Table 3
Comparison of nucleotide sequences between HEV isolates recovered from patients with hepatitis E and those from pigs in the country where the patients lived

Country	HEV isolate from		Nucleotide sequence		HEV genotype
	Human	Pig	Length compared (nt)	Identity (%)	
USA	US2	Meng	7242	91.8	3
Taiwan	TW8E-2	TW11SW	304	99.0	4
Spain	VH2	E11	304	94.0	3
China	T1	G8	300	99.3	4
Japan	HE-JA1 (Hokkaido)	swJ13-1 (Hokkaido)	7240	99.0	4
	HE-JA18 (Hokkaido)	swJL145 (Hokkaido, raw liver[a])	824	100	4
	HE-JA9 (Fukushima)	swJ18-3 (Akita)	412	98.8	3 (IIIjp[b] [3b][c])
	HE-JA4 (Hokkaido)	swJL234 (Hokkaido, raw liver[a])	824	98.9–100	3 (IIIus[b] [3a])
	HE-JA7 (Iwate)	swJIW9 (Iwate)	412	98.8	3 (IIIus [3a])
	HEV-Sendai (Miyagi)	swJ791 (Tochigi)	412	98.3	3 (IIIsp[b] [3e])

[a] HEV isolate recovered from raw pig liver sold in grocery stores as food in Hokkaido.
[b] Tentative naming of three different clusters for Japanese genotype 3 HEV isolates used only in the report by M. Takahashi et al. (2003).
[c] In accordance with the provisional designation of HEV subgenotypes by Lu et al. (2006).

patients with hepatitis E in Hokkaido. Of interest, one swine HEV isolate (swJL145) obtained from a packaged pig liver was 100% identical to the virus recovered from an 86-year-old patient who had contracted sporadic hepatitis E after ingestion of undercooked pig liver (Table 3). Retrospective interviews indicated that none of 22 randomly selected patients with acute hepatitis of non-E etiology at the same two hospitals reported having consumed pig livers and/or intestine before disease onset, suggesting that consumption of undercooked pig liver/intestine is a potential risk factor for HEV infection ($P < 0.01$) (Yazaki et al., 2003).

Tei et al. (2003) reported a small outbreak of hepatitis E in which four individuals in two families were infected by ingesting raw meat of an infected wild deer. An HEV sequence was detected from the leftover frozen deer meat, and found to have 99.7–100% nucleotide sequence identity to the viruses recovered from the four patients. Family members who ate no or little deer meat were not infected. Li et al. (2005) investigated a case of hepatitis E acquired after the person ate wild boar meat. Genotype 3 HEV RNA was detected in both the patient's serum and wild boar meat. These findings suggest that zoonotic food-borne transmission of HEV from wild boars and wild deer to humans plays an important role in the occurrence of cryptic hepatitis E in Japan. The HEV isolate (JMNG-Oki02C) that was identified from a wild mongoose in Okinawa, Japan, is 99.5% similar to a swine HEV isolate (swJOK1-1) obtained from a farm pig in Okinawa (Nakamura et al., 2006; Takahashi et al., 2005), suggesting the natural occurrence of cross-species infection of HEV between mongooses and pigs, similar to that between wild boar and deer (Takahashi et al., 2004b). To definitively assess the zoonotic risk of HEV infection in wild animals, it is important to isolate and fully characterize HEV strains from naturally infected animals, and to determine the prevalence of anti-HEV antibody in animal populations. Regarding HEV infection among wild deer, our recent study revealed that none of 132 wild deer in Japan including 2 deer with anti-HEV antibody had detectable HEV RNA (Sonoda et al., 2004), suggesting that HEV infection via consumption of raw meat or viscera from wild deer occurs very rarely. As for HEV in wild boars, although the prevalence of HEV antibody has been reported to be 25% (15/59) in Australia (Chandler et al., 1999) and 9% (3/35) in Japan (Sonoda et al., 2004), the genomic characteristics of boar HEV isolates are not fully understood. However, HEV RNA was detected in 3 (2.3%) of the 128 wild boars captured in 16 prefectures in Japan, and their full-length genomes have recently been characterized to be markedly heterogeneous (Nishizawa et al., 2005).

As of January 11, 2007, nucleotide sequences of 562 swine HEV isolates are retrievable from the GenBank/EMBL/DDBJ databases (Table 2). Among them, genotype 3 HEV accounts for 91% and genotype 4 HEV accounts for 9%. In addition, nucleotide sequences of 37 boar HEV isolates of genotypes 3 and 4, a deer HEV isolate of genotype 3, and a mongoose HEV isolate of genotype 3 are deposited. Although a single genotype 1 HEV isolate (CAM-3F15 [DQ145799]) recovered from swine feces in Cambodia and four genotype 1 HEV isolates (HEV66 [AY963777], HEV73 [AY963778], HEV77 [AY963779], and HEV78 [AY963780]) that are annotated to have been isolated from work horses in Cairo, Egypt, are deposited in the databases, detailed information on these isolates is not available.

5. Genomic variability of HEV

HEV sequences have been classified into four genotypes: genotype 1 consists of epidemic strains in developing countries in Asia and Africa; genotype 2 has been described in Mexico and Africa; genotype 3 HEV is widely distributed and has been isolated from sporadic cases of acute hepatitis E and/or domestic pigs in many countries in the world, except for countries in Africa; and genotype 4 contains strains from humans and/or domestic pigs exclusively in Asian countries (Table 1). A total of 75 entire or nearly entire HEV sequences are available for comparison, as of January 11, 2007 (Table 2). The phylogenetic tree constructed by the neighbor-joining method (Saitou and Nei, 1987) based on the entire genomic sequence of 75 HEV isolates indicates that 17 isolates segregate into genotype 1, 1 into genotype 2, 25 into genotype 3, and 32 into genotype 4 (Fig. 2). Pairwise comparison of these 75 HEV isolates over the entire genome reveals an inter-genotype difference of

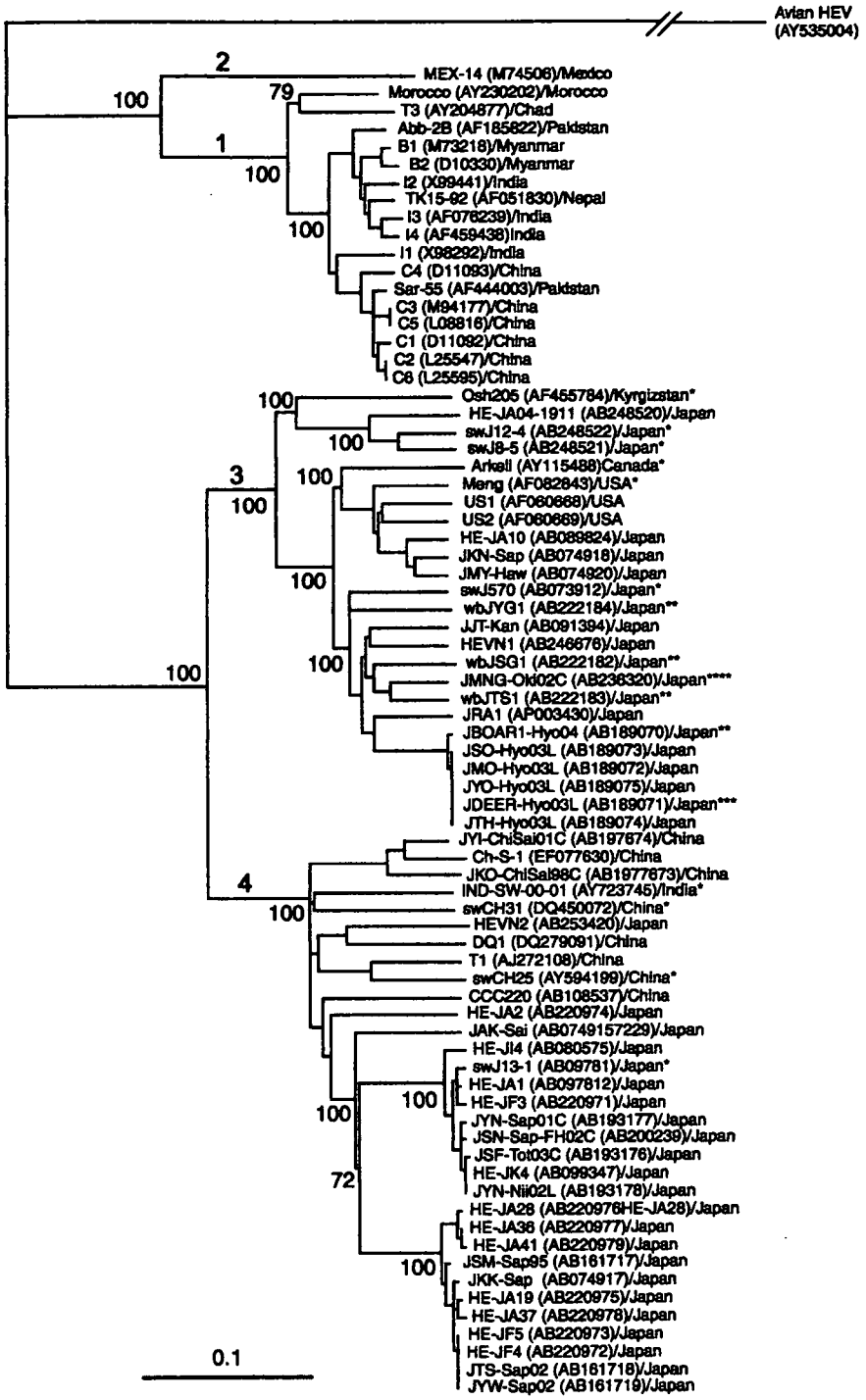

Fig. 2. Phylogenetic tree constructed by the neighbor-joining method based on the full-length nucleotide sequences of 75 HEV isolates, using an avian HEV isolate (accession no. AY535004, Huang et al., 2002) as an outgroup. Fifty-four human isolates, 8 swine isolates, 4 boar isolates, 1 deer isolate and 1 mongoose isolate whose entire or nearly entire sequence has been reported were included for comparison, with the GenBank accession number in parentheses. After the slash, the name of the country where the HEV strain was isolated is shown. Asterisks indicate isolates obtained from pigs (*), wild boars (**), a deer (***) and a mongoose (****), respectively. Bootstrap values are indicated for the major nodes as a percentage of the data obtained from 1000 resamplings (Felsenstein, 1985). Bar, 0.1 substitutions per site.

23.6–27.7%. Among the four genotypes of HEV, genotype 1 has up to 11.8% of intra-genotype diversity, while genotype 3 and genotype 4 show a wide range of intra-genotype diversity (0–19.3% and 0.1–17.0%, respectively). Notably, genotype 3 has a wider degree of intra-genotype variability than genotype 4 ($12.9 \pm 4.8\%$ versus $10.9 \pm 5.6\%$, $P = 0.0028$ [Mann–Whitney U-test]), corroborating the recent review article by Lu et al. (2006) who reported that genotype 3 can be provisionally

divided into 10 subgenotypes (3a–3j) but genotype 4 into 7 subgenotypes (4a–4g), with a difference of 12.1–18.0% over the entire genome, 11.5–20.0% within the 5′ end sequence of ORF1 (287 nt), 11.4–22.8% within the 3′ end sequence of ORF1 (307 nt), 12.6–19.8% within the 301-nt sequence at the central portion of ORF2, and 12.9–19.3% within the 3′ end sequence of ORF2 (318 nt) among the subgenotypes in each genotype.

As for genotype 2, the entire nucleotide sequence has been determined for only the single Mexican strain (MEX-14) (Huang et al., 1992), and partial nucleotide sequences of 359–451 nt at the 3′ terminus of ORF2 have been determined for 16 African strains circulating in CAR, Chad, the DRC, Egypt, Namibia and Nigeria (Buisson et al., 2000; Maila et al., 2004; Nicand et al., 2005). Pairwise comparison of the common 179-nt ORF2 sequence indicates that the 16 African isolates differ from each other by up to 10.3% and from the Mexican isolate by 12.3–16.8%, and that the inter-genotype difference among the four distinct genotypes is 18.5–21.9%. The phylogenetic tree constructed based on the 179-nt ORF2 sequence (Fig. 3) also suggests that genotype 2 HEV strains segregate into at least two subgenotypes, i.e., Mexican and African subgenotypes. Upon comparison of the 179-nt sequence, the 17 genotype 1 isolates differ from each other by up to 13.4%, 25 genotype 3 isolates by up to 21.2%, and 30 genotype 4 isolates by up to 20.7%. Although accumulation of the full-length sequences of genotype 2 HEV isolates circulating in Africa is needed, genotype 2 HEV strains may be less heterogeneous than genotype 3 and 4 HEV strains, but may be more heterogeneous than genotype 1 HEV strains.

In Japan, polyphyletic HEV strains of genotypes 3 and 4 have been isolated not only from humans and domestic pigs but also from boars, a deer, and a mongoose captured in the wild (Li et al., 2005; Nakamura et al., 2006; Nishizawa et al., 2005; Sonoda et al., 2004; Takahashi et al., 2004b; Tei et al., 2003), and genotype 3 HEV isolates are provisionally classified into three phylogenetic clusters, with the highest nucleotide identity being 94.4–100% between human and swine isolates in each cluster (M. Takahashi et al., 2003). Entire or nearly entire genomic sequences have been determined for 20 genotype 3 HEV isolates of Japan origin. Among these 20 isolates, 14 isolates obtained from humans, pigs, wild boars, a wild deer and a wild mongoose segregated into a cluster (provisionally designated as cluster IIIjp, M. Takahashi et al., 2003) (Fig. 2), which corresponded to subgenotype 3b (Lu et al., 2006) consisting of predominantly Japan-indigenous strains represented by the JRA1 isolate of human origin (AP003430) and swJ570 of swine origin (AB073912), and 3 human HEV isolates were classifiable into a second cluster (cluster IIIus) which corresponded to subgenotype 3a (Lu et al., 2006) with HEV isolates homologous to those (US1 and US2) in the United States. In addition, one human (HE-JA04-1911) and two swine HEV isolates (swJ8-5 and swJ12-4) that are classifiable into a third cluster (cluster IIIsp/subgenotype 3e, Lu et al., 2006) and are 84.2–87.2% similar to Spanish HEV isolates in the 304-nt ORF2 sequence, were recently found to be most closely related to human and swine HEV isolates circulating in the United Kingdom among the HEV isolates reported outside Japan (Inoue et al., 2006a,b). In the United Kingdom, sporadic cases of hepatitis E (Ijaz et al., 2005; McCrudden et al., 2000; Wang et al., 2001) and the existence of a close genetic relationship between human and swine HEV strains (Banks et al., 2004) have been reported. As swine are one of the major reservoirs of HEV (Meng, 2003; Smith, 2001; M. Takahashi et al., 2003), it may be conceivable that UK isolate-like HEV isolates belonging to cluster IIIsp/subgenotype 3b and US isolate-like HEV isolates belonging to cluster IIIus/subgenotype 3a entered Japan via importation of pigs from the United Kingdom and the United States, respectively, in the past. This speculation is supported by the historical evidence that the Japanese government started to import several kinds of Yorkshire pigs and Berkshire pigs from the United Kingdom in 1900 and from the United States in 1921 in order to introduce excellent domestic pigs for food (http://www.pig-pins.or.jp/youton/shiryo.html). Evidence that swine HEV can be imported through international trading of pigs has been reported in Taiwan (Wu et al., 2002). UK isolate-like and US isolate-like HEV isolates have been recovered from pigs in 4 and 21, respectively, of 56 swine farms with viremic pigs in Japan (Okamoto et al., 2001; M. Takahashi et al., 2003; Takahashi et al., 2005).

