TABLE IV. Comparison of Demographic Characteristics and Laboratory Parameters Between Patients Infected With HEV With U at Nt 3148 or Those With Another Nucleotide at Nt 3148

	Nt 3148		
	U (n = 8)	C or G (n = 40)	*P* value[a]
Age (years)	58.5 ± 8.9	57.7 ± 12.0	0.7085
Male [number (%)]	8 (100)	33 (82.5)	0.2532
Peak ALT (IU/l)	2,814 ± 1,009	2,459 ± 1,237	0.2685
Peak total bilirubin (mg/dl)	16.5 ± 10.3	9.6 ± 8.3	0.0721
Lowest prothrombin activity (%)	51.8 ± 29.1	76.8 ± 25.9	**0.0293**
High HEV load[b] [number (%)]	3 (37.5)	10 (25.0)	0.3704

The demographic characteristics and laboratory parameters of patients with type E acute or fulminant hepatitis were studied [Mizuo et al., 2002, 2005; Suzuki et al., 2002; Kuno et al., 2003; Yajima et al., 2003; Yazaki et al., 2003; Sainokami et al., 2004; Saitoh et al., 2004; Yamamoto et al., 2004; Hijioka et al., 2005]. Twenty-seven patients were infected with genotype 4 HEV and 21 patients were infected with genotype 3 HEV.

[a]*P* value that is statistically significant is indicated in bold face.

[b]With HEV RNA titer of $\geq 10^5$ copies/ml at the first examination.

pregnancy [Harrison, 1999; Purcell and Emerson, 2001; Smith, 2001] or 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 type E fulminant hepatitis. Hepatitis B virus variants with mutations in the precore region [Kosaka et al., 1991; Liang et al., 1991; Omata et al., 1991; Terazawa 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 significant numbers of nucleotide substitutions in the 5′-untranslated region of the hepatitis A virus genome [Fujiwara et al., 2001, 2002]. Recently, it was suggested that the severity of hepatitis E is influenced by the genotype of HEV, based on the finding that patients infected with genotype 4 HEV tend to have more severe disease than those with genotype 3 HEV in Japan [Mizuo et al., 2005]. The mortality rate of hepatitis E in developing countries where genotype 1 HEV prevails is reported to be about 1% [Purcell and Emerson, 2001]. 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. Genotype 4 HEV is unique in that there is an insertion of a single nucleotide (U) at nt 5159, which affects both ORF2 and ORF3 (Fig. 2A). The ORF2 of genotype 4 HEV overlaps ORF1 by one nt, whereas ORF2 in all reported isolates of genotypes 1–3 begins 41 nt downstream of ORF1. The first initiation codon of ORF3 in genotype 4 HEV isolates is 28 nt downstream of ORF1, in contrast with ORF3 in reported isolates of genotypes 1–3 which overlaps ORF1 by one nt. Consequently, genotype 4 HEV has an additional 14 codons in ORF2. The predicted size of ORF3 of genotype 4 HEV at the 5′-terminal portion is nine codons shorter than that of reported isolates of genotypes 1–3 [Wang et al., 2000; Takahashi et al., 2003]. The uniqueness of the genotype 4 HEV genome may explain, at least in part, its association with the severe form of hepatitis E.

Amino acid substitutions in viral proteins that are related with altered pathogenesis have been well documented [Brack et al., 1998; Raychaudhuri et al., 1998; Lum et al., 2003; Glenn and Novembre, 2004]. The viral RNA 5′- and 3′-untranslated region sequences can also affect the expression of disease symptom [Slobodskaya et al., 1996; Brack et al., 1998; Bryant et al., 2005]. In the present study, amino acid changes in the coding regions or nucleotide substitutions in the 5′- and 3′-untranslated regions, that may be associated with the development of fulminant hepatitis, were not observed among patients who were infected with genotype 4 HEV and diagnosed with fulminant hepatitis. However, a silent substitution of U at nt 3148, that is located within the RNA helicase domain of ORF1, was observed significantly more frequently among genotype 4 HEV isolates than among isolates of the other genotypes ($P < 0.0001$), and among HEV isolates obtained from patients with fulminant hepatitis than among those obtained from patients with acute hepatitis ($P = 0.0006$). The results suggest that U3148 in the HEV genome is associated with progression to fulminant hepatitis or the severe form of hepatitis E. The underlying reason for the association of a silent substitution at nt 3148 in the HEV genome with the progression to fulminant hepatitis remains unknown. Two possible explanations are as follows. One explanation is that the silent substitution at nt 3148 may influence the efficiency of replication of HEV. Nt 3148 is located at the RNA helicase domain [Koonin et al., 1992] and the particular nucleotide at nt 3148 may alter the secondary structure of the genome, thereby affecting the expression of RNA helicase. The secondary structure of the RNA genome with U3148 may be favorable for translation of the RNA helicase and RNA polymerase whose coding region is located downstream of the RNA

helicase domain, as discussed previously [Hirata et al., 2003]. Another explanation is that the nucleotide sequence containing nt 3148 may regulate the transcription of the subgenomic mRNA of the HEV genome. It was reported that in the liver of cynomolgus macaques infected with HEV, there was a subgenomic mRNA that was shorter than the entire genome and had a common 3′-end with genomic RNA [Tam et al., 1991; Yarbough et al., 1991]. The 22-nt sequence including nt 3148 was conserved among all four genotypes, supporting the latter explanation. As the 5′-end of the subgenomic mRNA has not been determined as yet, it is uncertain whether nt 3148 can affect the transcription of the mRNA. As to whether the sequence including nt 3148 plays a role at the genomic level, the 15-nt sequence including U3148 is homologous to the sequence 5′ UGCYAUUGAGCAGGC 3′ (nt 67–81) in the methyltransferase domain of ORF1, which is well conserved among the genotypes, and further investigation may be warranted.

Of interest, a plant virus, *Apple stem grooving virus* (ASGV), that contained a single silent substitution in the coding region, did not induce the symptoms in host plants that are characteristic of the wild-type virus [Hirata et al., 2003]. As the substitution did not affect the abundance of mRNA transcribed from the downstream, the mechanism of symptom attenuation is under discussion. Its genome consists of a single-stranded, positive-sense RNA of 6.5 kb that is 5′-capped and 3′-polyadenylated. Some features of the ASGV genome resemble those of the HEV genome, suggesting that a silent substitution in the HEV genome may also affect the symptoms in the host. HEV RNA replication occurred in primate cell cultures transfected with in vitro transcripts of an infectious cDNA clone of the HEV genome [Emerson et al., 2004b]. Studies using a mutagenized genotype 4 HEV with U3148 that is constructed in vitro, may elucidate the mechanism by which the silent substitution of U3148 leads to progression to fulminant hepatitis.

In conclusion, the results of this study suggest that a silent substitution of U at nt 3148 in genotype 4 HEV is associated closely with the occurrence of fulminant hepatitis. As the number of patients with type E fulminant hepatitis is limited, accumulation of patients with type E fulminant hepatitis not only in Japan but also in other countries and extensive clinical and virological analyses of a large number of such cases are needed in future studies to evaluate our proposal. Studies on the mechanism by which the silent substitution of U3148 leads to progression to fulminant hepatitis may elucidate a novel determinant of disease severity of HEV infection.

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REFERENCES

Brack K, Frings W, Dotzauer A, Vallbracht A. 1998. A cytopathogenic, apoptosis-inducing variant of hepatitis A virus. J Virol 72:3370–3376.

Bryant JE, Vasconcelos PF, Rijnbrand RC, Mutebi JP, Higgs S, Barrett AD. 2005. Size heterogeneity in the 3′ noncoding region of South American isolates of yellow fever virus. J Virol 79:3807–3821.

Coursaget P, Buisson Y, N'Gawara MN, Van Cuyck-Gandre H, Roue R. 1998. Role of hepatitis E virus in sporadic cases of acute and fulminant hepatitis in an endemic area (Chad). Am J Trop Med Hyg 58:330–334.

Donati MC, Fagan EA, Harrison TJ. 1997. Sequence analysis of full length HEV clones derived directly from human liver in fulminant hepatitis E. In: Rizzetto M, Purcell RH, Gerin JL, Verme G, editors. Viral hepatitis and liver disease. Torino: Edizioni Minerva Medica. pp 313–316.

Emerson SU, Anderson D, Arankalle A, Meng XJ, Purdy M, Schlauder GG, Tsarev SA. 2004a. *Hepevirus*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA, editors. Virus taxonomy: VIIIth report of the ICTV. London: Elsevier/Academic Press. pp 853–857.

Emerson SU, Nguyen H, Graff J, Stephany DA, Brockington A, Purcell RH. 2004b. In vitro replication of hepatitis E virus (HEV) genomes and of an HEV replicon expressing green fluorescent protein. J Virol 78:4838–4846.

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′ nontranslated region of hepatitis A virus RNA: Strains from fulminant hepatitis have fewer nucleotide substitutions. Gut 51:82–88.

Glenn AA, Novembre FJ. 2004. A single amino acid change in gp41 is linked to the macrophage-only replication phenotype of a molecular clone of simian immunodeficiency virus derived from the brain of a macaque with neuropathogenic infection. Virology 325:297–307.

Harrison TJ. 1999. Hepatitis E virus—An update. Liver 19:171–176.

Hijioka S, Sato Y, Iwashita Y, Indou Y. 2005. A case of acute hepatitis E who had a history of frequent ingestion of raw meat and viscera from wild deer and boars (in Japanese). Jpn J Gastroenterol 102:723–728.

Hirata H, Lu X, Yamaji Y, Kagiwada S, Ugaki M, Namba S. 2003. A single silent substitution in the genome of *Apple stem grooving virus* causes symptom attenuation. J Gen Virol 84:2579–2583.

