those who clear the infection spontaneously (Kamar et al., 2008). Data on T cell subpopulations were not available in this patient group. The HEV RNA viraemic patient in this study who received tacrolimus for prophylaxis of organ rejection had a total lymphocyte cell count below the normal range. However, this patient cleared the HEV infection spontaneously, indicating that the T cell count in this patient was not heavily suppressed.

The principal transmission route for HEV-3 is supposedly foodborne, mainly from pig products that are insufficiently cooked, although environmental transmission routes from irrigation water or living in close contact with animals are also known risk factors common to both healthy and immunologically vulnerable persons (Van der Poel, 2014). Transfusion transmitted HEV infections (TTI) have been described in case reports and larger observational studies (Hewitt et al., 2014), and TTI in multi-transfused patients may also explain a higher HEV RNA prevalence among immunocompromised patients, who often receive multiple transfusions during the course of a transplant process. However, in this study, TTI as the cause of active HEV infection was refuted by look-back procedures.

In the assessment of factors associated with anti-HEV positivity, old age and non-HIV status were significantly associated with anti-HEV positivity, whereas individuals living with HIV were not. The lower odds of anti-HEV positivity among HIV-infected individuals compared with immunocompromised patients without HIV and the similar odds of anti-HEV positivity compared with age- and sex-matched blood donors are in concordance with other studies of HIV-infected individuals and co-infection with HEV. A case–control study performed in southern France showed that