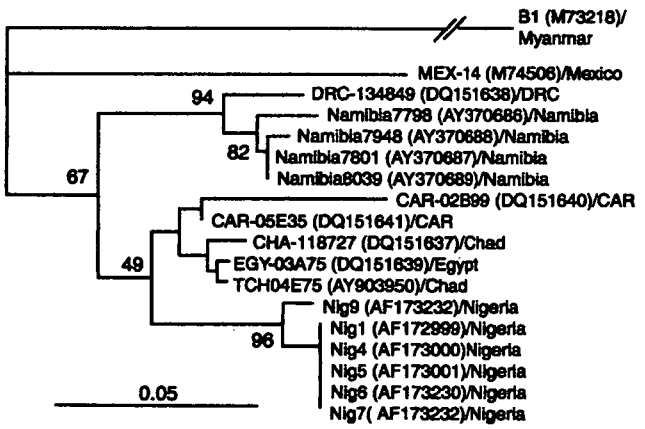

Fig. 3. Phylogenetic tree constructed by the neighbor-joining method based on the common 179-nt ORF2 sequences of genotype 2 HEV isolates, using a genotype 1 HEV isolate (B1, accession no. M73218, Tam et al., 1991) as an outgroup. The isolate names are followed by the accession number in parentheses, and the name of the country where the HEV strain was isolated after the slash. Bootstrap values are indicated for the major nodes as a percentage of the data obtained from 1000 resamplings (Felsenstein, 1985). Bar, 0.05 substitutions per site.

As for genotype 4 HEV strains circulating in Japan, the entire or nearly entire genomic sequence has been determined for 23 HEV isolates including one swine isolate, swJ13-1, whose entire sequence is 99% identical to that of the HE-JA1 isolate of human origin (Table 3). Among them, 21 HEV isolates segregate to a cluster consisting of all Japanese isolates (Fig. 2), which corresponds to subgenotype 4c (Lu et al., 2006). These 21 HEV isolates differ from each other by up to 12.6%, but the majority of them are classifiable into two mini-clusters (Fig. 2): one includes 11 isolates that have been circulating exclusively in Hokkaido, Japan and differ by only up to 3.0%, and the other includes 9 isolates that were identified not only in Hokkaido but also in other areas on Honshu Island of Japan (Tochigi, Ibaraki, Niigata, and Tottori) and differ by only up to 3.4%. The two other genotype

4 HEV isolates, i.e., the HE-JA2 isolate (for accession no., see Fig. 2) obtained in Hokkaido and the HEVN2 isolate obtained in Okinawa, differ from each other by 15.8% over the entire genome, and differ from the 21 other Japanese genotype 4 isolates by 13.2–14.5% and 15.5–16.5%, respectively. These results suggest that Japanese genotype 4 HEV isolates are classifiable into at least four subgroups, and that extremely heterogeneous HEV strains are circulating in Japan.

6. Clinical significance of HEV genotype

It is generally thought that the severity of hepatitis E depends on host factors of the infected patients such as pregnancy (Purcell and Emerson, 2001a; Harrison, 1999; Smith, 2001) and aging (Harrison, 1999). The mortality rate among pregnant women who acquired hepatitis E is as high as 20%. In addition, the presence of an underlying disease may influence the severity of hepatitis E (Mizuo et al., 2005). However, similar to other known hepatitis viruses, viral factors may play a role in the pathogenesis of HEV-associated fulminant hepatitis. Hepatitis B virus variants with mutations in the precore region (Kosaka et al., 1991; Liang et al., 1991; Omata et al., 1991; Yotsumoto et al., 1992) and/or the core promoter (Sato et al., 1995) have been implicated in fulminant hepatitis. Possible associations were also suggested between the severity of hepatitis A and nucleotide substitutions in the 5′ UTR of the hepatitis A virus genome (Fujiwara et al., 2001, 2002). Our recent study suggests that the severity of hepatitis E is affected by the genotype of HEV, based on the finding that patients infected with genotype 4 HEV tend to have more severe disease than those infected with genotype 3 HEV in Japan (Mizuo et al., 2005). The features of 32 patients with sporadic acute or fulminant hepatitis E living in Hokkaido, Japan, who were infected with HEV of genotype 3 or 4, are compared in Table 4. Of note, HEV genotype 4 tended to be associated with the severe form of hepatitis E. Severe or fulminant hepatitis developed in 9 (36%) and 2 patients (8%) among the 25 patients infected with HEV genotype 4, respectively, but in none of the 7 patients infected with HEV genotype 3 (Table 4). Even though the HEV isolates are genetically heterogeneous even within the same genotype, the 25 patients with HEV genotype 4 had a significantly higher peak alanine aminotransferase (ALT) level and a significantly lower level of lowest prothrombin activity than the 7 patients with HEV genotype 3. The mortality rate of hepatitis E in developing countries where genotype 1 HEV prevails is reported to be about 1% (Purcell and Emerson, 2001a). As for genotype 2 HEV, no fulminant cases have been reported thus far. In Japan where HEV isolates of genotypes 3 and 4 circulate, 4 (14.8%) of 27 patients with genotype 4 HEV and 1 (4.8%) of 21 patients with genotype 3 HEV died due to fulminant hepatitis, indicating that genotype 4 HEV may be more closely associated with the development of fulminant hepatitis than HEV of other genotypes. Our limited data from a patient who suffered from coinfection with HEV genotypes 3 and 4 suggested that genotype 4 had a higher HEV load in the circulation than genotype 3 (Takahashi et al., 2002). If this is common for HEV virions circulating in humans, it may explain why patients with genotype 4 appear to have a higher incidence of fulminant hepatitis than those with genotype 3. However, more data will be needed with studies conducted in other countries with other patients in order to confirm what was observed in the case of Japanese patients and to draw a plausible conclusion regarding the relationship between the severity of hepatitis E and the genotype of HEV.

Table 4
Comparison of various features between patients with HEV genotype 3 and those with HEV genotype 4

Feature	Patients with HEV of		*P*-value
	Genotype 3 ($n=7$)	Genotype 4 ($n=25$)	
Age (year)	46.9 ± 15.0	59.3 ± 13.4	NS
Male %	71.4	84.0	NS
Physical findings [no. (%)]			
Jaundice	4 (57.1)	23 (92.0)	NS
Hepatomegaly	0	6 (24.0)	NS
Laboratory data			
Total bilirubin (mg/dl)			
At first examination	3.0 ± 2.6	8.6 ± 6.7	0.0022
At peak	5.8 ± 5.7	11.7 ± 9.1	NS
ALT (IU/L)			
At the initial examination	1294 ± 954	2363 ± 1493	0.0373
At peak	1577 ± 886	2590 ± 1380	0.0338
AST (IU/L)			
At the initial examination	1041 ± 817	1963 ± 1488	0.0453
At peak	1310 ± 836	2098 ± 1384	NS
Lowest PT percent	94.9 ± 20.4	72.5 ± 27.0	0.0340
Lowest PT percent of ≤40% [no. (%)]	0	7 (28.0)	NS
Severe hepatitis [number (%)][a]	0	9 (36.0)	NS
Fulminant hepatitis [number (%)]	0	2 (8.0)	NS

Abbreviations (normal range): total bilirubin (0.2–1.2 mg/dl); ALT, alanine aminotransferase (6–43 IU/l); AST, aspartate aminotransferase (11–40 IU/l); PT, prothrombin activity (80–120%). NS, not significant.
[a] With peak total bilirubin level of ≥20 mg/dl and/or lowest PT% of ≤40%.

7. Quasispecies nature and evolution of HEV

Upon comparison of the 75 HEV isolates whose entire or nearly entire sequence has determined, the inter-genotype difference of the deduced amino acid sequence of ORF2 which encodes the capsid protein of HEV, was only 6.5–11.7%. The observed high degree of conservation of the amino acid sequence of the capsid protein among distinct genotypes, correlates with the little antigenic diversity; thus, there is only a single serotype of HEV. However, despite this limited amino acid heterogeneity, a significant degree of nucleic acid variability has been observed among different isolates from different regions of the world, as mentioned above. The molecular basis of this genetic variability may be the high error rate of the viral RNA-dependent RNA polymerase and the absence of proofreading mechanisms: the mutation frequency of a variety of different RNA viruses ranges from 10^{-4} to 10^{-5} substitutions per base per round of copying (Domingo, 1996). Based on the assumption that JKK-Sap00 (isolation date: 10 November 2000), JYW-Sap02 (30 August

2002) and JTS-Sap02 (14 September 2002) are descendants of JSM-Sap95 (28 March 1995), all of which were isolated in Hokkaido and differed from each other by 0.056–1.050%, the mutation rate of HEV has been estimated to be $1.40–1.72 \times 10^{-3}$ base substitution per site per year (Takahashi et al., 2004a).

Quasispecies have mainly been described in persistent virus infections such as those due to human immunodeficiency virus type 1 (Wolinsky et al., 1996; Zhu et al., 1993) and hepatitis C virus (Farci et al., 2000; Martell et al., 1992; Okamoto et al., 1992; Weiner et al., 1991) during which virus populations develop a high degree of sequence variation within each infected individual (Sanchez-Palomino et al., 1996). They are less common in viruses causing acute self-limited infections, such as dengue virus (W.K. Wang et al., 2002) and hepatitis A virus (Sanchez et al., 2003). HEV epidemics are mainly caused by a common source of contamination, usually drinking water resources. Although the spread of HEV among humans is assumed to be clonal according to a "one outbreak, one strain" scheme, the quasispecies nature of epidemic HEV was demonstrated in a retrospective analysis of both inter- and intra-patient diversity using 23 serum samples collected during a water-borne outbreak that occurred in 1986–1987 in Algeria (Grandadam et al., 2004). However, the extent of the sequence variation of HEV in vivo and its relationship to disease severity remain unknown.

The reason why HEV strains of genotypes 1 and 2 have less genomic variability than HEV strains of genotypes 3 and 4 remains to be elucidated. HEV strains of genotypes 1 and 2 often cause outbreaks or epidemics of hepatitis as a result of efficient transmission via the fecal–oral route, usually by contaminated water or food supply (Purcell and Emerson, 2001a). In contrast, HEV variants of genotypes 3 and 4 are predominantly maintained among animal species such as domestic pigs and only occasionally infect humans; this is most likely due to inefficient cross-species transmission of these variants. Maintenance of HEV strains of genotypes 3 and 4 among animal species would contribute to the long-term circulation of HEV in particular geographic regions and independent evolution of the virus in specific animal species. Therefore, differences in the degree of viral divergence among genotypes of HEV may reflect different transmission patterns.

To investigate the genetic changes in HEV strains in the community, we compared the 412-nt sequence within ORF2 of HEV among HEV isolates recovered from 48 patients in 1997, 16 patients in 1999, 14 patients in 2000, and 38 patients in 2002 in Kathmandu valley of Nepal (Shrestha et al., 2003, 2004). All 116 HEV-viremic samples were typed as genotype 1, and further as subgenotype 1a ($n = 85$, 73%), 1c ($n = 29$, 25%), and mixed infection of 1a and 1c ($n = 2$, 2%): subgenotype 1c was detected only in 1997. Genetic variability was observed among HEV strains and even among HEV strains of the same subtype (1a) obtained each year in the years of 1997, 1999, 2000, and 2002. When phylogenetic analysis of the 87 subtype 1a isolates was performed, they further segregated into five clusters, with two predominant clusters of 1a–2 and 1a–3: the annual frequency of cluster 1a–2 isolates decreased from 63% in 1997, 50% in 1999, 7% in 2000 and no cases in 2002; cluster 1a–3 isolates were observed in all 4 years and its annual frequency increased from 5% in 1997 to 95% in 2002. Of the remaining three clusters, cluster 1a–1 was detectable only in 1997 and clusters 1a–4 and 1a–5 emerged in 2000 and 2002, respectively. These results indicate that the genetic changes and takeover of HEV strains may contribute to the genetic variability of HEV in the community. The fact that no significant amino acid substitutions were recognized in the HEV strains isolated during a 5-year period suggests that genomic mutations of HEV may occur naturally in infected individuals without immunological pressure from the host, and that selective forces that do not allow amino acid substitutions may be involved in the observed pattern of divergence. Taking into account that partial sequencing of a selected genomic region was employed, a definitive picture of the biological significance of these and other possible changes in the entire genome needs to be obtained from more in-depth studies.

8. Conclusion

Recent studies have revealed that hepatitis E is a zoonosis and multiple HEV strains with significant sequence divergence are circulating in both humans and animals throughout the world. Molecular epidemiological studies on HEV infection in Nepal suggested that the genetic changes and takeover of particular HEV strains may contribute to the genetic variability of HEV in the community. In Japan where some people have peculiar habits of ingesting undercooked, or even raw, meat or viscera of animals, it is suggested that zoonotic food-borne transmission of HEV from not only domestic pigs but also wild boars and wild deer to humans plays an important role in the occurrence of cryptic hepatitis E. However, further clinical, epidemiological and virological studies are needed to elucidate the extent of genomic heterogeneity of HEV strains circulating in the world, the various and possibly region-dependent modes of HEV transmission, and the association of HEV genotype with disease severity and its underlying mechanism.