Ina Y. 1994. ODEN: A program package for molecular evolutionary analysis and database search of DNA and amino acid sequences. Comput Appl Biosci 10:11–12.

Kato M, Taneichi K, Matsubayashi K. 2004. A mini-outbreak of HEV infection in those who enjoyed *Yakiniku* party: One died of fulminant hepatitis (in Japanese). Kanzo 45:688.

Koonin EV, Gorbalenya AE, Purdy MA, Rozanov MN, Reyes GR, Bradley DW. 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 USA 89:8259–8263.

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.

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

Liu Z, Chi B, Takahashi K, Mishiro S. 2003. A genotype IV hepatitis E virus strain that may be indigenous to Changchun, China. Intervirology 46:252–256.

Lum JJ, Cohen OJ, Nie Z, Weaver JG, Gomez TS, Yao XJ, Lynch D, Pilon AA, Hawley N, Kim JE, Chen Z, Montpetit M, Sanchez-Dardon J, Cohen EA, Badley AD. 2003. Vpr R77Q is associated with long-term nonprogressive HIV infection and impaired induction of apoptosis. J Clin Invest 111:1547–1554.

Meng XJ. 2003. Swine hepatitis E virus: Cross-species infection and risk in xenotransplantation. Curr Top Microbiol Immunol 278:185–216.

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.

Nanda SK, Yalcinkaya K, Panigrahi AK, Acharya SK, Jameel S, Panda SK. 1994. Etiological role of hepatitis E virus in sporadic fulminant hepatitis. J Med Virol 42:133–137.

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.

Ohnishi S, Kang JH, Maekubo H, Takahashi K, Mishiro S. 2003. A case report: Two patients with fulminant hepatitis E in Hokkaido, Japan. Hepatol Res 25:213–218.

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.

Page RD. 1996. TreeView: An application to display phylogenetic trees on personal computers. Comput Appl Biosci 12:357–358.

Purcell RH, Emerson SU. 2001. Hepatitis E virus. In: Knipe DM, Howley PM, Griffin DE, Martin MA, Lamb RA, Roizman B, Straus SE, editors. Fields virology, 4th edition. Philadelphia, PA: Lippincott Williams and Wilkins. pp 3051–3061.

Raychaudhuri G, Govindarajan S, Shapiro M, Purcell RH, Emerson SU. 1998. Utilization of chimeras between human (HM-175) and simian (AGM-27) strains of hepatitis A virus to study the molecular basis of virulence. J Virol 72:7467–7475.

Sainokami S, Abe K, Kumagai I, Miyasaka A, Endo R, Takikawa Y, Suzuki K, Mizuo H, Sugai Y, Akahane Y, Koizumi Y, Yajima Y, Okamoto H. 2004. Epidemiological and clinical study of sporadic acute hepatitis E caused by indigenous strains of hepatitis E virus in Japan compared with acute hepatitis A. J Gastroenterol 39:640–648.

Saitoh N, Doyama H, Omori T, Yamada S, Shimazaki H. 2004. A domestic case of acute sporadic hepatitis E identified in Hokuriku district (in Japanese). Kanzo 45:268–273.

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

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 GG, Mushahwar IK. 2001. Genetic heterogeneity of hepatitis E virus. J Med Virol 65:282–292.

Sheikh A, Sugitani M, Kinukawa N, Moriyama M, Arakawa Y, Komiyama K, Li TC, Takeda N, Ishaque SM, Hasan M, Suzuki K. 2002. Hepatitis E virus infection in fulminant hepatitis patients and an apparently healthy population in Bangladesh. Am J Trop Med Hyg 66:721–724.

Slobodskaya OR, Gmyl AP, Maslova SV, Tolskaya EA, Viktorova EG, Agol VI. 1996. Poliovirus neurovirulence correlates with the presence of a cryptic AUG upstream of the initiator codon. Virology 221:141–150.

Smith JL. 2001. A review of hepatitis E virus. J Food Prot 64:572–586.

Suzuki K, Aikawa T, Okamoto H. 2002. Fulminant hepatitis E in Japan. N Engl J Med 347:1456.

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 JH, 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 K, Toyota J, Karino Y, Kang JH, Maekubo H, Abe N, Mishiro S. 2004. Estimation of the mutation rate of hepatitis E virus based on a set of closely related 7.5-year-apart isolates from Sapporo, Japan. Hepatol Res 29:212–215.

Takahashi K, Okada K, Kang JH, Karino Y, Ichida T, Matsuda H, Ohnishi S, Toyota J, Yamagiwa S, Maekubo H, Abe N, Mishiro S. 2005. A lineage of hepatitis E virus within genotype IV, associated with severe forms of hepatitis (in Japanese). Kanzo 46:389–390.

Tam AW, Smith MM, Guerra ME, Huang CC, Bradley DW, Fry KE, Reyes GR. 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.

Terazawa S, Kojima M, Yamanaka T, Yotsumoto S, Okamoto H, Tsuda F, Miyakawa Y, Mayumi M. 1991. Hepatitis B virus mutants with precore-region defects in two babies with fulminant hepatitis and their mothers positive for antibody to hepatitis B e antigen. Pediatr Res 29:5–9.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680.

Wang Y, Zhang H, Ling R, Li H, Harrison TJ. 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.

Yajima Y, Takahashi N, Yamagishi H, Kodama A, Miyazaki A, Sugiyama K, Eda Y, Okamoto H. 2003. An acute hepatitis case domestically infected with a hepatitis E virus whose nucleotide sequence showed a high similarity to that from a domestic swine in Japan (in Japanese). Jpn J Gastroenterol 100:454–458.

Yamamoto T, Suzuki H, Toyota T, Takahashi M, Okamoto H. 2004. Three male patients with sporadic acute hepatitis E in Sendai, Japan, who were domestically infected with hepatitis E virus of genotype III or IV. J Gastroenterol 39:292–298.

Yarbough PO, Tam AW, Fry KE, Krawczynski K, McCaustland KA, Bradley DW, Reyes GR. 1991. Hepatitis E virus: Identification of type-common epitopes. J Virol 65:5790–5797.

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.

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.

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Short Communication

Molecular tracing of Japan-indigenous hepatitis E viruses

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The ancestor(s) of apparently Japan-indigenous strains of *Hepatitis E virus* (HEV) was probably of foreign origin, but it remains unclear when and from where it made inroads. In this study, 24 genotype 3 and 24 genotype 4 HEV strains recovered in Japan each showed a significant cluster, clearly distinct from those of foreign strains, in the phylogenetic tree constructed from an 821 nt RNA polymerase gene fragment. The evolutionary rate, approximately $0{\cdot}8 \times 10^{-3}$ nucleotide substitutions per site per year, enabled tracing of the demographic history of HEV and suggested that the ancestors of Japan-indigenous HEV had made inroads around 1900, when several kinds of Yorkshire pig were imported from the UK to Japan. Interestingly, the evolutionary growth of genotype 3 in Japan has been slow since the 1920s, whereas genotype 4 has spread rapidly since the 1980s. In conclusion, these data suggest that the indigenization and spread of HEV in Japan were associated with the popularization of eating pork.

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Transmission of *Hepatitis E virus* (HEV) occurs primarily by the faecal–oral route through contaminated water supplies in developing countries (Purcell & Emerson, 2001). Additionally, increasing evidence has indicated that hepatitis E is a zoonosis (Harrison, 1999; Kabrane-Lazizi *et al.*, 1999; Meng *et al.*, 1997, 1998, 2002; Nishizawa *et al.*, 2003; Okamoto *et al.*, 2001; Tei *et al.*, 2003; Yazaki *et al.*, 2003). It has recently been suggested that zoonotic, food-borne transmission of HEV from domestic pigs, wild boars or wild deer to humans plays an important role in the occurrence of domestic infections of hepatitis E in Japan, where people have unique habits of ingesting raw fish (sushi or sashimi) and uncooked or undercooked meat (also organ meats, such as raw liver) (Matsuda *et al.*, 2003; Tamada *et al.*, 2004). Thus, it seems that HEV infection is now autochthonous in Japan. It remains unclear, however, when and from where the ancestral HEV strains made inroads and have spread in

The GenBank/EMBL/DDBJ accession numbers for the HEV nucleotide sequences reported in this paper are shown in Fig. 1.

Supplementary tables are available in JGV Online.

