Systematic Serological Testing for Hepatitis E Virus in Kidney Transplant Recipients

Valérie Moal,a,b Tristan Legris,a Anne Motte,a Henri Vacher-Coponat,a–d Lucie Fages,a Noémie Jourde-Chiche,a–d Patrick Borentain,a Dominique Jaubert,a René Gerolami,c,e Philippe Colson,b,c–e
Centre de Néphrologie et Transplantation Rénale, Assistance Publique–Hôpitaux de Marseille, Marseille, France; URMI'TE, UMR6 CNRS 198, Aix-Marseille Université, Marseille, France; Fondation HU Méditerranée Infection, Pôle des Maladies Infectieuses et Tropicales Clinique et Biologique, Fédération de Bactériologie-Hygiène-Virologie, Centre Hospitalo-Universitaire Timone, Assistance Publique–Hôpitaux de Marseille, Marseille, France; Aix-Marseille Université, Marseille, France; Service d’Hépato-Gastroentérologie, Assistance Publique–Hôpitaux de Marseille, Marseille, France.

Hepatitis E virus (HEV) genotype 3 is endemic in Europe and hyperendemic in southern France. Recent reports of a high prevalence of HEV RNA in blood donations and in culinary specialties from this geographical area confirmed the endemicity of HEV and sources of viral transmission in this geographical area. HEV causes acute and chronic hepatitis in solid organ transplant recipients. Since March 2012, we have implemented systematic HEV serological testing in our cohort of kidney transplant recipients (KTRs) in Marseille in southeastern France. The aim of our study was to assess HEV exposure in this cohort between March 2012 and May 2014. During these 27 months, we found that 39% of the patients who underwent kidney transplantation had an anti-HEV IgG response using a sensitive microplate enzyme immunoassay. This seroprevalence was approximately 43% at both 1 and 8 years after, using the same assay. In addition, systematic HEV serological testing detected 6 cases of HEV infection among 578 KTRs (1%) during the 27 months of the study, with 5 at an acute stage and 1 at a chronic stage. In conclusion, continuous HEV monitoring in this population is useful for better understanding the epidemiology of HEV in France, because these patients are a well-monitored population. Moreover, HEV monitoring in KTRs is clinically relevant because HEV represents a clinical threat in these patients. Nevertheless, HEV serological testing may be more fruitful for identifying HEV infections when performed in cases of biological liver abnormalities than when performed systematically.
exposure in our cohort of KTRs at Marseille University Hospital. In this study, we analyzed the patterns of HEV serologies that were systematically performed concurrently with routine blood sampling on the day of the transplantation, 1 year after kidney transplantation (KT), and at each annual or biennial general health assessment after.

MATERIALS AND METHODS

Patients. All of the KTRs included in the study were those followed up at Marseille University Hospital and systematically tested for HEV at our institution, which performs approximately 120 KT/year (3.9% of those performed in France [26]) and follows a cohort of approximately 1,600 KTRs (4.3% of the French KTRs [26]). The patients who received transplants at our institution received immunosuppressive induction therapy. Their initial maintenance immunosuppressive regimen consisted of prednisone, antimitabolite, and calcineurin inhibitor. The standard protocol for reducing the immunosuppressant doses consisted of progressive decreases in the dose of prednisone and the target whole-blood trough level of the calcineurin inhibitor. The antimitabolite dose was decreased according to the drug tolerance. At Marseille University Hospital, we perform a general health assessment for each KTR at the end of the first year posttransplantation and then every year or 2 years after. KTRs had the choice to perform their assessment in or outside our institution. In March 2012, systematic HEV serological testing was added to the blood analyses of this general health assessment and those performed on the day of the transplantation. In addition, the alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (GGT) levels were ideally measured for each blood sample collected during the first year posttransplantation and three times per year thereafter. Acute HEV infection was defined as resolution within 6 months after diagnosis. Chronic HEV infection was defined by the persistence of HEV RNA detected beyond 6 months.

