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研究報告の概要

○本邦におけるアラニンアミノトランスフェラーゼ高値供血者の無症候性E型肝炎感染の現況

E型肝炎ウイルス(HEV)の無症候性感染の現況は完全には調査されていない。本試験では、日本赤十字血液センターでアラニンアミノトランスフェラーゼ(ALT)高値(61-476 IU/L)を示した献血者のボランティア6700名から血清検体を採取し、ELISA を用いて抗HEV IgG、IgM、IgAの有無を調べ、nested RT-PCR を用いてHEV RNAを検査した。全体として、479名(7.1%)の供血者が抗HEV IgG陽性であり、そのうち8名は抗HEV IgM陽性、7名は抗HEV IgA陽性であった。抗HEV IgM および(または)抗HEV IgA陽性患者9名のうち、6名にHEV RNAが検出された。残り6691名から得た血清10検体のミニプールでさらにHEV RNAを調べたところ、3名がHEV RNA陽性であった(うち1名は抗HEV IgG陰性)。ALT値で層別化した場合、ALT≧201 IU/Lの供血者109名のHEV RNAの陽性率は、ALT 61-200 IU/Lの供血者6591名と比較して有意に高かった(2.8% vs. 0.1%, P < 0.0001)。ウイルス血症を発症した供血者9名から得られたHEV分離ウイルスは遺伝子型3に分類され、85.6～98.5%の一致度があり、オープンリーディングフレーム 2の412-ヌクレオチド配列における日本固有HEV株 (JRA1)との相同性は87.3～93.9%であった。本試験は、ALT≧201 IU/Lの日本人の約3%はさまざまなHEV株の無症候性感染を有することを示している。

使用上の注意記載状況・その他参考事項等

白血球除去赤血球「日赤」
照射白血球除去赤血球「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
ALT≧201 IU/Lの日本人の約3%はさまざまなHEV株の無症候性感染を有することが示されたとの報告である。日本赤十字社では、献血血液のALT検査を行い、61 IU/L以上の血液を排除している。	日本赤十字社では、厚生労働省科学研究「本邦に於けるE型肝炎の診断・予防・疫学に関する研究班」と共同して、献血者におけるHEV感染の疫学調査を行っている。北海道における輸血HEV感染報告を受け、試験的に北海道では研究的NATを行うなど安全対策を実施している。今後もHEV感染の実態に関する情報の収集及び安全対策に努める。

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Ongoing Subclinical Infection of Hepatitis E Virus Among Blood Donors With an Elevated Alanine Aminotransferase Level in Japan

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Ongoing subclinical infection of hepatitis E virus (HEV) has not been fully studied. In the present study, serum samples were collected from 6700 voluntary blood donors with an elevated alanine aminotransferase (ALT) level of 61-476 IU/l at a Japanese Red Cross Blood Center, and were tested for the presence of IgG, IgM and IgA classes of antibodies to HEV (anti-HEV) by in-house ELISA and HEV RNA by nested RT-PCR. Overall, 479 blood donors (7.1%) were positive for anti-HEV IgG, including 8 donors with anti-HEV IgM and 7 donors with anti-HEV IgA. Among the nine donors with anti-HEV IgM and/or anti-HEV IgA, six had detectable HEV RNA. The presence of HEV RNA was further tested in 10-sample minipools of sera from the remaining 6691 donors, and three donors including one without anti-HEV IgG were found to be positive for HEV RNA. When stratified by ALT level, the prevalence of HEV RNA was significantly higher among the 109 donors with ALT ≥201 IU/l than among the 6591 donors with ALT of 61–200 IU/l (2.8% vs. 0.1%, $P < 0.0001$). The HEV isolates obtained from the nine viremic donors segregated into genotype 3, shared a wide range of identities of 85.6–98.5% and were 87.3–93.9% similar to the Japan-indigenous HEV strain (JRA1), in the 412-nucleotide sequence of open reading frame 2. This study suggests that approximately 3% of Japanese individuals with ALT ≥201 IU/l have ongoing subclinical infection with various HEV strains. ***J. Med. Virol. 79: 734–742, 2007.*** © 2007 Wiley-Liss, Inc.

