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研究報告の概要

○病棟におけるE型肝炎ウイルスの患者間感染の分子学的エビデンス
血液疾患病棟で急性白血病の33才の男性が急性肝炎を発症し、患者の血漿及び糞便検体からE型肝炎ウイルス(HEV)遺伝子が検出されHEV感染症と診断された。患者にHEV流行地域への旅行歴、野生動物・ペットとの接触歴及び生肉・貝類の摂食歴はなく、また、複数回の輸血を受けていたが供血者検体の検査結果はHEV RNA陰性であった。
この病棟には、急性E型肝炎を発症し、ほぼ1年間にわたって血液と糞便の両方にHEVを排出した44才のリンパ腫の男性患者がおり、最後の病棟滞在時期がHEVに感染した患者と重なった。
PCRの結果、2人の患者のHEVはいずれもgenotype 3fに属し、シーケンスの同一性は97.8% ～98.6%であった。
2人の患者は地理的に異なった地域に住み、HEVの共通感染源に暴露されていなかったため、2人が同時に病棟に滞在した間に感染が起こったことが示唆される。
病棟での遡及的調査で一般的な衛生予防措置上の重大な違反は確認されなかったが、(1) 免疫抑制患者はウイルスに感染しやすい、(2) 感染患者は長期間にわたり二次感染につながるHEVを排出する、(3) ウイルスは無機物表面で長期間生存する、(4) HEVに対してワクチンは利用できないことから、我々は、免疫抑制患者が治療される病棟でE型肝炎の症例が発生した場合には、一般的な衛生予防措置は強化されなければならないと結論する。

使用上の注意記載状況・その他参考事項等

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血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
急性白血病の33才の男性がE型肝炎を発症し、HEV遺伝子検査の結果、重複する時期に同じ病棟に入院していた別のE型肝炎患者から感染したことが示唆されたとの報告である。 免疫抑制状態にある患者では、食物、輸血以外の経路によるHEV伝播の可能性についても、配慮する必要があるものと考える。 HEVは脂質膜のないRNAウイルスである。本剤の製造工程にはコーン分画及び液状加熱の2つのウイルス除去・不活化工程が含まれている。疫学的に見て、血漿分画製剤で最も長い歴史を持つアルブミンでは世界的にHEV感染の報告はないことから、本剤の安全性は確保されていると考える。	本剤の安全性は確保されていると考えるが、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。なお、日本赤十字社では、北海道における輸血後HEV感染報告を受け、献血者の疫学調査や、北海道で研究的NATを実施している。

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ceftriaxone, and benzylpenicillin were administered empirically. MRI revealed meningeal enhancement around the brainstem, contiguous with a markedly edematous cervical cord and "sugar coating" of the entire cervico-thoraco-lumbar cord (figure 1). A clinical diagnosis of necrotizing varicella myelitis with meningoencephalitis was made. Progressive bulbar palsy and respiratory failure developed. Because of the extremely poor prognosis, the patient was palliated, and she died 60 h after arrival at our institution.

Varicella-zoster virus (VZV) was detected by PCR of the patient's CSF and skin vesicle specimens. Postmortem examination confirmed extensive infarction and necrosis of the entire spinal cord due to necrotizing vasculitis in association with a lymphocytic meningitis.

This fulminant presentation of VZV necrotizing myelitis has been reported infrequently in profoundly immunosuppressed HIV-infected individuals in the pre-ART era [1–4]. To our knowledge, this is the first occurrence in a moderately immunosuppressed individual in the post-ART era. Its occurrence shortly after a change in ART raises the possibility that this is a manifestation of VZV immune restoration disease (IRD).

VZV complications involving the CNS are estimated to occur in 2% of patients with HIV/AIDS, with 4 other recognized variants, including multifocal encephalitis, ventriculitis, focal necrotizing myelitis, and vasculopathy that leads to cerebral infarction [2, 4–6]. Prognosis of VZV necrotizing meningomyelitis is extremely poor, with a median survival of 16 days [7].

The diagnosis of IRD is usually contingent on a clear response to ART with ≥1-log reduction in HIV RNA level [8]. Assessment of HIV RNA level was not performed, but a significant decrease is highly likely, given the initiation of 2 new classes of ART 2 weeks before presentation. The patient's moderate immunosuppression and decrease in CD4+ T cell count after the change of ART regimen does not preclude IRD [9]. Compartmentalization of VZV IRD in the CNS has been suggested and may explain the profound CNS changes in the absence of significant rash or systemic symptoms [10].

