

Characteristics of Autochthonous Hepatitis E Virus Infection in Solid-Organ Transplant Recipients in France

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(See the article by Colson et al, on pages 825–834, and the editorial commentary by Purcell and Emerson, on pages 819–821.)

Background. Hepatitis E virus (HEV) infections can lead to chronic hepatitis in immunocompromised patients. We have investigated the risk factors for HEV infection among solid-organ transplant recipients and the characteristics of these infections.

Methods. We performed serological tests, quantified the virus, and genotyped the virus in plasma samples. We performed a case-control study with HEV-infected patients and control participants matched for sex and age who were recruited from a population of solid-organ transplant recipients with no markers of HEV infection.

Results. We investigated 38 consecutive cases of HEV genotype 3 infection. Twenty-two (58%) of these 38 patients developed a chronic infection. The acute-phase aminotransferase levels were higher in the patients who cleared the virus than in those who developed chronic infections. The anti-HEV immunoglobulin G and immunoglobulin M profiles and HEV RNA concentration in patients who cleared the virus were similar to those in patients who developed a chronic infection. A logistic regression analysis of 37 case patients and 148 control participants indicated that the only factor independently associated with HEV infection was the consumption of game meat (68% of case patients vs 47% of control participants; odds ratio, 2.32; 95% confidence interval, 1.04–5.15).

Conclusion. Immunocompromised patients should avoid eating insufficiently cooked game meat or pork products so as to reduce the risk of HEV infection and chronic liver disease.

Hepatitis E is epidemic and endemic in many developing countries, where it is usually spread by contaminated water due to poor sanitation [1]. Increasing numbers of sporadic cases of acute hepatitis E have also been reported in many industrialized countries, where it is now considered to be an emerging infectious disease [2, 3]. Hepatitis E virus (HEV) genotypes 1 and

2 are the principal causes of waterborne hepatitis E in developing countries, whereas autochthonous cases in industrialized countries are caused by genotypes 3 and 4 [4–6]. HEV genotypes 3 and 4 are widely distributed in animals, and there is growing evidence that hepatitis E is a zoonotic disease [6, 7]. Domestic pigs, wild boar, and deer seem to be the main reservoirs for HEV. However, there are still questions about the mechanism by which the virus is transmitted to humans. Direct exposure to infected animals has been identified as an important route of HEV transmission. The prevalence of anti-HEV antibodies is higher among veterinarians and farmers who work with pigs than among blood donors [8, 9]. There has been a report of a pig owner being infected by his pet pig [10]. Eating undercooked game meat is also associated with HEV infection [11, 12]. Finally, exposure to a contaminated environment

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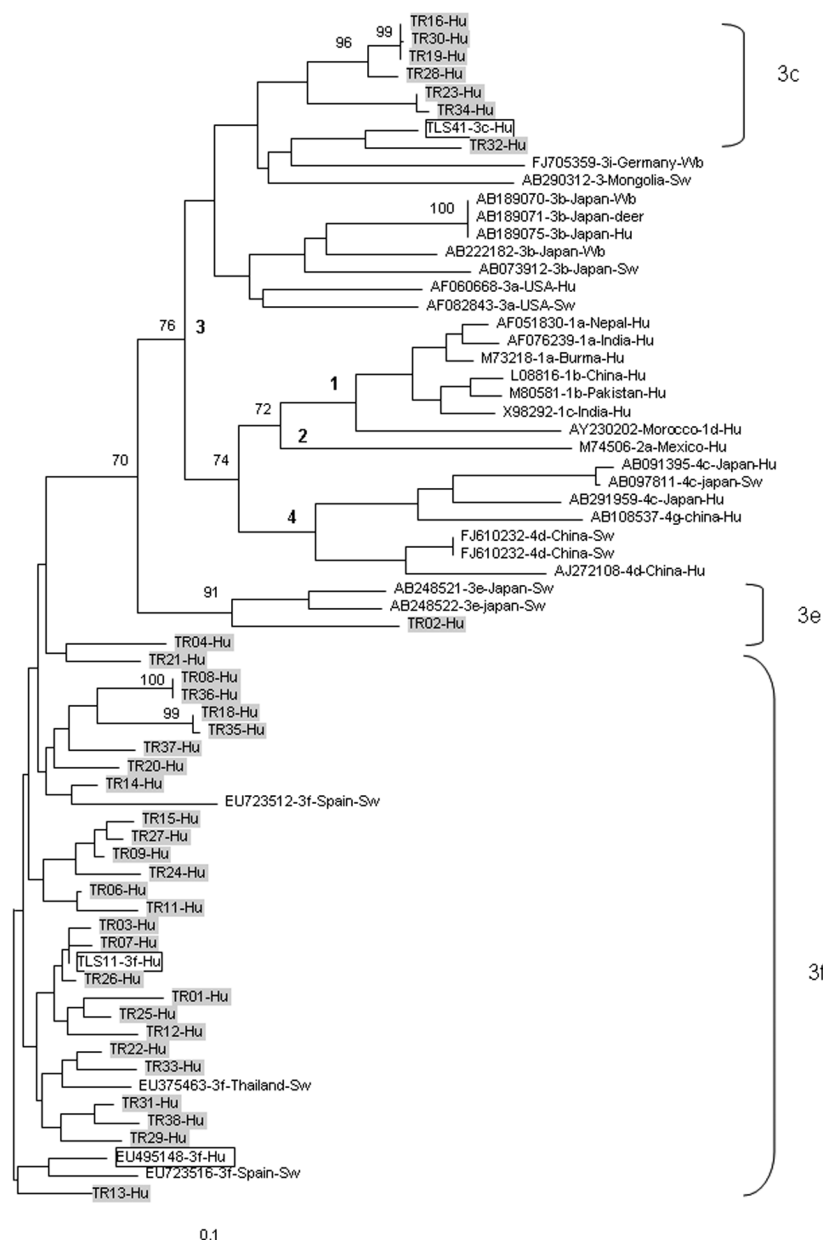