Methods. We analyzed HEV serology results performed systematically at Marseille University Hospital between 1 March 2012 and 31 May 2014 in the setting of blood analyses (i) on the day of the transplantation a few hours before, (ii) 1 year posttransplantation, and (iii) at each annual or biennial general health assessment after. In the third case, when the KTRs had more than one systematic HEV serological test, we took into account the most recent serology result. Serological testing for anti-HEV IgG and IgM responses was performed during the study period with two anti-HEV MEIAs. From 1 March 2012 to 27 January 2013, we used Adaltis MEIAs (Eligen; Adaltis, Casalecchio di Reno, Italy). Whereas a high anti-IgM sensitivity was demonstrated for these MEIAs, the sensitivity for anti-HEV IgG detection was demonstrated to be far higher with the Wantai MEIAs (Wantai Biologic Pharmacy Enterprise, Beijing, People’s Republic of China) (27). This led us to the replacement for the purpose of HEV serological testing at our clinical microbiology laboratory of the Adaltis assays by the Wantai assays, which were then used since 28 January 2013. The tests were performed according to the manufacturers’ instructions. The results were positive for a signal-to-cutoff ratio (SCR) of >1.1, negative for an SCR of <0.9, and around the positivity threshold for an SCR of ≥0.9 and ≤1.1. Serum samples were tested for HEV RNA in the cases of IgM positivity using a real-time reverse transcriptase PCR assay targeting a fragment of the open reading frame 2 (ORF2) region of the HEV genome, and by sequencing, as described previously (28). The estimated detection limit of the assay was 2.7 log_{10} copies/mL HEV RNA detection in serum indicated an ongoing infection. Isolated anti-HEV IgG detection indicated a past infection, and IgM positivity indicated a recent infection or a false-positive result in case of absence of concurrent HEV RNA. The HEV RNA genotype was determined by phylogenetic reconstruction with the most appropriate model using the MEGA 6 software (http://www.megasoftware.net/), HEV sequences of a known genotype and subtype, and the two best matches from GenBank for each of the sequences obtained here (28).

Nucleotide sequence accession numbers. The HEV sequences obtained in our laboratory are available from GenBank under accession numbers KP298697 to KP298704.

RESULTS

HEV IgG prevalence at the time of kidney transplantation. A total of 262 KTs were performed between 1 March 2012 and 31 May 2014. On the day of transplantation, the serum was available for analysis for 221 of these KT. Two patients received 2 kidney transplants during the study period. Among the 219 KTRs, 80 (37%) were women and 139 (63%) were men. The median age of the patients at KT was 55 years (range, 17 to 83 years). Anti-HEV IgG testing was performed for the first 76 KT cases of the study period using the Adaltis assay and for the last 145 KT cases of the study period using the Wantai assay. Anti-HEV IgG was present in 34% (75/221) of the serum samples. The prevalence of HEV IgG was 25% (19/76) using the Adaltis assay and 39% (56/145) using the Wantai assay. Twelve of these 75 anti-HEV IgG-positive serum samples also tested positive for anti-HEV IgM but not for HEV RNA. One patient had isolated anti-HEV IgM and no HEV RNA in the serum.

HEV IgG prevalence after kidney transplantation. (i) One year posttransplantation. We performed 142 KTs in 141 patients between 1 March 2012 and 31 May 2013. At the end of the study (on 31 May 2014), 8 KTRs had lost their transplant, 1 had lost his transplant but was retransplanted, and 2 died before the end of their first posttransplantation year. Among the 131 living KTRs with a functioning transplant, we analyzed those for whom systematic HEV serological testing was performed before the transplantation and at 1 year posttransplantation. Thus, we analyzed 79 patients (28 women and 51 men) whose median age at HEV testing was 52 years (range, 22 to 74 years) and who were transplanted a mean ± standard deviation time of 402 ± 423 days (range, 317 to 503 days) before (Fig. 1).