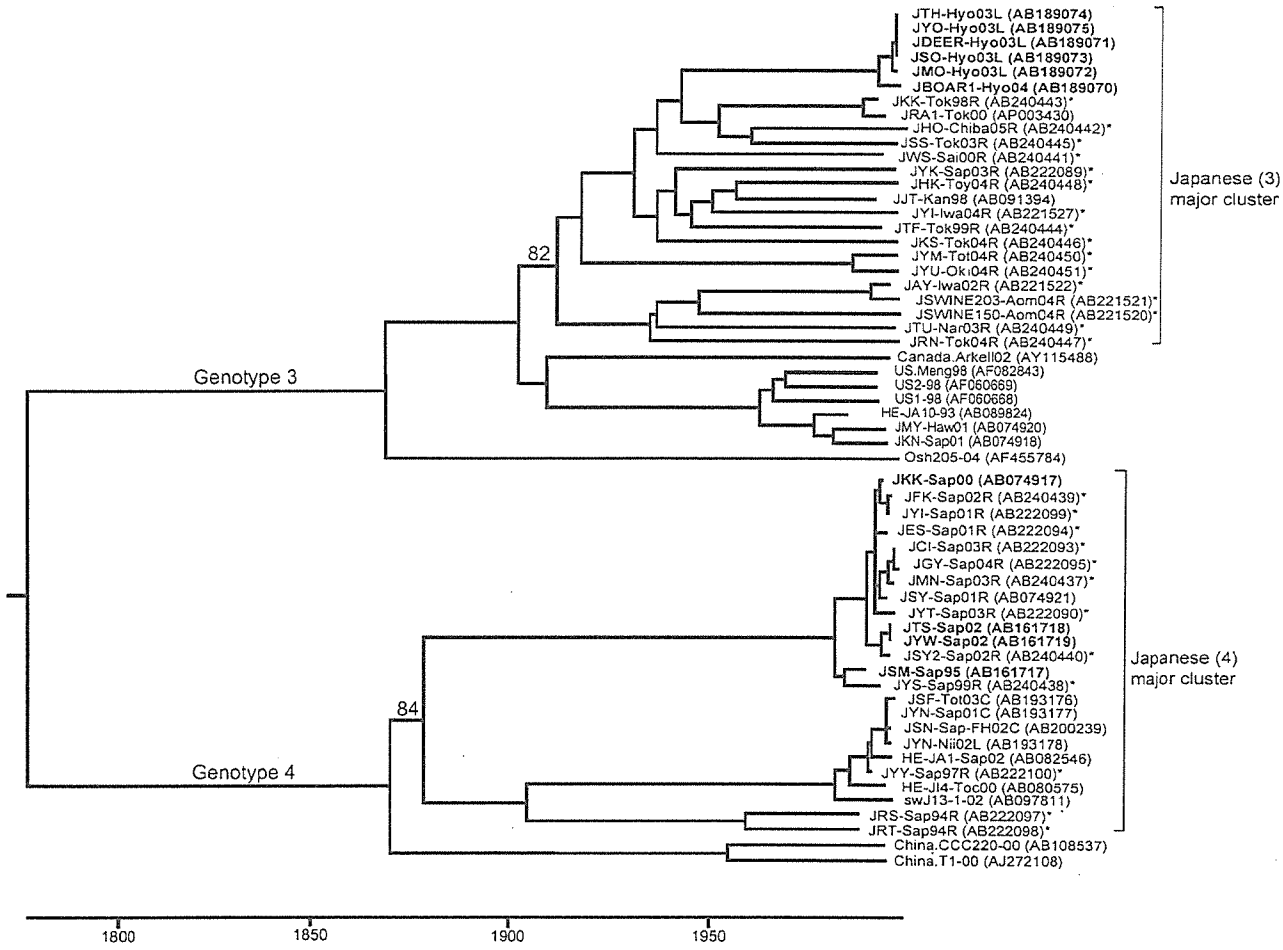

Fig. 1. Phylogenetic tree of the partial RNA polymerase region of the HEV genome. Twenty-four genotype 3 and 24 genotype 4 strains in Japan showed each significant cluster to have a high bootstrap value and to be distinct from other reference sequences (USA, Canada and Japanese minor strains in genotype 3; Chinese strains in genotype 4). Genetic distances have been transformed into a time scale of years by using estimates of the molecular clock ($0{\cdot}84 \times 10^{-3}$ nucleotide substitutions per site per year). Ten strains in bold are used for linear regression in Fig. 2. Strain names are followed by prefecture or city names in Japan: Hyo, Hyogo; Tok, Tokyo; Sai, Saitama; Sap, Sapporo; Iwa, Iwate; Kan, Kanagawa; Oki, Okinawa; Aom, Aomori; Nar, Nara; Tot, Tottori; Nii, Niigata; Toc, Tochigi; Toy, Toyama. Asterisks indicate strains that were newly sequenced in this study.

Japan. In this study, we first estimated the evolutionary rate of HEV by using Japan-indigenous genotype 3 and genotype 4 strains, which were phylogenetically distinct from the other strains in foreign countries. Then, based on this evolutionary rate, we traced the demographic history of HEV in Japan.

For linear-regression analyses within significant clusters, two independent datasets were applied: one was a Hyogo cluster (genotype 3) with JMO-Hyo03L, JTH-Hyo03L, JSO-Hyo03L, JYO-Hyo03L, JDEER-Hyo03L (these five isolates were obtained in April 2003) and JBOAR1-Hyo04 (April 2004) (Takahashi *et al.*, 2004a), and another was a Sapporo cluster (genotype 4) with JSM-Sap95 (March 1995), JKK-Sap00 (November 2000), JYWSap02 (August 2002) and JTS-Sap02 (September 2002) (Takahashi *et al.*, 2004b). GenBank accession numbers for these strains are given in Fig. 1. To elucidate the epidemiological history of the HEV population in Japan, 48 known and newly sequenced HEV strains ($n=24$ for each of genotype 3 and 4) were used for molecular-evolutionary analyses. The nucleotide sequences of 28 strains for the molecular-clock analyses were determined in this study (the other 20 sequences dealt with in this paper were available from GenBank).

Nucleic acids were extracted from serum samples (50 µl) by using a commercial Smitest EX-R & D kit (Genome Science) and precipitated in a 2 ml tube. The pellet was air-dried for 15 min and then suspended in 10 µl autoclaved distilled water containing 10 U RNase inhibitor ml^{-1} (TaKaRa

Shuzo). A sequence spanning 821 nt in the RNA-dependent RNA polymerase region (corresponding to nt 3961–4781 of the prototype Burmese HEV strain; GenBank accession no. M73218), including the GDD motif, was amplified by PCR in three overlapping regions with 20-mer primers deduced from known HEV sequences. Reverse transcription was performed at 50 °C for 60 min with the Thermo-Script RT system (Invitrogen), and the first- and second-round PCRs were carried out in the presence of Platinum *Taq* DNA Polymerase High Fidelity (Invitrogen). The final products were sequenced in an ABI 377 DNA sequencer (PE Biosystems) with an ABI Prism BigDye kit (Applied Biosystems). The sequences determined were utilized to confirm HEV genotypes and to construct phylogenetic trees. The reliability of the phylogenetic tree was assessed by bootstrap-resampling tests.

A reconstructed tree was built on the RNA polymerase region by using a heuristic maximum-likelihood (ML) topology search with stepwise addition and nearest neighbour-interchange algorithms. Tree likelihood scores were calculated by using the HKY85 model (Hasegawa *et al.*, 1985) with the molecular clock enforced, using PAUP version 4.0b8. Using the estimated topology, all possible root positions were evaluated under a single-rate dated-tips (SRDT) model with the computer software TipDate v1.2 and the root that yielded the highest likelihood was adopted (Rambaut, 2000). The program provided an ML estimate of the rate and also the associated date of the most recent common ancestor of the sequences, using a model that assumed a constant rate of nucleotide substitution. The molecular clock was tested by a likelihood-ratio test between the SRDT model and a general unconstrained branch-length model [different-rate (DR) model].

For estimates of demographic history, a non-parametric function $N(t)$, also known as a skyline plot, was obtained by transforming the coalescent intervals of an observed genealogy into a piecewise plot that represented an effective population size through time (Pybus *et al.*, 2001; Pybus & Rambaut, 2002). A parametric ML was estimated by several models with the computer software GENIE v3.5 to build a statistical framework for inferring the demographic history of a population on phylogenies reconstructed from sampled DNA sequences (Pybus & Rambaut, 2002). This model assumes a continuous epidemic process in which the viral transmission parameters remain constant through time. Model fitting was evaluated by likelihood-ratio tests of the parametric ML estimates (Lemey *et al.*, 2003; Pybus *et al.*, 2003; Tanaka *et al.*, 2005). Approximate 95 % confidence intervals for the parameters were estimated by using the likelihood-ratio test statistics.

A phylogenetic tree in the partial RNA polymerase region of the HEV genome is represented in Fig. 1. A functional gene, such as the RNA polymerase gene, is suitable for molecular-evolutionary analyses based on the neutral theory, because the substitution of functional genes is based on the neutral theory. The 24 genotype 3 and 24 genotype 4 strains in Japan showed a significant cluster with a high bootstrap value, which was the major Japanese cluster distinct from other strains found in foreign countries by molecular-evolutionary analyses. Such a significant cluster is suitable for the following coalescent analysis. Additionally, the tree topology based on the RNA polymerase region, including functional genes, was quite similar to that based on complete genomes (data not shown).

To determine the evolutionary rate of HEV, the 48 Japan-indigenous HEV strains (Fig. 1) were subjected to further molecular-evolutionary analyses. The molecular-evolutionary rate was estimated by two independent methods. In brief, linear-regression analyses using highly similar strains, i.e. six genotype 3 strains in Hyogo and four genotype 4 strains in Sapporo, indicated that a molecular-evolutionary rate was $(0{\cdot}81–0{\cdot}88) \times 10^{-3}$ nucleotide substitutions per site per year (Fig. 2). Second, TipDate (v1.2) was used to compare the DR model with the single-rate (SR) and SRDT models. The SRDT model provided an adequate fit to the data ($P > 0{\cdot}05$; see Supplementary Table S1, available in JGV Online). Based on the SRDT model, the mean rate of nucleotide substitutions was estimated to be $(0{\cdot}81–0{\cdot}94) \times 10^{-3}$ nucleotide