Figure 1. Phylogenetic tree constructed by the neighbor-joining method, based on the open reading frame 2 region of the hepatitis E virus (HEV) genome (189 nucleotides). The phylogenetic analysis includes (1) HEV sequences recovered from solid-organ transplant recipients (*gray background*), (2) HEV sequences from patients who are not solid-organ transplant recipients and who live in the same area (*black outline*), and (3) GenBank reference sequences. HEV sequences are labeled as follows: name of sequence, genotype, country of origin, and host (Hu, human; Wb, wild boar; Sw, swine [domestic pig]).

may be a possible route of transmission. The HEV genome has been found in sewage water and rivers in Europe [13, 14].

HEV infection generally runs a self-limiting symptomatic or asymptomatic course with acute hepatitis, but fulminant hepatitis may occur, especially in pregnant women or people with an underlying liver disease [1, 4, 15]. Until recently, it was thought that HEV infection was not responsible for chronic hepatitis. However, it has recently been shown that HEV can lead to chronic infection in solid-organ transplant (SOT) re-

cipients [16–18], in hematology patients who are given chemotherapy [19–22], and in human immunodeficiency virus (HIV)-positive patients [23, 24]. Liver fibrosis can develop very rapidly in immunocompromised patients, leading to liver cirrhosis within just a few years after infection [17, 25, 26].

The development of anti-HEV antibodies and the HEV RNA plasma concentration have been described in immunocompetent patients [27–29], but little is known about HEV markers in immunocompromised patients. Patients who develop a

Table 1. Bivariate and Multivariate Analyses of Exposure and Potential Risk Factors for Hepatitis E Virus Infection among Solid-Organ Transplant Recipients and Age- and Sex-Matched Control Participants

Analysis, variable	No. (%) of case patients	No. (%) of control participants	P	OR (95% CI)
Bivariate analysis				
Living in rural area (<20,000 inhabitants)	28 (74)	96 (65)	.23	1.65 (0.72–3.78)
Living in an apartment	8 (21.6)	47 (31.8)	.22	0.59 (0.25–1.40)
Living in a house	26 (70.3)	94 (63.5)	.44	1.35 (0.62–2.97)
Living on a farm	2 (5.4)	6 (4)	.71	1.35 (0.26–7.02)
Living in another home (camping, institution, etc)	1 (2.7)	1 (0.7)	.28	4.08 (0.24–67.95)
Having a septic tank	10 (28.5)	39 (27.6)	.91	1.04 (0.45–2.38)
Household animals				
Overall	19 (51)	90 (61)	.27	0.66 (0.32–1.38)
Cats	10 (27)	35 (23.6)	.66	1.19 (0.52–2.71)
Dogs	13 (35)	62 (43)	.45	0.75 (0.35–1.59)
Eating beef	36 (97)	142 (96)	.99	1.01 (0.11–9.4)
Eating pork	35 (97)	121 (87)	.08	5.20 (0.65–41.36)
Eating horse meat	14 (41)	42 (32)	.33	1.46 (0.67–3.2)
Eating fowl	37 (100)	145 (100)
Eating game meat	23 (67)	62 (47)	.03	2.3 (1.04–5.22)
Eating pork product (sausage, pate, etc)	35 (97)	123 (83)	.03	6.82 (0.86–53.9)
Eating raw meat	11 (30)	47 (31)	.81	0.91 (0.41–1.99)
Eating oysters	24 (65)	84 (59)	.38	1.40 (0.64–3.04)
Eating mussels	34 (100)	112 (77)	.002	10 (1.25–79.7)
Eating shellfish	19 (65)	65 (47)	.09	2.01 (0.86–4.70)
Eating raw vegetables	34 (89)	131 (90)	.38	1.94 (0.42–8.99)
Drinking bottled water	31 (97)	118 (91)	.25	3.15 (0.38–25.56)
Drinking tap water	27 (93)	108 (87)	.91	0.93 (0.28–3.06)
Drinking well water	0 (0)	10 (9)	.09	...
Drinking spring water	12 (41.3)	36 (34.9)	.64	1.31 (0.56–3.06)
Gardening	16 (51)	59 (40)	.20	1.59 (0.76–3.30)
Having a vegetable garden	12 (32.4)	30 (20.3)	.11	1.88 (0.84–4.21)
Practicing a sport	16 (44)	67 (45)	.92	0.96 (0.46–2.01)
Hunting	5 (13.5)	10 (6.7)	.17	2.15 (0.68–6.8)
Camping	5 (13.5)	19 (13)	.92	1.05 (0.36–3.04)
Multivariate analysis				
Eating game meat	23 (67)	62 (47)	.03	2.3 (1.04–5.22)