References

Agrawal, S., Gupta, D., Panda, S.K., 2001. The 3′ end of hepatitis E virus (HEV) genome binds specifically to the viral RNA-dependent RNA polymerase (RdRp). Virology 282, 87–101.

Amon, J.J., Drobeniuc, J., Bower, W.A., Magana, J.C., Escobedo, M.A., Williams, I.T., Bell, B.P., Armstrong, G.L., 2006. Locally acquired hepatitis E virus infection, El Paso, Texas. J. Med. Virol. 78, 741–746.

Arankalle, V.A., Goverdhan, M.K., Banerjee, K., 1994. Antibodies against hepatitis E virus in Old World monkeys. J. Viral. Hepat. 1, 125–159.

Arankalle, V.A., Chobe, L.P., Joshi, M.V., Chadha, M.S., Kundu, B., Walimbe, A.M., 2002. Human and swine hepatitis E viruses from Western India belong to different genotypes. J. Hepatol. 36, 417–425.

Aye, T.T., Uchida, T., Ma, M.Z., Iida, F., Shikata, T., Zhung, H., Win, K.M., 1992. Complete nucleotide sequence of a hepatitis E virus isolated from the Xinjiang epidemic (1986–1988) of China. Nucleic Acids Res. 20, 3512.

Balayan, M.S., Andjaparidze, A.G., Savinskaya, S.S., Ketiladze, E.S., Braginsky, D.M., Savinov, A.P., Poleschuk, V.F., 1983. Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal–oral route. Intervirology 20, 23–31.

Balayan, M.S., Usmanov, R.K., Zamyatina, N.A., Djumalieva, D.I., Karas, F.R., 1990. Brief report: experimental hepatitis E infection in domestic pigs. J. Med. Virol. 32, 58–59.

Banks, M., Bendall, R., Grierson, S., Heath, G., Mitchell, J., Dalton, H., 2004. Human and porcine hepatitis E virus strains, United Kingdom. Emerg. Infect. Dis. 10, 953–955.

Bi, S.L., Purdy, M.A., McCaustland, K.A., Margolis, H.S., Bradley, D.W., 1993. The sequence of hepatitis E virus isolated directly from a single source during an outbreak in China. Virus Res. 28, 233–247.

Buisson, Y., Grandadam, M., Nicand, E., Cheval, P., van Cuyck-Gandre, H., Innis, B., Rehel, P., Coursaget, P., Teyssou, R., Tsarev, S., 2000. Identification of a novel hepatitis E virus in Nigeria. J. Gen. Virol. 81, 903–909.

Cacopardo, B., Russo, R., Preiser, W., Benanti, F., Brancati, G., Nunnari, A., 1997. Acute hepatitis E in Catania (eastern Sicily) 1980–1994. The role of hepatitis E virus. Infection 25, 313–316.

Chandler, J.D., Riddell, M.A., Li, F., Love, R.J., Anderson, D.A., 1999. Serological evidence for swine hepatitis E virus infection in Australian pig herds. Vet. Microbiol. 68, 95–105.

Cheng, G.B., Meng, J.H., 2004. Identification of 5′ capped structure and 3′ terminal sequence of hepatitis E virus isolated from Morocco. World J. Gastroenterol. 10, 2045–2049.

Cooper, K., Huang, F.F., Batista, L., Rayo, C.D., Bezanilla, J.C., Toth, T.E., Meng, X.J., 2005. Identification of genotype 3 hepatitis E virus (HEV) in serum and fecal samples from pigs in Thailand and Mexico, where genotype 1 and 2 HEV strains are prevalent in the respective human populations. J. Clin. Microbiol. 43, 1684–1688.

Dawson, G.J., Mushahwar, I.K., Chau, K.H., Gitnick, G.L., 1992. Detection of long-lasting antibody to hepatitis E virus in a US traveller to Pakistan. Lancet 340, 426–427.

Domingo, E., 1996. Biological significance of viral quasispecies. Viral Hep. Rev. 2, 247–261.

Donati, M.C., Fagan, E.A., Harrison, T.J., 1997. Sequence analysis of full length HEV clones derived directly from human liver in fulminant hepatitis E. In: Rizzetto, M., Purcell, R.H., Gerin, J.L., Verme, G. (Eds.), Viral Hepatitis and Liver Disease. Edizioni Minerva Medica, Torino, pp. 313–316.

Drobeniuc, J., Favorov, M.O., Shapiro, C.N., Bell, B.P., Mast, E.E., Dadu, A., Culver, D., Iarovoi, P., Robertson, B.H., Margolis, H.S., 2001. Hepatitis E virus antibody prevalence among persons who work with swine. J. Infect. Dis. 184, 1594–1597.

Emerson, S.U., Anderson, D., Arankalle, A., Meng, X.J., Purdy, M., Schlauder, G.G., Tsarev, S.A., 2004. Hepevirus. In: Fauquet, C.M., Mayo, M.A., Maniloff, J., Desselberger, U., Ball, L.A. (Eds.), Virus Taxonomy: VIIIth report of the ICTV. Elsevier/Academic Press, London, pp. 851–855.

Erker, J.C., Desai, S.M., Schlauder, G.G., Dawson, G.J., Mushahwar, I.K., 1999. A hepatitis E virus variant from the United States: molecular characterization and transmission in cynomolgus macaques. J. Gen. Virol. 80, 681–690.

Farci, P., Shimoda, A., Coiana, A., Diaz, G., Peddis, G., Melpolder, J.C., Strazzera, A., Chien, D.Y., Munoz, S.J., Balestrieri, A., Purcell, R.H., Alter, H.J., 2000. The outcome of acute hepatitis C predicted by the evolution of the viral quasispecies. Science 288, 339–344.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Fujiwara, K., Yokosuka, O., Fukai, K., Imazeki, F., Saisho, H., Omata, M., 2001. Analysis of full-length hepatitis A virus genome in sera from patients with fulminant and self-limited acute type A hepatitis. J. Hepatol. 35, 112–119.

Fujiwara, K., Yokosuka, O., Ehata, T., Saisho, H., Saotome, N., Suzuki, K., Okita, K., Kiyosawa, K., Omata, M., 2002. Association between severity of type A hepatitis and nucleotide variations in the 5′ non-translated region of hepatitis A virus RNA: strains from fulminant hepatitis have fewer nucleotide substitutions. Gut 51, 82–88.

Garkavenko, O., Obriadina, A., Meng, J., Anderson, D.A., Bernard, H.J., Schroeder, B.A., Khudyakov, Y.E., Fields, H.A., Croxson, M.C., 2001. Detection and characterization of swine hepatitis E virus in New Zealand. J. Med. Virol. 65, 525–529.

Gouvea, V., Hoke Jr., C.H., Innis, B.L., 1998a. Genotyping of hepatitis E virus in clinical specimens by restriction endonuclease analysis. J. Virol. Methods 70, 71–78.

Gouvea, V., Snellings, N., Popek, M.J., Longer, C.F., Innis, B.L., 1998b. Hepatitis E virus: complete genome sequence and phylogenetic analysis of a Nepali isolate. Virus Res. 57, 21–26.

Graff, J., Torian, U., Nguyen, H., Emerson, S.U., 2006. A bicistronic subgenomic mRNA encodes both the ORF2 and ORF3 proteins of hepatitis E virus. J. Virol. 80, 5919–5926.

Grandadam, M., Tebbal, S., Caron, M., Siriwardana, M., Larouze, B., Koeck, J.L., Buisson, Y., Enouf, V., Nicand, E., 2004. Evidence for hepatitis E virus quasispecies. J. Gen. Virol. 85, 3189–3194.

Haqshenas, G., Shivaprasad, H.L., Woolcock, P.R., Read, D.H., Meng, X.J., 2001. Genetic identification and characterization of a novel virus related to human hepatitis E virus from chickens with hepatitis-splenomegaly syndrome in the United States. J. Gen. Virol. 82, 2449–2462.

Harrison, T.J., 1999. Hepatitis E virus—an update. Liver 19, 171–176.

Herrera, J.L., 1993. Hepatitis E as a cause of acute non-A, non-B hepatitis. Arch. Intern. Med. 153, 773–775.

Hino, K., Kondo, T., Niwa, H., Uchida, T., Shikata, T., Rikahisa, T., Mizuno, K., 1991. A small epidemic of enterically transmitted non-A, non-B acute hepatitis. Gastroenterol. Jpn. 26, 139–141.

Hirano, M., Ding, X., Tran, H.T., Li, T.C., Takeda, N., Sata, T., Nakamura, S., Abe, K., 2003a. Prevalence of antibody against hepatitis E virus in various species of non-human primates: evidence of widespread infection in Japanese monkeys (Macaca fuscata). Jpn. J. Infect. Dis. 56, 8–11.

Hirano, M., Ding, X., Li, T.C., Takeda, N., Kawabata, H., Koizumi, N., Kadosaka, T., Goto, I., Masuzawa, T., Nakamura, M., Taira, K., Kuroki, T., Tanikawa, T., Watanabe, H., Abe, K., 2003b. Evidence for widespread infection of hepatitis E virus among wild rats in Japan. Hepatol. Res. 27, 1–5.

Hsieh, S.Y., Meng, X.J., Wu, Y.H., Liu, S.T., Tam, A.W., Lin, D.Y., Liaw, Y.F., 1999. Identity of a novel swine hepatitis E virus in Taiwan forming a monophyletic group with Taiwan isolates of human hepatitis E virus. J. Clin. Microbiol. 37, 3828–3834.

Huang, C.C., Nguyen, D., Fernandez, J., Yun, K.Y., Fry, K.E., Bradley, D.W., Tam, A.W., Reyes, G.R., 1992. Molecular cloning and sequencing of the Mexico isolate of hepatitis E virus (HEV). Virology 191, 550–558.

Huang, F.F., Haqshenas, G., Guenette, D.K., Halbur, P.G., Schommer, S.K., Pierson, F.W., Toth, T.E., Meng, X.J., 2002. Detection by reverse transcription-PCR and genetic characterization of field isolates of swine hepatitis E virus from pigs in different geographic regions of the United States. J. Clin. Microbiol. 40, 1326–1332.

Huang, F.F., Sun, Z.F., Emerson, S.U., Purcell, R.H., Shivaprasad, H.L., Pierson, F.W., Toth, T.E., Meng, X.J., 2004. Determination and analysis of the complete genomic sequence of avian hepatitis E virus (avian HEV) and attempts to infect rhesus monkeys with avian HEV. J. Gen. Virol. 85, 1609–1618.

Inoue, J., Nishizawa, T., Takahashi, M., Aikawa, T., Mizuo, H., Suzuki, K., Shimosegawa, T., Okamoto, H., 2006a. Analysis of the full-length genome of genotype 4 hepatitis E virus isolates from patients with fulminant or acute self-limited hepatitis E. J. Med. Virol. 78, 476–484.

Inoue, J., Takahashi, M., Ito, K., Shimosegawa, T., Okamoto, H., 2006b. Analysis of human and swine hepatitis E virus (HEV) isolates of genotype 3 in Japan that are only 81–83% similar to reported HEV isolates of the same genotype over the entire genome. J. Gen. Virol. 87, 2363–2369.

Ijaz, S., Arnold, E., Banks, M., Bendall, R.P., Cramp, M.E., Cunningham, R., Dalton, H.R., Harrison, T.J., Hill, S.F., Macfarlane, L., Meigh, R.E., Shafi, S., Sheppard, M.J., Smithson, J., Wilson, M.P., Teo, C.G., 2005. Non-travel-associated hepatitis E in England and Wales: demographic, clinical, and molecular epidemiological characteristics. J. Infect. Dis. 192, 1166–1172.

Ishikawa, K., Matsui, K., Madarame, T., Sato, S., Oikawa, K., Uchida, T., 1995. Hepatitis E probably contracted via a Chinese herbal medicine, demonstrated by nucleotide sequencing. J. Gastroenterol. 30, 534–538.

Kabrane-Lazizi, Y., Fine, J.B., Elm, J., Glass, G.E., Higa, H., Diwan, A., Gibbs Jr., C.J., Meng, X.J., Emerson, S.U., Purcell, R.H., 1999a. Evidence for widespread infection of wild rats with hepatitis E virus in the United States. Am. J. Trop. Med. Hyg. 61, 331–335.

Kabrane-Lazizi, Y., Meng, X.J., Purcell, R.H., Emerson, S., 1999b. Evidence that the genomic RNA of hepatitis E virus is capped. J. Virol. 73, 8848–8850.

Koizumi, Y., Isoda, N., Sato, Y., Iwaki, T., Ono, K., Ido, K., Sugano, K., Takahashi, M., Nishizawa, T., Okamoto, H., 2004. Infection of a Japanese patient by genotype 4 hepatitis E virus while traveling in Vietnam. J. Clin. Microbiol. 42, 3883–3885.

Koonin, E.V., Gorbalenya, A.E., Purdy, M.A., Rozanov, M.N., Reyes, G.R., Bradley, D.W., 1992. Computer-assisted assignment of functional domains in the nonstructural polyprotein of hepatitis E virus: delineation of an additional group of positive-strand RNA plant and animal viruses. Proc. Natl. Acad. Sci. U.S.A. 89, 8259–8263.

Korkaya, H., Jameel, S., Gupta, D., Tyagi, S., Kumar, R., Zafrullah, M., Mazumdar, M., Lal, S.K., Xiaofang, L., Sehga, L.D., Das, S.R., Sahal, D., 2001. The ORF3 protein of hepatitis E virus binds to Src homology 3 domains and activates MAPK. J. Biol. 276, 42389–42400.

Kosaka, Y., Takase, K., Kojima, M., Shimizu, M., Inoue, K., Yoshiba, M., Tanaka, S., Akahane, Y., Okamoto, H., Tsuda, F., Miyakawa, Y., Mayumi, M., 1991. Fulminant hepatitis B: induction by hepatitis B virus mutants defective in the precore region and incapable of encoding e antigen. Gastroenterology 100, 1087–1094.

Kuno, A., Ido, K., Isoda, N., Satoh, Y., Ono, K., Satoh, S., Inamori, H., Sugano, K., Kanai, N., Nishizawa, T., Okamoto, H., 2003. Sporadic acute hepatitis E of a 47-year-old man whose pet cat was positive for antibody to hepatitis E virus. Hepatol. Res. 26, 237–242.

