Cluster of Cases of Acute Hepatitis Associated with Hepatitis E Virus Infection Acquired in The Netherlands

Marc-Alain Widdowson,1,2 Wim J. M. Jaspers,3 Wim H. M. van der Poel,2 Froukje Verschoor,2 Ana Maria de Roda Husman,2 H. L. J. Winter,4 Hans L. Zaaier,5 and Marion Koopmans2

1European Programme for Intervention Epidemiology and Training and 2National Institute for Public Health and the Environment, Bilthoven, 3St. Lucas Hospital, Winschoten, 4Regional Public Health Laboratory for Groningen and Drenthe, Groningen, and 5Sanquin–Central Laboratory of the Blood Transfusion Service, Amsterdam, The Netherlands

Increasing evidence suggests that hepatitis E virus (HEV) infection may occur in developed countries and that swine may act as a reservoir. We report a cluster of 2 confirmed cases and 1 presumptive case of hepatitis associated with HEV. The typed strain from 1 case was related to HEV strains found in North America and Europe, and it was also related to a cluster of swine HEV strains found in The Netherlands. Our findings indicate that locally acquired HEV infections in industrialized countries may be overlooked. Routine testing for HEV infection in patients with acute hepatitis in The Netherlands should be considered before a diagnosis of autoimmune hepatitis is reached and steroid therapy is initiated.

Hepatitis E virus (HEV) is a major cause of enterically transmitted non-A, non-B hepatitis in poor countries, often presenting as large outbreaks of hepatitis in which fecally contaminated water is usually implicated [1]. HEV infection is also considered to be a major cause of sporadic viral hepatitis in these countries, but the source of infection is rarely found [2]. Clinical cases of HEV infection in developed countries have generally been associated with travel to tropical or subtropical countries [3, 4]. Serosurveys with recently developed assays for HEV antibody, however, have consistently indicated a low seroprevalence of antibodies to HEV (1%–5%) in countries where HEV infection is not thought to be endemic [4–8]. There have also been an increasing number of reports from Europe and the United States of sporadic hepatitis attributable to HEV but not associated with travel [4, 9–14], leading to suggestions that HEV may be endemic at low levels in developed countries. As yet, however, no outbreak of HEV infection has been reported from developed countries. Strains of HEV related to those that infect humans have been found in swine in The Netherlands [15] and in the United States [16], which raises the possibility that swine may act a reservoir of infection for humans.

On 2 July 2001, a regional microbiological laboratory in the north of The Netherlands notified the National Institute for Public Health of 3 elderly people who presented to the same local hospital with acute hepatitis and that swine may act as a reservoir. We describe the investigation of this cluster of 3 cases of hepatitis.

METHODS

Epidemiology. Clinical records of the 3 patients were reviewed and additional blood and stool samples were obtained. Patients were interviewed at home with a questionnaire to gather information on relevant exposures. Informed consent was obtained from all 3 pa-
patients. The water company that supplied all 3 patients’ homes was contacted about possible problems concerning the water quality in the months preceding the patients’ illnesses.

**Laboratory studies.** All patients had negative results of routine laboratory tests for IgM antibodies to hepatitis A virus, for hepatitis B surface antigen and antibodies to hepatitis B core antigen, for IgM antibodies to Epstein-Barr virus and cytomegalovirus, and for IgG antibodies to hepatitis C virus and Coxiella burnetti.

Three commercial EIAs were used for serological diagnosis of hepatitis E: 2 assays that tested for IgG antibodies (HEV IgG ELISA [Genelabs Diagnostics] and Abbott HEV EIA [Abbott Laboratories]) and 1 that tested for IgM antibodies (HEV IgM ELISA [Genelabs Diagnostics]). All tests were done twice, and only repeatedly reactive samples were considered to be positive for any 1 test, as recommended by the manufacturers. For confirmation, the first sample obtained from each patient was tested by use of an immunoblot assay (Mikrogen) featuring 4 recombinant (Escherichia coli) HEV antigens.

RNA was extracted from serum and stool samples. RT-PCR techniques were used to detect and sequence a 197-bp segment from open-reading frame (ORF) 2 of the HEV genome followed by phylogenetic analysis, by means of methods described elsewhere [15].

**RESULTS**

**Clinical characteristics.** Two cases (cases 1 and 3) occurred in women aged 80 and 82 years, respectively, and the other (case 2) occurred in an 84-year-old man. Dates of onset of symptoms of jaundice and/or vomiting were 8 December 2000 for case patient 1, 14 May 2001 for case patient 2, and 30 May 2001 for case patient 3. Both case patients 1 and 3 presented with jaundice, and case patient 3 additionally presented with nausea and vomiting. Case patients 1 and 3 were hospitalized for 9 and 7 days, respectively, and they received supportive treatment. Case patient 2 presented with nausea and vomiting but not jaundice and was treated as an outpatient. Liver enzyme and total bilirubin levels were elevated in all patients. According to their medical records, the 2 patients with icterus (case patients 1 and 3) took 2 months to recover completely, whereas the nonicteric male patient (case patient 2) recovered in 3 weeks.