NOTE. CI, confidence interval; OR, odds ratio.

chronic infection could have a specific virological pattern that differs from that of the patients who clear the virus. This study was performed to identify the risk factors for HEV infection among SOT recipients and to investigate the biological factors in patients with and patients without persistent HEV infections.

PATIENTS, MATERIALS, AND METHODS

Patients. We identified prospectively 38 patients with an acute HEV infection among the SOT recipients attending our outpatient and inpatient clinics from January 2004 through June 2009 who had an unexplained acute high level of liver enzyme activity. The initial outcomes of the first 15 patients have been reported elsewhere [18]. Acute HEV infection was defined by the presence of elevated levels of liver enzymes (ie, alanine

aminotransferase [ALT], aspartate aminotransferase [AST], γ -glutamyl transpeptidase [γ GT], and alkaline phosphatase) and the presence of HEV RNA in the plasma. Other causes of acute or chronic hepatitis (including hepatitis A, B, and C virus, Epstein-Barr virus, and cytomegalovirus infections) were excluded by means of serological and molecular tests. The 38 patients (32 men and 6 women) were 24 kidney recipients, 2 simultaneous kidney-pancreas recipients, and 12 liver transplant recipients, whose ages ranged from 28 to 78 years (median, 48 years). Two kidney transplant recipients and 1 liver transplant recipient had experienced previous graft rejections and needed retransplantation. All 38 patients live in southwestern France, 28 (74%) of them in a rural area; 35 patients are of French origin and 3 are of Spanish origin. Chronic in-

Table 2. Biological and Virological Characteristics during the Acute Phase of Illness of Solid-Organ Transplant Recipients Infected with Hepatitis E Virus (HEV)

Variable	All patients (n = 38)	Patients infected with genotype 3f (n = 27)	Patients infected with genotype 3c (n = 7)	Patients infected with genotype 3e or nontypable strains (n = 4)	P
ALT level, IU/L	168 (18–922)	169 (18–922)	158 (35–731)	183 (22–518)	NS
AST level, IU/L	93 (20–436)	90 (30–436)	108 (21–345)	147 (20–235)	NS
AP level, IU/L	266 (66–7775)	273 (71–1924)	207 (157–7775)	215 (66–450)	NS
γGT level, IU/L	136 (38–3482)	136 (38–1495)	179 (55–1582)	288 (60–3482)	NS
Total bilirubin level, μmol/L	15 (6–277)	14 (6–54)	17 (8–277)	19 (9–40)	NS
HEV RNA concentration, log copies/mL	6.3 (2.7–7.8)	6.5 (5.1–7.8)	6.3 (3.4–6.7)	2.7 (2.7–6.3)	NS
No. (%) of patients with anti-HEV IgM positivity	28 (80)	21 (87)	6 (86)	1 (25)	NS
No. (%) of patients with anti-HEV IgG positivity	12 (34)	7 (29)	4 (57)	1 (25)	NS
No. (%) of patients with chronic infection	22 (58)	15 (55)	6 (85)	1 (25)	NS

NOTE. Data are median (range) values, unless otherwise indicated. ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; γGT, γ-glutamyl transpeptidase; IgG, immunoglobulin G; IgM, immunoglobulin M; NS, not significant.

fection was defined as the presence of HEV RNA in the plasma for >6 months. The grade and stage of chronic hepatitis were assessed on the basis of liver biopsy specimens according to the Metavir classification [30]. The rate of fibrosis progression was defined as the ratio of the difference in fibrosis stage in Metavir units and the time between 2 liver biopsies.

Case-control study. We matched each of 37 SOT recipients who had an autochthonous HEV infection for sex and age (2 main factors associated with HEV infection in industrialized countries [2]) with 4 control participants who had no markers of HEV infection. The control participants were SOT recipients with no detected plasma HEV RNA or anti-HEV immunoglobulin G (IgG) and immunoglobulin M (IgM). All case and control patients completed a questionnaire on their living conditions, their food and drink intakes, and their leisure activities. One liver transplant recipient with an HEV infection died of septic shock before completing the questionnaire.