Kwo, P.Y., Schlauder, G.G., Carpenter, H.A., Murphy, P.J., Rosenblatt, J.E., Dawson, G.J., Mast, E.E., Krawczynski, K., Balan, V., 1997. Acute hepatitis E by a new isolate acquired in the United States. Mayo Clin. Proc. 72, 1133–1136.

Li, T.C., Chijiwa, K., Sera, N., Ishibashi, T., Etoh, Y., Shinohara, Y., Kurata, Y., Ishida, M., Sakamoto, S., Takeda, N., Miyamura, T., 2005. Hepatitis E virus transmission from wild boar meat. Emerg. Infect. Dis. 11, 1958–1960.

Liang, T.J., Hasegawa, K., Rimon, N., Wands, J.R., Ben-Porath, E., 1991. A hepatitis B virus mutant associated with an epidemic of fulminant hepatitis. N. Engl. J. Med. 324, 1705–1709.

Lu, L., Drobeniuc, J., Kobylnikov, N., Usmanov, R.K., Robertson, B.H., Favorov, M.O., Margolis, H.S., 2004. Complete sequence of a Kyrgyzstan swine hepatitis E virus (HEV) isolated from a piglet thought to be experimentally infected with human HEV. J. Med. Virol. 74, 556–562.

Lu, L., Li, C., Hagedorn, C.H., 2006. Phylogenetic analysis of global hepatitis E virus sequences: genetic diversity, subtypes and zoonosis. Rev. Med. Virol. 16, 5–36.

Magden, J., Takeda, N., Li, T., Auvinen, P., Ahola, T., Miyamura, T., Merits, A., Kaariainen, L., 2001. Virus-specific mRNA capping enzyme encoded by hepatitis E virus. J. Virol. 75, 6249–6255.

Maila, H.T., Bowyer, S.M., Swanepoel, R., 2004. Identification of a new strain of hepatitis E virus from an outbreak in Namibia in 1995. J. Gen. Virol. 85, 89–95.

Mansuy, J.M., Peron, J.M., Abravanel, F., Poirson, H., Dubois, M., Miedouge, M., Vischi, F., Alric, L., Vinel, J.P., Izopet, J., 2004. Hepatitis E in the south west of France in individuals who have never visited an endemic area. J. Med. Virol. 74, 419–424.

Martell, M., Esteban, J.I., Quer, J., Genesca, J., Weiner, A., Esteban, R., Guardia, J., Gomez, J., 1992. Hepatitis C virus (HCV) circulates as a population of different but closely related genomes: quasispecies nature of HCV genome distribution. J. Virol. 66, 3225–3229.

McCrudden, R., O'Connell, S., Farrant, T., Beaton, S., Iredale, J.P., Fine, D., 2000. Sporadic acute hepatitis E in the united kingdom: an underdiagnosed phenomenon? Gut 46, 732–733.

Mechnik, L., Bergman, N., Attali, M., Beergabel, M., Mosenkis, B., Sokolowski, N., Malnick, S., 2001. Acute hepatitis E virus infection presenting as a prolonged cholestatic jaundice. J. Clin. Gastroenterol. 33, 421–422.

Meng, X.J., 2000. Novel strains of hepatitis E virus identified from humans and other animal species: is hepatitis E a zoonotic? J. Hepatol. 33, 842–845.

Meng, X.J., 2003. Swine hepatitis E virus: cross-species infection and risk in xenotransplantation. Curr. Top. Microbiol. Immunol. 278, 185–216.

Meng, X.J., Purcell, R.H., Halbur, P.G., Lehman, J.R., Webb, D.M., Tsareva, T.S., Haynes, J.S., Thacker, B.J., Emerson, S.U., 1997. A novel virus in swine is closely related to the human hepatitis E virus. Proc. Natl. Acad. Sci. U.S.A. 94, 9860–9865.

Meng, X.J., Halbur, P.G., Shapiro, M.S., Govindarajan, S., Bruna, J.D., Mushahwar, I.K., Purcell, R.H., Emerson, S.U., 1998. Genetic and experimental evidence for cross-species infection by swine hepatitis E virus. J. Virol. 72, 9714–9721.

Meng, X.J., Wiseman, B., Elvinger, F., Guenette, D.K., Toth, T.E., Engle, R.E., Emerson, S.U., Purcell, R.H., 2002. Prevalence of antibodies to hepatitis E virus in veterinarians working with swine and in normal blood donors in the United States and other countries. J. Clin. Microbiol. 40, 117–122.

Mitsui, T., Tsukamoto, Y., Suzuki, S., Yamazaki, C., Masuko, K., Tsuda, F., Takahashi, M., Tsatsralt-Od, B., Nishizawa, T., Okamoto, H., 2005. Serological and molecular studies on subclinical hepatitis E virus infection using periodic serum samples obtained from healthy individuals. J. Med. Virol. 76, 526–533.

Mizuo, H., Suzuki, K., Takikawa, Y., Sugai, Y., Tokita, H., Akahane, Y., Itoh, K., Gotanda, Y., Takahashi, M., Nishizawa, T., Okamoto, H., 2002. Polyphyletic strains of hepatitis E virus are responsible for sporadic cases of acute hepatitis in Japan. J. Clin. Microbiol. 40, 3209–3218.

Mizuo, H., Yazaki, Y., Sugawara, K., Tsuda, F., Takahashi, M., Nishizawa, T., Okamoto, H., 2005. Possible risk factors for the transmission of hepatitis E virus and for the severe form of hepatitis E acquired locally in Hokkaido, Japan. J. Med. Virol. 76, 341–349.

Nakamura, M., Takahashi, K., Taira, K., Taira, M., Ohno, A., Sakugawa, H., Arai, M., Mishiro, S., 2006. Hepatitis E virus infection in wild mongooses of Okinawa, Japan: demonstration of anti-HEV antibodies and a full-genome nucleotide sequence. Heaptol. Res. 34, 137–140.

Nicand, E., Armstrong, G.L., Enouf, V., Guthmann, J.P., Guerin, J.P., Caron, M., Nizou, J.Y., Andraghetti, R., 2005. Genetic heterogeneity of hepatitis E virus in Darfur, Sudan, and neighboring Chad. J. Med. Virol. 77, 519–521.

Nishizawa, T., Takahashi, M., Mizuo, H., Miyajima, H., Gotanda, Y., Okamoto, H., 2003. Characterization of Japanese swine and human hepatitis E virus isolates of genotype IV with 99% identity over the entire genome. J. Gen. Virol. 84, 1245–1251.

Nishizawa, T., Takahashi, M., Endo, K., Fujiwara, S., Sakuma, N., Kawazuma, F., Sakamoto, H., Sato, Y., Bando, M., Okamoto, H., 2005. Analysis of the full-length genome of hepatitis E virus isolates obtained from wild boars in Japan. J. Gen. Virol. 86, 3321–3326.

Okamoto, H., Kojima, M., Okada, S., Yoshizawa, H., Iizuka, H., Tanaka, T., Muchmore, E.E., Peterson, D.A., Ito, Y., Mishiro, S., 1992. Genetic drift of hepatitis C virus during an 8.2-year infection in a chimpanzee: variability and stability. Virology 190, 894–899.

Okamoto, H., Takahashi, M., Nishizawa, T., Fukai, K., Muramatsu, U., Yoshikawa, A., 2001. Analysis of the complete genome of indigenous swine hepatitis E virus isolated in Japan. Biochem. Biophys. Res. Commun. 289, 929–936.

Okamoto, H., Takahashi, M., Nishizawa, T., 2003. Features of hepatitis E virus infection in Japan. Intern. Med. 42, 1065–1071.

Omata, M., Ehata, T., Yokosuka, O., Hosoda, K., Ohto, M., 1991. Mutations in the precore region of hepatitis B virus DNA in patients with fulminant and severe hepatitis. N. Engl. J. Med. 324, 1699–1704.

Pina, S., Buti, M., Cotrina, M., Piella, J., Girones, R., 2000. HEV identified in serum from humans with acute hepatitis and in sewage of animal origin in Spain. J. Hepatol. 33, 826–833.

Purcell, R.H., Emerson, S.U., 2001a. Hepatitis E virus. In: Knipe, D.M., Howley, P.M., Griffin, D.E., Lamb, R.A., Martin, M.A., Roizman, B., Straus, S.E. (Eds.), Fields Virology, fourth ed. Lippincott Williams & Wilkins, Philadelphia, PA, pp. 3051–3061.

Purcell, R.H., Emerson, S.U., 2001b. Animal Models of hepatitis A and E. ILAR J. 42, 161–177.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.

Sanchez, G., Bosch, A., Gomez-Mariano, G., Domingo, E., Pinto, R.M., 2003. Evidence for quasispecies distributions in the human hepatitis A virus genome. Virology 315, 34–42.

Sanchez-Palomino, S., Olivares, I., Yuste, E., Richman, D.D., Lopez-Galindez, C., 1996. Random important alterations in HIV-1 viral quasispecies after antiviral treatment. Antivir. Ther. 1, 225–236.

Sato, S., Suzuki, K., Akahane, Y., Akamatsu, K., Akiyama, K., Yunomura, K., Tsuda, F., Tanaka, T., Okamoto, H., Miyakawa, Y., Mayumi, M., 1995. Hepatitis B virus strains with mutations in the core promoter in patients with fulminant hepatitis. Ann. Intern. Med. 122, 241–248.

Schlauder, G.G., Dawson, G.J., Erker, J.C., Kwo, P.Y., Knigge, M.F., Smalley, D.L., Rosenblatt, J.E., Desai, S.M., Mushahwar, I.K., 1998. The sequence