KEY WORDS: hepatitis E virus; subclinical infection; PCR; genotype; phylogenetic analysis

INTRODUCTION

Hepatitis E is an acute disease that is endemic in many developing countries of Asia and Africa where sanitation is suboptimal, and is also endemic in many industrialized countries including the United States, European countries and Japan [Harrison, 1999; Purcell and Emerson, 2001; Smith, 2001; Emerson and Purcell, 2003; Okamoto et al., 2003]. Hepatitis E virus (HEV), the causative agent of hepatitis E, is a positive-sense RNA virus without an envelope and is classified as the sole member of the genus *Hepevirus* in the family *Hepeviridae* [Emerson et al., 2004]. Its genome is approximately 7.2 kilobases in length and contains three open reading frames (ORFs: ORF1, ORF2 and ORF3) flanked by short untranslated regions [Tam et al., 1991]. ORF1 is the largest of the three and encodes viral non-structural proteins. ORF2 encodes the capsid protein and ORF3 encodes a small protein that undergoes phosphorylation [Koonin et al., 1992; Zafrullah et al., 1997]. Extensive genomic diversity has been noted among HEV isolates and HEV sequences have been classified into four genotypes (genotypes 1–4) [Schlauder and Mushahwar, 2001]. Genotype 1 HEV has been responsible for a number of waterborne epidemics of

The nucleotide sequence data reported in this study have been assigned DDBJ/EMBL/GenBank accession numbers AB288357–AB288365.

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hepatitis E in Asia and Africa. Although HEV of genotype 2 has been detected less frequently, it was responsible for outbreaks in Mexico in 1986–1987 [Velazquez et al., 1990] and has been implicated in sporadic infections in Africa [Buisson et al., 2000]. On the other hand, genotypes 3 and 4 HEV cause sporadic cases of acute hepatitis but have not been found to be responsible for epidemics in humans; these infections seem to be zoonotic and both genotypes have been detected in pigs (genotype 3 worldwide, and genotype 4 in Asia), which may constitute the major reservoir of genotypes 3 and 4 [Harrison, 1999; Meng, 2005; Lu et al., 2006].

Polyphyletic HEV strains of genotypes 3 and 4 are circulating in Japan [Takahashi et al., 2001, 2002; Mizuo et al., 2002; Inoue et al., 2006] and HEV has been recognized as an important causative agent of sporadic acute hepatitis of non-A, non-B, non-C etiology in this country [Mizuo et al., 2002; Okamoto et al., 2003]. It has been reported that food-borne transmission of HEV may occur through ingestion of raw or undercooked meat including liver and intestine from infected swine, deer or boar [Matsuda et al., 2003; Tei et al., 2003; Yazaki et al., 2003]. Imported hepatitis E and transfusion-transmitted HEV infection have also been documented [Koizumi et al., 2004; Matsubayashi et al., 2004; Mitsui et al., 2004]. Furthermore, a high prevalence of IgG class antibodies to HEV (anti-HEV IgG) among healthy individuals, most likely due to past subclinical HEV infection, has been reported in some regions in Japan [Li et al., 2000; Tanaka et al., 2001, 2005; Mitsui et al., 2004, 2005], and HEV-viremic subjects have been identified among symptom-free blood donors with an elevated alanine aminotransferase (ALT) level [Fukuda et al., 2004]. However, it remains unknown whether and, if so, how frequently recent subclinical HEV infection is occurring in Japan. Therefore, in an attempt to estimate the prevalence of recent subclinical HEV infection in Japan stratified by ALT level, anti-HEV antibodies and HEV RNA were assayed in serum samples obtained from Japanese voluntary blood donors with an elevated ALT level of $\geq$61 IU/l who are likely to have ongoing HEV infection.

MATERIALS AND METHODS

Serum Samples

Approximately 2.1% of voluntary blood donors had an elevated ALT level of 61 IU/l or greater at the Japanese Red Cross Saitama Blood Center, Japan, between April 2003 and March 2006. During this period, serum samples were collected from a total of 6700 voluntary blood donors (age, 35.7 ± 10.6 [mean ± standard deviation, SD] years; 6051 men and 649 women) with an elevated ALT level of 61–476 (range; 88.9 ± 34.6, mean ± SD) IU/l. The Blood Center is located in Saitama Prefecture, a prefecture in the central part of mainland Honshu of Japan.

All 6700 donors were negative for hepatitis B surface antigen and antibodies to hepatitis C virus (HCV), human immunodeficiency virus (HIV) types 1 and 2, and human T-lymphotropic virus type 1, as well as hepatitis B virus DNA, HCV RNA and HIV type 1 RNA by the nucleic acid amplification test using Roche's Multiplex reagent [Mine et al., 2003]. Serum samples obtained from repeat donors during the study period were excluded: that is, each sample was obtained from a unique individual.