Laboratory investigations. Serological analyses were performed with the EIAgen HEV IgG kit and EIAgen HEV IgM kit (Adaltis) as recommended by the manufacturer. Viral RNA was detected in blood samples by real-time polymerase chain reaction with a hydrolysis probe [31]. The plasma concentration of HEV RNA was measured by the same real-time polymerase chain reaction assay, and plasmid standards were obtained by amplifying a 189-nucleotide fragment of the open reading frame 2 gene of HEV genotype 3. The plasmid standard was prepared with the Topo TA Cloning kit for sequencing (Invitrogen); it was titrated by means of limiting dilution. The HEV RNA concentrations were determined at the acute phase when the ALT activity level peaked. We also sequentially quantified plasma HEV RNA concentration during the chronic phase (6 months after the ALT level peaked; $n = 3$ –11).

The genotype was determined by sequencing a 189-nucleotide fragment within the open reading frame 2 gene [5]. Phy-

logenetic trees were created by use of the neighbor-joining method (Kimura 2-parameter method). Phylogenetic analyses were performed with genotype information on reference sequences based on the HEV classification proposed by Lu et al [6] (GenBank accession numbers: EU220992–EU221003, FJ665420–FJ665430, GU196319–GU196330, and FJ665979).

Statistical analyses. Stata software (version 9.2; StataCorp) was used for the statistical analyses. The characteristics of individuals were compared using the χ^2 test for categorical variables and the Mann-Whitney U test for continuous variables. A statistically significant difference was defined as a P value of <.05. We determined the prevalence of exposure to specific factors of case patients and control participants by bivariate matched analysis and calculated the Mantel-Haenszel odds ratios and 95% confidence intervals. We used the McNemar χ^2 test to examine categorical risks for disease in pairs of case patients and control participants and a logistic model clustered on pairing to estimate the effect of multiple variables. The dependent variable was HEV infection, and the explanatory variables were factors found to be associated with disease in the bivariate analysis. Variables related to disease at $P < .1$ in bivariate comparisons were included in the logistic regression model.

RESULTS

Phylogenetic analysis of HEV strains. We identified 38 SOT recipients seen from January 2004 through June 2009 as having an HEV infection on the basis of the presence of HEV RNA in their blood plasma. The HEV genotype was determined for 35 of the patients. Of these, 27 were infected with genotype 3f, 7 with genotype 3c, and 1 with genotype 3e (Figure 1). There was a close genetic relationship (>99% nucleotide sequence identity) between 3 genotype 3c strains: TR16, TR30, and TR19.

Table 3. Factors Associated with Development of Chronic Hepatitis E Virus (HEV) Infection among Solid-Organ Transplant Recipients

Acute-phase variables	Patients without chronic infection (n = 16)	Patients with chronic infection (n = 22)	P
Time between transplantation and HEV infection, months	60 (1–168)	43 (3–169)	NS
No. of patients with graft rejection	1	2	NS
No. of patients who used calcineurin inhibitors	15	14	NS
No. of patients who used PSIs	2	8	NS
No. of patients who used MPA	10	16	NS
No. of patients who used steroids	12	11	NS
ALT level, IU/L	259 (22–922)	151 (18–874)	.03
AST level, IU/L	151 (20–340)	78 (21–436)	.05
AP level, IU/L	310 (66–1924)	255 (71–7775)	NS
γGT level, IU/L	262 (60–2337)	124 (38–3482)	NS
Total bilirubin level, μmol/L	16 (6–54)	14 (6–277)	NS
No. of patients with HEV genotype			
3f	12	15	NS
3c	1	6	NS
Other	3	1	NS
HEV RNA plasma concentration, log copies/mL	6.3 (2.7–7.85)	6.3 (4.55–7.76)	NS
No. (%) of patients with anti-HEV IgM positivity	10 (67)	18 (90)	NS
No. (%) of patients with anti-HEV IgG positivity	2 (13)	10 (50)	.06
CD4 ⁺ cell count, cells/μL	457 (162–1119)	389 (45–1146)	NS
CD8 ⁺ cell count, cells/μL	424 (166–836)	367 (98–1094)	NS
CD19 ⁺ cell count, cells/μL	41 (9–437)	43 (17–360)	NS

NOTE. Data are median (range) values, unless otherwise indicated. ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; γGT, γ-glutamyl transpeptidase; IgG, immunoglobulin G; IgM, immunoglobulin M; MPA, mycophenolic acid; NS, not significant; PSI, proliferating signal inhibitor.

We also identified 2 clusters of genotype 3f strains. The open reading frame 2 nucleotide sequences of the TR08 and TR36 strains were 100% identical, and those of the TR18 and TR35 strains were 98.4% identical. The close genetic relationship between the genotype 3c strains and that between the TR18 and TR35 strains of genotype 3f were confirmed by phylogenetic analyses with the use of a 1230-nucleotide fragment within the open reading frame 1 part of the HEV genome (data not shown).