and phylogenetic analysis of a novel hepatitis E virus isolated from a patient with acute hepatitis reported in the United States. J. Gen. Virol. 79, 447–456.
Schlauder, G.G., Desai, S.M., Zanetti, A.R., Tassopoulos, N.C., Mushahwar, I.K., 1999. Novel hepatitis E virus (HEV) isolates from Europe: evidence for additional genotypes of HEV. J. Med. Virol. 57, 243–251.
Schlauder, G.G., Frider, B., Sookoian, S., Castano, G.C., Mushahwar, I.K., 2000. Identification of 2 novel isolates of hepatitis E virus in Argentina. J. Infect. Dis. 182, 294–297.
Schlauder, G.G., Mushahwar, I.K., 2001. Genetic heterogeneity of hepatitis E virus. J. Med. Virol. 65, 282–292.
Shrestha, S.M., Shrestha, S., Tsuda, F., Nishizawa, T., Gotanda, Y., Takeda, N., Okamoto, H., 2003. Molecular investigation of hepatitis E virus infection in patients with acute hepatitis in Kathmandu, Nepal. J. Med. Virol. 69, 207–214.
Shrestha, S.M., Shrestha, S., Tsuda, F., Nishizawa, T., Takahashi, M., Gotanda, Y., Okamoto, H., 2004. Genetic changes in hepatitis E virus of subtype 1a in patients with sporadic acute hepatitis E in Kathmandu, Nepal, from 1997 to 2002. J. Gen. Virol. 85, 97–104.
Smith, J.L., 2001. A review of hepatitis E virus. J. Food Prot. 64, 572–586.
Sonoda, H., Abe, M., Sugimoto, T., Sato, Y., Bando, M., Fukui, E., Mizuo, H., Takahashi, M., Nishizawa, T., Okamoto, H., 2004. Prevalence of hepatitis E virus (HEV) infection in wild boars and deer and genetic identification of a genotype 3 HEV from a boar in Japan. J. Clin. Microbiol. 42, 5371–5374.
Takahashi, K., Iwata, K., Watanabe, N., Hatahara, T., Ohta, Y., Baba, K., Mishiro, S., 2001. Full-genome nucleotide sequence of a hepatitis E virus strain that may be indigenous to Japan. Virology 287, 9–12.
Takahashi, M., Nishizawa, T., Yoshikawa, A., Sato, S., Isoda, N., Ido, K., Sugano, K., Okamoto, H., 2002. Identification of two distinct genotypes of hepatitis E virus in a Japanese patient with acute hepatitis who had not travelled abroad. J. Gen. Virol. 83, 1931–1940.
Takahashi, K., Kang, J.H., Ohnishi, S., Hino, K., Miyakawa, H., Miyakawa, Y., Maekubo, H., Mishiro, S., 2003. Full-length sequences of six hepatitis E virus isolates of genotypes III and IV from patients with sporadic acute or fulminant hepatitis in Japan. Intervirology 46, 308–318.
Takahashi, M., Nishizawa, T., Miyajima, H., Gotanda, Y., Iita, T., Tsuda, F., Okamoto, H., 2003. Swine hepatitis E virus strains in Japan form four phylogenetic clusters comparable with those of Japanese isolates of human hepatitis E virus. J. Gen. Virol. 84, 851–862.
Takahashi, K., Toyota, J., Karino, Y., Kang, J.H., Maekubo, H., Abe, N., Mishiro, S., 2004a. Estimation of the mutation rate of hepatitis E virus based on a set of closely related 7.5-year-apart isolates from Sapporo, Japan. Hepatology 29, 212–215.
Takahashi, K., Kitajima, N., Abe, N., Mishiro, S., 2004b. Complete or near-complete nucleotide sequences of hepatitis E virus genome recovered from a wild boar, a deer, and four patients who ate the deer. Virology 330, 501–505.
Takahashi, M., Nishizawa, T., Tanaka, T., Tsatsralt-Od, B., Inoue, J., Okamoto, H., 2005. Correlation between positivity for immunoglobulin A antibodies and viraemia of swine hepatitis E virus observed among farm pigs in Japan. J. Gen. Virol. 86, 1807–1813.
Tam, A.W., Smith, M.M., Guerra, M.E., Huang, C.C., Bradley, D.W., Fry, K.E., Reyes, G.R., 1991. Hepatitis E virus (HEV): molecular cloning and sequencing of the full-length viral genome. Virology 185, 120–131.
Tei, S., Kitajima, N., Takahashi, K., Mishiro, S., 2003. Zoonotic transmission of hepatitis E virus from deer to human beings. Lancet 362, 371–373.
Tsarev, S.A., Emerson, S.U., Reyes, G.R., Tsareva, T.S., Legters, L.J., Malik, I.A., Iqbal, M., Purcell, R.H., 1992. Characterization of a prototype strain of hepatitis E virus. Proc. Natl. Acad. Sci. U.S.A. 89, 559–563.
Tsarev, S.A., Binn, L.N., Gomatos, P.J., Arthur, R.R., Monier, M.K., van Cuyck-Gandre, H., Longer, C.F., Innis, B.L., 1999. Phylogenetic analysis of hepatitis E virus isolates from Egypt. J. Med. Virol. 57, 68–74.
Tyagi, S., Korkaya, H., Zafrullah, M., Jameel, S., Lal, S.K., 2002. The phosphorylated form of the ORF3 protein of hepatitis E virus interacts with its non-glycosylated form of the major capsid protein ORF2. J. Biol. Chem. 277, 22759–22767.
van Cuyck, H., Juge, F., Roques, P., 2003. Phylogenetic analysis of the first complete hepatitis E virus (HEV) genome from Africa. FEMS Immunol. Med. Microbiol. 39, 133–139.
van Cuyck-Gandre, H., Zhang, H.Y., Tsarev, S.A., Warren, R.L., Caudill, J.D., Snellings, N.J., Begot, L., Innis, B.L., Longer, C.F., 2000. Phylogenetically distinct hepatitis E viruses in Pakistan. Am. J. Trop. Med. Hyg. 62, 187–189.
Wang, Y., Ling, R., Erker, J.C., Zhang, H., Li, H., Desai, S., Mushahwar, I.K., Harrison, T.J., 1999. A divergent genotype of hepatitis E virus in Chinese patients with acute hepatitis. J. Gen. Virol. 80, 169–177.
Wang, Y., Zhang, H., Ling, R., Li, H., Harrison, T.J., 2000. The complete sequence of hepatitis E virus genotype 4 reveals an alternative strategy for translation of open reading frames 2 and 3. J. Gen. Virol. 81, 1675–1686.
Wang, Y., Levine, D.F., Bendall, R.P., Teo, C.G., Harrison, T.J., 2001. Partial sequence analysis of indigenous hepatitis E virus isolated in the United Kingdom. J. Med. Virol. 65, 706–709.
Wang, Y.C., Zhang, H.Y., Xia, N.S., Peng, G., Lan, H.Y., Zhuang, H., Zhu, Y.H., Li, S.W., Tian, K.G., Gu, W.J., Lin, J.X., Wu, X., Li, H.M., Harrison, T.J., 2002. Prevalence, isolation, and partial sequence analysis of hepatitis E virus from domestic animals in China. J. Med. Virol. 67, 516–521.
Wang, W.K., Lin, S.R., Lee, C.M., King, C.C., Chang, S.C., 2002. Dengue type 3 virus in plasma is a population of closely related genomes: quasispecies. J. Virol. 76, 4662–4665.
Weiner, A.J., Brauer, M.J., Rosenblatt, J., Richman, K.H., Tung, J., Crawford, K., Bonino, F., Saracco, G., Choo, Q.L., Houghton, M., Han, J.H., 1991. Variable and hypervariable domains are found in the regions of HCV corresponding to the flavivirus envelope and NS1 proteins and the pestivirus envelope glycoproteins. Virology 180, 842–848.
Wolinsky, S.M., Korber, B.T., Neumann, A.U., Daniels, M., Kunstman, K.J., Whetsell, A.J., Furtado, M.R., Cao, Y., Ho, D.D., Safrit, J.T., 1996. Adaptive evolution of human immunodeficiency virus-type 1 during the natural course of infection. Science 272, 537–542.
Worm, H.C., Schlauder, G.G., Wurzer, H., Mushahwar, I.K., 2000. Identification of a novel variant of hepatitis E virus in Austria: sequence, phylogenetic and serological analysis. J. Gen. Virol. 81, 2885–2890.
Wu, J.C., Chen, C.M., Chiang, T.Y., Tsai, W.H., Jeng, W.J., Sheen, I.J., Lin, C.C., Meng, X.J., 2002. Spread of hepatitis E virus among different-aged pigs: two-year survey in Taiwan. J. Med. Virol. 66, 488–492.
Yazaki, Y., Mizuo, H., Takahashi, M., Nishizawa, T., Sasaki, N., Gotanda, Y., Okamoto, H., 2003. Sporadic acute or fulminant hepatitis E in Hokkaido, Japan, may be food-borne, as suggested by the presence of hepatitis E virus in pig liver as food. J. Gen. Virol. 84, 2351–2357.
Yin, S., Purcell, R.H., Emerson, S.U., 1994. A new Chinese isolate of hepatitis E virus: comparison with strains recovered from different geographical regions. Virus Genes 9, 23–32.
Yotsumoto, S., Kojima, M., Shoji, I., Yamamoto, K., Okamoto, H., Mishiro, S., 1992. Fulminant hepatitis related to transmission of hepatitis B variants with precore mutations between spouses. Hepatology 16, 31–35.
Zanetti, A.R., Schlauder, G.G., Romano, L., Tanzi, E., Fabris, P., Dawson, G.J., Mushahwar, I.K., 1999. Identification of a novel variant of hepatitis E virus in Italy. J. Med. Virol. 57, 356–360.
Zhai, L., Dai, X., Meng, J., 2006. Hepatitis E virus genotyping based on full-length genome and partial genomic regions. Virus Res. 120, 57–69.
Zhu, T., Mo, H., Wang, N., Nam, D.S., Cao, Y., Koup, R.A., Ho, D.D., 1993. Genotypic and phenotypic characterization of HIV-1 patients with primary infection. Science 261, 1179–1181.

J Gastroenterol 2007; 42:911–917
DOI 10.1007/s00535-007-2109-x

Journal of Gastroenterology

Evaluation of anti-hepatitis E virus (HEV) immunoglobulin A in a serological screening for HEV infection

Abeer Elkady[1], Yasuhito Tanaka[1], Fuat Kurbanov[1], Noboru Hirashima[2], Masaya Sugiyama[1], Anis Khan[1], Hideaki Kato[3], Akihiko Okumura[4], and Masashi Mizokami[1]

[1]Department of Clinical Molecular Informative Medicine, Nagoya City University Graduate School of Medical Sciences, 1 Kawasumi, Mizuho-ku, Nagoya 467-8601, Japan
[2]Kasadera Hospital, Nagoya, Japan
[3]Department of Forensic Medical Science, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan
[4]Division of Gastroenterology, Department of Internal Medicine, Aichi Medical University, School of Medicine, Nagakute, Japan

Background. Several formulations of serological diagnostic kits were developed recently in Japan for detecting hepatitis E virus (HEV) infection. The present study was conducted to evaluate a novel anti-HEV serological kit based on detection of class A immunoglobulin antibody (anti-HEV IgA). ***Methods.*** Serum samples from 81 acute hepatitis (AH) and 112 chronic hepatitis (CH) patients were tested for anti-HEV IgG, anti-HEV IgM, and anti-HEV IgA by enzyme immunoassay, and HEV RNA was detected by reverse transcription-polymerase chain reaction. ***Results.*** Eight of 81 (9.9%) AH patients were positive for anti-HEV IgG; 6/81 (7.4%) were positive for anti-HEV IgM; and 3/81 (3.7%) were positive for anti-HEV IgA. HEV RNA was detected only in two patients, and both were positive for anti-HEV IgA and negative for hepatitis A, B, and C virus markers. Of 112 CH patients, reactivity to anti-HEV IgM and anti-HEV IgG was found in two and four patients, respectively. None of these six patients was positive for anti-HEV IgA or HEV RNA. For these six CH patients, serial serum samples stored during the clinical follow-up (1994–2003) were further subjected to anti-HEV IgG, IgM, IgA, and HEV RNA examinations. None of the examined stored samples was reactive for anti-HEV IgA or HEV RNA despite reactivity to anti-HEV IgM and IgG. ***Conclusions.*** Serological examination for anti-HEV IgA together with IgM and IgG allows sensitive and specific determination of acute or past infection with HEV. Although its prevalence is low, HEV infection must be investigated in acute hepatitis patients even in nonendemic HEV countries.

Key words: hepatitis E virus, IgA antibody

Received: July 6, 2007 / Accepted: August 20, 2007
Reprint requests to: M. Mizokami

Introduction

Acute hepatitis E is caused by a single positive-stranded RNA virus of approximately 7.2 kb called hepatitis E virus (HEV).[1,2] The primary route of HEV transmission is fecal–oral. HEV infection is endemic in the developing countries of Africa, the Middle East, and Southeast and Central Asia.[3] In industrialized countries, acute hepatitis E has been reported sporadically in individuals without a history of traveling to HEV-endemic areas.[4–7] Zoonotic transmission of HEV also plays an important role in infection with HEV, particularly in developed countries.[8–13]

A short period of clinically asymptomatic viremia (1–2 weeks) is characteristic in acute hepatitis E infection. The onset of acute antibody response, as indicated by emerging of anti-HEV immunoglobulin M (IgM) and IgG in the serum, is usually manifested by the typical acute hepatitis syndrome.[14] The anti-HEV IgM titer usually declines to baseline within 3–6 months after the symptomatic phase.[15] The anti-HEV IgG remains detectable in the serum for a longer period, from 1 to 13 years, as reported by different studies.[16]

No chronic cases or carrier state of HEV infection has been reported,[17] and the infection is usually associated with acute self-limiting and mild illness, particularly in endemic regions.[18]

A subclinical form of HEV infection was recently reported among symptom-free individuals with evidence of viremia and fecal shedding.[19] Lack of an acute antibody response, as shown by the absence of anti-HEV IgM, was also characteristic in these viremic but apparently healthy individuals.[20,21]

Serologic tests are necessary to establish a definite diagnosis of viral hepatitis. Specific tests for IgM and IgG antibodies to HEV have been developed and are commercially available in Europe, Asia, and Canada. Current tests are capable of detecting anti-HEV IgM in

up to 90% of acute infections if a serum sample is obtained 1–4 weeks after the onset of disease.[22,23] However, currently available serologic tests vary greatly in sensitivity and specificity.[24] Various reports have indicated the occurrence of false-positive or nonspecific reactions during the serological detection of class IgM antibody.[25] A molecular approach based on reverse transcription-polymerase chain reaction (RT-PCR) is useful for detecting HEV in blood and feces during the acute phase of infection. However, the sensitivity of the technique depends on a proper match between the HEV strain and the PCR primers.[26] Furthermore, because the very short period of viremia and the high cost of RT-PCR are limitations, serologic approaches are still used for screening examinations.

Immunoglobulin A (IgA) is another class of antibody, which is elicited during the acute stage of different viral infections, including HEV infection.[27,28] The aim of our study was to evaluate a recently developed serological method based on detection of anti-HEV IgA for the diagnosis of HEV infection in acute hepatitis (AH) patients in Japan. In addition, we aimed to reveal subclinical forms of HEV infection among chronic hepatitis (CH) patients during a follow-up of about 8 years.

Patients and methods

Patients

Serum samples were collected consecutively from 81 AH patients (41 men; mean age, 42.7 years; range, 20–78 years) who were hospitalized between 1994 and 2003. The AH patients were classified into four clinical groups according to the etiological agent: 17 cases of acute hepatitis A group (AHA) (11 men; mean age, 43.1 years; range, 21–66 years), 12 cases of acute hepatitis B group (AHB) (eight men; mean age, 31.6 years; range, 20–59 years), and three cases of acute hepatitis C group (AHC) (one man; mean age, 67.7 years; range, 56–78 years). Forty-nine patients negative for hepatitis A, B, and C virus markers were assigned to a non-A, non-B, and non-C hepatitis group (NABC) (21 men; mean age, 43.8 years; range, 20–72 years). At Nagoya City University, a database query was performed to identify serum samples derived from CH patients, spanning as long a follow-up time as possible. Initially 112 CH patients were selected, including 34 with chronic hepatitis B (CHB), 74 with chronic hepatitis C (CHC), two with primary biliary cirrhosis, one with autoimmune hepatitis, and one with non-alcoholic fatty liver disease. Further, 53 retrospective samples were examined, spanning a total of 48 years of follow-up, from six patients who were found positive for any of the HEV serological markers.

Serological methods

A chemiluminescence enzyme immunoassay was used to detect antibodies to hepatitis C virus (anti-HCV; Ortho Clinical Diagnostics, Tokyo, Japan), hepatitis B surface antigen (HBsAg), hepatitis B core (anti-HBc) IgM, and hepatitis A virus (anti-HAV) IgM (Lumipulse, Fujirebio, Tokyo, Japan).

Detection of anti-HEV IgM, IgG, and IgA

Anti-HEV IgG and anti-HEV IgM were measured by enzyme-linked immunosorbent assay with a Viragent HEV-Ab Kit (Cosmic, Tokyo, Japan) by using purified recombinant HEV open reading frame (ORF) 2 protein, according to the instruction manual. A positive reaction was defined as an optical density (OD) of ≥0.13 for anti-HEV IgG and ≥0.30 for anti-HEV IgM. Anti-HEV IgA was measured by a previously described method.[29] Test samples with OD ≥0.642 were considered positive for anti-HEV IgA.[29]

Detection of HEV RNA

Nucleic acids were extracted from serum samples (50 µl) with a commercial kit (Smitest EX-R&D; Genome Science, Fukushima, Japan) and precipitated in a 2-ml tube. The nucleotide sequence of HEV was reverse-transcribed to cDNA with random primers, and PCR was performed with the cDNA obtained, with a specific set of screening primers targeting a partial nucleotide sequence of the ORF1 region of the HEV genome, as described previously.[30]

Sequencing and phylogenetic analysis of HEV isolates

For the purpose of sequence and phylogenetic analysis of HEV RNA-positive cases, PCR for amplification of a 326-nucleotide sequence of the ORF1 region of the HEV genome was performed with another set of previously described primers.[31] PCR products of ORF1 of the HEV genome were directly sequenced with a Prism BigDye kit (Applied Biosystems, Foster City, CA, USA) in an ABI 3100 DNA automated sequencer. Sequences were aligned with the CLUSTALX software program.[32] A phylogenetic tree was constructed by the neighbor-joining method with six-parameter distance correction.[33] Bootstrap values were determined on 1000 resampling tests of the data set.

Sequence data

The nucleotide sequence data reported in this study will appear in the DDBJ/EMBL/GenBank nucleotide

sequence database with the accession numbers AB329585 and AB329586.

Ethical considerations

This study was conducted in accordance with the guidelines of the Declaration of Helsinki and its subsequent amendments. Informed consent was obtained from all patients.

