International Journal of Infectious Diseases 86 (2019) 114–121

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Detection and characterization of hepatitis E virus genotype 3 in HIV-infected patients and blood donors from southern Brazil

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ARTICLE INFO

Article history:
Received 29 May 2019
Received in revised form 21 June 2019
Accepted 30 June 2019
Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Hepatitis E virus
HEV
Seroprevalence
HEV RNA
HIV
Blood donors

ABSTRACT

Background: Hepatitis E virus genotype 3 (HEV-3) infection usually causes self-limited acute hepatitis. In immunosuppressed patients, HEV-3 infection can rapidly progress to chronic hepatitis and cirrhosis. In southern Brazil, data on HEV seroprevalence are scarce.
Methods: Testing for HEV RNA and antibodies (anti-HEV) was performed for 320 HIV-infected patients followed at the HIV/AIDS Service of the Federal University of Rio Grande between 2012 and 2013, as well as 281 blood donor samples obtained in 2015. Variables associated with anti-HEV positivity were assessed by multivariable logistic regression analysis.
Results: HIV and blood donor groups showed similar HEV seroprevalence (6.7% and 7.1%, respectively). Risk factors associated with anti-HEV detection were older age, marital status, a higher number of sexual partners, poor sanitation, and alcohol use (HIV group), and living in a rural area (blood donors). HEV RNA was detected in eight serum samples from HIV-infected patients and in one blood donor, who was also positive for anti-HEV IgM and IgG.
Conclusions: The prevalence rates of HEV infection were comparable between HIV-seropositive patients who were not severely immunocompromised and blood donors. The blood donor's HEV isolate showed high similarity with swine HEV strains from Brazilian herds in the same region, thus indicating a potential risk of foodborne and parenteral transmission via blood transfusion.

Introduction

Hepatitis E virus (HEV) genotypes 1 and 2 infect only humans, and are frequently associated with waterborne outbreaks of hepatitis E in African and Asian countries (Abe et al., 2006; Rein et al., 2012; Stoszek et al., 2006; World Health Organization, 2010), where the main route of transmission is fecal–oral. HEV genotypes 3, 4, and 7 infect humans and several animal species. Human HEV genotype 3 (HEV-3) infections are frequently asymptomatic and are primarily related to the consumption of raw or undercooked pork or game meat (Dalton et al., 2008; Meng, 2011). HEV can also be transmitted through solid organ and blood products derived from asymptomatic HEV-infected individuals. Moreover, HEV-3 can evolve into chronic hepatitis in immunocompromised patients, such as solid organ transplant recipients, patients with hematological malignancies, and patients at an advanced stage of human immunodeficiency virus (HIV) disease (Alric et al., 2010; Dalton et al., 2009; Kamar et al., 2008).

In Brazil, HEV-3 is the only genotype identified (Gardinali et al., 2012a; Lopes Dos Santos et al., 2010; Vasconcelos et al., 2015), and anti-HEV IgG prevalence rates vary from 0 to 38% (Bortoliero et al., 2006; Carrilho et al., 2005; Hardtke et al., 2018; Lyra et al., 2005; Martins et al., 2014; Passos-Castilho et al., 2016; Silva et al., 2012; Trinta et al., 2001; Vitral et al., 2014)

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https://doi.org/10.1016/j.ijid.2019.06.027

among specific populations. However, the associated risk factors are not well known. Currently, only two studies have assessed HIV-infected individuals (Ferreira et al., 2018; Salvio et al., 2018). The aim of the present study was to investigate the occurrence and seroprevalence of HEV infection and associated risk factors in HIV-infected individuals and blood donors from the southernmost part of the country, a region where the consumption of pork meat is widespread.

Materials and methods

Study population

A cross-sectional study involving HIV-infected patients was conducted in Rio Grande, a port city of 200 000 inhabitants in the southernmost part of Brazil. Serum samples were obtained prospectively from 360 patients as part of the routine clinical procedure of the HIV/AIDS Service of the Federal University of Rio Grande (FURG), between December 2012 and April 2013. In addition, plasma samples collected between October and November 2015 from 281 blood donors were retrieved from the blood bank of the hospital of Santa Casa do Rio Grande (SCRG). Testing for HEV RNA and antibodies was performed at Laboratório de Desenvolvimento Tecnológico em Virologia – IOC/Fiocruz. The sample size calculation considered a confidence level of 95% and estimated prevalence rates of $8 \pm 3\%$ in HIV-infected patients and $3 \pm 2\%$ in blood donors.