Epidemiological characteristics of HEV infections. None of the patients had a travel history before the onset of the disease, which indicates that their hepatitis E was autochthonous. None of them declared having any direct contact with a potential animal reservoir (pigs, wild boar, or deer). No seasonal periodicity was observed: 12 cases occurred during the first trimester, 10 during the second trimester, 8 during the third trimester, and 8 during the last trimester ($P = .7$). The cases of genotype 3f infection that occurred from 2004 through 2009 were uniformly distributed (4 cases occurred in 2004, 4 in 2005, 7 in 2006, 2 in 2007, 6 in 2008, and 4 in 2009), but there were no cases of genotype 3c infection from 2004 through 2006. The first 2 cases of genotype 3c infection occurred in 2007, and the remaining 5 cases occurred in 2008. The proportion of 3c in-

fections was greater after 2006 than that before 2006, compared with the distribution of other genotypes ($P = .02$).

We studied 37 SOT recipients with autochthonous hepatitis E and 148 matched control SOT recipients. They all completed a questionnaire that examined their living conditions, food and drink intakes, and leisure activities (Table 1). Bivariate analysis showed that the consumption of game meat (hunted animals such as wild boar, deer, and hare; 68% of case patients vs 47% of control participants; $P = .03$), the consumption of food products made with pork (pate, sausages, etc; 97% of case patients vs 84% of control participants; $P = .03$), and the consumption of mussels (100% of control participants vs 78% of control patients; $P = .002$) were associated with autochthonous HEV infections (Table 1). Logistic regression analysis indicated that the consumption of game meat was independently associated with an autochthonous HEV infection in the SOT recipients in southwestern France (68% of case patients vs 47% of control participants; odds ratio, 2.32; 95% confidence interval, 1.04–5.15). No epidemiological characteristics were associated with infection with a particular genotype, including genotypes 3f and 3c.

Characteristics of acute-phase HEV infections. The demographic characteristics of the patients did not influence the

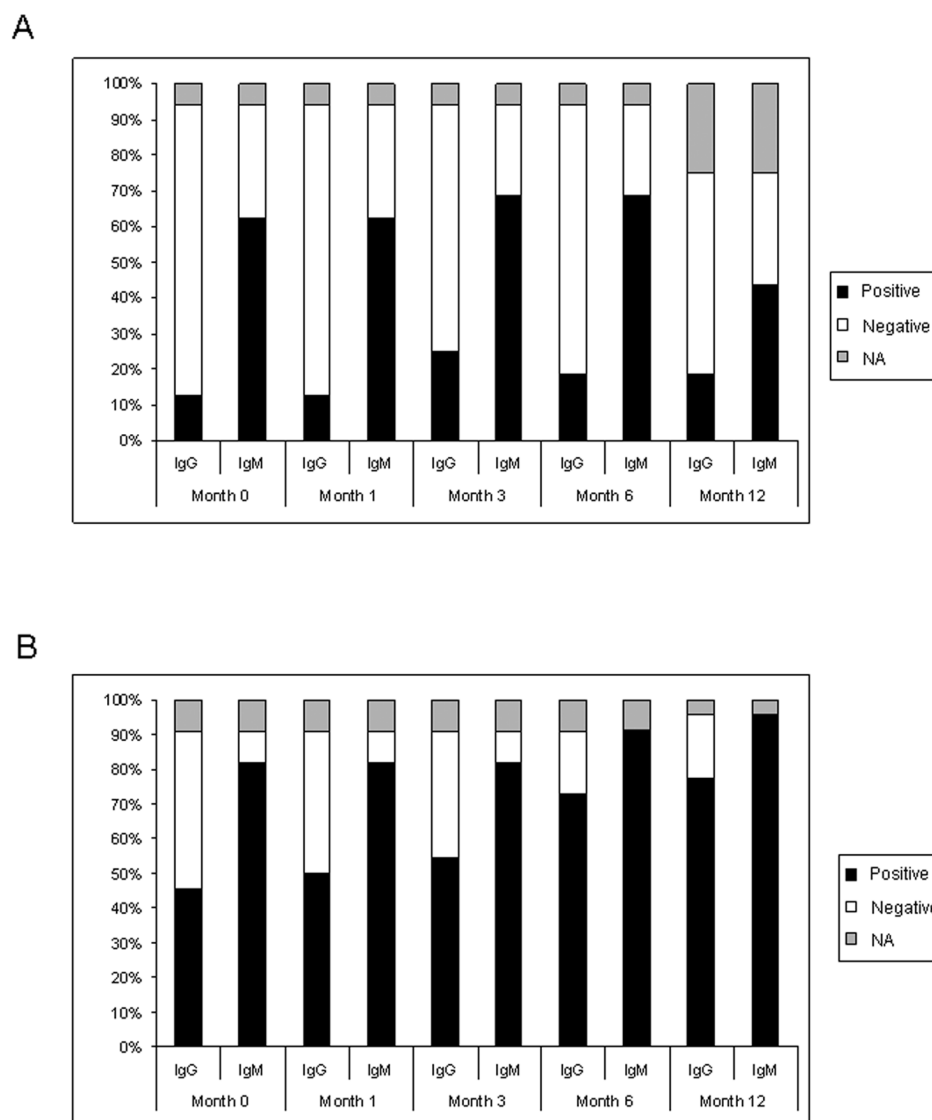


Figure 2. Serial serological results obtained for the 16 solid-organ transplant recipients with a resolving hepatitis E virus infection (*A*) and for the 22 solid-organ transplant recipients whose infection evolved to a chronic infection (*B*). IgG, immunoglobulin G; IgM, immunoglobulin M; NA, not available.