Detection of Antibodies to HEV

To detect anti-HEV IgG, anti-HEV IgM and anti-HEV IgA, enzyme-linked immunosorbent assay (ELISA) was performed using purified recombinant ORF2 protein of HEV genotype 4 that had been expressed in the pupae of silkworm [Mizuo et al., 2002], as described previously [Takahashi et al., 2005]. The optical density (OD) of each sample was read at 450 nm. The cut-off value used for the anti-HEV IgG, anti-HEV IgM, and anti-HEV IgA assays was 0.175, 0.440, and 0.642, respectively [Takahashi et al., 2005]. Samples with OD values for anti-HEV IgG, IgM, or IgA equal to or greater than the respective cut-off value were considered to be positive for anti-HEV IgG, IgM, or IgA, respectively. The specificity of the anti-HEV assays was verified by absorption with the same recombinant ORF2 protein that was used as the antigen probe. Briefly, if the OD value of the tested sample was less than 30% of the original value after absorption with the recombinant ORF2 protein, the sample was considered to be positive for anti-HEV.

Detection of HEV RNA

In serum samples with anti-HEV IgM and/or anti-HEV IgA, reverse transcription (RT)-polymerase chain reaction (PCR) was performed for detection of HEV RNA using nested primers targeting the ORF2 region as described previously [Mizuo et al., 2002]. The size of the amplification product of the first-round PCR was 506 base pairs (bp), and that of the second-round PCR was 457 bp. The nested RT-PCR assay that we used is capable of amplifying all four known genotypes of HEV strains reported thus far [Mizuo et al., 2002; Takahashi et al., 2003b; Yazaki et al., 2003]. The specificity of the RT-PCR assay was verified by sequence analysis as described below. The sensitivity of the RT-PCR assay was assessed as described previously [Mizuo et al., 2002]. For serum samples that were negative for HEV RNA when 100 µl of serum samples was used, total RNA was extracted from 500 µl of serum, reverse transcribed, and then subjected to the nested PCR as described above. To extract RNA from 500 µl of serum, test serum diluted two-fold in saline was centrifuged at 287,582 × *g* at 4°C for 2 hr in a TLA-100.2 rotor (Beckman Coulter K.K., Tokyo, Japan), and the resulting pellet was suspended in 100 µl of saline and subjected to the RT-PCR assay. To confirm the reproducibility, this assay was performed in duplicate.

As for serum samples without anti-HEV IgM and anti-HEV IgA, 10 µl each from 10 serum samples were pooled,

and each pool was tested for HEV RNA by the above-mentioned RT-PCR assay. If a pool was positive for HEV RNA, the 10 serum samples of that pool were individually tested for the presence of HEV RNA. This RT-PCR assay was performed using both 10 and 100 μl of each serum sample, and reproducibility was confirmed.

Sequence Analysis of PCR Products

The amplification products were sequenced directly on both strands using the BigDye Terminator Cycle Sequencing Ready Reaction Kit on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequence analysis was performed using Genetyx-Mac Version 12.2.7 (Genetyx Corp., Tokyo, Japan) and ODEN Version 1.1.1 from the DNA Data Bank of Japan (DDBJ: National Institute of Genetics, Mishima, Japan) [Ina, 1994]. Sequence alignments were generated by CLUSTAL W (version 1.8) [Thompson et al., 1994]. A phylogenetic tree was constructed by the neighbor-joining method [Saitou and Nei, 1987] based on the partial nucleotide sequence of the ORF2 region (412 nucleotides [nt]). Bootstrap values were determined on 1000 resamplings of the data sets [Felsenstein, 1985].

Statistical Analysis

Statistical analyses were performed using the χ^2-test for comparison of proportions between two groups. Differences were considered to be statistically significant at $P < 0.05$.

RESULTS

Age- and Sex-Specific Prevalence of Anti-HEV Antibodies

A total of 6700 serum samples obtained from apparently healthy blood donors with an elevated ALT level were tested for the presence of anti-HEV IgG. Anti-HEV IgG was detected in 7.1% (479/6700) of the tested population including 7.0% of the 6051 male donors and 8.6% of the 649 female donors, the difference not being significant (Table I). The prevalence of anti-HEV IgG generally increased with age among both the male and female donors, and was significantly higher among donors aged ≥30 years than among those aged <30 years in total (8.8% vs. 2.9%, $P < 0.0001$), in the males (8.6% vs. 2.9%, $P < 0.0001$) and in the females (10.7% vs. 2.5%, $P = 0.0013$). The 479 serum samples with anti-HEV IgG were tested for anti-HEV IgM and anti-HEV IgA. Among them, anti-HEV IgM was detected in eight samples (1.7%) and anti-HEV IgA in seven samples (1.5%). In total, nine samples were positive for anti-HEV IgM and/or anti-HEV IgA (Table II).