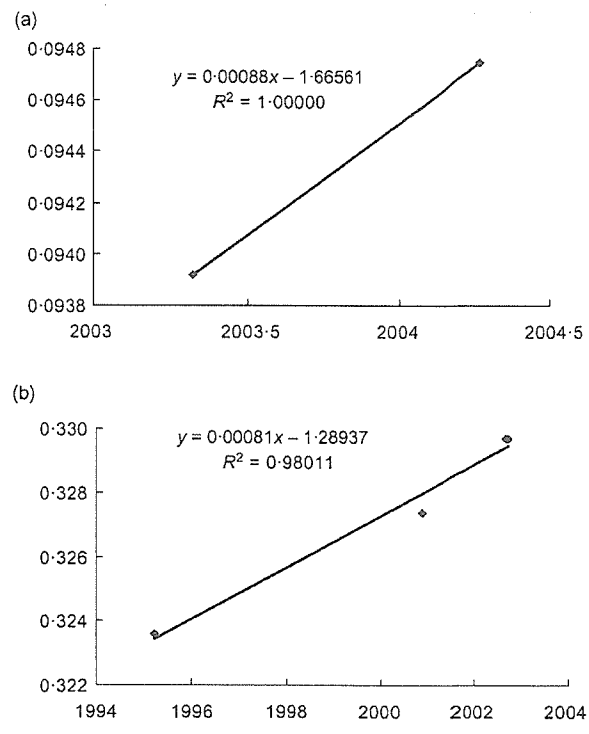

Fig. 2. Linear-regression analyses within the partial RNA polymerase region for evolutionary rate of HEV. (a) The evolutionary rate of genotype 3 in the Hyogo cluster is estimated to be $0{\cdot}88 \times 10^{-3}$ nucleotide substitutions per site per year; (b) the evolutionary rate of genotype 4 in the Sapporo cluster is estimated to be $0{\cdot}81 \times 10^{-3}$ nucleotide substitutions per site per year.

substitutions per site per year, which was similar to the rate for *Hepatitis C virus* (Ina *et al.*, 1994; Tanaka *et al.*, 2002). When we used $0{\cdot}84 \times 10^{-3}$ nucleotide substitutions per site per year, which was based on all 48 sequences (24 genotype 3 and 24 genotype 4), the time of the most recent common ancestor of Japan-indigenous genotype 3 was estimated to be in the 1900s (95 % confidence interval, 1902–1917) and that of genotype 4 was approximately in the 1880s (1881–1898) (Fig. 1).

Based on the phylogenetic tree, the effective number of HEV infections through time, $N(t)$, was analysed by using a skyline plot for the Japan-indigenous HEV strains. The parameters for several models in GENIE v3.5 were examined (see Supplementary Table S2, available in JGV Online). Time t was then transformed to year by using the constant rate ($0{\cdot}84 \times 10^{-3}$ nucleotide substitutions per site per year), assuming the collecting time to be the present. Fig. 3 shows the skyline plots and population growth for the HEV strains, according to a specific demographic model in GENIE v3.5 with three parameters and a piecewise-expansion growth model, which was evaluated by likelihood-ratio testing (Ina *et al.*, 1994; Lemey *et al.*, 2003; Pybus *et al.*, 2003; Tanaka *et al.*, 2005). Our estimates of the effective numbers of HEV infections showed a transition from constant size to exponential growth in the 1920s (95 % confidence interval, 1916–1930) among the genotype 3 population (Fig. 3a), whereas the rapid exponential growth among the genotype 4 population was dated in the 1980s (1978–1990) (Fig. 3b).

Because the natural course of HEV infection in human beings and animals is usually transient, not persistent as in the cases of hepatitis B and C viruses, it is almost impossible to estimate the molecular-evolutionary rate of HEV by using serial samples from an individual host. However, even though HEV does not persist in individual hosts, it could persist in the community by hopping from host to host successively. The first study attempting to estimate the number of synonymous mutations per synonymous site (k_s) of *Hepatitis A virus* (HAV) was reported by Sánchez *et al.* (2003). The estimated k_s values from HAV strains isolated from a clam-associated outbreak varied from 0·038 for VP0 to 0·29 for VP1. Similarly, we estimated the evolutionary rate of HEV by using Japan-indigenous genotype 3 and genotype 4 strains isolated over time. The rate was estimated to be approximately $0{\cdot}8 \times 10^{-3}$ nucleotide substitutions per site per year by two independent methods, which was around half of our previously estimated rate (Takahashi *et al.*, 2004b). One of the reasons is that the molecular-evolutionary rate would depend on estimated genes; the previous report (Takahashi *et al.*, 2004b) used complete sequences, whereas this study used only RNA polymerase sequences. Another reason is that the previous extrapolation of substitution rate on pairwise (direct) comparisons can give overestimates of the molecular clock and hence divergent times of HEV species, as reported previously (Ina *et al.*, 1994). Based on the molecular clock, we traced the demographic history of HEV in Japan and the indigenization time was suggested to be similar (approx. 1900), but the spread time was quite different, between HEV genotypes 3 and 4 (1920s versus 1980s). Interestingly, in addition, the evolutionary growth of genotype 3 has been quite slow since the 1920s, whereas genotype 4 strains have spread rapidly in Sapporo since the 1980s.

Zoonosis has been implicated in HEV transmission. The first animal strain of HEV to be isolated and characterized was a swine HEV from a pig in the USA in 1997 (Meng *et al.*, 1997). Since then, many swine HEV strains, which exhibit extensive genetic heterogeneity, have been identified worldwide and shown to be genetically related closely to strains of human HEV (Chandler *et al.*, 1999; Hsieh *et al.*, 1999; Huang *et al.*, 2002; Okamoto *et al.*, 2001; Wang *et al.*, 2002). Recent findings suggested an interspecies HEV transmission between boar and deer in their wild life (Takahashi *et al.*, 2004a) and that both animals might serve as an infection source for human beings. More recently, wild mongoose was newly added to the list of HEV-reservoir animals in Japan

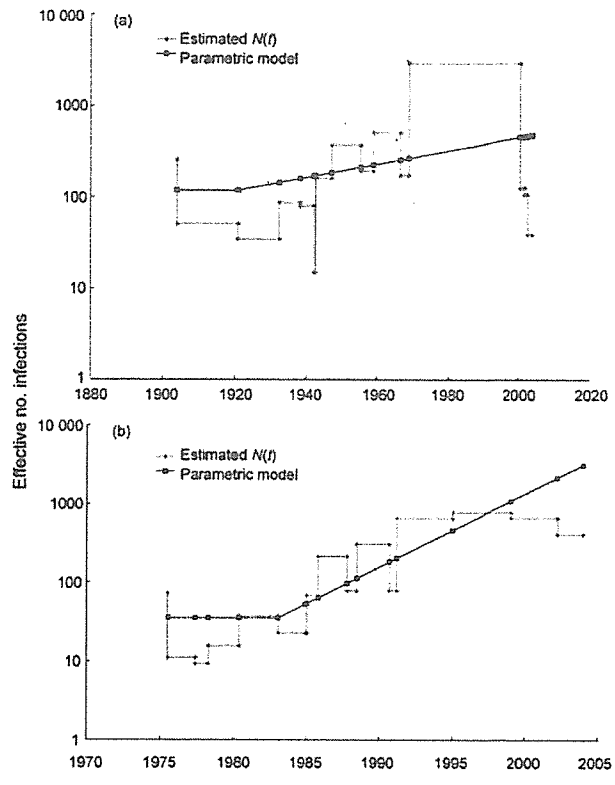

Fig. 3. ML estimates of $N(t)$ on the effective number of (a) HEV genotype 3 and (b) HEV genotype 4 infections in Japan. The parametric model is indicated by the black line and stepwise plots by the grey line, which represents corresponding non-parametric estimates of $N(t)$ (number as a function of time). Genetic distances have been transformed into a time scale of years by using estimates of the molecular clock in the partial RNA polymerase region of HEV.

(Nakamura *et al.*, 2006). Notwithstanding the importance of these wild animals, pigs for food must be the major reservoirs of HEV: a recent Japanese study indicated that anti-HEV antibodies were detected in 1448 (58 %) of 2500 pigs from 2 to 6 months of age at 25 commercial swine farms in Japan (Takahashi *et al.*, 2003). The importance of transmission of HEV from pigs to humans was further supported by a recent field study in Indonesia: Muslim people, for whom it is a taboo to eat or contact pigs, were significantly less frequently positive for anti-HEV than Hindu people (2·0 vs 20 %) (Surya *et al.*, 2005).

Our molecular-evolutionary analyses suggested that HEV entered Japan around 1900. If we have traced the origin of Japan-indigenous HEV correctly back to about 100 years ago, what happened at that time in relevance to HEV's indigenization? Several kinds of Yorkshire pig were imported for the first time in the history of Japan from the UK in 1900, by the Japanese government's policy to introduce excellent domestic animals for food in Western countries to Japan, as a measure to nutritionally strengthen the people (especially soldiers) of this formerly vegetarian country. Since then, the Yorkshire pigs have been propagated in Japan and, in the 1930s, thousands of pigs were reported all over Japan (http://okayama.lin.go.jp/history/2-3-1-2.htm), suggesting that the domestic spread of HEV might have been associated with the popularization of pigs for food in Japan. Indeed, a previous phylogenetic analysis of a 304 bp nucleotide sequence (ORF2) obtained from the two UK swine strains showed a close relationship with Japanese swine strains in genotype 3 (Banks *et al.*, 2004), indicating that Japanese genotype 3 may have been imported from the UK. On the other hand, Japanese genotype 4 strains were related phylogenetically to Asian strains in Taiwan and China. As the HEV found in wild boars living in the Iriomote Island, near Taiwan, was of genotype 4 (unpublished results), the source of Japanese genotype 4 might be from Taiwan or the mainland of China. Note that a phylogenetic analysis showed that the Japanese swine and human HEV strains segregated into four clusters [three genotype 3 clusters (one major Japanese and two minor clusters) and one genotype 4 cluster], with the highest nucleotide identity being 94·4–100 % between swine and human strains in each cluster (Takahashi *et al.*, 2003), suggesting that swine have served as one of the most important reservoirs for HEV to be transmitted to humans. The possible risk factor for transmission of HEV was to have eaten uncooked or undercooked pig liver and/or intestine 1–2 months before the onset of hepatitis E in Hokkaido, Japan (Mizuo *et al.*, 2005). Such eating habits, which are particularly unique to those living in Hokkaido (Sapporo is one of the big cities there) in recent decades, might be one of the reasons that HEV has been widespread in this area since 1990, as supported by our molecular-evolutionary analyses in this study.