biological or virological characteristics of the HEV-infected patients. All the patients except 2 had elevated ALT and AST levels at the acute phase. The 2 patients with normal ALT and AST levels had an abnormal level of γ GT activity. The median ALT level was 168 IU/L (range, 18–922 IU/L), and the median AST level was 93 IU/L (range, 20–436 IU/L). The ALT activity levels of patients infected with genotype 3f (median, 169 IU/L) were similar to those of patients infected with genotype 3c (median, 158 IU/L; $P = .71$). Similarly, the AST activity levels of patients infected with genotype 3f (median, 90 IU/L) were similar to those of patients infected with genotype 3c (median, 108 IU/L; $P = .60$) (Table 2). Only 9 (24%) of 38 patients had an abnormal total bilirubin concentration. The alkaline phosphatase

activity level was abnormal in 17 (45%) of 38 patients. The patients infected with genotypes 3f and 3c had similar total bilirubin concentrations, alkaline phosphatase levels, and γ GT levels.

We quantified the HEV RNA concentration in the plasma of 35 patients at the acute phase of infection. The median HEV RNA concentration was 6.3 log copies/mL (range, 2.7–7.8 log copies/mL). There was no correlation between the HEV RNA concentration during the acute phase and the ALT activity level. The HEV RNA concentrations in patients infected with genotype 3f (median, 6.5 log copies/mL) and in those infected with genotype 3c (median, 6.3 log copies/mL; $P = .18$) were similar (Table 2).

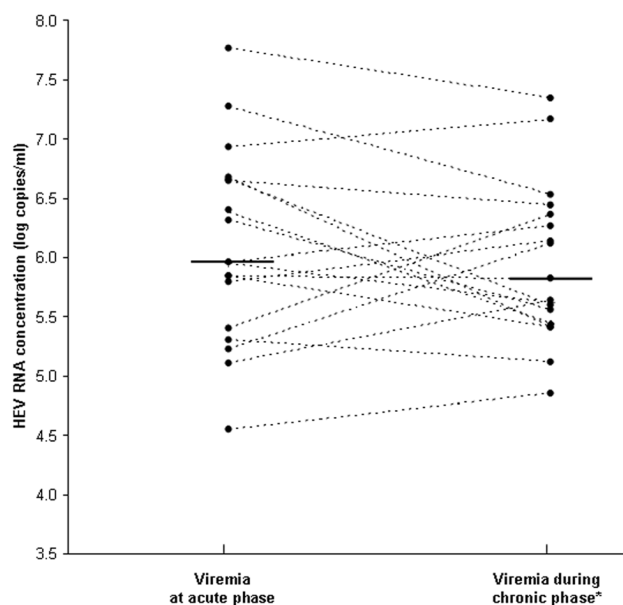


Figure 3. Acute-phase and chronic-phase hepatitis E virus (HEV) RNA concentrations in plasma of patients chronically infected with HEV ($n = 19$). The horizontal lines indicate the median HEV RNA concentrations. *Values represent the mean of the HEV RNA concentrations during the chronic phase 6 months after the alanine aminotransferase level peaked.

Serological tests were performed during the acute phase on samples from 35 patients. Anti-HEV IgM was detected in samples from 28 (80%) of 38 patients, but anti-HEV IgG was detected in samples from only 12 (34%) of 38 patients. Seven patients tested negative for anti-HEV IgG and IgM during the acute phase. Three of these patients tested positive for anti-HEV IgM and IgG 1–6 months after the HEV infection was diagnosed, but the remaining 4 patients always tested negative for these antibodies. Twelve patients tested positive for anti-HEV IgG and IgM at the acute phase. Sixteen patients were anti-HEV IgM positive but anti-HEV IgG negative, but anti-HEV IgG was detected in samples from 5 of these 16 patients after 1–7 months (Figure 2). No specific anti-HEV IgG and IgM profile was associated with any HEV genotype (Table 2). Patients with resolving HEV infections continued to test positive for anti-HEV IgM for 12–29 months after the infection was resolved.

Factors at diagnosis associated with HEV persistence.

Twenty-two (58%) of 38 patients developed a chronic infection. The median delay between transplantation and HEV infection was 53 months (range, 1–169 months). The median delay between transplantation and HEV infection was 60 months for the patients who had resolving infection and 43 months for those who developed a chronic infection (Table 3). The occurrence of graft rejection did not influence the development of chronic infection. The ALT levels at diagnosis of patients

who had a resolving infection (median, 259 IU/mL) were higher than those of patients who developed a chronic infection (median, 151 IU/mL; $P = .03$). Similarly, the AST levels at diagnosis of patients who had a resolving infection (median, 151 IU/mL) were higher than those of patients who developed a chronic infection (median, 78 IU/mL; $P = .05$). Of the 27 patients infected with genotype 3f, 15 (55%) developed a chronic infection, as did 6 (85%) of the 7 patients infected with genotype 3c. The plasma HEV RNA concentrations in patients who cleared the infection (median, 6.3 log copies/mL) and in those who developed a chronic infection (median, 6.3 log copies/mL; $P = .40$) were similar. The presence of anti-HEV IgG and IgM during the acute phase was not associated with clearance of the virus. The counts of CD4⁺ cells, CD8⁺ cells, and CD19⁺ cells of patients who cleared the virus and those of patients who developed a chronic infection were identical, as were the frequencies of use of calcineurin inhibitors, proliferating signal inhibitors, mycophenolic acid, and steroids (Table 3).