Detection of HEV RNA Among All 6700 Blood Donors

Among the nine serum samples with anti-HEV IgM and/or anti-HEV IgA, five samples tested positive for HEV RNA when RT-PCR was performed with a sample volume of both 10 and 100 μl, and one sample was positive for HEV RNA with a sample volume of 500 μl (Group A in Table II). Among 661 10-sample pools and nine 9-sample pools, three 10-sample pools were positive for HEV RNA. The 30 serum samples of the 3 pools that had been positive for HEV RNA were tested individually for the presence of HEV RNA, and 3 samples (nos. 5503, 8177 and 7369 in Groups B and C in Table II) were found to be positive for HEV RNA in two distinct volumes of 10 and 100 μl. Consequently, 9 (0.1%) of the 6700 samples were found to be viremic for HEV in the present study. When stratified by the presence of anti-HEV antibodies, HEV RNA was detectable in 6 (66.7%) of the 9 donors with anti-HEV IgM and/or anti-HEV IgA, 2 (0.4%) of the 470 donors with anti-HEV IgG but without anti-HEV IgM or anti-HEV IgA, and 1 (0.02%) of the 6221 donors without any serological markers of HEV infection.

Prevalence of Anti-HEV and HEV RNA, Stratified by ALT Level

In the present study, 479 donors with anti-HEV IgG were found, including 371 (7.2%) with an ALT level of 61–100 IU/l, 96 (6.6%) with an ALT level of 101–200 IU/l, and 12 (11.0%) with an ALT level of ≥201 IU/l (Table III). The prevalence of anti-HEV IgG was higher among donors with an ALT level of ≥201 IU/l than among those with an ALT level of 61–200 IU/l, although the difference was not statistically significant (11.0% vs. 7.1%, $P = 0.1148$). As for the prevalence of HEV RNA, there was a significant difference between donors with an ALT level of ≥201 IU/l and those with an ALT level of 61–200 IU/l in total (2.8% vs. 0.1%, $P < 0.0001$), in males (2.1% vs. 0.1%, $P < 0.0001$) and in females (8.3% vs. 0%, $P < 0.0001$).

Genetic Analysis of HEV Isolates Recovered from Nine Viremic Donors

The nine HEV isolates recovered from the transiently viremic donors were named HE-JSB1217, HE-JSB1564, HE-JSB1582, HE-JSB4175, HE-JSB5503, HE-JSB6151, HE-JSB7017, HE-JSB7369, and HE-JSB8177, respectively, with the prefix of HE-JSB followed by the ID no. of each sample. The 412-nt sequence of ORF2 of these HEV isolates were determined and compared with each other and with that of known HEV isolates of genotypes 1–4. These nine HEV isolates were markedly variable, sharing nucleotide identities ranging from 85.6% to 98.5%. However, they were all close to the prototype Japanese isolate of genotype 3 (JRA1 [accession no. AP003430]) with nucleotide identities of 87.3–93.9%, and were only 78.1–80.7%, 75.2–76.6%, and 78.2–80.5% similar to the B1 isolate (M73218) of genotype 1, MEX-14 isolate (M74506) of genotype 2, and T1 isolate (AJ272108) of genotype 4, respectively. When the nine HEV isolates obtained in the present study were compared with 412 other reported genotype 3 isolates whose common 299-to-412-nt ORF2 sequence is available as of December 2006, each of them was closest to a human or swine HEV

TABLE I. Age- and Sex-Dependent Prevalence of Anti-HEV Antibodies

	Total			Male			Female		
		Anti-HEV			Anti-HEV			Anti-HEV	
Age (years)	N	IgG-class	IgM- and/or IgA-class	N	IgG-class	IgM- and/or IgA-class	N	IgG-class	IgM- and/or IgA-class
16–19	439	13 (3.0%)	0	397	11 (2.8%)	0	42	2 (4.8%)	0
20–29	1414	40 (2.8%)	0	1294	38 (2.9%)	0	120	2 (1.7%)	0
30–39	2736	183 (6.7%)	4 (0.1%)	2541	164 (6.5%)	3 (0.1%)	195	19 (9.7%)	1 (0.5%)
40–49	1319	126 (9.6%)	3 (0.2%)	1202	114 (9.5%)	3 (0.2%)	117	12 (10.3%)	0
50–59	651	93 (14.3%)	1 (0.2%)	520	77 (14.8%)	1 (0.2%)	131	16 (12.2%)	0
60–70	141	24 (17.0%)	1 (0.7%)	97	19 (19.6%)	1 (1.0%)	44	5 (11.4%)	0
Total	6700	479 (7.1%)	9 (0.1%)	6051	423 (7.0%)	8 (0.1%)	649	56 (8.6%)	1 (0.2%)