HEV serological testing at 1 year posttransplantation was performed using Wantai assays in all cases (Fig. 1). Anti-HEV IgG was present in 44% of the KTRs (n = 35). Three of these 35 anti-HEV IgG-positive KTRs were also anti-HEV IgM positive or had anti-HEV IgM around the positivity threshold, but they harbored no detectable HEV RNA in their serum. Among the KTRs in whom anti-HEV IgG was detected 1 year posttransplantation with the Wantai assay, 50% (n = 17) were anti-HEV IgG negative on the day of the transplantation using the Adaltis assay. These results suggested either IgG seroconversion 1 year posttransplantation or a false-negative result on the Adaltis test on the day of the transplantation. Using the Wantai IgG assay, we retrospectively tested 14 serum samples that were collected at the day of transplantation and were still available for 14 of these 17 KTRs. The 14 serum samples that tested negative for anti-HEV IgG using the Adaltis assay on the day of the transplantation were anti-HEV IgG positive using the Wantai assay. Based on the results from previous studies (27, 29), the results of IgG testing obtained with the Adaltis test for these 14 serum samples collected at the time of transplantation were considered false negative. In addition, 12 of these 17 KTRs had no detectable HEV RNA in the serum 1 year posttransplantation, and for the 5 remaining KTRs, retrospective HEV RNA testing could not be performed due to the absence of available serum. No IgG seroconversion was observed when using the same assay at the time of transplantation and 1 year thereafter. In contrast, 5 of the 18 KTRs (28%) who were anti-HEV IgG positive
using the Adaltis assay at the time of transplantation presented IgG seroreversion 1 year after. One of these patients concurrently presented IgM seroreversion.

(ii) More than 1 year posttransplantation. Systematic HEV serological testing was performed for 538 serum samples from 499 different patients, at a median time of 8.2 years after transplantation (range, 1.9 to 32.2 years), in the setting of annual or biennial general health assessments, excluding those performed at 1 year posttransplantation. Of these 499 KTRs, 181 (36%) were women and 318 (64%) were men. The median age of the KTRs at time of the HEV testing was 56 years (range, 20 to 87 years). Anti-HEV IgGs were tested in 189 cases using the Adaltis assay and in 310 cases using the Wantai assay (Table 1). The prevalence of anti-HEV IgG was 16% (30/189) when HEV testing was performed using the Adaltis assay between 1 March 2012 and 27 January 2013, and it was 42% (131/310) using the Wantai assay between 28 January 2013 and 31 May 2014.

(iii) Diagnosis of ongoing HEV infection. HEV RNA was detected in the serum samples from 6 of the 578 tested KTRs. No case of HEV infection was diagnosed during the first year posttransplantation. Fourteen of the 161 KTRs with anti-HEV IgG at >1 year posttransplantation also had anti-HEV IgM, and 4/14 had detectable HEV RNA in the serum (cases 1 to 4). Six of the 161

**TABLE 1** Anti-HEV IgG and IgM prevalences in kidney recipients transplanted for >1 year, according to serological assay used and year of testing

<table>
<thead>
<tr>
<th>Response by year of testing</th>
<th>Adaltis (n = 189)</th>
<th>Wantai (n = 310)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>173</td>
<td>0</td>
</tr>
<tr>
<td>IgG positive</td>
<td>27 (16)</td>
<td>79 (16)</td>
</tr>
<tr>
<td>IgM positive</td>
<td>7 (4)</td>
<td>4 (2)</td>
</tr>
<tr>
<td>IgM and HEV RNA positive</td>
<td>2 (29)</td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td>16</td>
<td>195</td>
</tr>
<tr>
<td>IgG positive</td>
<td>3 (19)</td>
<td>79 (16)</td>
</tr>
<tr>
<td>IgM positive</td>
<td>1 (6)</td>
<td>4 (2)</td>
</tr>
<tr>
<td>IgM and HEV RNA positive</td>
<td>0 (0)</td>
<td>1 (25)</td>
</tr>
<tr>
<td>2014</td>
<td>0</td>
<td>115</td>
</tr>
<tr>
<td>IgG positive</td>
<td>52 (45)</td>
<td></td>
</tr>
<tr>
<td>IgM positive</td>
<td>6 (3)</td>
<td></td>
</tr>
<tr>
<td>IgM and HEV RNA positive</td>
<td>1 (17)</td>
<td></td>
</tr>
<tr>
<td>Total IgG-positive KTRs*</td>
<td>30 (16)</td>
<td>131 (42)</td>
</tr>
</tbody>
</table>