In conclusion, based on our present data, the indigenization and domestic spread of HEV in Japan are proposed to have been associated with the importation and popularization of pigs for food in Japan. However, there still remains a possibility of different scenarios. Another animal(s) might have carried the virus to Japan: for example, mongoose was imported from India to Japan in 1910 (Nakamura *et al.*, 2006).

Acknowledgements

Contributions of authors are as follows: Y. T. performed molecular-clock analyses and wrote the manuscript; K. T. amplified and sequenced viral isolates; E. O. helped Y. T. with the molecular-clock analyses; Y. K., J.-H. K., K. S., A. M., A. H., H. M., H. S., Y. A. and T. K. provided HEV RNA-positive sera to K. T. for sequence determinations; M. M. supervised the molecular-clock analyses; and S. M. designed the study and helped Y. T. to write the manuscript. This work was supported in part by grants from the Ministry of Health, Labour and Welfare of Japan (200400676A) and from the United States–Japan Collaborative Medical Science Program (Hepatitis Panel). We greatly appreciate Dr Oliver G. Pybus (Department of Zoology, University of Oxford, Oxford, UK) for his enlightening advice on molecular-evolutionary analyses using GENIE v3.5.

References

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.

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.

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

Hasegawa, M., Kishino, H. & Yano, T. (1985). Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *J Mol Evol* **22**, 160–174.

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, 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.

Ina, Y., Mizokami, M., Ohba, K. & Gojobori, T. (1994). Reduction of synonymous substitutions in the core protein gene of hepatitis C virus. *J Mol Evol* **38**, 50–56.

Kabrane-Lazizi, Y., Fine, J. B., Elm, J. & 7 other authors (1999). Evidence for widespread infection of wild rats with hepatitis E virus in the United States. *Am J Trop Med Hyg* **61**, 331–335.

Lemey, P., Pybus, O. G., Wang, B., Saksena, N. K., Salemi, M. & Vandamme, A.-M. (2003). Tracing the origin and history of the HIV-2 epidemic. *Proc Natl Acad Sci U S A* **100**, 6588–6592.

Matsuda, H., Okada, K., Takahashi, K. & Mishiro, S. (2003). Severe hepatitis E virus infection after ingestion of uncooked liver from a wild boar. *J Infect Dis* **188**, 944.

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.

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. *Hepatol Res* (in press).

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.

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.

Purcell, R. H. & Emerson, S. U. (2001). Hepatitis E virus. In *Fields Virology*, 4th edn, pp. 3051–3061. Edited by D. M. Knipe, P. M. Howley, D. E. Griffin, R. A. Lamb, M. A. Martin, B. Roizman & S. E. Straus. Philadelphia, PA: Lippincott Williams & Wilkins.

Pybus, O. G. & Rambaut, A. (2002). GENIE: estimating demographic history from molecular phylogenies. *Bioinformatics* 18, 1404–1405.

Pybus, O. G., Charleston, M. A., Gupta, S., Rambaut, A., Holmes, E. C. & Harvey, P. H. (2001). The epidemic behavior of the hepatitis C virus. *Science* 292, 2323–2325.

Pybus, O. G., Drummond, A. J., Nakano, T., Robertson, B. H. & Rambaut, A. (2003). The epidemiology and iatrogenic transmission of hepatitis C virus in Egypt: a Bayesian coalescent approach. *Mol Biol Evol* 20, 381–387.

Rambaut, A. (2000). Estimating the rate of molecular evolution: incorporating non-contemporaneous sequences into maximum likelihood phylogenies. *Bioinformatics* 16, 395–399.

Sánchez, G., Bosch, A. & Pintó, R. M. (2003). Genome variability and capsid structural constraints of hepatitis A virus. *J Virol* 77, 452–459.

Surya, I. G. P., Kornia, K., Suwardewa, T. G. A., Mulyanto Tsuda, F. & Mishiro, S. (2005). Serological markers of hepatitis B, C, and E viruses and human immunodeficiency virus type-1 infections in pregnant women in Bali, Indonesia. *J Med Virol* 75, 499–503.

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., Kitajima, N., Abe, N. & Mishiro, S. (2004a). 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, K., Toyota, J., Karino, Y., Kang, J.-H., Maekubo, H., Abe, N. & Mishiro, S. (2004b). Estimation of the mutation rate of hepatitis E virus based on a set of closely related 7.5-year-apart isolates from Sapporo, Japan. *Hepatol Res* 29, 212–215.

Tamada, Y., Yano, K., Yatsuhashi, H., Inoue, O., Mawatari, F. & Ishibashi, H. (2004). Consumption of wild boar linked to cases of hepatitis E. *J Hepatol* 40, 869–870.

Tanaka, Y., Hanada, K., Mizokami, M., Yeo, A. E. T., Shih, J. W.-K., Gojobori, T. & Alter, H. J. (2002). A comparison of the molecular clock of hepatitis C virus in the United States and Japan predicts that hepatocellular carcinoma incidence in the United States will increase over the next two decades. *Proc Natl Acad Sci U S A* 99, 15584–15589.

Tanaka, Y., Hanada, K., Orito, E. & 8 other authors (2005). Molecular evolutionary analyses implicate injection treatment for schistosomiasis in the initial hepatitis C epidemics in Japan. *J Hepatol* 42, 47–53.

Tei, S., Kitajima, N., Takahashi, K. & Mishiro, S. (2003). Zoonotic transmission of hepatitis E virus from deer to human beings. *Lancet* 362, 371–373.

Wang, Y.-C., Zhang, H.-Y., Xia, N.-S. & 11 other authors (2002). Prevalence, isolation, and partial sequence analysis of hepatitis E virus from domestic animals in China. *J Med Virol* 67, 516–521.

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.

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＜原　著＞

本邦に於けるE型肝炎ウイルス感染の統計学的・疫学的・ウイルス学的特徴：全国集計254例に基づく解析

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北村　庸雄[21)]　正木　尚彦[22)]　松林　圭二[23)]　松田　裕之[24)]　松井　淳[25)]
道堯浩二郎[26)]　三原　弘[27)]　宮地　克彦[28)]　宮川　浩[29)]　水尾　仁志[30)]
持田　智[31)]　森山　光彦[32)]　西口　修平[33)]　岡田　克夫[34)]　齋藤　英胤[35)]
佐久川　廣[36)]　柴田　実[37)]　鈴木　一幸[38)]　高橋　和明[4)]　山田剛太郎[39)]
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要旨：極く最近まで殆んど不明状態にあった我国のE型肝炎の実態を明らかにする目的で，我々は全国から総数254例のE型肝炎ウイルス（HEV）感染例を集め，統計学的・疫学的・ウイルス学的特徴を求めてこれを解析した．その結果，[i] HEV感染は北海道から沖縄まで全国津々浦々に浸透していること；[ii] 感染者の多くは中高年（平均年齢約50歳）で，且つ男性優位（男女比約3.5対1）であること；[iii] 我国に土着しているHEVはgenotype 3とgenotype 4であるが，後者は主に北海道に偏在していること；[iv] 年齢と肝炎重症度との間に相関があること；[v] Genotype 3よりはgenotype 4による感染の方が顕性化率も重症化率も高いこと；[vi] 発生時期が無季節性であること；[vii] 集積症例全体の約30%は動物由来食感染，8%は輸入感染，2%は輸血を介する感染に帰せしめ得たものの，過半の症例（約60%）に於いては感染経路が不明のままであること；等の知見を得た．

索引用語：　E型肝炎　E型肝炎ウイルス　疫学　日本

1）一心病院，2）相川内科病院，3）甲府市立甲府病院，4）東芝病院，5）武蔵野赤十字病院，6）富山大学三内，7）広島大学分子病態制御内科，8）国立療養所東京病院，9）東京逓信病院，10）国保中央病院，11）順天堂大学静岡病院，12）関西医大三内，13）日立総合病院，14）昭和大学二内，15）手稲渓仁会病院，16）札幌厚生病院，17）豊川市民病院，18）北海道大学二内，19）鳥取大学二内，20）市立加西病院，21）順天堂大学浦安病院，22）国立国際医療センター，23）北海道赤十字血液センター，24）松田内科医院，25）至聖病院，26）愛媛大学三内，27）高岡市民病院，28）大阪医大二内，29）帝京大学溝口，30）勤医協中央病院，31）埼玉医大消化器肝臓内科，32）日本大学消化器肝臓内科，33）兵庫医大肝胆膵科，34）鳥取県立中央病院，35）慶應大学消化器内科，36）ハートライフ病院，37）NTT東日本関東病院，38）岩手医大一内，39）川崎医大川崎病院，40）岡山済生会総合病院，41）板橋中央総合病院，42）北見赤十字病院，43）国立病院機構長崎医療センター

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Table 1 Remarkable predominance of male over female, irrespective of severity of the disease.

Gender	Total n = 243	Disease categories			
		Subclinical n = 71	AH[a] n = 135	ASH[a] n = 21	FH[a] n = 16
Female	55(23%)	18(34%)	29(21%)	4(19%)	4(25%)
Male	188(77%)	53(66%)	106(79%)	17(81%)	12(75%)
F/M ratio	1 / 3.4	1 / 2.9	1 / 3.7	1 / 4.3	1 / 3

[a]Abbreviations : AH, acute hepatitis ; ASH, acute severe hepatitis (defined by prolonged prothrombin time, i.e., PT value < 40%) ; FH, fulminant hepatitis.

Table 2 Age of the subjects, possibly influencing clinical manifestations.