TABLE II. Detection of HEV RNA Among Three Categories of Blood Donors With Elevated ALT Level

					Anti-HEV (OD_{450} value)			HEV RNA[a]		
ID no.	Age (years)/ sex	ALT (IU/l)	AST (IU/l)	γ-GTP (IU/l)	IgG-class	IgM-class	IgA-class	10 µl	100 µl	500 µl
Group A ($n = 9$) with anti-HEV IgG with anti-HEV IgM and/or anti-HEV IgA										
1217	34/M	94	37	149	2.583 (+)	1.818 (+)	1.676 (+)	+[b]	+	NT[c]
1564	48/M	61	32	86	2.343 (+)	1.709 (+)	2.648 (+)	+	+	NT
1582	51/M	101	52	89	2.404 (+)	0.917 (+)	1.310 (+)	+	+	NT
4175	68/M	261	145	154	1.709 (+)	2.566 (+)	1.130 (+)	+	+	NT
6151	49/M	128	41	607	1.364 (+)	2.237 (+)	2.442 (+)	+	+	NT
7017	36/F	224	95	362	1.401 (+)	1.313 (+)	0.348 (−)	−	−	+
1304	38/M	181	67	104	>3.000 (+)	>3.000 (+)	0.825 (+)	−	−	−
3243	35/M	170	78	334	0.404 (+)	0.470 (+)	0.017 (−)	−	−	−
7667	47/M	66	37	45	0.350 (+)	0.044 (−)	0.868 (+)	−	−	−
Group B ($n = 470$) with anti-HEV IgG but without anti-HEV IgM nor anti-HEV IgA										
5503	34/M	77	55	20	0.394 (+)	0.091 (−)	0.138 (−)	+	+	NT
8177	41/M	82	53	197	0.193 (+)	0.042 (−)	0.061 (−)	+	+	NT
Group C ($n = 6221$) without anti-HEV IgG										
7369	48/M	276	210	219	0.006 (−)	0.016 (−)	0.011 (−)	+	+	NT

[a]HEV RNA was assayed using the indicated volume of serum sample.
[b]+, positive for HEV RNA; −, negative for HEV RNA.
[c]NT, not tested.

TABLE III. Prevalence of Anti-HEV IgG and HEV RNA Among Voluntary Blood Donors With Elevated ALT Level, Stratified by ALT Level

	Total			Male			Female		
ALT (IU/l)	N	Anti-HEV IgG	HEV RNA	N	Anti-HEV IgG	HEV RNA	N	Anti-HEV IgG	HEV RNA
61–100	5131	371 (7.2%)	4 (0.1%)	4635	331 (7.1%)	4 (0.1%)	496	40 (8.1%)	0
101–200	1460	96 (6.6%)	2 (0.1%)	1319	83 (6.3%)	2 (0.2%)	141	13 (9.2%)	0
201–476	109	12 (11.0%)	3 (2.8%)	97	9 (9.3%)	2 (2.1%)	12	3 (25.0%)	1 (8.3%)
Total	6700	479 (7.1%)	9 (0.1%)	6051	423 (7.0%)	8 (0.1%)	649	56 (8.6%)	1 (0.2%)

isolate of Japan origin. That is, HE-JSB1564, HE-JSB5503, and HE-JSB8177 had the highest identity of 99.0%, 98.5% and 98.3%, respectively, with HE-JBD2 (AB154829). The HE-JSB4175 isolate was closest to HE-JA9 (AB082565, 97.8%), HE-JSB1217 and HE-JSB1582 to HE-JHD1988 (AB175485, 94.9% and 93.7%, respectively), HE-JSB7369 to HE-JA21 (AB115542, 94.2%), HE-JSB6151 to G3-4531-Swine (DQ079632, 94.4%), and HE-JSB7017 to HE-JHD1980 (AB175484, 93.4%).