The study was approved by the local research ethics committees (FURG: 008/2013 and SCRG: 023/2015).

Data collection

Demographic, socio-economic, and clinical data were obtained using a pre-coded structured questionnaire. All patients participating in this study did so voluntarily and signed an informed consent form. Socio-demographic variables included age, sex, skin color, civil status, education, per capita income, residence (urban or rural), access to mains drinking water, and sewage. Behavioral variables included number of sexual partners in the last year, sexual preference, tattoo, illegal inhaled and/or injected drugs, and consumption of raw or undercooked pork or seafood. Among HIV-infected patients, harmful use of alcohol was assessed using the Alcohol Use Disorders Identification Test (AUDIT) questionnaire with a cutoff of 15/16 (World Health Organization, 2001). Clinical and laboratory variables included the duration of HIV infection, use of highly active antiretroviral therapy (HAART), alanine and aspartate aminotransferase (ALT and AST) levels, hepatitis B surface antigen (HBsAg) and antibodies to hepatitis C virus (anti-HCV) results, HIV viral load ($\log_{10}$ copies/ml), and CD4+ cell count (cells/mm^3).

HEV RNA and antibody detection

HEV RNA was detected by quantitative one-step reverse transcription polymerase chain reaction (qRT-PCR) (Jothikumar et al., 2006) and by HEV-ORF1-designed conventional RT-PCR (Huang et al., 2002; Wang et al., 1999). The conventional RT-PCR reactions were performed in a single tube using the SuperScript III One-Step RT-PCR System (Invitrogen, Carlsbad, CA, USA) and ORF1 designed primers, resulting in fragments of 287 bp.