Age in yrs	Total n = 242	Disease categories			
		Subclinical n = 70	AH n = 135	ASH n = 21	FH n = 16
Less than 40	63(26%)	38(54%)[a]	21(16%)	3(14%)	1(6%)
40 to 59	105(43%)	20(29%)	70(52%)	11(53%)	4(25%)
60 or more	74(31%)	12(17%)	44(32%)	7(33%)	11(69%)[b]
Mean ± SD	50.1 ± 15.6	42.3 ± 15.9[c]	52.8 ± 14.4	52.8 ± 15.6	58.9 ± 10.1[d]

[a] $P < 0.001, 0.003, < 0.001$ against "AH", "ASH", "FH" respectively; [b] $P = 0.010, < 0.001$ against "AH", "Subclinical" (Chi square test). [c] $P < 0.001, 0.009, < 0.001$ against "AH", "ASH", "FH" (t test) ; [d] $P = 0.047$ against "AH" (Welch test).

緒　言

我国や西欧諸国は，アジア・アフリカの熱帯亜熱帯地域諸国と異なり，E型肝炎が頻発する地域ではないから，相当数の症例を集積するには時間と手間がかかる故，100例以上の症例を纏めて解析した報告は，我々の知る限り英文であれ和文であれ一報だに存在しない．我々は，約3年の歳月をかけて，共著者の夫々が過去およびリアルタイムに経験した症例の情報と検体を持ち寄り，更にはこれに我国から学会や論文で発表された症例の情報をも追加し，2006年1月末までに総数254例の，国内で経験されたHEVヒト感染例を集積することを得た．かほどの多数例を纏めて解析した仕事は未見であるし，聊か興味深い知見も得られたので，以下にそれを報告する．

方　法

症例の任意登録

共著者の夫々が，過去及び現在進行形で経験したHEV感染例について，地域，年齢，性，発病年，発病月，病型診断(Subclinical, Acute Hepatitis, Acute Severe Hepatitis, Fulminant Hepatitisのいずれか)，経過中最高ALT値，経過中最高総ビリルビン値，経過中最延長プロトロンビン時間値，ウイルス学的診断根拠(HEV RNA陽性，あるいはIgM抗体・IgG抗体共陽性)，HEV genotype，推定あるいは確定された感染経路，海外渡航歴の有無，等の情報を任意登録した．2006年1月末の時点で，この『任意登録』によって集積し得た症例数はn＝206である．尚，HEV RNAが陽性でありながらgenotypingが未施行であった症例については，可能な限り検体の入手に努力し，sequencingを行った(方法後出)．

既報告例の引用登録

国内学会での過去の報告例については，抄録から上記調査項目に相当するデータを拾い集めた．論文発表例[1)~16)]については，一部は，当該論文著者自身から上記調査項目に相当するデータを任意登録して貰ったが，それが不可能であった場合には論文中の記載から該当データを引用登録した．この『引用登録』によって集積し得た症例数はn＝48であり，そのうち最古の症例

Table 3 Geographical distribution of HEV genotypes, showing a significant predominance of type-3 over type-4 in the areas other than Hokkaido.

Areas[a]	Total n = 228	HEV genotype			
		1	2	3	4
Hokkaido	123	—	—	58(47%)	65(53%)[b]
Tohoku	18	—	—	17(94%)	1(6%)
Kanto-Koshin'etsu	48	5(10%)[c]	—	31(65%)	12(25%)
Chuhbu-Hokuriku	8	1(9%)[c]	—	7(91%)	—
Kinki	10	1(10%)[c]	—	9(90%)	—
Chuh-Shikoku	10	1(10%)[c]	—	7(70%)	2(20%)
Kyushu-Okinawa	11	—	—	9(82%)	2(18%)

[a] Japan was divided, from northeast to southwest, into 7 areas, each of which includes the following prefectures. "Hokkaido" : Hokkaido alone. "Tohoku" : Aomori, Iwate, Miyagi, Akita, Yamagata, and Fukushima. "Kanto-Koshin'etsu" : Ibaraki, Tochigi, Gunma, Saitama, Chiba, Tokyo, Kanagawa, Shizuoka, Yamanashi, Nagano, and Niigata. "Chuhbu-Hokuriku" : Toyama, Ishikawa, Fukui, Gifu, Aichi, and Mie. "Kinki" : Shiga, Kyoto, Osaka, Hyogo, Nara, and Wakayama. "Chuh-Shikoku" : Tottori, Shimane, Okayama, Hiroshima, Yamaguchi, Kagawa, Ehime, Tokushima, and Kochi. "Kyushu-Okinawa" : Fukuoka, Saga, Nagasaki, Kumamoto, Oita, Miyazaki, Kagoshima, and Okinawa.

[b] $P < 0.001$ against other areas (Chi square test).

[c] All but one were from cases of imported infection.

Table 4 HEV genotype and clinical manifestation : the severer the disease the higher the frequency of genotype 4.

HEV genotype		Disease categories		
		Subclinical	AH	ASH + FH
1	(n = 7)	—	6	1
2	(n = 0)	—	—	—
3	(n = 135)	52	76	7
4	(n = 78)	7	48	23
	Rate of type-4[a]	7/59(12%)	48/130(37%)	23/31(74%)

[a] $P < 0.001$ between "Subclinical" and "AH" as well as between "AH" and "ASH+FH" (Chi square test).

は1979年に発生したものであった[13].

HEV genome 塩基配列解析

ORF1内の異なる3領域の，それぞれ69 nt[17]，326 nt[18]，821 nt[19]の全てあるいは少なくとも一つの断片をPCRで増幅し，direct sequencingすることによりgenotypeを決定した．

統計学的有意差検定

群間の比率の差や平均値の差の有意性検定の為に用いた統計学的方法は各々のTableの脚注の中に記す．

結　果

HEV感染者の居住地

居住地情報が得られた症例の地域別内訳（括弧内は例数）は，北海道（n＝130），岩手（15），宮城（1），山形（1），福島（1），茨城（4），栃木（3），群馬（1），埼玉（6），千葉（6），東京（23），神奈川（5），静岡（1），山梨（1），長野（1），新潟（2），富山（3），石川（1），愛知（3），京都（1），大阪（2），奈良（2），兵庫（5），鳥取（4），岡山（4），広島（2），愛媛（2），福岡（1），長崎（12），熊本（1），大分（2），沖縄（3），であった．

HEV感染者の性と年齢

性別情報不明あるいは病型情報不明であった11名を除いた243名に基づく，性差の成績をTable 1に示す．同様に，年齢不詳あるいは病型情報不明の12名を除外

Table 5 Liver function test levels differed by HEV genotype.

Parameters[a]	Genotype 3			Genotype 4			P
	n	mean	SD	n	mean	SD	
peak ALT (IU/L)	101	1676	1390	75	3048	2501	< 0.001[b]
peak T.B. (mg/dL)	80	7.1	8.6	71	11.8	8.9	0.01[c]
nadir P.T. (%)	74	79.6	26.3	67	63.3	27.7	< 0.001[c]

[a] Abbreviations : ALT, alanine amino transferase ; T.B., total bilirubin ; P.T., prothrombin time. [b] By Welch. [c] By *t* test.

Table 6 Month when the infection occurred, suggesting that there was no seasonality.

Month	Number of cases	Adjusted number
January	20	
February	16	
March	21	20[a]
April	37	24[b]
May	11	
June	17	
July	20	
August	22	
September	26	21[c]
October	17	
November	23	
December	18	

[a] Of the 21 cases in March, 2 were infected simultaneously by eating the same *namagimo* (raw liver) of wild boar [21] while the other 19 were exposed to respective infection-sources, and hence the number of independent infections should be 20, not 21 ; [b] Similarly, of the 37 cases in April, 11 were from a mini-outbreak that occurred after wild boar barbecue party [20] and 4 were from deer-*sashimi* sharing [22] ; [c] Of the 26 in September also included 6 individuals from a mini-outbreak [10].

した242名に基づく，年齢分布の成績をTable 2に示す．

男性優位，中高年優位が一見して顕著であるのみならず，不顕性感染群と顕性感染群(特に劇症肝炎群)との間に，年齢分布の顕著な有意差が認められた．即ち，高齢になるほど重症化率が高かった．逆に云えば，不顕性感染群には若年者が多く存在した．

Genotype 分布の地域差

HEV genotypeが判明した228例について，居住地(全国を北海道，東北，関東甲信越，中部北陸，近畿，中国四国，九州沖縄の7ブロックに分割)ごとのgenotype分布をTable 3に示す．

北海道以外の地域ではgenotype 3が圧倒的多数を占めたが，北海道に於いてはgenotype 3と4がほぼ同数存在した．Genotype 1が検出された8名中7名はインド（n＝4），バングラデシュ（2），ネパール（1）への渡航歴を有していた．

HEV genotype と肝炎重症度との相関

Genotype情報及び病型診断情報の両方が得られた220例について，病型ごとのgenotype分布をTable 4に示す．

Genotype 4の頻度が，不顕性感染群から急性肝炎群へ，更には重症肝炎群(急性肝炎重症型＋劇症肝炎)へと有意差を以て上昇（12%→37%→74%）していた．同様に，Table 5に見る如く，経過中最高ALT値，経過中最高総ビリルビン値，経過中最延長プロトロンビン時間値のいずれもが，genotype 4の相対的高病原性を示唆する所見を示した．

季節性

発生月が判明した247例の集計結果をTable 6に示す．

4月が突出して高い発生例数（n＝37）を示したが，そのうちの11例(於長崎)[20]及び2例(於鳥取)[21]は夫々同一感染源による小規模集団感染に属するものであった故，11 cases→1 incidence，2 cases→1 incidenceとして，互いに独立する感染発生件数をカウントし直すと，4月のそれはn＝24に減少した．同様に，3月と9月にも夫々1件ずつの小規模集団感染事例[10][22]が含まれていた．かくて，互いに独立する感染発生件数（Table 6に於ける"adjusted number")で比較する限り，顕著な月別変動は存在しなかった．

Table 7 Routes of transmission.