Necrotizing myelitis is a devastating complication of VZV. In the context of immunosuppression, necrotizing myelitis may represent a new manifestation of VZV IRD.

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Molecular Evidence of Patient-to-Patient Transmission of Hepatitis E Virus in a Hematology Ward

TO THE EDITOR—A 33-year-old man receiving treatment for acute leukemia in a hematological ward developed acute hepatitis (aspartate aminotransferase level, 1215 IU/L; alanine aminotransferase level, 2960 IU/L). Test results for viral markers (i.e., anti-hepatitis A virus IgM, hepatitis B virus surface antigen and DNA, anti-hepatitis C virus antibodies, and hepatitis C virus RNA) were negative; nonviral causes of liver disease, such as autoimmunity, toxic or iatrogenic hepatitis, and metabolic disorders, were excluded. A diagnosis of hepatitis E virus (HEV) infection was made after the detection of the HEV genome in plasma and stool samples from the patient [1]. Anti-HEV IgG was detected 2 weeks after the onset of the illness and persisted throughout.

The patient had not traveled in areas where HEV was endemic and declared that he had had no contact with wild or domestic animals. He had not eaten raw meat or shellfish. No symptomatologic cases of hepatitis E had been reported in his family or in nurses and medical staff during the same period. The patient had received many transfusions from blood

donors. Because HEV can be transmitted through transfusion [2], all donors' samples were tested and had negative results for HEV RNA.

Medical records from the hematology ward indicated that a 44-year-old man with lymphoma had developed acute hepatitis E 1 year earlier. This patient was hospitalized repeatedly for short periods during that year until his lymphoma was cured. The patient did not recover after the acute phase of hepatitis, and he excreted HEV in both blood and stool for almost a year. His last stay in the ward overlapped with that of the other patient who was infected with HEV.

We therefore looked for a link between the HEV strains from the 2 patients with use of samples that were collected at the time of diagnosis of acute hepatitis E. PCR products amplified from 3 distinct regions of the HEV genome were sequenced. Both strains belonged to HEV genotype 3f. Phylogenetic analyses including HEV sequences from local and GenBank reference strains indicated that the strains from the 2 patients were closely related. The nucleotide identity of the 3 HEV sequences from the 2 patients was 97.8%–98.6%. Both strains also harbored the same insertion in the ORF1 hypervariable region that differed from the reference sequences. Because the 2 patients lived 250 km apart in 2 geographically distinct areas and had not been exposed to a common source of HEV, transmission probably occurred during their overlapping stays in the hospital that occurred 3 weeks prior to the onset of hepatitis E in the patient with acute leukemia.

A retrospective audit of the ward identified no major breaches of universal hygiene precautions. However, a lapse in strict hygiene procedures could be the cause of HEV contamination through enteric transmission, because HEV can persist for weeks on inanimate surfaces [3]. Parenteric iatrogenic transmission has also been suggested [4].

We conclude that universal hygiene precautions must be reinforced when cases of hepatitis E occur in medical wards where immunosuppressed patients are treated. There are several reasons for reinforced precautions: (1) immunosuppressed patients are highly susceptible to viral infections; (2) infected patients excrete HEV for a prolonged time, which results in a high risk of secondary transmission; (3) the virus persists for long periods on inanimate surfaces; and (4) no vaccine is available against HEV, although a phase-2 vaccine trial has had recent success [5].

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医薬品 研究報告 調査報告書

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販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社)					

研究報告の概要

○日本のALT高値献血者のE型肝炎ウイルス陽性率についての全国調査
背景:我々は日本における輸血後E型肝炎感染症例2例を報告したが、日本の献血者のE型肝炎ウイルス(HEV)陽性率は十分明らかになっていない。
試験デザインおよび方法:すべての赤十字血液センターから、ALT高値のため献血不適となった献血者の血液検体を収集し、HEV試験に供した。
結果:北海道のALT高値(500 IU/L超)献血者41名では、8検体(19.5%)にHEV RNAが検出された。日本全土のALT高値(200 IU/L超)献血者1,389名では、HEV RNA、IgM-HEV抗体、IgG-HEV抗体陽性検体数が、それぞれ15(1.1%)、14(1.0%)、45(3.2%)であった。RNA陽性献血者はほとんど男性であり、日本のどの地域にも認められたが、北海道を含む東日本の方が多く、西日本の方が少ない傾向であった。HEV RNA陽性であった23検体のうち、19検体はgenotype 3、4検体はgenotype 4であった。分離株9株のDNA配列は、既知のブタHEV分離株と98.5%以上の相同性を示した。ALT値61～199IU/Lの献血者1,062名では、IgM-HEV抗体およびIgG-HEV抗体陽性検体の割合はそれぞれ0.1および2.7%であったが、これらの検体はHEV RNA陰性であった。
結論:日本各地のALT高値献血者にHEVマーカー(HEV RNAおよび抗HEV抗体)が認められ、いずれのマーカーとも、東日本の方が西日本より高かった。