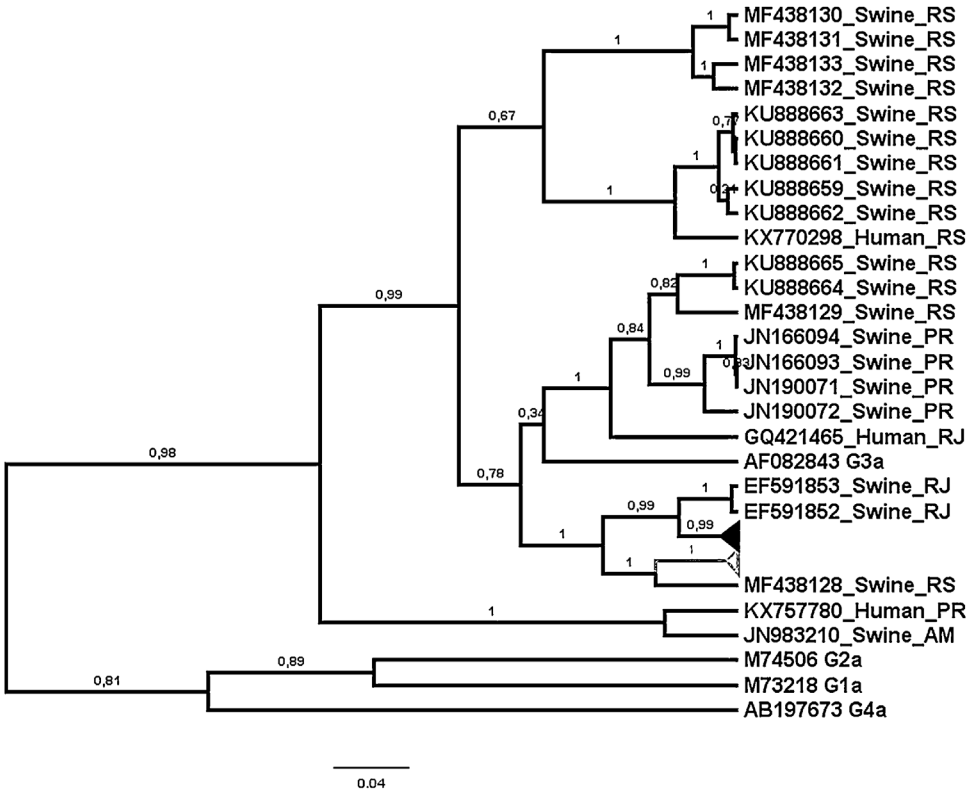

Figure 1. Phylogenetic analysis of the HEV strain from human blood donor. The Bayesian phylogenetic tree was constructed by using partial nucleotide sequence of ORF1. For each sequence, the GenBank accession number, host from which it was isolated and the state of origin are shown. Collapsed clades in black and grey are isolates from the states of Minas Gerais (KY907039-KY907064) and Amazonas (JN983190-JN983207), respectively. Tree root was composed of reference representatives of genotypes 1, 2, 3 and 4. Posterior probabilities (pp) are shown at the branch label. Numerical value ≥0.7 indicates the pp replicates that supported the interior branch. Newly described HEV sequence in this study is indicated by "*". Brazilian states = Amazonas (AM); Minas Gerais (MG); Paraná (PR); Rio de Janeiro (RJ); Rio Grande do Sul (RS). Scale bar indicates evolutionary distance of 0.04 substitutions per position in the sequence.

Anti-HEV IgM and IgG were detected using the *recom*Well HEV IgM/IgG ELISA and the *recom*Line HEV IgG/IgM immunoblot assays (Mikrogen Diagnostik, Neuried, Germany). All samples with a positive or borderline result by ELISA were retested in duplicate.

Sequencing and phylogenetic analysis

The fragments obtained from a partial genomic region of ORF1 were subjected to direct sequencing using the dideoxynucleotide chain termination method with the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit 1 and the ABI PRISM 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) (Otto et al., 2008). A multiple sequence alignment of 207 bp was constructed to include all ORF1 Brazilian HEV isolates (swine: **JN983190**–**JN983207**, **KY907039**–**KY907064**, **JN166093**, **JN166094**, **JN190071**, **KU888659**–**KU888665**, **MF438128**–**MF438135**; human: **GQ421465** and **KX757780**) and HEV single representatives of reference genotypes 1 (**M73218**), 2 (**M74506**), 3 (**AF082843**), and 4 (**AB197673**) (Smith et al., 2016). Nucleotide identity was calculated with the Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990). The phylogenetic analysis was based on Bayesian inference using the Bayesian Markov chain Monte Carlo (MCMC) statistical framework, implemented in BEAST v1.8.1 package (Drummond et al., 2012). Substitution model TrNef + G was selected using the jModeltest program (Posada, 2008).

Statistical analysis

The statistical analysis was performed using Stata (StataCorp LP, College Station, TX, USA). Poisson regression was used for the multivariate analysis with a robust calculation of variances and using a backward stepwise analysis. Prevalence ratios (PR) and their respective 95% confidence intervals (CI) were calculated. All variables were entered following a three-level hierarchical model (Victora et al., 1997). The first level contained the socio-demographic variables, the second level contained the behavioral variables, and the third level contained the clinical and laboratory variables. The variables were removed from the model according to the *p*-value, with variables with $p < 0.05$ retained for adjustment at the next level. The significance test used was the Wald test. A *p*-value of <0.05 for a two-tailed test was used as the criterion of significance for all statistical tests.

Results

The overall prevalence of anti-HEV IgG in HIV-infected patients was 6.7% (24/360) and in blood donors was 7.1% (20/281); the difference between these two groups was not significant ($p > 0.05$). Samples that tested repeatedly positive or borderline by enzyme immunoassay (EIA) were further evaluated by immunoblot (IB) assay, with positive results for 26 HIV-infected patients and 16 blood donor samples. Samples that resulted positive or inconclusive by IB were also tested for anti-HEV IgM by EIA, and further evaluated by IB; three HIV-infected patients and one blood donor were confirmed anti-HEV IgM-positive.

Among 360 HIV-infected patients, none had HEV RNA detectable by ORF1-nested RT-PCR, but eight (2.2%) had a positive result by ORF3-qRT-PCR, with a mean viral load of 2506.4 ± 4210.1 copies/ml. Serial samples from these eight patients obtained previously or subsequently all tested negative for anti-HEV IgM and IgG.

One blood donor was found to be anti-HEV (IgG and IgM) and HEV RNA positive at the time of the blood donation (Figure 1). This HEV-3 strain (GenBank accession number **KX770298**) was highly similar to HEV isolates from the state of Rio Grande do Sul (nucleotide identity of 94–96%), but less similar to the autochthonous human case reported in Rio de Janeiro (85%).