Routes	Number of cases (%)	With direct evidence	With indirect evidence
Contact with animal	1(0.5%)	—	1[a]
Blood transfusion	5(2.3%)	5[b]	—
Travel and Import	17(7.9%)	—	13[c]
Zoonotic food-borne	68(31%)	5[d]	26[e]
Unknown	125(58%)	—	—

[a] Patient's pet cat was anti-HEV positive [8]. [b] Complete matching of HEV sequences between donor and recipient was observed in each case [11),13)]. [c] Nucleotide sequences of HEV from the patients were more homologous to those in the visited countries (India, Thailand, Nepal, Pakistan, Bangladesh, China) than those in Japan. [d] Complete matching of HEV sequences between patients and left-over animal meats [16),22)]. [e] Shown in literature [7),10),20),21)].

感染経路

感染経路を確定あるいは推定し得た症例は，全体の約40%でしかなかった（Table 7）.

5例の輸血感染（1例は愛知県，1例は東京，3例は北海道）は全て，ドナーと受血者の間でHEV RNA sequenceの一致が確認された直接証明例である．一方，他の感染経路（animal contact, travel and import, zoonotic food-borne）に於いては，感染源と感染者のHEV塩基配列が一致するとの直接証拠が得られたのは，シカからの感染[22]とイノシシからの感染[16]の2事例5名のみであって，その他は全て間接証拠からの推定である．感染源であると確定あるいは推定された動物種は，ブタ（症例数n＝44），イノシシ（15），シカ（5），動物種不明（2）であった.

北海道と本州以南で感染経路を比較すると，輸入感染の頻度に顕著な差が認められた：北海道1/130(0.8%) vs 本州以南16/124（13%）.

考　察

本邦を含む先進工業地域諸国からは初出と思われる，この200例を越えるHEV感染例の解析から得られた成績の中には，幾つかの興味深い知見が含まれている.

先ず，感染者のデモグラフィーに関しては，従来の教科書におしなべて“a disease of young adults”と記載されていたのに反し，本研究の成績は「中高年男性の病気」であることを強く示唆した．少数例ではあるが同様の成績が我国からも（A型肝炎に比較してE型肝炎患者は高齢で男性優位）[23]フランスからも（男女比約4対1，平均年齢約50歳）[24]も報告されているので，従来の教科書や常識が依拠していた流行地に於ける疫学と，これから明らかにされるであろう非流行地に於ける疫学の間に，相当の差異があるものと考えられる.

肝炎重症化の因子についても然りである．流行地に於ける観察から，妊娠第三期に於ける感染が従前唯一の重症化因子として認識されて来たが，非流行地である日本に於ける本研究の集計例の中に妊婦例は一例だに存在せず，寧ろ，加齢とHEV genotype 4が，新たな重症化因子として浮き彫りになった.

特に，HEV genotype 4とdisease severityとの間の有意な相関は，従前未報告の新知見であり，本研究の成果の中で最も特筆に値するものである．即ち，重症肝炎例にgenotype 4が多く見られるとの報告は従来から存在したが[15)24)]，病原性の強弱に関するgenotype 3と4との間の差異を統計学的有意差を以て示したのは，本報告が初めてである．両genotypesの間にはゲノム構造上も若干の差異がある（genotypes 1, 2, 3ではORF1とORF3が別フレーム上にあるが，genotype 4に於いては同一フレーム）し，増殖速度の差異（genotype 3<genotype 4）を示唆する所見（姜貞憲，松林圭二他，unpublished results）も得られているから，本研究が示唆したgenotype 4の相対的高病原性について，今後その機序が次第に明らかにされて行くと思われる.

HEV感染に於けるzoonotic transmissionの重要性は，特に我国からの多数の報告[7)8)10)14)16)20)~22)25)]により広く認識されるところとなった．その所為もあり，今回の全国集計に任意登録された症例の多くに於いては，動物由来感染を疑うための問診が相当積極的に為されていたが，それでもなお，zoonotic transmissionで説明し得る症例は全体の約30%でしかなかった．輸入感染例の約3倍もの頻度で動物由来感染が存在するということ自体が，新しく且つ刮目すべき知見ではあったものの，もっと重要な知見は，集計症例全体の約60%もが感染経路不明のまま残されたという事実の方だったかもし

れない．何故なら，それにより，我々が未だ把握していない感染経路の存在をも念頭に置いた今後の研究の必要性が示されたからである．そして，その目的の為には，特に北海道に於いて一層積極的な調査を行うことが望まれる．北海道で経験される輸入感染の頻度（0.8%）は本州以南でのそれ（13%）の10分の1以下でしかないという事実が，道内の感染源の重要性を何よりも雄弁に物語っているからである．

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文　献

1) 藤吉　誠，荘司貞志，近藤朝明，他．胆汁うっ滞像を認めたE型肝炎に不顕性小型アメーバ感染を合併した1例．日本消化器病学会雑誌　1992；89：2804—2807
2) 安藤文英，小柳年正，酒井好古．国内で感染し発症した散発性E型急性肝炎と思われる1邦人例．肝臓　1994；35：560—565
3) 三浦英明，古橋修介，山田春木．腸チフスによる敗血症を併発したE型急性肝炎重症型の1例．肝臓　2001；42：133—137
4) 佐藤　慎，井戸健一，礒田憲夫，他．海外渡航歴のないE型急性肝炎の1例．肝臓　2002；43：332—335
5) Mizuo H, Suzuki K, Takikawa 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—3218
6) Takahashi M, Nishizawa T, Yoshikawa A, 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—1940
7) Yazaki Y, Mizuo H, Takahashi M, 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—2357
8) Kuno A, Ido K, Isoda N, et al. Sporadic acute hepatitis E of a 47-year-old man whose pet cat was positive for antibody to hepatitis E virus. Hepatol Res 2003; 26: 237—242
9) 齊藤奈津子，土山寿志，大森俊明，他．北陸地方で確認された散発性E型急性肝炎の1例．肝臓　2004；45：268—273
10) 加藤　将，種市幸二，松林圭二．焼肉店での会食後に発生したE型肝炎ウイルス集団感染：うち1例は劇症肝炎で死亡．肝臓　2004；45：688
11) Matsubayashi K, Nagaoka Y, Sakata H, et al. Transfusion-transmitted hepatitis E caused by apparently indigenous hepatitis E virus strain in Hokkaido, Japan. Transfusion 2004; 44: 934—940
12) Kumagai M, Nishii Y, Koizumi Y, et al. Domestic infection of hepatitis E in Japan. Intern Med 2004; 43: 807—810
13) Mitsui T, Tsukamoto Y, Yamazaki C, et al. Prevalence of hepatitis E virus infection among hemodialysis patients in Japan: evidence for infection with a genotype 3 HEV by blood transfusion. J Med Virol 2004; 74: 563—572
14) Hijikata S, Satoh Y, Iwashita Y, et al. A case of hepatitis E who had a history of frequent ingestion of raw meat and viscera from wild deer and boars. J Jpn Soc Gastroenterol 2005; 102: 723—728
15) Mizuo H, Yazaki Y, Sugawara K, et al. 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 2005; 76: 341—349
16) Li TC, Chijiwa K, Sera N, et al. Hepatitis E virus transmission from wild boar meat. Emerging Infect Dis 2005; 11: 1258—1260
17) Takahashi K, Kang JH, Ohnishi S, 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—318
18) Takahashi K, Kang JH, Ohnishi S, et al. Genetic heterogeneity of hepatitis E virus recovered from Japanese patients with acute sporadic hepatitis. J Infect Dis 2002; 185: 1342—1345
19) Tanaka Y, Takahashi K, Orito E, et al. Molecular tracing of Japan-indigenous hepatitis E viruses. J Gen Virol 2006; 87: 949—954
20) Tamada Y, Yano K, Yatsuhashi H, et al. Consumption of wild boar linked to cases of hepatitis E. J Hepatol 2004; 40: 869—870
21) Matsuda H, Okada K, Takahashi K, et al. Severe

hepatitis E virus infection after ingestion of uncooked liver from a wild boar. J Infect Dis 2003; 188: 944

22) Tei S, Kitajima N, Takahashi K, et al. Zoonotic transmission of hepatitis E virus from deer to human beings. Lancet 2003; 362: 371—373

23) Sainokami S, Abe K, Kumagai I, et al. Epidemiological and clinical study of sporadic acute hepatitis E caused by indigenous strains of hepatitis E virus in Japan compared with acute hepatitis A. J Gastroenterol 2004; 39: 702—703

24) Mansuy JM, Peron JM, Abravanel F, et al. Hepatitis E in the south west of France in individuals who have never visited an endemic area. J Med Virol 2004; 74: 419—424

25) 高橋和明，岡田克夫，姜　貞憲，他．重症肝炎との関連性濃厚なE型肝炎ウイルスgenotype IV内の広域分布型一系統：鳥取，新潟，札幌から得られた3本の完全長および1本の準完全長HEV塩基配列．肝臓　2005；46：389—390

Demographic, epidemiological, and virological characteristics of hepatitis E virus infections in Japan based on 254 human cases collected nationwide

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To know the reality of hepatitis E virus (HEV) infections in Japan, quite obscure until a few years ago, we have collected a total of 254 human cases of HEV infection, and analyzed for demographic, epidemiological, and virological characteristics. As a result, we now know [i] HEV has penetrated nationwide from Hokkaido to Okinawa; [ii] hepatitis E is a disease of middle-aged people (approx. 50 years old in average) with a predominance of male over female (approx. 3.5 vs 1); [iii] HEV strains of genotype 3 and 4 are autochthonous in Japan, but the latter is present almost exclusively in Hokkaido; [iv] the older the age the severer the disease; [v] HEV genotype 4 is associated with more obvious and severer clinical manifestations than genotype 3; [vi] no seasonality in its incidence; and [vii] transmission routes remain obscure in most cases (approx. 60%), whereas about 30%, 8%, and 2% are ascribable to zoonotic food-borne transmission, imported infection, and via blood transfusion, respectively.