Results

Incidence of anti-HEV antibody and HEV RNA among acute hepatitis patients

Table 1 summarizes the clinical characteristics and prevalence of anti-HEV antibody in 81 AH patients with different viral etiologies. Serological assays for detection of anti-HEV antibodies, including anti-HEV IgG, anti-HEV IgM, and anti-HEV IgA, in serum samples collected from 81 AH patients revealed that, overall, eight of 81 samples (9.9%) were positive for anti-HEV IgG. Of these, four (23.5%) were from the AHA group, and four (8.2%) from the NABC AH group. Six of 81 (7.4%) patients were positive for anti-HEV IgM: one (5.9%) in the AHA group and five (10.2%) in the NABC AH group. Three of 81 samples (3.7%) were positive for anti-HEV IgA, and all were from the NABC AH group (3/49; 6.1%). HEV RNA was detected in two samples (2.5%), both from the NABC group. No patients from either the AHB or the AHC groups showed reactivity to any anti-HEV antibodies.

Serum samples of six AH patients exhibited positivity for an HEV marker (Table 2). Among the six serum samples positive for anti-HEV IgM, only two were also positive for both HEV RNA and anti-HEV IgA (AH1 and AH2) (Table 2). Another sample was also positive for anti-HEV IgA, with no detectable HEV RNA (AH3) (Table 2). Sample AH3 was obtained from the patient 35 days after hospitalization.

The three remaining samples, found positive for anti-HEV IgM but negative for both HEV RNA and anti-HEV IgA (AH4, AH5, and AH6), showed serological evidence of infection with HAV, Epstein-Barr virus, and cytomegalovirus virus, respectively.

Incidence of anti-HEV antibodies and HEV RNA among chronic hepatitis patients

To investigate the serological presence of hepatitis E markers in CH patients, serum samples from 112 CH patients were screened for anti-HEV IgG, anti-HEV IgM, and anti-HEV IgA. Table 3 summarizes the incidence of HEV antibodies (anti-HEV IgG, anti-HEV IgM, and anti-HEV IgA) in serum samples collected from 112 CH patients with different etiological agents. Six of 112 samples (5.4%) were positive for either anti-HEV IgG or anti-HEV IgM, and none showed reactivity for both together. Four of 112 samples (3.6%) were positive for anti-HEV IgG; three belonged to the CHC group, and one to the CHB group (Table 3). Only two of 112 samples (1.8%) were positive for anti-HEV IgM, and both belonged to the CHC group (Table 3). However, no CH patients were positive for anti-HEV IgA or HEV RNA.

Retrospective screening for anti-HEV antibody and HEV RNA in stored serum samples of chronic hepatitis patients with any anti-HEV antibody

Serial serum samples stored during the follow-up in Nagoya City University Hospital (1994–2003) were retrospectively retrieved for the six CH patients who exhibited reactivity to any of the anti-HEV antibodies. The serial serum samples were further subjected to anti-HEV IgG, IgM, and IgA examinations (Table 4). Two CH patients (CHC1 and 2) were positive for anti-HEV

Table 1. Prevalence of hepatitis E virus markers among acute hepatitis patients enrolled in this study

	Total (n = 81)	AHA (n = 17)	AHB (n = 12)	AHC (n = 3)	NABC (n = 49)
Age[a]	42.7 ± 15.6	43.1 ± 12.0	31.6 ± 11.4	67.7 ± 11.1	43.8 ± 15.9
Sex (male)[b]	41 (50.6)	11 (64.7)	8 (66.7)	1 (33.3)	21 (42.9)
AST (IU/ml)[a]	1585.4 ± 2340.8	3036.8 ± 2781.0	1038.2 ± 707.6	305.7 ± 474.9	1244.0 ± 2275.4
ALT (IU/ml)[a]	1751.6 ± 2015.9	3087.1 ± 2703.4	1982.3 ± 1285.7	449.7 ± 732.9	1282.7 ± 1665.2
TB (mg/dl)[a]	5.3 ± 7.1	4.1 ± 2.5	8.6 ± 7.3	1.3 ± 0.3	5.3 ± 8.2
anti-HEV IgG[b]	8 (9.9)	4 (23.5)	0	0	4 (8.2)
anti-HEV IgM[b]	6 (7.4)	1 (5.9)	0	0	5 (10.2)
anti-HEV IgA[b]	3 (3.7)	0	0	0	3 (6.1)
HEV RNA[b]	2 (2.5)	0	0	0	2 (4.1)

AHA, acute hepatitis A; AHB, acute hepatitis B; AHC, acute hepatitis C; NABC, non-A, non-B, non-C hepatitis; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TB, total bilirubin; HEV, hepatitis E virus

[a] Mean ± SD

[b] n (%)

Table 2. Characteristics of acute hepatitis patients positive for any of anti-HEV antibody

Case No.	Sex	Age	Diagnosis	Sampling day[a]	ALT	Anti-HEV IgG	Anti-HEV IgM	Anti-HEV IgA	HEV RNA
AH1	M	23	NABC	6	2997	>2 (+)	1.89 (+)	>3 (+)	(+)
AH2	M	32	NABC	3	1920	>2 (+)	1.99 (+)	>3 (+)	(+)
AH3	M	60	NABC	35	2049	>2 (+)	1.17 (+)	1.44 (+)	(−)
AH4	M	44	AHA	2	3336	.42 (+)	>2 (+)	0.199 (−)	(−)
AH5	F	21	NABC (EBV)	2	467	<0.05 (−)	0.88 (+)	0.076 (−)	(−)
AH6	M	35	NABC (CMV)	3	376	0.08 (−)	1.35 (+)	0.159 (−)	(−)
AH7	M	43	AHA	2	944	1.01 (+)	0.11 (−)	0.090 (−)	(−)
AH8	M	60	AHA	1	3029	0.37 (+)	0.27 (−)	0.158 (−)	(−)
AH9	M	45	AHA	2	163	0.73 (+)	0.05 (−)	0.056 (−)	(−)
AH10	M	48	NABC	8	1455	0.20 (+)	<0.05 (−)	0.055 (−)	(−)

AH, acute hepatitis; EBV, Epstein-Barr virus; CMV, cytomegalovirus
[a] Days after hospitalization

Table 3. Incidence of anti-hepatitis E antibody among different clinical groups of chronic hepatitis patients

	Anti-HEV IgG	Anti-HEV IgM	Anti-HEV IgA	HEV RNA
CHB (n = 34)	1 (2.9)	0	0	0
CHC (n = 74)	3 (4.1)	2 (2.7)	0	0
PBC (n = 2)	0	0	0	0
AIH (n = 1)	0	0	0	0
NAFLD (n = 1)	0	0	0	0
Total (n = 112)	4 (3.6)	2 (1.8)	0	0

CHB, chronic hepatitis B; CHC, chronic hepatitis C; PBC, primary biliary cirrhosis; AIH, autoimmune hepatitis; NAFLD, nonalcoholic fatty liver disease

IgM at the time of screening for anti-HEV antibody. Interestingly, all of the stored serum samples of CHC1 examined showed reactivity to anti-HEV IgM with stable high OD values, from 1.12 in 1994 to 1.79 in 2003, and none of the serial samples was positive for anti-HEV IgA or HEV RNA. A similar trend was observed for CHC2; the optical density values for anti-HEV IgM remained low and constant in all stored samples examined, excluding those from 1994, 1997, and 1998, which showed OD values of less than the cutoff value for anti-HEV IgM positivity (<0.05, 0.15, and 0.24, respectively) (Table 4). None of the examined serum samples of the four anti-HEV IgG-positive patients showed reactivity to anti-HEV IgM or anti-HEV IgA. A serum sample from CHC6 was reactive for anti-HEV IgG only at the time point of the initial screening. HEV RNA was undetectable in the sera of the four CH patients with anti-HEV IgG on examination of the retrospective serial serum samples collected during 1994–2003 (Table 4). Fluctuating levels of alanine aminotransferase (ALT) were observed sporadically in the stored samples throughout the retrospective screening period in the six CH patients. Elevation of ALT might be related to underlying infection with hepatitis B virus in HBV3 and HCV in HCV1, 2, and 4–6, rather than HEV, as indicated by undetectable HEV RNA in the corresponding stored samples.

Phylogenetic analysis of HEV strains isolated from acute hepatitis E patients

The phylogenetic relation of the HEV strains isolated from the two acute hepatitis E patients (Table 2; AH1 and AH2) with previously published strains retrieved from DDBJ/GenBank is shown in Fig. 1. The phylogenetic tree, constructed using 326 nucleotide ORF1 sequences of the HEV genome, revealed that both of the strains clustered with HEV strains of genotype 1 isolated from HEV-endemic areas of Asia, including Nepal, Myanmar, Pakistan, and India (Fig. 1). One patient (AH1), a Japanese man living in Toyokawa, Aichi, Japan, had a history of traveling to India before the onset of symptoms, indicating that the HEV strain of AH1 was imported from India and was not an indigenous HEV strain. The other patient (AH2) was a

Table 4. Follow-up serum samples (1993–2003) of chronic hepatitis patients who showed positivity to any of anti-HEV antibody

Case No Age Sex		1994	1995	1996	1997	1998	1999	2000	2001	2002	2003
CHC1	ALT	118	157	298				166		127	145
60	IgG	(–)	(–)	(–)				(–)		(–)	(–)
F	IgM	1.12	0.73	0.92				1.95		1.97	1.79
	IgA	(–)	(–)	(–)				(–)		(–)	(–)
	HEV RNA	(–)	(–)	(–)				(–)		(–)	(–)
CHC2	ALT	47	184	164	73	106	100	84	70	82	50
29	IgG	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)
M	IgM	(–)	0.61	0.46	(–)	(–)	0.42	0.38	0.38	0.38	0.46
	IgA	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	HEV RNA	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)
CHB3	ALT	29	38	34	210	44		179		33	28
49	IgG	0.60	0.51	0.49	0.50	0.59		0.48		0.50	0.57
M	IgM	(–)	(–)	(–)	(–)	(–)		(–)		(–)	(–)
	IgA	(–)	(–)	(–)	(–)	(–)		(–)		(–)	(–)
	HEV RNA	(–)	(–)	(–)	(–)	(–)		(–)		(–)	(–)
CHC4	ALT	46	88	57	86	85	73	28	27	37	36
63	IgG	0.30	0.24	0.32	0.30	0.37	0.35	0.41	0.42	0.50	0.50
F	IgM	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	IgA	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	HEV RNA	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)
CHC5	ALT	63	56	62	19		94	164	289	311	191
50	IgG	0.70	0.52	0.56	0.48		0.44	0.41	0.43	0.44	0.49
M	IgM	(–)	(–)	(–)	(–)		(–)	(–)	(–)	(–)	(–)
	IgA	(–)	(–)	(–)	(–)		(–)	(–)	(–)	(–)	(–)
	HEV RNA	(–)	(–)	(–)	(–)		(–)	(–)	(–)	(–)	(–)
CHC6	ALT	41	36	49	33	35	31	40	48	30	33
76	IgG	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	0.74
F	IgM	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	IgA	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	HEV RNA	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)

CHC, chronic hepatitis C; CHB, chronic hepatitis B

Bangladeshi living in Nagoya, Aichi, Japan, who had a history of traveling to Bangladesh before onset of the disease.

Discussion

A sensitive and specific method is needed for screening for HEV infection in acute hepatitis patients. To evaluate anti-HEV IgA as a specific diagnostic tool for HEV infection, we examined serum samples collected from AH patients and found that anti-HEV IgM was present in the sera of six of 81 AH patients (7.4%). Among these six patients, we detected anti-HEV IgA in three (AH1–AH3), and we detected HEV RNA in two of these three patients, indicating that patients AH1–AH3 had acute HEV infection. Our results suggest that anti-HEV IgA is a specific diagnostic marker for detecting recent HEV infection in patients with acute hepatitis, and that the combination of testing for the three anti-HEV antibodies (IgA, IgG, and IgM) can be a sensitive and specific screening tool for the diagnosis of HEV infection in acute hepatitis patients. In patient AH3, the serum sample was obtained t 35 days after hospitalization, which may explain why HEV RNA was undetectable in this sample despite the positivity for all three HEV serological markers, including anti-HEV IgA. In a previous follow-up study, HEV RNA disappeared from sera 15–59 days after disease onset, whereas anti-HEV IgA remained detectable for much longer (50–144 days after disease onset).[29]

Previously, anti-HEV IgA was detected in the sera of all 68 molecularly diagnosed acute hepatitis E patients.[29] Furthermore, Nagasaki et al.[34] detected anti-HEV IgA and anti-HEV IgG in an acute hepatitis E patient at the time of admission, but no anti-HEV IgM. This patient was initially misdiagnosed as having autoimmune hepatitis of acute onset before the HEV infection was found.[34] The longer duration of anti-HEV IgA in sera compared with anti-HEV IgM and HEV RNA may be an additional advantage of an anti-HEV IgA assay for screening for HEV infection among acute hepatitis patients.[25,29,35] However, another study demonstrated that the sensitivity of an anti-HEV IgA assay for diagnosing HEV infec-

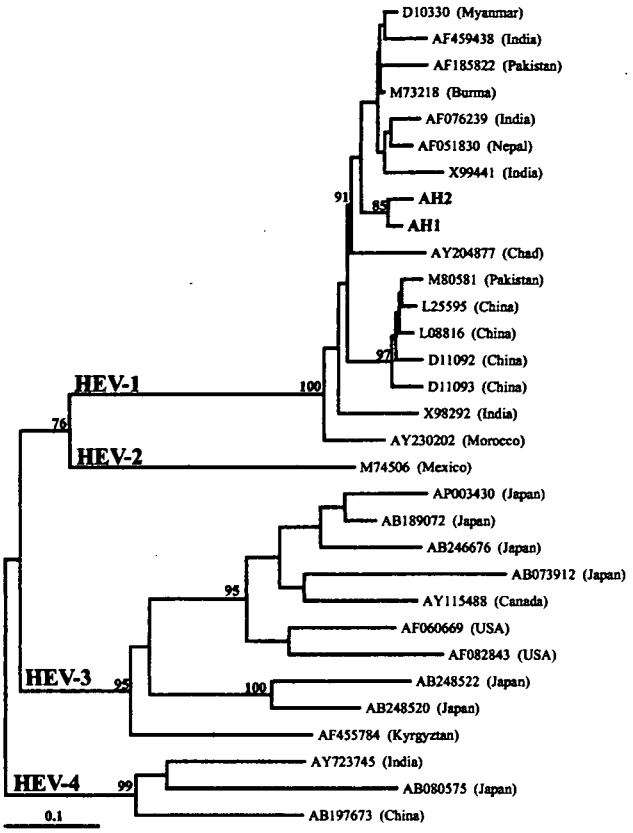

Fig. 1. Phylogenetic tree constructed by the neighbor-joining method based on the partial nucleotide sequence of the open reading frame 1 region (326 nt) of 29 reported hepatitis E virus (HEV) strains of genotypes 1–4. The two HEV strains (AH1 and AH2) that were found in the present study are indicated in bold. Reference sequences retrieved from GenBank/EMBL/DDBJ are indicated with their accession number, and the origin of each reference sequence is indicated in parentheses. The outgroup consists of human HEV strains (AY455784, AB080575, AB197673) isolated from India, Japan, and China respectively. Bootstrap values are indicated as a percentage obtained from 1000 resampling tests

tion may depend on the HEV genotype and the antigen type used in the serological assay.[36] Further prospective studies in several countries are needed to clarify the usefulness of the anti-HEV IgA assay, because RT-PCR is too laborious and expensive to be used routinely in developing countries where HEV infection is endemic.