The characteristics of the individuals studied are shown in Table 1. The crude seroprevalence of anti-HEV IgG among HIV-infected patients was significantly higher only among individuals aged over 40 years ($p < 0.01$), those who were less educated ($p < 0.05$), and those who reported the harmful use of alcohol ($p < 0.05$). No difference in seroprevalence was observed according to the CD4+ cell count. In the blood donor group, the prevalence was higher only among the less educated individuals ($p < 0.05$), those residing in rural areas ($p < 0.01$), and those whose homes were not served by mains drinking water ($p < 0.05$).

After adjustment, the probability of the outcome in the HIV-infected patient group for the first level was three times greater in individuals older than 40 years, twice as high in individuals with a partner, and 60% lower in individuals living in houses connected to sewer pipes. At the second level, the harmful use of alcohol remained associated, increasing the likelihood of the outcome by 2.5-fold. In the model adjusted for the group of blood donors, the only factor significantly associated with the outcome was that the subject lived in a rural area; this characteristic increased the risk of a positive HEV serology by more than 500% (Table 2).

Discussion

In the southern region of Brazil, only limited data are available on the prevalence of HEV infection, with reports of prevalence in blood donors in the states of Paraná and Santa Catarina (Bortoliero et al., 2006; Passos-Castilho et al., 2016) and in swine herds in Rio Grande do Sul and Paraná (Gardinali et al., 2012b; Heldt et al., 2016; Vasconcelos et al., 2015). This cross-sectional study was conducted to determine HEV prevalence in HIV-infected patients, a population with a probable increased risk for developing chronic HEV infection, and in blood donors as a control (low risk) group. In this

Table 1
Characteristics of the study groups.

Variables	HIV-infected patients ($n = 360$)	Blood donors ($n = 281$)	
	Mean ± SD/*n* (%)	Mean ± SD/*n* (%)	
Age (years)	42.31 ± 11.52	33.65 ± 11.37	***
Male sex	183 (50.8)	211 (75.1)	***
White skin color	247 (68.6)	230 (81.9)	***
Education (years)	7.16 ± 3.74	10.92 ± 1.08	***
Per capita income (MW)	1.03 ± 0.94	2.13 ± 4.15	***
Partner	179 (49.7)	209 (74.4)	***
Urban residence	328 (91.1)	262 (93.2)	
Mains drinking water	347 (96.4)	267 (95.0)	
Sewage lines	177 (49.2)	152 (54.1)	
Sexual partners ≥3	59 (16.4)	30 (10.7)	*
Homo/bisexual male	49 (13.6)	1 (0.4)	***
Prior tattoo	125 (34.7)	112 (39.9)	
Inhaled drugs	119 (33.1)	30 (10.7)	***
Injected drugs	50 (13.9)	1 (0.4)	***
Raw/undercooked meat	152 (42.2)	119 (42.3)	
Raw/undercooked pork	65 (18.1)	19 (6.8)	***
Raw/undercooked seafood	64 (17.8)	60 (21.4)	
Harmful use of alcohol	27 (7.9)[a]	–	
HBsAg	11 (3.1)[b]	0 (0.0)	**
Anti-HCV antibodies	32 (8.9)[b]	0 (0.0)	***
Length of HIV infection (years)	7.89 ± 5.27	–	
Using HAART	320 (88.9)	–	
Length of HAART use (years)	5.63 ± 4.37	–	
Nadir CD4+ cells (cells/mm^3)	187.92 ± 174.49	–	
CD4+ cells (cells/mm^3)	569.29 ± 321.09	–	
Undetectable HIV VL	258 (71.7)	–	

SD, standard deviation; MW, minimum wage; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HAART, highly active antiretroviral therapy; VL, viral load. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
[a] $n = 343$.
[b] $n = 358$.

Table 2
Prevalence and adjusted prevalence ratios (aPR) for factors associated with anti-HEV IgG antibodies in the study groups.