* KTRs, kidney transplant recipients.
KTRs presenting with anti-HEV IgG for > 1 year posttransplantation had also anti-HEV IgM around the positivity threshold, and 1/6 had detectable HEV RNA in the serum (case 5). Finally, systematic HEV serological testing showed isolated anti-HEV IgM in 4/499 cases, and in one case, HEV RNA was detected in the serum (case 6).

At time of HEV infection diagnosis, the median patient age was 55 years (range, 52 to 61 years), and the median time since transplantation was 3 years (range, 3 to 8.8 years) (Table 2). The HEV infection was asymptomatic in 5 of the 6 cases (83%). Asthenia and abdominal pain were reported in case 1. At the time of HEV infection diagnosis, the liver enzyme levels were most often moderately increased, except in case 6, in whom they were within the usual ranges. This case is of particular interest because anti-HEV IgM and serum HEV RNA were detected just before the onset of hepatitis. Retrospectively, 4 of the 6 cases were found to present with moderate transaminitis or increased GGT level, which did not lead to testing for HEV in three cases. The fourth patient (case 5) presented with a chronic GGT level increase due to sarcoidosis with liver involvement, and an HEV infection had been repeatedly ruled out before annual systematic serological testing. An epidemiological investigation of HEV infections showed that no patient received a blood product transfusion in the months preceding HEV infection. No patient was a hunter or was professionally exposed to animals. Case 1 reported contact with sewage the month before the hepatitis onset, whereas case 1 consumed raw pig liver sausage, and another patient consumed cooked pig liver (Table 3). In case 1, previous HEV serology performed using the Adaltis assay in June 2012 (2 years before HEV infection diagnosis) indicated a past exposure profile, but this IgG positivity was

### Table 2: Characteristics of HEV-infected patients between March 2012 and May 2014 at Marseille University Hospital

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (yr)</th>
<th>Gender&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Time since kidney transplantation (yr)</th>
<th>ALT level (IU/liter)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>GGT level (IU/liter)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Total bilirubinemia (µmol/liter)&lt;sup&gt;d&lt;/sup&gt;</th>
<th>HEV RNA (log&lt;sub&gt;10&lt;/sub&gt; copies/ml)</th>
<th>IgM</th>
<th>IgG</th>
<th>HEV genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>61</td>
<td>M</td>
<td>8.8</td>
<td>N</td>
<td>118</td>
<td>N</td>
<td>5.3</td>
<td>+</td>
<td>+</td>
<td>3f</td>
</tr>
<tr>
<td>2</td>
<td>51</td>
<td>M</td>
<td>7.3</td>
<td>157</td>
<td>186</td>
<td>N</td>
<td>7.1</td>
<td>+</td>
<td>+</td>
<td>3f</td>
</tr>
<tr>
<td>3</td>
<td>54</td>
<td>M</td>
<td>3</td>
<td>70</td>
<td>93</td>
<td>N</td>
<td>6.2</td>
<td>+</td>
<td>+</td>
<td>3i</td>
</tr>
<tr>
<td>4</td>
<td>56</td>
<td>F</td>
<td>8</td>
<td>39</td>
<td>N</td>
<td>N</td>
<td>5.0</td>
<td>+</td>
<td>+</td>
<td>3f</td>
</tr>
<tr>
<td>5</td>
<td>56</td>
<td>M</td>
<td>3.8</td>
<td>174</td>
<td>523</td>
<td>N</td>
<td>3.4</td>
<td>T&lt;sup&gt;e&lt;/sup&gt;</td>
<td>+</td>
<td>3e</td>
</tr>
<tr>
<td>6</td>
<td>52</td>
<td>M</td>
<td>3</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>5.3</td>
<td>+</td>
<td>−</td>
<td>3f</td>
</tr>
</tbody>
</table>