Characteristics of chronic HEV infections. The acute-phase HEV RNA concentration and the mean of the HEV RNA concentrations measured during the chronic phase were compared among 19 patients. The acute-phase HEV RNA concentrations (median, 6 log copies/mL) and the chronic-phase concentrations (median, 5.8 log copies/mL; $P = .37$) were similar (Figure 3). Anti-HEV IgM was detected throughout the chronic phase in samples from all the chronically infected patients. Two liver biopsies were performed for each of 16 patients (median

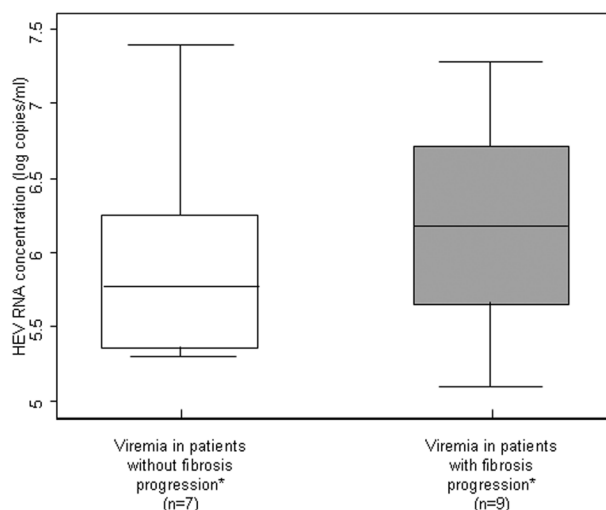


Figure 4. Chronic-phase hepatitis E virus (HEV) RNA concentrations in plasma of patients according to the progression of liver fibrosis. Data are presented as box plots in which 50% of the values lie within the box. The horizontal lines through the middle of the boxes indicate the median values. The top and bottom of each box are the first and the third quartiles, respectively. *Values represent the mean of the HEV RNA concentrations during the chronic phase 6 months after the alanine aminotransferase level peaked.

time between biopsies, 21 months). The overall rate of liver fibrosis progression was 0.6 ± 0.7 Metavir units per year. We identified 2 groups of patients according to the evolution of fibrosis during follow-up. Fibrosis did not progress in 7 patients: the fibrosis score at the first biopsy was similar to that at the second biopsy. Fibrosis progressed in 9 patients: the fibrosis score at the second biopsy had increased by at least 1 point above that at the first biopsy. The chronic-phase ALT levels in patients whose liver fibrosis did not progress (median, 132 IU/L) and those in patients whose liver fibrosis did progress (median, 87 IU/L; $P = .26$) were similar. The chronic-phase HEV RNA concentrations in patients with no liver fibrosis progression (median, 5.8 log copies/mL) and those in patients with progression (median, 6.2 log copies/mL; $P = .51$) were also similar (Figure 4).

DISCUSSION

We performed a case-control study to investigate the factors associated with HEV infection in SOT recipients living in southwestern France. Multivariate analysis indicated that the consumption of game meat was the only factor associated with HEV infection in this population. Two main HEV genotypes were identified, genotypes 3f and 3c, but the plasma HEV RNA concentrations, serological profiles, and frequencies of progression to a chronic infection were all similar between these 2 genotypes.

The majority of the patients studied were infected with HEV genotype 3f, which is also the most prevalent genotype in southwestern France [32]. HEV genotype 3c infections were reported only after 2006, which indicates that this genotype has emerged recently in southwestern France. Until now, chronic infections have been associated only with HEV genotype 3, which is the main genotype found in industrialized countries [17, 19, 22–25]. Chronic infection with the genotypes found in tropical and subtropical areas, genotypes 1 and 2, remains to be investigated. Phylogenetic analyses based on 2 distinct regions of the HEV genome demonstrated that 3 HEV genotype 3c strains and 2 genotype 3f strains were genetically very similar. We have looked for a possible epidemiological link between the patients infected with genetically close HEV strains. The temporal relationships and medical records of 2 patients infected with genotype 3c were compatible with nosocomial transmission, as described elsewhere [20]. However, no epidemiological link was found for the other patients. Therefore, the diet and the environment are potential sources of infection.