Variables	HIV-infected patients ($n = 360$)				Blood donors ($n = 281$)			
	n (%)	aPR	95% CI	*p*-Value	*n* (%)	aPR	95% CI	*p*-Value
Age (years)[a,d]				0.01				0.7
18–40	6 (3.6)	1			12 (5.7)	1		
>40	20 (10.4)	3.14	1.31–7.55		4 (5.6)	0.77	0.22–2.74	
Sex[a]				0.4				0.4
Female	11 (6.2)	1			2 (2.9)	1		
Male	15 (8.2)	1.38	0.68–2.08		14 (6.6)	1.87	0.92–7.08	
Skin color[a]				0.6				0.2
White	17 (6.9)	1			12 (5.2)	1		
Black/other	9 (8.0)	1.27	0.56–2.90		4 (7.8)	2.36	0.72–7.79	
Education (years)[a]				0.06				0.4
0–8	23 (9.3)	1			8 (10.7)	1		
>8	8 (2.7)	0.32	0.10–1.04		8 (3.9)	0.61	0.19–1.92	
Per capita income (MW)[a]				0.2				0.3
≤1	21 (9.0)	1			6 (7.2)	1		
>1	5 (4.0)	0.52	0.20–1.37		10 (5.1)	1.98	0.55–7.08	
Partner[a,d]				0.04				0.2
No	9 (5.0)	1			2 (2.8)	1		
Yes	17 (9.4)	2.27	1.06–4.88		14 (6.7)	2.29	0.56–9.32	
Residence[a,e]				0.5				0.001
Urban	24 (7.3)	1			11 (4.2)	1		
Rural	2 (6.3)	0.64	0.15–2.69		5 (26.3)	6.26	2.42–16.22	
Drinking water mains[a]				0.8				0.9
No	1 (7.7)	1			3 (21.4)	1		
Yes	25 (7.8)	1.31	0.21–8.04		13 (4.9)	1.09	0.25–4.82	
Sewer pipes[a,d]				0.02				0.9
No	18 (9.9)	1			10 (6.6)	1		
Yes	8 (4.5)	0.40	0.18–0.89		6 (4.7)	0.87	0.25–3.06	
Sexual partners[b,d]				0.04				0.3
0–2	19 (6.3)	1			15 (6.0)	1		
≥3	7 (11.8)	2.25	1.02–5.00		1 (3.3)	0.38	0.07–2.13	
Tattoo[b]				0.6				0.5
No	17 (7.2)	1			12 (7.1)	1		
Yes	9 (7.2)	1.23	0.58–2.61		4 (3.6)	0.69	0.21–2.21	
Inhaled drugs[b]				0.1				0.9
No	19 (7.9)	1			15 (6.0)	1		
Yes	7 (5.9)	0.50	0.20–1.25		1 (3.3)	0.86	0.11–6.81	
Injected drugs[b]				0.2				–
No	21 (6.8)	1			16 (5.7)	–		
Yes	5 (10.0)	1.64	0.73–1.42		0 (0.0)	–	–	
Raw meat[b]				0.9				0.2
No	17 (8.2)	1			7 (4.3)	1		
Yes	9 (5.9)	1.01	0.46–2.24		9 (7.6)	1.75	0.69–4.46	
Raw pork[b]				0.2				0.9
No	23 (7.8)	1			14 (5.3)	1		
Yes	3 (4.6)	0.34	0.07–1.51		2 (10.5)	0.91	0.15–5.35	
Raw seafood[b]				0.6				0.5
No	22 (7.4)	1			14 (6.3)	1		
Yes	4 (6.3)	0.77	0.29–2.08		2 (3.3)	0.63	0.15–2.73	
Harmful use of alcohol[b,d]				0.03				–
No	20 (6.3)	1			–	–	–	
Yes	5 (18.5)	2.58	1.08–6.19		–	–	–	
Anti-HCV antibodies[c]				0.8				–
No	21 (6.4)	1			16 (5.7)	–	–	
Yes	4 (12.5)	0.86	0.27–2.72		0 (0.0)	–	–	
Length of HIV infection (years)[c]				0.2				–
0–8	10 (5.1)	1			–	–	–	
>8	16 (9.8)	1.73	0.82–3.65		–	–	–	
Using HAART[c]				0.2				–
No	3 (7.5)	1			–	–	–	
Yes	23 (7.2)	0.38	0.09–1.63		–	–	–	
Nadir CD4+ cells (cells/mm^3)[c]				0.4				–
<200	16 (7.4)	1			–	–	–	
≥200	10 (7.0)	0.40	0.30–1.62		–	–	–	
CD4+ cells (cells/mm^3)[c]				1				–
<500	14 (8.1)	1			–	–	–	
≥500	12 (6.4)	0.99	0.38–2.66		–	–	–	
Detectable HIV VL[c]				0.5				–
No	20 (7.8)	1			–	–	–	
Yes	6 (5.9)	0.67	0.23–1.95		–	–	–	