**Cases diagnosed using systematic HEV serological HEV testing**

**Cases diagnosed in the presence of biological hepatitis**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (yr)</th>
<th>Gender&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Time since kidney transplantation (yr)</th>
<th>ALT level (IU/liter)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>GGT level (IU/liter)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Total bilirubinemia (µmol/liter)&lt;sup&gt;d&lt;/sup&gt;</th>
<th>HEV RNA (log&lt;sub&gt;10&lt;/sub&gt; copies/ml)</th>
<th>IgM</th>
<th>IgG</th>
<th>HEV genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34</td>
<td>M</td>
<td>3</td>
<td>96</td>
<td>65</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>2</td>
<td>58</td>
<td>F</td>
<td>7.2</td>
<td>63</td>
<td>262</td>
<td>N</td>
<td>6.9</td>
<td>+</td>
<td>+</td>
<td>3i</td>
</tr>
<tr>
<td>3</td>
<td>71</td>
<td>M</td>
<td>6</td>
<td>61</td>
<td>50</td>
<td>NT</td>
<td>4.2</td>
<td>+</td>
<td>+</td>
<td>3i</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Case</th>
<th>Cause of testing</th>
<th>Date of HEV infection diagnosis (mo/day/yr)</th>
<th>Time between HEV infection diagnosis and liver biological disturbances (days)</th>
<th>Status at last follow-up</th>
<th>Consumed pig liver sausage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Systematic</td>
<td>05/20/2014</td>
<td>32</td>
<td>Acute hepatitis</td>
<td>Yes, after 3.2 mo</td>
</tr>
<tr>
<td>2</td>
<td>Systematic</td>
<td>10/24/2012</td>
<td>33</td>
<td>Acute hepatitis</td>
<td>Yes, after 8 wk of ribavirin</td>
</tr>
<tr>
<td>3</td>
<td>Systematic</td>
<td>11/15/2013</td>
<td>856</td>
<td>Chronic hepatitis</td>
<td>Yes, after 7 wk of ribavirin</td>
</tr>
<tr>
<td>4</td>
<td>Systematic</td>
<td>04/10/2012</td>
<td>None</td>
<td>Chronic hepatitis</td>
<td>Yes, after 3 wk of ribavirin</td>
</tr>
<tr>
<td>5</td>
<td>Systematic</td>
<td>03/01/2012</td>
<td>Chronic increase in GGT, 51&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Chronic hepatitis</td>
<td>Yes, after 9 wk of ribavirin</td>
</tr>
<tr>
<td>6</td>
<td>Systematic</td>
<td>09/10/2012</td>
<td>None</td>
<td>Acute hepatitis</td>
<td>Yes, after 10 wk</td>
</tr>
<tr>
<td>7</td>
<td>Increase in ALT and GGT&lt;sup&gt;d&lt;/sup&gt;</td>
<td>01/17/2013</td>
<td>None</td>
<td>Acute hepatitis</td>
<td>Yes, after 13 wk</td>
</tr>
<tr>
<td>8</td>
<td>Increase in ALT and GGT&lt;sup&gt;d&lt;/sup&gt;</td>
<td>01/11/2013</td>
<td>Chronic increase in GGT&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Chronic hepatitis</td>
<td>Yes, after 8 wk of ribavirin</td>
</tr>
<tr>
<td>9</td>
<td>Increase in ALT and GGT&lt;sup&gt;d&lt;/sup&gt;</td>
<td>05/02/2013</td>
<td>227</td>
<td>Acute hepatitis</td>
<td>Yes, after 5.5 mo</td>
</tr>
</tbody>
</table>

<sup>a</sup> M, male; F, female.
<sup>b</sup> ALT, alanine aminotransferase level (reference values, 8 to 40 IU/liter); N, normal.
<sup>c</sup> GGT, gamma-glutamyl transpeptidase level (reference values, 10 to 50 IU/liter).
<sup>d</sup> Total bilirubin (reference values, <21 µmol/liter). NT, not tested.
<sup>e</sup> T, at threshold value.