Bivariate analysis indicated that the infected patients ate mussels more frequently than did the control participants. The consumption of shellfish was associated with an outbreak of HEV among passengers on a cruise ship [33]. The HEV genotype 3 genome has been identified in river bivalves in Japan

[34], and HEV RNA has been detected in sewage water and rivers in France and other European countries [13, 14]. However, the detection of HEV RNA in mussels has never been reported in Europe. The infected patients ate pork meat products more frequently than did the control participants. Several of these pork products, especially sausages, are prepared with pig liver in Southern France, and HEV genotypes 3f and 3e have been found among piglets on farms [35]. Finally, our bivariate and multivariate analyses have identified the consumption of game meat as a factor associated with autochthonous HEV infection. Hunting was found to be the only factor associated with the presence of anti-HEV antibodies among blood donors in southwestern France [36]. A German study also identified the consumption of wild boar meat and offal as independent factors associated with HEV infection [37]. HEV infections due to the consumption of wild boar or deer meat have also been documented [11, 12, 38]. HEV genotype 3f was recently found in wild boar in Southern France [39], as was genotype 3c (F.L.-A. et al, unpublished data, 2008).

The ALT levels in acute-phase SOT recipients varied (0.5–26 times the normal value), as did the AST levels (0.6–13 times the normal value). In contrast, the ALT and AST levels in immunocompetent patients with a genotype 3 HEV infection were higher than those among SOT recipients (4–150 and 1.5–151 times the normal value, respectively) [32]. Although icterus was observed in 68% of immunocompetent patients [32], only 24% of the SOT recipients had abnormal total bilirubin concentrations. Two of our patients had normal aminotransferase levels at the onset of the disease but their γ GT levels were abnormal. Any abnormal liver enzyme activity in this population should therefore lead to a check for an HEV infection. However, we may have missed other cases of HEV infection among patients with normal liver enzyme activity.

Although a high HEV load has been associated with more severe disease among pregnant women [29, 40], we found no correlation between the acute-phase HEV RNA concentration and the aminotransferase levels, the total bilirubin concentration, or the evolution to a chronic infection in our population.

Most (80%) of the SOT recipients tested positive for anti-HEV IgM during the acute phase. Thus, the results of the IgM test among immunocompromised patients and those among immunocompetent patients appear to be very similar [28]. Anti-HEV IgM persisted for 12–29 months in patients with a resolving infection, whereas it has been reported that anti-HEV IgM generally remains detectable in samples from immunocompetent patients for 2 weeks to 3 months after the acute phase [41]. Anti-HEV IgM also persisted in all of our patients who had a chronic infection. Specific IgM antibodies also persist in patients chronically infected with hepatitis delta virus [42] and hepatitis C virus (HCV) [43, 44]. The IgG and IgM se-

rological kits are both microplates coated with the same HEV-specific synthetic antigens. For samples from immunocompetent patients, the performance of the EIAgen HEV IgM kit (Adaltis) was similar to that of HEV IgM enzyme-linked immunosorbent assay kit (version 3.0; MP Diagnostics, formerly Genelabs) [28]. Both tests were fairly sensitive (90% and 88%, respectively) and extremely specific (100% and 99.5%, respectively). Tests of samples from 23 immunocompromised patients showed that the EIAgen HEV IgM kit (Adaltis) and the IgM enzyme-linked immunosorbent assay kit (version 3.0; MP Diagnostics) had similar sensitivity (82% and 70%, respectively; F.L.-A. et al, unpublished data, 2009). However, other serological kits may be better at diagnosing HEV infections [24].

We also investigated the biological, immunological, and pharmacological factors involved in the persistence of HEV in immunocompromised patients. We found that the aminotransferase levels in patients whose illness progressed to chronic hepatitis were lower than those in patients who cleared the virus. This is consistent with the natural course of infections with other hepatitis viruses, such as HCV [45]. The subsets of immune cells in patients who resolved the infection and in those who developed a chronic infection were also similar. An in-depth study of the host response is needed to identify the immune factors associated with the clearance of the virus, because the results of a previous study with fewer patients indicated that the total lymphocyte and CD4 lymphocyte counts were lower in patients who developed a chronic infection [18].

As reported elsewhere for kidney transplant recipients with a chronic HCV infection [46], the pattern of fibrosis progression in SOT recipients with a chronic HEV infection varied. The fibrosis progressed more rapidly in SOT recipients infected with HEV (0.6 ± 0.7 Metavir units per year) than in the kidney transplant recipients infected with HCV (0.09 ± 0.03 Metavir units per year) [46]. The mechanisms underlying this phenomenon need further investigation.

Unfortunately, no vaccine is yet available for HEV, but successful phase II vaccine trials have been reported [47]. Once available, vaccination could be useful for patients on transplant waiting lists, as is vaccination against other hepatotropic viruses [48].

In conclusion, the consumption of game meat is the main factor associated with the transmission of HEV to SOT recipients in southwestern France. Direct contact with a contaminated environment could be another route of transmission. Thorough cooking of game meat and pork products and improved information on HCV transmission would help minimize the risk of HEV infection. These preventive measures are particularly important because of the high risk of chronic, severe liver disease. Further studies are needed to elucidate the factors involved in the persistence of HEV in this population.

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