We found two patients positive for anti-HEV IgM in the absence of anti-HEV IgA and anti-HEV IgG (CHC1 and 2) among CH patients. We did not detect HEV RNA in the serum of either case. Moreover, when we examined the serum samples of these two patients retrospectively (1994–2003) for the presence of anti-HEV IgM only, the results were the same, indicating a nonspecific reaction during the detection of anti-HEV IgM in these two cases. Such a false-positive reaction may occur during the detection of anti-HEV IgM owing to serologic cross-reaction with other antigenically related conditions.[25,37] Interestingly, a recent report showed that two patients without history of hepatic dysfunction or hepatitis were positive for anti-HEV IgM; these may be false positives, because the patients were negative for anti-HEV IgG, anti-HEV IgA, and HEV RNA.[38] Another important point is that in all samples examined retrospectively from these two patients were negative for anti-HEV IgA, despite the nonspecific reaction for anti-HEV IgM. This result suggests that anti-HEV IgA is a useful marker for specific discrimination of acute HEV cases.

Serological evidence of past infection with HEV was established in four (3.6%) of 112 CH patients enrolled in the present study. A recent report revealed the presence of frequent past HEV infection among blood donors with elevated ALT levels, most of which were subclinical.[21] In another study that examined serial serum samples collected periodically from two subjects over a short period, the anti-HEV IgA assay was also capable of diagnosing recent subclinical forms of HEV infection even in the absence of viremia.[20] Screening for HEV in patients with underlying chronic liver disease, even in non-HEV-endemic areas, is important and recommended, because superinfection with HEV in those patients might be associated with liver decompensation and a poor outcome.[39] Furthermore, patients with subclinical cases of HEV infection may serve as potential human reservoirs of the disease, particularly in HEV-endemic areas.[40,41]

In conclusion, anti-HEV IgA is more specific than anti-HEV IgM for screening for HEV infection among acute hepatitis patients, and examination for anti-HEV IgA as well as anti-HEV IgM and anti-HEV IgG allows sensitive and specific discrimination of acute and past infection with HEV. Since acute HEV infection was established in 6.1% of AH patients with NABC etiology, serological examinations for HEV infection should be performed in AH patients with unknown etiology, even in nonendemic HEV countries.

Acknowledgments. We gratefully thank Dr. Hiroaki Okamoto for his help in examining the anti-HEV IgA. This study was supported by a Grant-in-Aid from the Ministry of Health, Labour and Welfare of Japan.

References

1. Balayan MS, Andjaparidze AG, Savinskaya SS, Ketiladze ES, Braginsky DM, Savinov AP, et al. Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal–oral route. Intervirology 1983;20:23–31.
2. Reyes GR, Purdy MA, Kim JP, Luk KC, Young LM, Fry KE, et al. Isolation of a cDNA from the virus responsible for enterically transmitted non-A, non-B hepatitis. Science 1990;247:1335–9.

3. Labrique AB, Thomas DL, Stoszek SK, Nelson KE. Hepatitis E: an emerging infectious disease. Epidemiol Rev 1999;21:162–79.
4. Erker JC, Desai SM, Schlauder GG, Dawson GJ, Mushahwar IK. A hepatitis E virus variant from the United States: molecular characterization and transmission in cynomolgus macaques. J Gen Virol 1999;80(Pt 3):681–90.
5. Kwo PY, Schlauder GG, Carpenter HA, Murphy PJ, Rosenblatt JE, Dawson GJ, et al. Acute hepatitis E by a new isolate acquired in the United States. Mayo Clin Proc 1997;72:1133–6.
6. Mizuo H, Suzuki K, Takikawa Y, Sugai Y, Tokita H, Akahane Y, et al. Polyphyletic strains of hepatitis E virus are responsible for sporadic cases of acute hepatitis in Japan. J Clin Microbiol 2002;40:3209–18.
7. Takahashi M, Nishizawa T, Yoshikawa A, Sato S, Isoda N, Ido K, et al. Identification of two distinct genotypes of hepatitis E virus in a Japanese patient with acute hepatitis who had not travelled abroad. J Gen Virol 2002;83:1931–40.
8. Kabrane-Lazizi Y, Fine JB, Elm J, Glass GE, Higa H, Diwan A, et al. Evidence for widespread infection of wild rats with hepatitis E virus in the United States. Am J Trop Med Hyg 1999;61:331–5.
9. Meng XJ, Halbur PG, Shapiro MS, Govindarajan S, Bruna JD, Mushahwar IK, et al. Genetic and experimental evidence for cross-species infection by swine hepatitis E virus. J Virol 1998; 72:9714–21.
10. Nishizawa T, Takahashi M, Mizuo H, Miyajima H, Gotanda Y, Okamoto H. Characterization of Japanese swine and human hepatitis E virus isolates of genotype IV with 99 % identity over the entire genome. J Gen Virol 2003;84:1245–51.
11. Yazaki Y, Mizuo H, Takahashi M, Nishizawa T, Sasaki N, Gotanda Y, et al. Sporadic acute or fulminant hepatitis E in Hokkaido, Japan, may be food-borne, as suggested by the presence of hepatitis E virus in pig liver as food. J Gen Virol 2003;84:2351–7.
12. Tanaka Y, Takahashi K, Orito E, Karino Y, Kang JH, Suzuki K, et al. Molecular tracing of Japan-indigenous hepatitis E viruses. J Gen Virol 2006;87:949–54.
13. Tei S, Kitajima N, Takahashi K, Mishiro S. Zoonotic transmission of hepatitis E virus from deer to human beings. Lancet 2003; 362:371–3.
14. Zhang JZ, Im SW, Lau SH, Chau TN, Lai ST, Ng SP, et al. Occurrence of hepatitis E virus IgM, low avidity IgG serum antibodies, and viremia in sporadic cases of non-A, -B, and -C acute hepatitis. J Med Virol 2002;66:40–8.
15. Favorov MO, Khudyakov YE, Mast EE, Yashina TL, Shapiro CN, Khudyakova NS, et al. IgM and IgG antibodies to hepatitis E virus (HEV) detected by an enzyme immunoassay based on an HEV-specific artificial recombinant mosaic protein. J Med Virol 1996; 50:50–8.
16. Khuroo MS, Kamili S, Dar MY, Moecklii R, Jameel S. Hepatitis E and long-term antibody status. Lancet 1993;341:1355.
17. Khuroo MS, Saleem M, Teli MR, Sofi MA. Failure to detect chronic liver disease after epidemic non-A, non-B hepatitis. Lancet 1980;2:97–8.
18. Meky FA, Stoszek SK, Abdel-Hamid M, Selim S, Abdel-Wahab A, Mikhail N, et al. Active surveillance for acute viral hepatitis in rural villages in the Nile Delta. Clin Infect Dis 2006;42:628–33.
19. Nicand E, Grandadam M, Teyssou R, Rey JL, Buisson Y. Viraemia and faecal shedding of HEV in symptom-free carriers. Lancet 2001;357:68–9.
20. Mitsui T, Tsukamoto Y, Suzuki S, Yamazaki C, Masuko K, Tsuda F, et al. Serological and molecular studies on subclinical hepatitis E virus infection using periodic serum samples obtained from healthy individuals. J Med Virol 2005;76:526–33.
21. Gotanda Y, Iwata A, Ohnuma H, Yoshikawa A, Mizoguchi H, Endo K, et al. Ongoing subclinical infection of hepatitis E virus among blood donors with an elevated alanine aminotransferase level in Japan. J Med Virol 2007;79:734–42.
22. Bryan JP, Tsarev SA, Iqbal M, Ticehurst J, Emerson S, Ahmed A, et al. Epidemic hepatitis E in Pakistan: patterns of serologic response and evidence that antibody to hepatitis E virus protects against disease. J Infect Dis 1994;170:517–21.
23. Arankalle VA, Chadha MS, Tsarev SA, Emerson SU, Risbud AR, Banerjee K, et al. Seroepidemiology of water-borne hepatitis in India and evidence for a third enterically-transmitted hepatitis agent. Proc Natl Acad Sci USA 1994;91:3428–32.
24. Mast EE, Alter MJ, Holland PV, Purcell RH. Evaluation of assays for antibody to hepatitis E virus by a serum panel. Hepatitis E Virus Antibody Serum Panel Evaluation Group. Hepatology 1998;27:857–61.
25. Tian DY, Chen Y, Xia NS. Significance of serum IgA in patients with acute hepatitis E virus infection. World J Gastroenterol 2006;12:3919–23.
26. Schlauder GG, Desai SM, Zanetti AR, Tassopoulos NC, Mushahwar IK. Novel hepatitis E virus (HEV) isolates from Europe: evidence for additional genotypes of HEV. J Med Virol 1999;57: 243–51.
27. Chau KH, Dawson GJ, Bile KM, Magnius LO, Sjogren MH, Mushahwar IK. Detection of IgA class antibody to hepatitis E virus in serum samples from patients with hepatitis E virus infection. J Med Virol 1993;40:334–8.
28. Nomura M, Imai M, Tsuda F, Furuta S, Akahane Y, Tachibana K, et al. Immunoglobulin A antibody against hepatitis B core antigen in the acute and persistent infection with hepatitis B virus. Gastroenterology 1985;89:1109–13.
29. Takahashi M, Kusakai S, Mizuo H, Suzuki K, Fujimura K, Masuko K, et al. Simultaneous detection of immunoglobulin A (IgA) and IgM antibodies against hepatitis E virus (HEV) Is highly specific for diagnosis of acute HEV infection. J Clin Microbiol 2005;43: 49–56.
30. Takahashi K, Kang JH, Ohnishi S, Hino K, Miyakawa H, Miyakawa Y, et al. Full-length sequences of six hepatitis E virus isolates of genotypes III and IV from patients with sporadic acute or fulminant hepatitis in Japan. Intervirology 2003;46:308–18.
31. Takahashi K, Kang JH, Ohnishi S, Hino K, Mishiro S. Genetic heterogeneity of hepatitis E virus recovered from Japanese patients with acute sporadic hepatitis. J Infect Dis 2002;185: 1342–5.
32. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994;22:4673–80.
33. Gojobori T, Ishii K, Nei M. Estimation of average number of nucleotide substitutions when the rate of substitution varies with nucleotide. J Mol Evol 1982;18:414–23.
34. Nagasaki F, Ueno Y, Kanno N, Okamoto H, Shimosegawa T. A case of acute hepatitis with positive autoantibodies who actually had hepatitis E virus infection. Hepatol Res 2005;32:134–7.
35. Tokita H, Harada H, Gotanda Y, Takahashi M, Nishizawa T, Okamoto H. Molecular and serological characterization of sporadic acute hepatitis E in a Japanese patient infected with a genotype III hepatitis E virus in 1993. J Gen Virol 2003;84:421–7.
36. Herremans M, Duizer E, Jusic E, Koopmans MP. Detection of hepatitis E virus-specific immunoglobulin a in patients infected with hepatitis E virus genotype 1 or 3. Clin Vaccine Immunol 2007;14:276–80.
37. Thomas DL, Yarbough PO, Vlahov D, Tsarev SA, Nelson KE, Saah AJ, et al. Seroreactivity to hepatitis E virus in areas where the disease is not endemic. J Clin Microbiol 1997;35:1244–7.
38. Goto K, Ito K, Sugiura T, Ando T, Mizutani F, Miyake Y, et al. Prevalence of hepatitis E virus infection in Japanese children. J Pediatr Gastroenterol Nutr 2006;42:89–92.
39. Hamid SS, Atiq M, Shehzad F, Yasmeen A, Nissa T, Salam A, et al. Hepatitis E virus superinfection in patients with chronic liver disease. Hepatology 2002;36:474–8.
40. Aggarwal R, Kamili S, Spelbring J, Krawczynski K. Experimental studies on subclinical hepatitis E virus infection in cynomolgus macaques. J Infect Dis 2001;184:1380–5.
41. Aggarwal R, Kini D, Sofat S, Naik SR, Krawczynski K. Duration of viraemia and faecal viral excretion in acute hepatitis E. Lancet 2000;356:1081–2.