### Table 3: Outcomes in HEV-infected patients between March 2012 and May 2014 at Marseille University Hospital

<table>
<thead>
<tr>
<th>Case</th>
<th>Cause of testing</th>
<th>Date of HEV infection diagnosis (mo/day/yr)</th>
<th>Time between HEV infection diagnosis and liver biological disturbances (days)</th>
<th>Status at last follow-up</th>
<th>Consumed pig liver sausage</th>
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<tr>
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<td>32</td>
<td>Acute hepatitis</td>
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<td>2</td>
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<td>33</td>
<td>Acute hepatitis</td>
<td>Yes, after 8 wk of ribavirin</td>
</tr>
<tr>
<td>3</td>
<td>Systematic</td>
<td>11/15/2013</td>
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</tr>
<tr>
<td>4</td>
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<td>04/10/2012</td>
<td>None</td>
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<td>Yes, after 3 wk of ribavirin</td>
</tr>
<tr>
<td>5</td>
<td>Systematic</td>
<td>03/01/2012</td>
<td>Chronic increase in GGT, 51&lt;sup&gt;e&lt;/sup&gt;</td>
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</tr>
<tr>
<td>6</td>
<td>Systematic</td>
<td>09/10/2012</td>
<td>None</td>
<td>Acute hepatitis</td>
<td>Yes, after 10 wk</td>
</tr>
<tr>
<td>7</td>
<td>Increase in ALT and GGT&lt;sup&gt;d&lt;/sup&gt;</td>
<td>01/17/2013</td>
<td>None</td>
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<tr>
<td>8</td>
<td>Increase in ALT and GGT&lt;sup&gt;d&lt;/sup&gt;</td>
<td>01/11/2013</td>
<td>Chronic increase in GGT&lt;sup&gt;*&lt;/sup&gt;</td>
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</tr>
<tr>
<td>9</td>
<td>Increase in ALT and GGT&lt;sup&gt;d&lt;/sup&gt;</td>
<td>05/02/2013</td>
<td>227</td>
<td>Acute hepatitis</td>
<td>Yes, after 5.5 mo</td>
</tr>
</tbody>
</table>

<sup>a</sup> HEV infection previously ruled out. GGT, gamma-glutamyl transpeptidase level (reference values, 10 to 50 IU/liter).
<sup>b</sup> Contact with sewage.
<sup>c</sup> Consumption of cooked pig liver in restaurant.
<sup>d</sup> ALT, alanine aminotransferase level (reference values, 8 to 40 IU/liter).
<sup>e</sup> Consumption of cooked wild boar liver and butchering of wild boar with bare hands.
not confirmed retrospectively using the Wantai assay and was considered a false-positive result. The patient, being a native of Corsica, where traditional pig liver sausages are commonly eaten, had been advised not to eat uncooked pig liver sausage. In 5 of the 6 cases, hepatitis E was diagnosed at an acute stage, and in case 3 at a chronic stage (Table 3). The HEV infection progressed toward chronicity in two of 4 cases (50%), with ribavirin treatment being initiated before the 6th month postinfection in one case (case 2) that was excluded to determine the rate of evolution toward a chronic HEV infection. We decreased the doses of immunosuppressive drugs in patients at the chronic stage before considering ribavirin treatment, except in case 4, who presented two important risk factors for acute allograft rejection (donor-specific antibodies and a second kidney transplant). Despite the reduction in immunosuppression, HEV infection persisted, and ribavirin treatment was introduced. In the 4 cases treated with ribavirin, hepatitis E infection resolved after a mean duration of 7 weeks (Table 3). Finally, in case 1, as determined retrospectively, the HEV RNA load in serum continuously decreased from the beginning of steroid therapy with a high cumulative dose of methylprednisolone (2,520 mg) to treat a suspected acute allograft rejection (Table 3).