The phylogenetic tree constructed based on the common 412-nt sequence within the ORF2 sequence confirmed that the nine HEV isolates obtained in the present study belonged to genotype 3, and that they segregated into the clusters consisting of Japanese HEV strains of the same genotype that had been recovered from humans, swine and wild boars, supporting the indigenous nature of these nine blood donor isolates (Fig. 1).

DISCUSSION

Recent studies have documented that sporadic acute hepatitis E does occur among individuals in industrialized countries with no history of travel to areas endemic for HEV [Kwo et al., 1997; Harrison, 1999; Mansuy et al., 2004; Ijaz et al., 2005; Waar et al., 2005; Amon et al., 2006; Preiss et al., 2006; Sadler et al., 2006]. In Japan, hepatitis E is rare compared with hepatitis A, but is occurring more frequently than previously thought [Mizuo et al., 2002; Okamoto et al., 2003]. As for the geographical distribution of hepatitis E in Japan, it was reported that there was wide variation with a higher prevalence in the northern part of Japan (Hokkaido Island and northern part of mainland Honshu) [Mizuo et al., 2002; Abe et al., 2006]. The Japanese Red Cross Saitama Blood Center is located in Saitama Prefecture, which is north of and adjacent to Metropolitan Tokyo. Only six cases of locally acquired sporadic acute hepatitis E have thus far been reported in this prefecture, in contrast with more than 100 cases in Hokkaido and 23 cases in Tokyo [Abe et al., 2006].

In the present study, 7.0% (468/6700) of the study population had anti-HEV IgG in the absence of IgM/IgA class anti-HEV and HEV RNA, which is much higher than expected. This finding suggests the presence of frequent past HEV infection among individuals living in the central part of Japan, most of which seem to be subclinical. HEV RNA was assayed in serum samples obtained from all 6700 donors, and the prevalence of ongoing subclinical HEV infection in three distinct groups of the study population according to the presence of class-specific HEV antibodies, was investigated. Among the nine donors with IgM and/or IgA class anti-HEV, six donors (66.7%) were found to be viremic for HEV. Furthermore, among the 6691 donors without anti-HEV IgM and anti-HEV IgA, three HEV-viremic donors with no signs or symptoms of hepatitis were found. In an attempt to detect HEV RNA in a large sample size, we first screened for present HEV infection by testing 10-sample minipools (each pool contained