HEV, hepatitis E virus; aPR, adjusted prevalence ratio; CI, confidence interval; MW, minimum wage; HCV, hepatitis C virus; HAART, highly active antiretroviral therapy; VL, viral load.

[a] First level.
[b] Secon d level.
[c] Third level.
[d] HIV-infected patient final model.
[e] Blood donor final model.

study, the anti-HEV IgG prevalence rates were similar in these two groups (6.7% vs. 7.1% by EIA and 7.2%, respectively).

Among HIV-infected patients, HEV seroprevalence rates ranging from 3% to 20% have been observed in the Americas (Crum-Cianflone et al., 2012; Fainboim et al., 1999; Sherman et al., 2014), and of 2% to 10% in blood donors from the southern and southeastern regions of Brazil (Bortoliero et al., 2006; Gonçales et al., 2000; Passos-Castilho et al., 2017, 2016; Trinta et al., 2001). These discrepancies may, in part, reflect different regional characteristics. Moreover, the performance of the serological tests employed may vary from assay to assay, taking into account the limitations related to their sensitivity and specificity (Avellon et al., 2015; Pas et al., 2013; Bendall et al., 2010). Whether HIV-infected individuals have a higher HEV seroprevalence than the general population is controversial. Women attending an anonymous HIV testing program presented a higher HEV seroprevalence (17.7%) when compared to blood donors (4.0%) from a southeastern Brazilian city. The authors attributed this finding to the low socioeconomic status of these women (Gonçales et al., 2000). Similarly, two other studies conducted in Argentina also noted a higher seroprevalence among HIV-infected individuals (6.6% and 7.3%) compared to blood donors (1.8%) and HIV-negative individuals (4.4%), possibly related to blood transmission (Fainboim et al., 1999) or to lower CD4 counts (Debes et al., 2016). However, other studies have found no differences in prevalence between these two population groups (Politou et al., 2015; Ramezani et al., 2013).

HEV infection is usually asymptomatic and self-limited, and viremia does not persist for longer than a month (Krain et al., 2014). Most of the studies investigating HEV viremia have been conducted among smaller-sized samples of HIV-infected patients presenting either elevated liver enzymes, low CD4+ cell counts (<200 cells/mm^3), or chronic liver disease. In such studies, the frequency of HEV RNA detection has varied from 0 to 4% (Crum-Cianflone et al., 2012; Hassing et al., 2014; Kaba et al., 2011; Keane et al., 2012; Merchante et al., 2015; Pischke et al., 2015; Rivero-Juarez et al., 2015; Sellier et al., 2011; Sherman et al., 2014). In the present study, eight (2.2%) out of the 360 HIV-infected patients had HEV RNA detectable by qRT-PCR; however, none of the serum samples obtained previously and subsequently remained positive, thereby excluding chronic infection. Moreover, none of them were positive by the conventional nested RT-PCR, neither for anti-HEV IgM nor for anti-HEV IgG.

Often, the detection of HEV RNA is not coincident with the presence of HEV IgM/IgG antibodies, and characterizes an immunological window (Baylis et al., 2012). In this study, five HIV-infected patients who tested positive or indeterminate for anti-HEV IgG antibodies and negative for HEV RNA, presented a positive or indeterminate test for the presence of anti-HEV IgM antibodies by EIA method. Of these, three were confirmed positive by IB method, thus suggesting a recent infection. Moreover, none of the serum samples obtained previously and subsequently had a positive result, thereby excluding chronic infection. Studies assessing HIV-infected patients have detected chronic hepatitis in approximately one-third of the viremic cases (Crum-Cianflone et al., 2012; Kaba et al., 2011; Kuniholm et al., 2016; Merchante et al., 2015; Rivero-Juarez et al., 2015; Sellier et al., 2011), mostly associated with severe immunodeficiency (CD4+ cell counts lower than 200 cells/mm^3) (Dalton et al., 2009; Debes et al., 2016; Kaba et al., 2011; Kuniholm et al., 2016; Merchante et al., 2015). In the present study, the population assessed had a significant previous immunosuppression history (mean nadir CD4+ cell count <200 cells/mm^3); however, at the time of recruitment, the mean CD4+ cell count was higher than 500 cells/mm^3.