It is worthy to note that three additional cases of HEV infections were diagnosed in our cohort of KTRs during the same period. The 3 KTRs presented with liver biological disturbances leading to the detection of anti-HEV IgM in all cases and HEV RNA in 2 cases (described in Tables 2 and 3 after the 6 cases of HEV infections diagnosed through systematic HEV serological testing).

**Phylogenetic analysis of HEV sequences.** The 8 HEV sequences obtained here belonged to genotypes 3i, 3f, or 3e, which were previously described in autochthonous cases in our geographical area and in Europe (Table 2 and Fig. 2) (28). The sequences from GenBank that were the most similar to these sequences were recovered from humans, pigs, and pig liver sausages from samples collected in France and Spain. The mean nucleotide identity with these best matches was 96.9% (range, 94.1 to 99.3%). Interestingly, the best matches for the HEV sequences recovered from cases 1, 3, and 8 were obtained from pig liver sausages, with nucleotide identities of 95.2 to 99.3%. Particularly, the HEV sequence obtained from case 1 from a serum sample collected on 20 May 2014 showed only one nucleotide difference with those described recently from a human (from a sample collected on 20 December 2013) and a pig liver sausage (collected on 6 December 2013) in a case of HEV foodborne transmission (30).

**DISCUSSION**

The present study allowed us to gain a better insight into HEV epidemiology in our cohort of KTRs at Marseille University Hospital, allowing continuity with previous works. Here, we assessed a strategy based on systematic HEV serological testing and that led to new diagnoses of HEV infections besides those triggered by liver biological perturbations. Systematic HEV serological testing with the most sensitive IgG MEIA, as established earlier (19, 27, 29), showed a more accurate picture of hepatitis E exposure in our cohort of KTRs than that seen previously (12, 25). The prevalence of HEV IgG (approximately 40%) that we observed is among the highest reported, regardless of the tested population or geographical area (see Table S1 in the supplemental material) (19, 31–35), and is in line with our previous data (12, 25). A previous national survey in France showed an increasing north-to-south gradient of acute hepatitis E infection (36). However, recent data from the French Blood Agency (13) showed that the Alpes-Méditerranéenne region, where Marseille is located, and the Midi-Pyrénées region, where the prevalence of HEV IgG in blood donors is the highest in Europe (52%) (19), were the regions with the third and fifth highest HEV RNA detection rates of blood donations, behind some regions in northern France. These data suggest that the epidemiology of HEV in France is still not fully understood. Of note, our study highlighted the stability of HEV seroprevalence according to the duration of kidney transplantation (up to 8 years postgraft), which suggests the absence of a major impact of kidney transplantation on HEV exposure.

We diagnosed HEV infections in 6 cases during a systematic posttransplant check-up by detecting anti-HEV IgM around or above the positivity threshold. HEV infections were ongoing, as confirmed by the presence of HEV RNA in the serum samples from these 6 cases. The rate of chronic HEV infection evolution was 60% (3/5 cases), as defined by the persistence of HEV RNA in the serum for >6 months. We identify three highlights of this case series. First, the HEV infections most likely could not have been diagnosed without systematic HEV serological testing in two cases, the case with normal liver enzyme levels (case 6) and the case with chronic liver biological disturbances, who was tested several times for HEV (case 5). Second, one case of infection resolved at the acute stage after the patient received high doses of steroids (case 1). At first glance, we would think that this immunosuppressive treatment would have worsened acute hepatitis E. In our case and in another case of acute hepatitis E recently reported by Sebode et al. (37) and in whom lower doses of steroids (up to 700 mg/day versus 100 mg/day) were used, steroid treatment did not seem to impair HEV clearance. Further observations are required to evaluate whether steroid treatment improves the outcome of infection. Finally, 4 cases out of 6 were treated with ribavirin for chronic and (one) acute HEV infection, and the infections resolved. This last point supports the recent suggestions of Hewitt et al. (38) to screen all transplant patients yearly for persistent HEV infection, with an option to treat those who are chronically infected, independent of the route of infection. Another lesson learned from this case series is that mild biological liver abnormalities lead nephrologists more often to order hepatitis B and C than hepatitis E testing, whereas in the same period as this study, we diagnosed only one case of hepatitis C relapse. Besides, we previously reported that HEV was the most frequent cause of autochthonous acute infections with hepatitis viruses diagnosed in adults in 2008 at Marseille University Hospital, being responsible for 51% of cases (39). The mandatory reporting of cases of hepatitis A, B, C, and E would provide a better understanding of the epidemiology and consequences in terms of the morbidity and mortality of these viruses in France.