Kanzo 2006; 47: 384—391

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特集・肝炎診療の最前線

肝炎治療の最前線－その実際

肝炎劇症化時の対応

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Summary

急性肝炎は本来，特別な治療を要さない予後良好の疾患であるが，1～2％が昏睡を発現し劇症肝炎となる。劇症化した場合の内科的救命率は急性型で約50％，亜急性型で約20％と極めて予後不良である。劇症化の予知・予防は困難だが，強い倦怠感，進行性の黄疸，肝濁音界の消失，プロトロンビン時間の延長，尿素窒素の低下などに注意し，劇症化が疑われた場合は専門施設への速やかな搬送を考慮する。劇症化した場合は，直ちに人工肝補助を中心とした集中治療を行いつつ，肝移植の準備も並行して進める。治療法，救命率，肝移植の必要性などを家族に説明する。

Key Words　劇症肝炎／急性肝不全／劇症化予知／人工肝補助／肝移植

劇症肝炎の定義と診断

急性肝炎のうち，重篤な肝機能障害により肝性脳症をはじめとする肝不全症状を呈する場合を劇症肝炎と言い，わが国では表1に示すような診断基準が定められている。この基準の特徴は，肝性脳症を客観的に判断が可能なⅡ度以上と定め，また，重篤な肝障害の客観的な指標としてプロトロンビン時間40％以下と定めていることである。

急性肝炎劇症化の頻度と予後

発生頻度は，日本では急性肝炎全体の約2％と言われている[1]が，成因によって異なり，A型肝炎では0.14～0.35％，B型肝炎では1～4％，いわゆる非A非B型肝炎では2.3～4.7％が劇症化すると報告されている[2~4]。

劇症肝炎の予後は発症から肝性昏睡発現までの期間により大きく異なることが知られており，この期間が10日以内の比較的予後良好な急性型と，11日以上の極めて予後不良

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表１　劇症肝炎の診断基準

劇症肝炎とは，肝炎のうち初発症状発現後８週以内に高度の肝機能障害に基づいて肝性昏睡Ⅱ度以上の脳症をきたし，プロトロンビン時間40%以下を示すものとする。そのうちには発病後10日以内に脳症が発現する急性型と，それ以後に発現する亜急性型がある。

（注１）先行する慢性肝疾患が存在する場合は劇症肝炎から除外する。ただし，Ｂ型肝炎の無症候性キャリアからの急性増悪例は劇症肝炎に含めて扱う。
（注２）薬物中毒，循環不全，妊娠性脂肪肝，Reye症候群など，肝炎を伴わない肝不全は劇症肝炎から除外する。
（注３）肝性脳症の昏睡度分類は犬山分類（1972年）に基づく。
（注４）成因分類は「難治性の肝疾患に関する研究班」の指針（2002年）に基づく。
（注５）プロトロンビン時間が40%以下を示す症例のうち，肝性脳症が認められないか，昏睡Ⅰ度以内の症例は急性肝炎重症型，初発症状出現から８週以降24週以内に肝性昏睡Ⅱ度以上の脳症を発現する症例は遅発性肝不全に分類する。これは劇症肝炎の類縁疾患であるが，診断に際しては除外して扱う。

（第12回犬山シンポジウム，1981年８月，第89回日本消化器病学会総会，2003年４月改訂より）

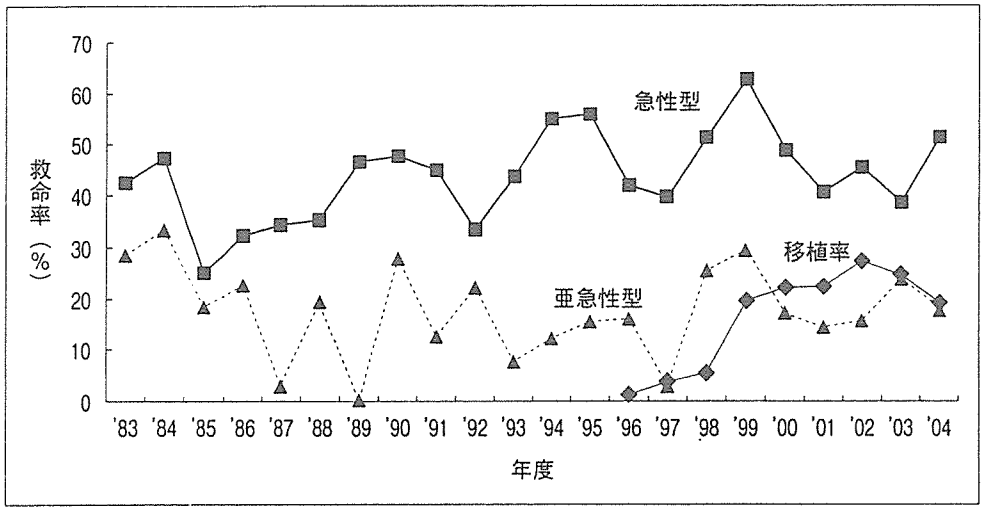

図１　劇症肝炎の病型別内科的救命率の変遷

な亜急性型に分類している。

急性肝炎は自己終息的な疾患であり，通常特殊な治療をせずに回復する予後良好な疾患であるが，劇症肝炎に至ると内科的救命率は全体として30～40%程度と極めて予後不良である。図１にわが国における劇症肝炎の内科的救命率の変遷を示す。急性型の予後はやや改善傾向を示すが，亜急性型は依然として予後不良であり，肝移植の適応である。

劇症化の機序

ウイルス肝炎における肝細胞障害機構として，感染細胞に表出されるウイルス抗原を，これに特異的な細胞障害性Ｔ細胞が攻撃することが想定されている。通常のウイルス性急性肝炎では，このような機構で巣状の壊死が形成されると考えられるが，劇症肝炎においてこの機構が広汎肝細胞死にまで至る機序

として，被感染者（宿主）の過剰な炎症・免疫反応やこれに伴う循環障害が想定されている。一方，性交渉や院内感染などにおいて，同じ感染源から複数の劇症肝炎患者が発生した事例がいくつか報告されたことから，宿主側の要因である免疫過剰反応のみならず特殊なウイルス株が肝炎の劇症化を引き起こす可能性が示唆された。このようなウイルス側の要因として，ウイルス遺伝子の変異による抗原性あるいは増殖力，蛋白転写活性の変化が想定され，B型肝炎ウイルスを中心に研究されている。

劇症化予知の試み

急性肝炎の予後が良好なのに反し，劇症化した場合の予後が極めて不良なことから，急性肝炎の段階で劇症化を早期に予知し，集中治療を行うことにより劇症化を阻止あるいは予後を改善しようとする試みが行われている。

急性肝炎の劇症化の徴候として古くから進行性の黄疸，強い全身倦怠感と食欲不振，悪心・嘔吐，肝性口臭，発熱，頻脈，出血傾向，浮腫，腹水，乏尿，肝濁音界の縮小（肝の萎縮），羽ばたき振戦などが挙げられており，注意して観察すべき症候である。

臨床検査としては，総ビリルビン高値や，総蛋白，アルブミン，プロトロンビン時間，尿素窒素，コリンエステラーゼ，総コレステロール，HDLコレステロールなどの低値に注意すべきである。

厚生労働省「難治性の肝・胆道疾患調査研究班」では，プロトロンビン時間80％以下を示した段階での多変量解析（多重ロジスティックモデル）による予知式（表2）を提唱し，プロスペクティブな検証を進めている。この式では，予測劇症化確率20％を専門施設への搬送基準，50％を人工肝補助などの特殊治療開始基準としている[5]。

表2　プロトロンビン時間(PT)80％以下を示した急性肝炎の劇症化予知式

選択変数	回帰係数	有意確率	オッズ比
ln(1+TB)	0.692	0.016	1.997
PT（%）	-0.065	0.000	0.937
年齢	1.388	0.001	4.009
成因	0.868	0.031	2.382
定数	-1.156		

年齢：0：50歳以下，1：51歳以上
成因：0：HAV，HCV，HEV，acute HBV，他のウイルス，薬剤
1：HBV carrier，成因不明

劇症化時の対応

1．家族への説明と特定疾患申請

劇症肝炎は若年者にも発症し急激かつ重篤な経過をたどるため，家族の動揺は大きく，家族への説明は極めて重要である。しかも現状の説明に終わることなく，近い将来予測される事態に対する対策をあらかじめ説明しておく必要がある。そのためには客観的な資料が必要であり，わが国でこれまで行われてきた全国集計のデータ[6]が有用である。これらの客観的な資料を基に，頻回にきめ細かく説明しておくことが患者およびその家族との信頼関係の確立に繋がり，人工肝補助や移植準備などに円滑に進むことができる。

具体的には，急性肝炎の段階では先に述べた予測劇症化確率と仮に劇症化した場合の特殊治療や肝移植の必要性などを簡単に説明し，劇症化が懸念される場合は専門の施設への搬送が必要な旨をあらかじめ説明する。劇症化した場合は，人工肝補助療法の必要性を説明し速やかに開始する。これと同時に，先に述べたわが国の内科的救命率および移植による