The detection of HEV RNA among blood donors cannot be regarded as a rare event since well-documented cases of blood transmission have been reported from industrialized countries, where genotype 3 (HEV-3) is predominant (Boxall et al., 2006; Colson et al., 2007; Matsubayashi et al., 2008). In this study, the finding of a viremic donation (HEV-3-positive) indicates a potential risk of transmission of HEV via transfusion in the population studied. However, the absence of detectable RNA in the respective recipient prevented us from confirming the transmission by sequencing and genetic comparison with the donor's HEV strain. The recipient of the single packed red blood cell unit, transfused 14 days after donation, was a 76-year-old male patient with gastric adenocarcinoma who underwent chemotherapy (oxaliplatin, fluorouracil, and folinic acid) 43 days after the transfusion. He had no signs or symptoms of hepatitis, and serial measurements of his liver enzymes, performed during the 6 months after the transfusion, remained at normal serum levels. His virological and serological screenings remained negative on day 226 post-transfusion. Indeed, seroconversion is not often observed among the recipients of HEV contaminated blood donations. This could be explained either by the low HEV viral load after the removal of a significant portion of the plasma, or by the potential neutralizing effect of the simultaneous presence of HEV antibodies in the blood donated.

HEV-3 is the only genotype detected so far in Brazil (da Costa Lana et al., 2014; de Souza et al., 2012; dos Santos et al., 2011, 2009; Gardinali et al., 2012a,b; Heldt et al., 2016; Lopes Dos Santos et al., 2010; Passos et al., 2013). In the present study, the blood donor's HEV-3 strain showed a high similarity with swine strains from the same Brazilian region. This finding supports the assumption that in Brazil the transmission of HEV to humans is mainly zoonotic (Lopes Dos Santos et al., 2010), as observed in many developed countries (Dalton et al., 2008; Meng, 2011; Ruggeri et al., 2013).

This study showed that the HEV seroprevalence was higher among older individuals in the HIV-infected patient group, even after adjustment; however, the analysis failed to establish this finding in the blood donor group. Some other studies have shown a positive correlation between age and HEV seroprevalence, similar to other enterically transmitted diseases, possibly due to cumulative exposure over time. In regions where genotypes 3 and 4 are predominant, the most affected age groups are middle-aged adults and the elderly (Avellon et al., 2015; Kmush et al., 2015; Lewis et al., 2010; Pas et al., 2013; Rapicetta et al., 2013; Verhoef et al., 2012). Thus, the lack of association for the donor group observed here may be explained by the lower mean age of this group.

In the HIV-infected patient group, a protective effect was observed when the person's residence was connected to sewage lines. This effect was not observed among blood donors, for whom the main risk factor was living in a rural area. Untreated water consumption, a lack of sewer pipes, and the improper handling of animals, including pigs and their waste, are all frequent in rural areas. Moreover, soil and nearby water contamination are thought to serve as a source of food contamination (Meng, 2011). These facts could explain the associations found in both groups.

The consumption of raw or undercooked meat or pork liver was not associated with the HEV seroprevalence in this study, neither in HIV-infected patients nor in blood donors. In developed countries, this behavior is often related to cases of hepatitis E and high specific antibody prevalence rates in the general population (Mansuy et al., 2011; Wichmann et al., 2008). This finding was also observed among HIV-infected individuals in England (Keane et al., 2012). A more careful assessment of this behavior, such as the frequency, quantity, and conditions of food preparation and consumption, may provide additional information.

Having a partner and three or more sexual partners in the last year were variables associated with a higher HEV seroprevalence in this study. The likelihood of interpersonal HEV transmission is low (Somani et al., 2003), and the available data do not demonstrate sexual transmission of the virus (Chau et al., 2001). However,

environmental fecal contamination in confined areas or even fomites may play a role in the process of viral dissemination from infected individuals (Rapicetta et al., 2013; Ruggeri et al., 2013). In this circumstance, the population density per household with intimate familiarity could play an important role.