**Laboratory findings.** The 3 patients had positive results for IgG antibodies to HEV by both commercial assays. A blood sample obtained from case patient 2 eight days after the onset of disease and a second sample obtained from case patient 3 three weeks after onset both tested positive for IgM antibody. A serum sample obtained from case patient 1 eight days after the onset of specific symptoms tested negative for IgM antibodies (table 1). EIA results that were positive for IgG were confirmed by use of the immunoblot assay in all 3 cases, but only 1 of the 2 EIA results positive for IgM was confirmed by use of the immunoblot assay.

Stool specimens were obtained from case patients 2 and 3 at 51 and 31 days after onset, respectively, and no virus was detected in either sample by RT-PCR. HEV RNA was detected by PCR in the acute-phase serum sample obtained from case patient 2. The sequence of the amplified segment of ORF2 was found to be closely related to 1 of the 2 subclusters of Dutch swine strains within genotype III (figure 1). There was 11.49% nucleotide difference from the closest Dutch porcine strain (NL-Swine15) and 6.75% nucleotide difference from the nearest human strain (Greece1).

**Epidemiologic investigation.** All 3 case patients lived ≤13 km from each other in the northeast of The Netherlands (figure 2). No obvious risk factor for infection was evident from the completed questionnaires. The case patients lived separately and

<table>
<thead>
<tr>
<th>Patient</th>
<th>Date of onset</th>
<th>Date on which blood sample was obtained</th>
<th>Abbott HEV EIA</th>
<th>HEV IgG ELISA</th>
<th>Immunoblot assay</th>
<th>HEV IgM ELISA</th>
<th>Immunoblot assay</th>
<th>RT-PCR result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8 Dec 2000</td>
<td>1 Feb 2001</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>ND</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 Jul 2001</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>−</td>
</tr>
<tr>
<td>2</td>
<td>14 May 2001</td>
<td>22 May 2001</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24 Jul 2001</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>−</td>
</tr>
<tr>
<td>3</td>
<td>30 May 2001</td>
<td>19 Jun 2001</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±e</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 Jul 2001</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>−</td>
</tr>
</tbody>
</table>

**NOTE.** ND, not done; +, positive; −, negative.

a All samples with initial positive results were tested a second time and in duplicate before the results were confirmed to be positive.
b Manufactured by Abbott Laboratories.
c Manufactured by Genelabs Diagnostics.
d Serum sample was used up.
e Weak reactivity was observed.
Figure 1. Unrooted tree phylogenetic showing relationships among selected human and pig strains of hepatitis E virus (HEV) based on a 197-bp sequence of open-reading frame (ORF) 2. The sequence from case 2 (NL-Groningen) is depicted with prototype Dutch swine strains (NL-Swine22 and NL-Swine15) along with foreign isolates that cluster with those sequences, as well as prototype isolates from different clusters. Numbers represent branch lengths, which are proportional to the evolutionary distance between sequences. US human and pig HEV strains: Us1, Us2, and US-Swine (GenBank accession nos. AF060668, AF060669, and AF082843, respectively); Greek human HEV strains: Greece1 and Greece2 (accession nos. AF110391 and AF110392, respectively); Argentinean human isolate: Argentina2 (accession no. AF264012); Burmese human isolates: Burma1 and Burma2 (accession nos. M73218 and D10330, respectively); Italian human strain: Italy1 (accession no. AF110390); Pakistani human strain: Pakistan (accession no. M80581); Mexican human strain: Mexico (accession no. M74506); Chinese human strains: China3, ChinaT1, and China4 (accession nos. D11092, AJ272108, and D11093, respectively); Indian human isolates: India1, India2, and India3 (accession nos. X98292, X99441, and AF124407, respectively); Nepalese human strain: Nepal (accession no. AF051830).

did not know each other. There was no history of travel outside The Netherlands in the 4 months before the onset of illness. The patients reported drinking tap water, and there was no history of eating shellfish. One patient had eaten out in a restaurant in the 3 months before the onset of illness. None of the case patients lived on a farm or near any livestock, and no problems with rats were reported. Case patient 1 had regular contact with a dog, case patient 2 had regular contact with a cat, and case patient 3 had occasional contact with a goat. None of the case patients had undergone any operations in the 3 months before the illness, nor had they received blood or blood products or any injections other than influenza vaccination. No family member or close contact of the case patients was reported to have been ill in the 3 months before the patient’s illness. The one water company that supplied all 3 homes reported no bacteriologic contamination of the water supply during routine testing (which was done in compliance with Dutch drinking water regulations) in the months preceding these cases.