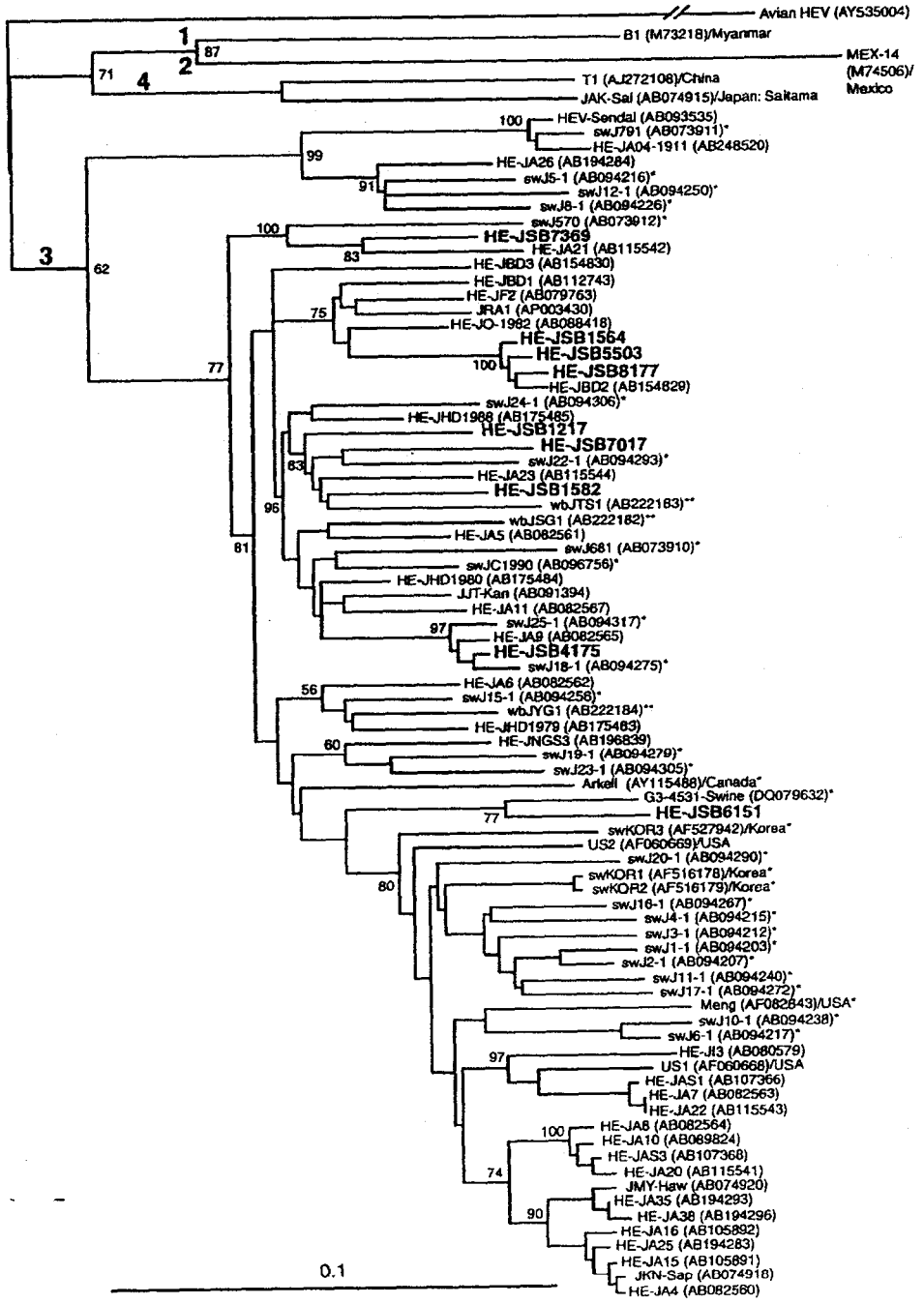

Fig. 1. Phylogenetic tree constructed by the neighbor-joining method based on the partial nucleotide sequence of the ORF2 region (412 nt) of 84 HEV isolates, using a chicken HEV (AY535004) as an outgroup. In addition to the HE-JSB1217, HE-JSB1564, HE-JSB1582, HE-JSB4175, HE-JSB5503, HE-JSB6151, HE-JSB7017, HE-JSB7369, and HE-JSB8177 isolates found in the present study which are indicated in bold type. 75 reported HEV isolates of genotypes 1–4 whose common 412-nt sequence is known are included for comparison and their accession nos. are shown in parentheses. Swine and wild boar HEV isolates are indicated with asterisks (* and **, respectively). Bootstrap values are indicated for the major nodes as a percentage obtained from 1000 resamplings of the data.

10 μl of serum from each of 10 subjects); then, for pools that were positive for HEV RNA, the individual serum samples were tested for the presence of HEV RNA. Despite the limited amount of serum from each sample that was tested, current subclinical HEV infection was recognized molecularly in three donors in this study. Two viremic donors (0.4%) were found among the 470 donors with anti-HEV IgG but without anti-HEV IgM nor anti-HEV IgA. Since serial serum samples were not available from these two viremic donors during the HEV infection, it is unclear whether they could not elicit acute antibodies of anti-HEV IgM and anti-HEV IgA.

However, the observation that HEV RNA is detectable in serum despite the absence of anti-HEV IgM and anti-HEV IgA is in agreement with our previous observations that two hemodialysis patients and one hospital employee who contracted subclinical HEV infection in 1979, 1980, or 2003, respectively, exhibited only anti-HEV IgG, although transient HEV viremia was observed [Mitsui et al., 2004, 2005]. Patients with HEV infection without an acute antibody response have also been reported [Caudill et al., 1994; Clayson et al., 1995].

Of particular note, one donor (0.02%) was found to have HEV RNA among the 6221 donors without serological markers of HEV infection in the present study. The precise reason why the viremic donor did not show an antibody response against HEV despite significant elevations of ALT (276 IU/l) and AST (210 IU/l) levels is unknown. However, 4 months before the blood sampling, he had elevated ALT (81 IU/l) and AST (165 IU/l) levels in the absence of HEV RNA in serum, suggesting that he contracted subclinical HEV infection on the background of an unexplained chronic liver disease. It was reported that neither anti-HEV IgG nor anti-HEV IgM was detectable in four symptom-free persons with evidence of HEV viremia who came into contact with patients with acute hepatitis E during an outbreak of hepatitis E [Nicand et al., 2001]. Aggarwal et al. [2001] conducted experimental studies on subclinical HEV infection in cynomolgus macaques, and reported that subclinical HEV infection in some animals was associated with failure of the development of an immune response, compared with animals with clinical HEV infection.