肝臓　48巻7号　338-340（2007）

＜短　報＞

動物種の如何を問わずE型肝炎ウイルス抗体を検出し得る簡便ELISA法

高橋　和明[1)]　安倍　夏生[1)]　道堯浩二郎[2)]　北嶋　直人[3)]
松井　高峯[4)]　津田　新哉[5)]　新井　雅裕[1)]　三代　俊治[1)*]

緒言：E型肝炎は人獣共通感染症（zoonosis）の一種として認知されており，本邦に於けるE型肝炎ウイルス（hepatitis E virus，HEV）感染の少なくとも四分の一は動物由来感染であると推定されている[1)]．ヒトがシカ及びイノシシから感染した事例には直接証拠が報告されているし[2)3)]，ブタからの感染にも極めて強力な間接証拠が提供されている．イノシシ，シカ，ブタ以外にも多くの動物種がE型肝炎ウイルス（HEV）のreservoirsとして存在する（例えば，ネパールのラット，エジプトのウマ，沖縄のマングースからはHEV RNAが採取されている）．従って，地球上に棲息する数多の動物種にHEVがいかに広く浸透しているかを知ることはHEV研究の重要課題の一つであり，その第一段階として各動物種に於けるHEV抗体の陽性率の調査がある．

而るに，従来のHEV抗体検出法[4)]には，調査対象となる動物種に特異的に対応する二次抗体を用意せねばならぬという困難が伴った．例えば，アライグマを対象にHEV抗体を検出しようとすれば，anti-raccoon IgG or IgM antibodiesを何處かから入手するか，さもなくば新規に作製する必要があった．この隘路を突破する目的で，我々は，動物種の如何を問わず一律にHEV抗体を検出し得る方法を案出した．

方法：HEV genotype 3のキャプシド蛋白をコードするORF2の一部（isolate name JTF-Tok99-c169のnt 1-1680）をpET100/D-TOPO vector（invitrogen）に組み込み，大腸菌で蛋白発現させ，MagneHis Protein Purification System（Promega）等により精製し，HEV抗体検出用の抗原として用いた．従来法に於いては二次抗体がトレーサーとして用いられているが，その代わりに，本法では上記抗原をperoxidaseで標識したものをトレーサーとして用い，ELISAの系を組んだ．即ち，Xiang et al[5)]が住血吸虫症の診断に用いた"double-antigen Sandwich assay"に於ける如く，固相リガンドもトレーサーリガンドも共に同一の抗原であること（antigen-antibody-antigen Sandwich）が本法の最大の特徴である（Fig. 1）．

結果：愛媛県で採取されたイノシシ由来血清（n＝400）を用い，従来法と本法による二重測定を行ったところ，大半の検体に於いて，本法によるoptical density（O.D.値）が従来法によるO.D.値を上回っていた（Fig. 2a）．兵庫県と北海道で採取されたシカ由来血清を対象に本法によるHEV抗体検出を試みたところ，O.D.値が1.0を越えるものを兵庫県検体では14本（7.8%）認めたのに対し，北海道検体では僅かに1本（1.3%）認めたのみであった（Fig. 2b）．

考察：上に示したイノシシとシカのみならず，ヒト及びマングース由来検体についても本法によるHEV抗体測定を試み，得られたO.D.値から「陽性」と判断し得る検体が多数存在することを我々は知った（data not shown here）．しかも，Fig. 2aが示唆する如く，本法で得られるO.D.値は従来法のそれを大半例に於いて遥かに上回っており，本法の感度の高さが示唆された．

しかし特異性には問題が残されている．高いO.D.値が得られたとしても，それが真にHEV抗体の存在によるものであるとの確かな証拠はない．例えば，Fig. 2aに於いて，従来法で0.5以下のO.D.値しか示さなかった検体の中には，本法で1.0以上のO.D.値を示したものが20本前後存在する．これらが真に「HEV抗体陽性」であるか否かは現時点では全く不明である．

而るに，Fig. 2bに示す如く，シカ血清のHEV抗体を本法で測定した時のO.D.値の分布が，兵庫県と北海道の間で明らかな差異を示した（兵庫県検体の方が高値

1）東芝病院研究部
2）愛媛大学光学医療診療部
3）市立加西病院消化器科
4）帯広畜産大学獣医学科病態獣医学講座病理学
5）中央農業総合研究センター病害防除部
*Corresponding author: shunji.mishiro@po.toshiba.co.jp

＜受付日2007年3月13日＞＜採択日2007年5月2日＞

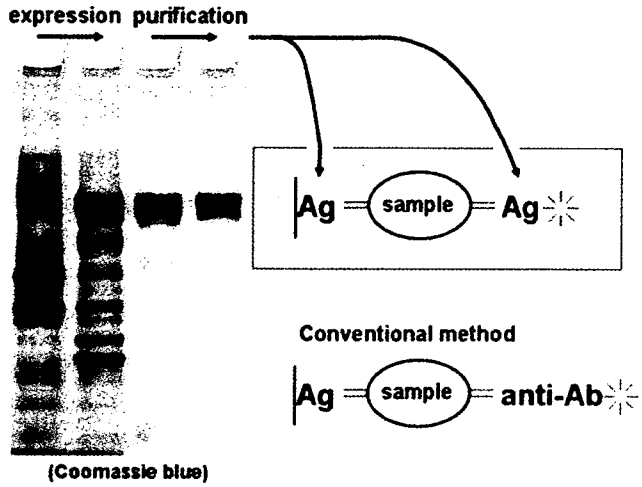

Fig. 1 Expression and purification of the HEV-ORF2 protein and the principle of the antigen-antibody-antigen Sandwich ELISA.

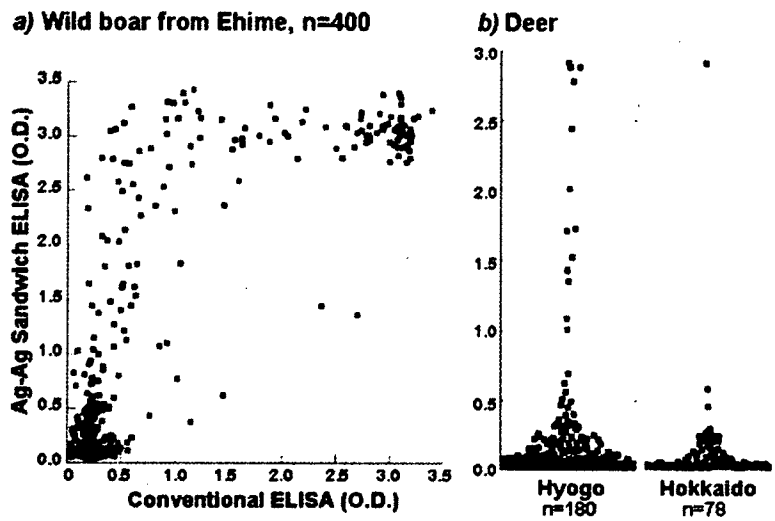

Fig. 2 Anti-HEV assay results with the new Sandwich ELISA. a) Correlation between the new ELISA system and the conventional one using sera from wild boars caught in Ehime. b) Comparison of the antibody titers between sera from deer in Hyogo and those from Hokkaido. O.D. denotes optical density.

方向にシフトしていた）ことは，本法の特異性に関して若干の示唆を与える．即ち，兵庫県の野生ジカからはHEV RNAが検出されヒトへの感染例も既に報告されているのに対し[2]，北海道の野生ジカ集団に於いてはHEV感染が皆無かあるいは極めて稀であるという知見が得られている（Li TC, personal communication）からである．本法によるHEV抗体測定結果は，然様な知見に全く矛盾しない．

特異性に関する詰めの検討（例えば非流行地健常人由来多数検体のアッセイ等）を残してはいるものの，動物種固有の免疫グロブリンに対する特異抗体を必要としない本法は，希少種を含む様々な動物種に於けるHEV抗体の検出の為には特に，有用且つ簡便な手段を提供するものである．Ag-Ab-Ag Sandwichという原理に基づく以上，IgGとIgMを区別して測定することは出来ないからヒト例に於ける臨床診断には有用でないが，ヒトに於ける疫学調査や宿主動物の探索には有用性を発揮し得ると考える．

索引用語：E型肝炎，E型肝炎ウイルス，HEV抗体，人獣共通感染症

文献：1）Abe T, Aikawa T, Akahane Y, et al. Kanzo 2006; 47: 384—391　2）Tei S, Kitajima N, Takahashi K, et al. Lancet 2003; 362: 371—373　3）Li TC, Chijiwa K, Sera N, et al. Emerg Infect Dis 2005; 11: 1958—1960　4）Takahashi M, Kusakai S, Mizuo H, et al. J Clin Microbiol 2005; 43: 49—56　5）Xiang X, Tianping W, Zhigang T. J Immunol Methods 2003; 280: 49—57

英文要旨

An easy ELISA for detecting antibodies to hepatitis E virus irrespective of animal species

Kazuaki Takahashi[1], Natsumi Abe[1], Kojiro Michitaka[2], Naoto Kitajima[3], Takane Matsui[4], Shinya Tsuda[5], Masahiro Arai[1], Shunji Mishiro[1]*

A partial ORF2 protein of hepatitis E virus (HEV) was expressed in *E. coli*, purified, and used as solid-phase ligand as well as tracer-ligand labeled with peroxidase, in an antigen-antibody-antigen Sandwich ELISA system, to detect antibodies to HEV. This new system showed higher sensitivity of detecting anti-HEV than conventional ELISA system which needs specific antibodies against IgG, IgM, or IgA of concerned animal species as tracer-ligands. Since our new ELISA system is universally applicable to any animal species without needing specific antibodies, it will be useful in further elucidating the animal reservoirs of HEV.

Key words: hepatitis E, hepatitis E virus, anti-HEV, zoonosis

Kanzo 2007; 48: 338—340

1) Toshiba General Hospital
2) Ehime University School of Medicine
3) Kasai City Hospital
4) Obihiro University of Agriculture and Veterinary Medicine
5) National Agricultural Research Center
*Corresponding author: shunjimishiro@po.toshiba.co.jp

日消誌 2007；104：219—225

—症例報告—

人工肝補助療法を施行し救命し得たgenotype IVのE型劇症肝炎の1例

藤山洋一　山岸由幸　菊池真大
下地顕一郎　加藤眞三　永田博司
齋藤英胤　日比紀文[1)]　三代俊治[2)]

要旨：症例は40歳男性．全身倦怠感，褐色尿が出現し，肝障害を認め入院．プロトロンビン時間の著明な低下，肝性昏睡II度を認め，E型肝炎ウイルス抗体高値にてE型劇症肝炎と診断した．保存血清より中国型genotype IV型のE型肝炎ウイルスが検出された．これまで海外にて感染し帰国後劇症化したE型肝炎の報告例はない．経過より脳症発症前から積極的に人工肝補助療法を導入し救命し得たと考えられ，貴重な症例であり報告した．

索引用語：E型肝炎，劇症肝炎，人工肝補助療法

はじめに

E型肝炎ウイルスは経口感染によって感染するRNAウイルスで，東南アジア，赤道周辺，中南米などで多く発生している．わが国では輸入感染症の1つと考えられていたが，2001年に海外渡航歴のない原因不明の急性肝炎症例から国内型HEV株が検出されて以降[1)]，国内型症例の報告が相次いでいる．E型肝炎は大部分が予後良好な疾患であるが，まれに劇症化し予後不良となる．E型劇症肝炎例の報告は少なく救命例は更に限られる．本症例は，臨床経過から脳症発症前より積極的に人工肝補助療法を導入したことにより救命し得たと考えられ，貴重な症例であり報告する．

I 症　例

患者：40歳，男性（会社員）．

主訴：全身倦怠感，褐色尿．

既往歴：特記すべきことなし，輸血歴（−）．

家族歴：家族内に肝疾患なし，父　高血圧．

生活歴：機会飲酒，生肉食なし，鍼治療歴なし，刺青・ピアスなし，常時服薬なし，不特定の同性，異性の性交渉なし．

現病歴：生来健康．2002年頃より仕事で中国を頻回に往来していた．2004年7月下旬より8月中旬まで中国山東省（ツーポー市）に出張で出かけていた．その間に体調不良は認めず，生物摂取，動物との接触，また漢方薬含め薬の内服もなかった．8月下旬より中国へ再渡航したが，翌日より上腹部痛，全身倦怠感と褐色尿を自覚し帰国．近医を受診した．血液検査上TB 5.0mg/dl，GOT 4180IU/l，GPT 5661IU/l，ALP 579IU/l，γ-GTP 426IU/lと黄疸，トランスアミナーゼの著明な上昇を認めたため同日入院となった．A，B，C型肝炎ウイルスマーカーは陰性であり，原因不明の急性肝炎として肝庇護剤投与にて経過観察されるも，PT%が25%まで低下したため精査加療目的にて当院へ転院となった．

入院時現症：

身長170.0cm，体重61.9kg，体温36.8℃，血圧120/70mmHg，脈拍60/分整，意識清明．眼球結膜黄染あり．上腹部にやや圧痛を認め，グル音やや低下，右鎖骨中線上に肝1横指触知，脾腫はなく，腹水なし，手掌紅斑なし，ばち指なし，クモ

1）慶應義塾大学医学部消化器内科
2）東芝病院研究部

Table 1. 当院入院時検査所見 1

【末梢血】		【生化学】	
WBC	900 /μl	TP	7.0 g/dl
RBC	492 万 /μl	ALB	3.7 g/dl
Hb	15.7 g/dl	TB	12.7 mg/dl
Plt	100×10^3 /μl	DB	9.1 mg/dl
		LDH	515 IU/l
【凝固】		AST	1307 IU/l
APTT	48.5 sec	ALT	3489 IU/l
PT	18 %	ALP	610 IU/l
PT-INR	2.79	γ-GTP	324 IU/l
FNG	170 mg/dl	Ch-E	225 IU/l
AT-III	42 %	AMY	70 IU/l
		UN	9.3 mg/dl
【新鮮尿】		Cr	0.6 mg/dl
PH	7.0	UA	6.2 mg/dl
Glu	(−)	Glu	100 mg/dl
Pro	(−)	TC	136 mg/dl
Bld	(−)	TG	111 mg/dl
Bil	(2 +)	NH_3	50 μmol/l
Uro	(±)	CRP	0.65 mg/dl
		AFP	4 ng/ml

Table 2. 当院入院時検査所見 2

【血清】		【免疫】	
HAV-IgM Ab	(−)	IgG	2020 mg/dl
HA-IgG Ab	(−)	IgA	572 mg/dl
HBs-Ag	(−)	IgM	671 mg/dl
HBe-Ag	(−)	抗核抗体	(−)
HBe-Ab	(−)	抗 M2 抗体	(−)
HBs-Ab	(−)		
HBc-IgM	(−)		
HBc-Ab	(−)	【HEV 関連】	
HCV-Ab	(−)	HEV-IgM	143 (< 30)
HCV-PCR	(−)	HEV-IgG	124 (< 13)
EB-IgM	(−)	HEV-PCR	(−) *1
EB-IgG	(+)	HEV-Genotype	IV 型*2 (JKS2-Tok04)
CMV-IgM	(−)		
CMV-IgG	(+)		
CMV-Antigenemia	(−)	【入院時予測劇症化確率】	
HIV1, 2-Ab	(−)		37.4 % *3

*1 (株) SRL にて測定 (VIRAGENT HEV-Ab. (株) 特殊免疫研究所)
*2 東芝病院研究部にて解析
*3 岩手医大第一内科および厚生労働省登録システムによる急性肝炎重症型劇症化予測式

状血管腫なし，羽ばたき振戦など神経学的異常所見は認めなかった．

入院時検査所見 (Table 1, 2)：

ビリルビン尿を認め，白血球は正常で，貧血は認めず，血小板は 10 万/μl と低下していた．凝固系では PT% が 18% と著明に低下し，また AT-