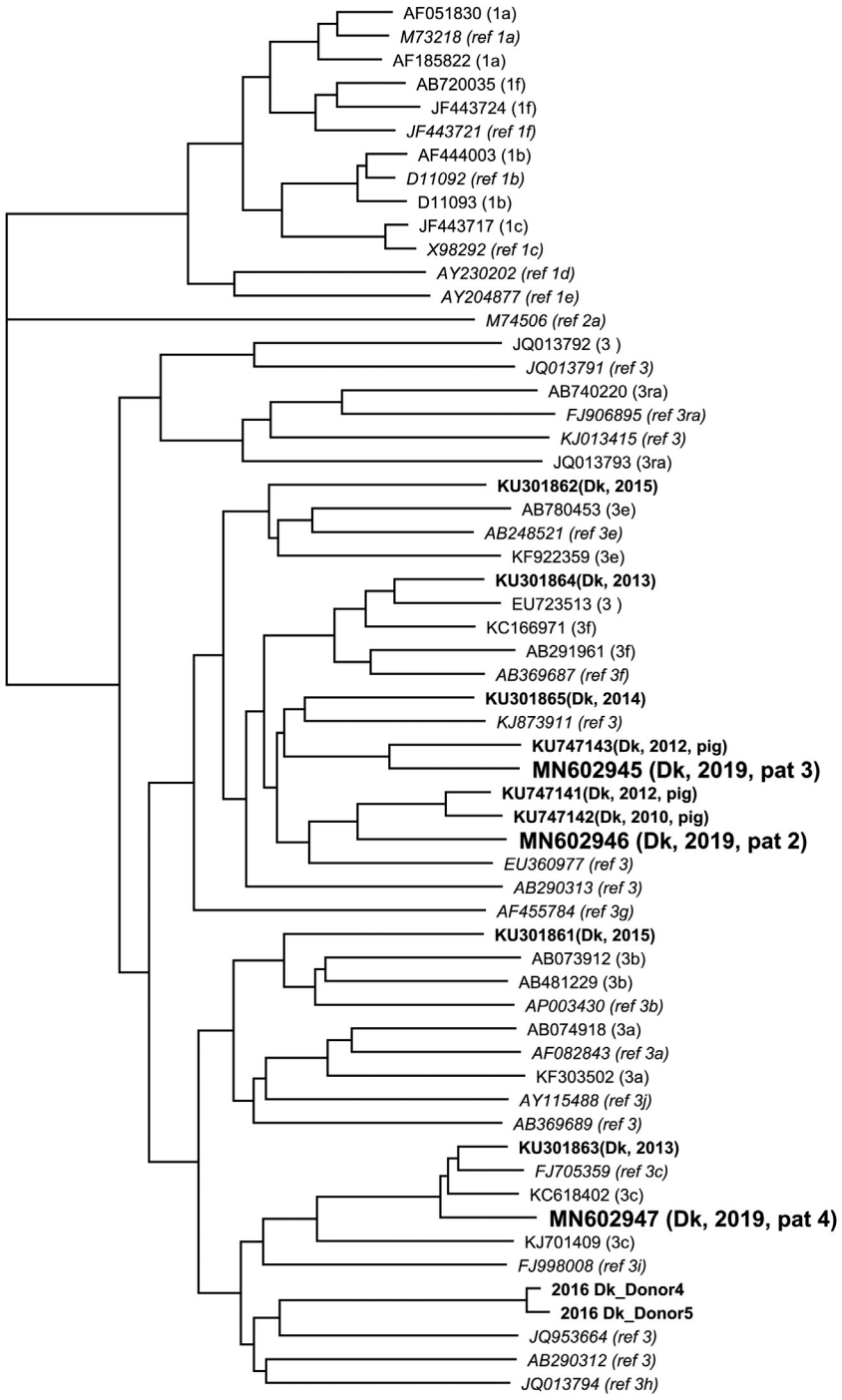

Figure 2. Phylogenetic tree of hepatitis E virus genotype 3 (HEV-gt3), including genotypes gt1 and gt2. Sequences of ORF2 from a 1390-bp fragment or smaller. Reference sequences (normal type) and sequences from Danish gt3 (bold type with year of diagnosis). gt3 from immunocompromised Danish patients (large font: Dk, 2019, pat 2–4), Danish blood donors (2016 Dk_Donor4–5), previously diagnosed symptomatic Danish patients (Dk, year), and from Danish pig livers (Dk, year, pig).

HIV-infected persons had lower anti-HEV seroprevalence (47.3%) than age- and sex-matched blood donors living in the same area (38.7%) (Abravanel et al., 2017). Correspondingly, Keane et al. found no difference between HIV-infected persons (9.4%) and age- and sex-matched non-HIV-infected patients (13.8%) living in England. The HIV-infected population in our study is supposedly well treated with combination antiretroviral therapy (c-ART) — in accordance with the general treatment for HIV across Denmark. We found no difference in CD4+T cell count between anti-HEV-positive and anti-HEV-negative individuals. In accordance with these results, HIV-infected patients in Argentina with a normal CD4+T cell count had an HEV seroprevalence similar to that of a non-HIV-infected immunocompetent population (Debes et al., 2016). The results indicate that persons living with HIV in Denmark do not have a higher risk of an HEV infection.

Additionally, neither transplantation history nor blood transfusion history was associated with anti-HEV positivity. The latter is in contrast to a recent study among patients with a diagnosis of

haematological malignancy, showing a linear increase in anti-HEV IgG seropositivity with the number of transfusions received during a 5-year period, suggesting HEV acquisition via blood transfusion (Ankcorn et al., 2019). These deviating results may be due to differences in unknown fractions of transfusion history outside the local hospital/region. However, we did not find any evidence of a correlation between the number of transfusions received and HEV seropositivity when restricting transfusion history to a 5-year period, as also reported by the authors of the UK study. Estimates of TTI HEV risk have suggested that exposure to 13 blood components from blood donors living in the same geographical area equals the background exposure of 1 year of daily living in the same area (Tedder et al., 2017).

We found a 65% increased risk of anti-HEV positivity per 10 years of increasing age. The anti-HEV frequencies of donors and patients in relation to age were similar, and the difference in seroprevalence was mainly explained by age, as described in earlier studies (Holm et al., 2015). When comparing different populations in cross-sectional anti-HEV seroprevalence studies, the median age and IQR of the populations investigated, or alternatively age-weighted seroprevalence, should be included in the judgement as well.

This study has strengths and limitations. This was a large cross-sectional study of immunocompromised patients investigated for HEV epidemiology and the first large HEV RNA investigation in Denmark among this patient category. Additionally, the ability to compare results with blood donor studies from the same country and during the same time period using identical testing methods provides a sound basis for investigating both common and differential transmission risks of HEV infection.

Limitations of the study include the absence of follow-up samples within the first year of follow-up, excluding any possible detection of HEV RNA persistence in HEV RNA-positive patients. Another limitation is that this investigation is a point cross-sectional study, in which the sampling time was random in relation to the course of the diagnosis causing the immunodeficiency. This may have resulted in an underestimation of the prevalence rates of HEV RNA and the number of detected chronic HEV infections. However, a recent study among HSCT (allogeneic and autologous) and SOT patients in the peri-transplant period in the UK did not detect higher prevalence rates (0.46%) or more chronic HEV infections compared with a randomly sampled study among allo-HSCT and SOT transplant patients from the UK as well (0.67%) (Reekie et al., 2018; Ankcorn et al., 2018).

In conclusion, the HEV RNA prevalence was 0.15% in a Danish cohort of immunocompromised patients. There were higher rates of 0.58% and 0.21% in the allogeneic HSCT and SOT patient subgroups, respectively. All HEV viraemic patients anti-HEV seroconverted spontaneously, and no evidence of symptomatic chronic HEV infections was found. Transfusion history was not associated with active or former HEV infections in this study. A high HEV seroprevalence rate of 22% in the PERSIMUNE cohort did not differ from the seroprevalence in Danish blood donors (10.9%) when adjusting for age and sex. The results support the food-borne/environmental transmission of HEV as the main transmission route common to both vulnerable immunocompromised patients and healthy blood donors. However, the possibility of HEV infection should be considered in TX patients with liver enzyme elevations.

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Conflict of interest

No conflicts of interest for any of the authors.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2019.11.014.

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