使用上の注意記載状況・その他参考事項等

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血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
日本全国でALT高値のため献血不適となった献血者の血液検体に、HEVマーカー(HEV RNAおよび抗HEV抗体)が認められ、いずれのマーカーとも東日本の方が西日本より高かったとの報告である。 HEVは脂質膜のないRNAウイルスである。本剤の製造工程にはコーン分画及び液状加熱の2つのウイルス除去・不活化工程が含まれている。疫学的に見て、血漿分画製剤で最も長い歴史を持つアルブミンでは世界的にHEV感染の報告はないことから、本剤の安全性は確保されていると考える。	本剤の安全性は確保されていると考えるが、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。なお、日本赤十字社では、北海道における輸血後HEV感染報告を受け、献血者の疫学調査や、北海道で研究的NATを実施している。

A nationwide survey for hepatitis E virus prevalence in Japanese blood donors with elevated alanine aminotransferase

Hidekatsu Sakata, Keiji Matsubayashi, Hiromi Takeda, Shinichiro Sato, Toshiaki Kato, Satoru Hino, Kenji Tadokoro, and Hisami Ikeda

BACKGROUND: Although we reported two cases of transfusion-transmitted hepatitis E in Japan, the prevalence of hepatitis E virus (HEV) in Japanese blood donors is not very clear.

STUDY DESIGN AND METHODS: Blood samples of donors who were deferred from donation because of elevated alanine aminotransferase (ALT) levels were collected from all Japanese Red Cross Blood Centers and subjected to HEV tests.

RESULTS: Among the 41 donors with elevated ALT levels higher than 500 IU per L in Hokkaido, HEV RNA was detected in 8 (19.5%) samples. In 1389 donor samples with ALT levels of higher than 200 IU per L in nationwide Japan, the numbers of positive HEV RNA, immunoglobulin M (IgM) anti-HEV, and immunoglobulin G (IgG) anti-HEV samples were 15 (1.1%), 14 (1.0%), and 45 (3.2%), respectively. Although RNA-positive donors were predominantly male and found in any geographic area of Japan, they tended to be higher in number in eastern Japan including Hokkaido and lower in number in western Japan. Of the 23 HEV-positive samples, 19 were Genotype 3 and 4 were Genotype 4. DNA sequences of the 9 isolates showed more than 98.5 percent homology with the known swine HEV isolates. In 1062 donor samples with ALT levels of 61 to 199 IU per L, the percentages of IgM and IgG anti-HEV–positive samples were 0.1 and 2.7 percent, respectively, although there was no HEV RNA–positive sample.

CONCLUSION: HEV markers (HEV RNA and anti-HEV) were detected in donors with elevated ALT levels who were widely distributed over Japan. The prevalence and incidence were higher in eastern Japan than in western Japan.

Although hepatitis E virus (HEV) is an emerging pathogen of enterically transmitted viral hepatitis in endemic areas, its infection is now recognized as a form of zoonosis in which swine, wild boar, and deer act as reservoirs for human infection in Japan.[1-8] HEV subgenomic sequencing studies have revealed a close relationship between the strains infecting humans and those infecting pigs. Accumulating evidence suggests that eating undercooked meat and viscera of pig and other animals is associated with a high risk of acquiring HEV infection. The HEV-infected individuals show transient viremia, which suggests the potential risk of a blood-borne route of HEV infection.[9-12] We previously reported two cases of transfusion-transmitted acute hepatitis E in Hokkaido, Japan.[9,12] In both cases, sequence analyses showed that the isolates of both donors and patients appeared to be identical. Moreover, HEV RNA has been reported to be present among some blood donors with elevated alanine aminotransferase (ALT) levels in Japan.[9,13,14] Although HEV was previously considered to be endemic only in developing countries, approximately 13 percent of the non-A, non-B, and non-C acute hepatitis cases were caused by HEV in Japan, a developed country.[15] However, no report has been available on a nationwide survey for HEV prevalence in Japan.