DISCUSSION

We report 3 cases of locally acquired acute hepatitis due to HEV in elderly patients. The cases occurred within 6 months of each other and in a small geographic area in The Netherlands. The HEV strain that infected 1 patient was shown to be closely related to swine HEV strains occurring in The Netherlands. Previous reports of HEV infection in developed countries have involved single, epidemiologically unrelated cases [4, 9–12].
Although neither viral RNA nor IgM antibodies were detected in the samples obtained from case patient 1, a presumptive diagnosis of HEV infection was made on the basis of the following findings: presence of IgG antibodies detected by 2 ELISAs with different HEV antigens, with results confirmed by IgG reactivity against all 4 HEV antigens in an immunoblot assay; clinical symptoms; exclusion of other infectious causes of hepatitis; and resolution of symptoms after receipt of supportive treatment that did not include steroids. The absence of detectable IgM antibodies in the sample obtained from case patient 1 eight weeks after the onset of disease is not unexpected, because more than one-half of the patients with confirmed HEV infection have been shown to test seronegative for IgM antibodies 1 month after the onset of disease [17, 18].

Unfortunately, however, the absence of viral sequence data for case patients 1 and 3 did not make it possible to link the cases by means of molecular methods and to confirm the existence of an outbreak of infection. Nonetheless, the epidemiologic characteristics of this cluster suggest a link, because the case patients lived very close to each other and were infected in the space of 6 months (2 were infected in the same month). The advanced age of the case patients is also curious, because the attack rate of clinical infection in outbreaks is usually reported to be highest among young men [1].

Although the case patients reported no sick human close contacts, they may have been infected unwittingly from another clinical case patient or from a patient with a subclinical infection. Subclinical HEV infection acquired in The Netherlands has already been reported elsewhere [4], and, in countries in which HEV infection is endemic, serological evidence suggests that there are at least twice as many subclinical cases as clinical cases [19]. The cases may have been part of ongoing person-to-person transmission in The Netherlands and only detected by chance. Person-to-person transmission, however, has been shown to be uncommon in areas of endemicity [20] and would be expected to be especially so in a country with good sanitation systems. Another possibility is the occurrence of self-limiting bursts of infection in the population with HEV strains from swine, imported human cases, or, conceivably, from imported foodstuffs. Foodborne transmission has been reported, especially in China [1], and shellfish have also been implicated in transmission [21]. Contaminated water, the usual source in developing countries, remains a possible source of infection for these cases. Although no bacteriologic contamination of the water supply was reported in the months preceding these cases, HEV may have nonetheless been present, especially since it is unknown whether water treatment processes are effective against HEV.

The nucleotide sequence of the infecting strain from case 2 places it among genotype III with American and European human and swine sequences, and it is closely related to strains from Dutch swine and from cases in humans in Greece [15]. Genotype III is clearly distinct from genotypes I, II, and IV, which contain sequences from the Indian subcontinent, Mexico, and China, respectively. This raises the possibility that these patients were infected with strains that are endemic in Europe—either strains that are circulating among humans or “spillover” infections from a swine reservoir. In The Netherlands, 22% of 115 tested swine herds were found to be infected with HEV strains that are related to human HEV strains [15]. Cross-infectivity of swine HEV strains with nonhuman primates and human strains with swine has also been shown experimentally [22, 23].

Few laboratories perform diagnosis of HEV infection in The Netherlands, with most HEV testing being performed by the Central Laboratory of the Blood Transfusion Service (Amsterdam). In part, this is because commercial tests for detection of HEV have been developed for use in countries with a high prevalence of HEV infection, and the test results can be difficult to interpret in countries where the prevalence of infection is low [24]. In the 18 months before our cases occurred, the regional laboratory had diagnosed no other cases of HEV infection from samples obtained from 32 patients with acute hepatitis. It is possible, however, that the cluster is an artifact, because after our first case of HEV infection was diagnosed, the regional laboratory and hospital physician may have been more inclined to perform HEV testing for other patients with acute hepatitis.

Regardless of whether these cases were related, they indicate that cases of locally acquired hepatitis caused by European strains of HEV may be occurring undiagnosed in The Nether-
lands. In cases of non-A, non-B acute hepatitis, routine testing for HEV infection should be considered (along with testing for Epstein-Barr virus, cytomegalovirus, and hepatitis C virus), even in the absence of history of foreign travel, and particularly before ruling out a diagnosis of viral hepatitis and considering a diagnosis of autoimmune hepatitis with possible steroid treatment. Commercial tests for reliable detection of HEV infection in areas of low prevalence need to be developed. Further studies are required to examine the epidemiology of HEV in developed countries and to determine whether swine strains of HEV can infect humans.

Acknowledgments

We thank Dr. J. Schellekens and Henk Hooft, for performing initial serological testing, Dr. R. Koot (Sanquin-Central Laboratory of the Blood Transfusion Service, Amsterdam), for performing confirmatory testing, and the municipal health authority of Groningen, for interviewing case patients.

References