In Japan, approximately 200 patients with clinical HEV infection and 80 patients with subclinical HEV infection who contracted the infection between 2001 and 2005 have been reported [Okamoto et al., 2003; Fukuda et al., 2004; Abe et al., 2006; Inoue et al., 2006]. However, the exact ratio of the number of cases of clinical HEV infection to that of subclinical HEV infection remains unknown. At the Japanese Red Cross Saitama Blood Center, 2.1% of voluntary blood donors had an elevated ALT level of 61 IU/l or greater during the period from April 2003 to March 2006. The population size of individuals ≥20 years of age in Saitama Prefecture was reported to be 5.68 million on January 1, 2005 (http://www.pref.saitama.lg.jp/). In the present study, 9 (0.13%) of 6700 individuals with an elevated ALT level of ≥61 IU/l had HEV viremia. Assuming that HEV viremia is detectable by RT-PCR for 1 month during acute HEV infection [Takahashi et al., 2003a, 2005], the annual number of cases of subclinical HEV infection in Saitama Prefecture is estimated to be approximately 2000. Although we cannot rule out the possibility that the number of cases with clinical HEV infection is underestimated, only one or two patients with hepatitis E have been reported per year in this prefecture. Therefore, it is assumed that less than 0.1% of HEV-infected cases exhibit clinical manifestation of the infection.

As the nine viremic donors identified in the present study had an elevated ALT level, the blood from the nine donors was not used for transfusion, suggesting that ALT testing may help prevent transfusion-transmitted HEV infection. As one of the nine infected donors had only a slightly elevated ALT level of 61 IU/l, it seems likely that even donors with a normal ALT level (≤60 IU/l) may have detectable HEV RNA. However, the prevalence of HEV RNA decreased with ALT level, and was significantly lower among the 5131 donors with ALT level of 61–100 IU/l than among the 109 donors with ALT of ≥201 IU/l (0.078% vs. 2.8%, $P < 0.0001$). It is reasonable to speculate that the prevalence of ongoing HEV infection among donors with a normal ALT level may be less than 0.078% in Saitama Prefecture. The proportion of such donors may be significantly small or negligible, according to the geographic region. Reflecting the high prevalence of clinical HEV infection, at least three cases of transfusion-transmitted hepatitis E have been reported in Hokkaido and one case in Tokyo [Matsubayashi et al., 2004; Abe et al., 2006], but none in Saitama Prefecture up to the present. As donors with normal ALT level were not tested for HEV viremia in the present study, we cannot conclude that the serum ALT level can be used to exclude blood donors with ongoing HEV infection. Based on the current study, however, we would consider that ALT testing is at least useful in part for exclusion of donors with HEV viremia with the aim of preventing transfusion-associated hepatitis E, although alcohol consumption and obesity should be taken into consideration as the major contributing factors to an elevated ALT level in blood donors.

Multiple HEV strains of genotype 3 or 4 have been isolated from Japanese patients with sporadic acute or fulminant hepatitis E as well as from farm pigs, wild boars, a wild deer and a mongoose in Japan [Mizuo et al., 2002; Takahashi et al., 2003a,b, 2004; Inoue et al., 2006; Nakamura et al., 2006]. Reflecting the polyphyletic nature of human and animal HEV isolates of Japan origin, the HEV isolates recovered from nine viremic donors in the present study, differed by 1.5–14.4% from each other, although they belonged to the same genotype (genotype 3) with the highest nucleotide sequence identity of 87.3–93.9% with the JRA1 isolate that is believed to be indigenous to Japan [Takahashi et al., 2001]. A human HEV strain of genotype 4 (JAK-Sai [AB074915]) has been isolated [Takahashi et al., 2002] in the same prefecture as that of the nine viremic donors, and it shares only 78.6–81.3% identities with the nine HEV isolates obtained from the viremic donors in the present study. These results further support the marked heterogeneity of the HEV genome and its wide distribution in Japan, even within a certain prefecture in this country.

In conclusion, nine blood donors with HEV viremia were identified among 6700 voluntary blood donors with an elevated ALT level at a blood center located in the central part of mainland Honshu of Japan where hepatitis E is low-endemic. This study indicates that approximately 0.1% of individuals with an elevated ALT

level and 3% of individuals with an ALT level of ≥201 IU/l have ongoing subclinical infection of various HEV strains, suggesting the frequent occurrence of subclinical HEV infection, although clinical HEV infection is rarely reported. A large study of individuals who do not have an elevated ALT level is needed to assess the exact frequency of subclinical HEV infection, taking into consideration the geographic region in Japan.

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