In the same period as the study, we diagnosed HEV infections in 3 cases because of liver biological disturbances by detecting serum anti-HEV IgM in all 3 of the cases and the presence of HEV RNA in 2 cases (Tables 2 and 3). These findings suggest that in KTRs, HEV testing seems more useful for diagnosing infections if liver biological disturbances exist rather than when this testing is performed systematically. As the incidence of HEV RNA positivity among 53,234 blood donations was recently reported to be 0.045% (1/2,218) in France and 0.087% (2/2,300) in southeastern France, and most viremic blood donors (92%) were concurrently...
FIG 2 Phylogenetic tree based on a 307-nucleotide partial sequence corresponding to nucleotides 6044 to 6336 of open reading frame 2 (ORF2) of the HEV genome (GenBank accession no. AF082843). The HEV sequences obtained in our laboratory are indicated by a black frame. The 2 sequences with the highest BLAST scores recovered from the NCBI GenBank nucleotide sequence database (indicated in bold type and labeled with BH [best BLAST hit], GenBank accession number, host, country, and year of sample collection or sequence submission) with the ORF2 fragment from HEV sequences obtained in this study have been incorporated into the phylogeny reconstruction, in addition to a set of partial ORF2 fragments from full-length HEV genomes that were downloaded from the Virus Pathogen Database (41) (labeled with genotype and subtype, GenBank accession number, host, country, and year of sample collection or sequence submission). Nucleotide alignments were performed using the MUSCLE software (http://www.ebi.ac.uk/Tools/msa/muscle/). The evolutionary history was inferred in the MEGA 6 software (http://www.megasoftware.net/) following the determination of the best nucleotide substitution models and a comparison of the output trees using the neighbor-joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree; the scale bar indicates the number of nucleotide substitutions per site. The evolutionary distances were computed using the Kimura 2-parameter method, and the unit is the number of base substitutions per site. The analysis involved 41 nucleotide sequences. Bootstrap values of >50% are labeled on the tree. The avian HEV sequence GenBank accession no. AM943646 was used as an outgroup.
negative for IgM and IgG responses to HEV (13), our results obtained for 838 systematic HEV testing are not unexpected.

Four of the 9 cases of hepatitis E that we diagnosed during the 27 months of the study reported eating wild boar liver, pig liver, or pig liver sausage. This demonstrates that the recommendations of not consuming even cooked pork liver products (fresh or dried liver sausage, dry liver, faggot and liver dumplings), wild boar products, or deer products (meat and offal) are probably not sufficiently disseminated by physicians and health authorities and/or not sufficiently followed by patients (15, 40). Nevertheless, the source of HEV remains unknown for the other patients. Further studies are necessary to identify additional sources and transmission routes of HEV.

In conclusion, systematic HEV serological testing showed evidence of previous exposure to this virus in 40% of the KTRs followed up in Marseille and allowed the diagnosis of 6 HEV acute or chronic infections. Continuous HEV monitoring in KTRs is useful to better understand the epidemiology of HEV in France, because KTRs are a well-studied and monitored population. Moreover, HEV monitoring in KTRs is clinically relevant because HEV represents a clinical threat in these patients. Nevertheless, HEV serological testing may be more fruitful for identifying HEV infections when performed in cases of biological liver abnormalities than when performed systematically. Finally, KTRs must be aware they should not eat pig liver sausage.

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We declare no conflicts of interest.

REFERENCES


Systematic HEV Testing in Kidney Transplantation

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