The use of injectable drugs and having tattoos are behaviors often observed among HIV-infected individuals and may predispose them to acquire parenterally transmitted pathogens, through contact with contaminated blood. Blood transmission of HEV is well documented (Hewitt et al., 2014); however, in this study, these behaviors were not associated with the presence of anti-HEV antibodies. The data available on this subject are conflicting and merit more extensive study, because some researchers have found a higher prevalence among injectable drug users (Gessoni and Manoni, 1996; Kmush et al., 2015; Sylvan, 1998), whereas others have not (Keane et al., 2012; Politou et al., 2015). The harmful use of alcohol significantly increased the risk of being HEV-seropositive. This finding is not consistent with the literature (Dalton et al., 2011; Jiang et al., 2010; Pineda et al., 2014; Boxall et al., 2006). Although alcohol consumption may not be directly responsible for the infection, its use may be related to other behaviors that expose the individual to HEV. For example, one study investigating an outbreak of hepatitis E that occurred on a cruise ship found a higher prevalence among individuals who consumed alcohol (Said et al., 2009).

The limitations of this study include those inherent to cross-sectional studies, which cannot determine a causal relationship between the selected variables and the studied outcomes. However, the possibility of reverse causality is improbable since the antibody production curve and transient course of viremia remained independent of the exposure factors studied. Another limitation is the accuracy of the diagnostic methods used, taking into account that the viremia and serum antibody levels were affected by time and the individual's immunological status. Another aspect to consider is the possibility of a lack of statistical power for some of the studied associations. The absence of association with the ingestion of raw meat and other behavioral variables could be explained by the lack of more precise details on these habits. Therefore, a more detailed assessment of some of the studied risk factors could offer better information.

Hepatitis E is still often underestimated in many countries including Brazil. The promotion of educational measures, improving health professionals' knowledge would be an essential tool from a diagnostic perspective. This study highlights the importance of adopting prevention measures related to both known and suspected risk factors for HEV exposure with the aim of reducing contagion. These procedures should be directed toward the general population and particularly toward individuals who are known to be more vulnerable to the virus, such as pregnant women and individuals with chronic liver disease or an impaired immune system.

In summary, comparable prevalence rates of anti-HEV IgG antibodies were found in the two study populations. Serological and virological evidence confirmed the occurrence of HEV infection in the city of Rio Grande, located in the southernmost part of Brazil. Further studies are necessary to identify other potential factors associated with its transmission. No cases of persistent HEV infection were identified among HIV-infected patients; however, studies targeted at individuals with a more severe immune deficiency could be useful and enable a better understanding of HEV pathogenesis in this group. In Brazil, data are not available that would allow it to be determined whether HEV transmission by blood transfusion represents a considerable risk for both immunocompetent and immunocompromised groups. This information is essential for health authorities to assess the need for the inclusion of molecular HEV testing in the routine protocol of blood donor screening.

Funding

This paper was supported by the following grants: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), grants awarded to MAP; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), grants awarded to LDM and NRG; Programa de Pós-Graduação em Ciências da Saúde – FURG, grants awarded to CMS; FIOCRUZ – PASTEUR Program, budget awarded to MAP within the framework of the call 2013/2015. Mikrogen Diagnostik donated one *recom*Line HEV IgG/IgM kit used in the present study. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of interest

The authors declare no competing interests.

Author contributions

C. Moss da Silva: study design, data collection, project administration, funding acquisition, investigation, data analysis, writing – original draft. J. Mendes de Oliveira: study design, investigation, data analysis, writing – review and editing. R.A. Mendoza-Sassi: data analysis, writing – original draft. A.S. Figueiredo: investigation, data analysis. L.D. da Mota: investigation. M.M. Nader: investigation. N.R. Gardinali: investigation. S.B.S. Salvador: investigation. Y.B. Kevorkian: investigation. M.A. Pinto: study design, project administration, funding acquisition, data analysis, supervision, writing – review and editing. A.M. Barral Martinez: study design, funding acquisition, supervision, writing – original draft.

Acknowledgements

The authors acknowledge FURG – HIV/AIDS Service and CRGS – Hemotherapy Service for the cooperation and support provided by their staff.

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