ABBREVIATIONS: B19 = human parvovirus B19; EBV = Epstein-Barr virus; HAV = hepatitis A virus; HEV = hepatitis E virus; JRC = Japanese Red Cross; RT = room temperature.

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Here we report the results of two studies. First, we studied the presence of HEV in plasma samples collected from blood donors showing extremely high ALT levels in Hokkaido, Japan. Subsequently, we expanded the area of investigation to nationwide and studied HEV prevalence in Japanese blood donor samples with elevated ALT levels obtained from all Japan.

MATERIALS AND METHODS

Blood donor samples with elevated ALT levels in Hokkaido

For the preliminary study, we studied the blood donors with elevated ALT levels of 500 IU per L and greater in Hokkaido. There were 1,049,566 blood donations in Hokkaido from April 2000 through March 2003. Of these, 23,827 (2.3%) were disqualified because of an elevated ALT level of 61 IU per L or greater, which was cutoff value in the Japanese Red Cross (JRC). Of these, 41 had an ALT level of 500 IU per L or greater (Table 1). The samples from these 41 donors enrolled in this study were stored below –20°C until testing. The tests for qualitative HEV RNA and/or for antibodies were performed as described below.

Blood donor samples with elevated ALT levels in nationwide Japan

All donor samples (n = 1389) with ALT levels higher than 200 (mean ± standard deviation [SD], 314 ± 249) IU per L were collected from all JRC Blood Centers over Japan between April 2003 and March 2004. In addition, 1062 donor samples with ALT levels of 61 to 199 IU per L were collected randomly from 3 blood centers (Hokkaido, Hiroshima and Fukuoka). The 47 blood centers were divided into eastern Japan (three blocks: Hokkaido, Miyagi, and Tokyo) and western Japan (four blocks: Aichi, Osaka, Okayama, and Fukuoka; Fig. 1). Hiroshima and Fukuoka blood centers belong to western Japan. The samples were subjected to real-time reverse transcription–polymerase chain reaction (RT-PCR) testing for the presence of HEV RNA and enzyme-linked immunosorbent assay (ELISA) for antibody tests against HEV as described below. The samples were kept frozen below –20°C until testing.

Real-time RT-PCR for HEV RNA detection and sequence analyses

Total nucleic acids were extracted from 200 µL of plasma sample using a virus spin kit (QIAamp MinElute, Qiagen K.K., Tokyo, Japan) according to the manufacturer's instructions. The 20-µL eluate was subjected to one-step real-time RT-PCR and quantitative assay for HEV RNA as described in our previous study.[12] The amplification products were then sequenced directly on both strands and were analyzed as described previously.[16] The amplification products of ORF2 (412 nucleotides) from HEV RNA–positive samples were sequenced and compared with those of reported swine HEV isolates from pigs or pig livers by using GenBank Basic Local Alignment Search Tool (BLAST) homology search at the National Center for Biotechnology Information server (http://www.ncbi.nlm.nih.gov).

The nucleotide sequence data reported in this article will appear in DDBJ/EMBL/GenBank nucleotide sequence databases with the Accession Numbers AB434132 for HRC-HE1, AB434133 for HRC-HE2, AB434134 for HRC-HE3, AB434135 for HRC-HE4, AB434136 for HRC-HE5, AB434137 for HRC-HE6, AB434138 for HRC-HE7, AB434139 for HRC-HE8, AB434140 for HRC-HE9, AB434141 for HRC-HE10, AB434142 for HRC-HE11, AB434143 for HRC-HE12, AB434144 for JRC-HE1, AB434145 for JRC-HE2, AB434146 for JRC-HE3, AB434147 for JRC-HE4, AB434148 for JRC-HE5, AB434149 for JRC-HE6, AB434150 for JRC-HE7, AB434151 for JRC-HE8, AB434152 for JRC-HE9, AB434153 for JRC-HE10, and AB434154 for JRC-HE11.

TABLE 1. ALT-disqualified donors from April 2000 through March 2003 in Hokkaido, Japan (total number of donors, 1,049,566)

	Number of donors with each ALT level (IU/L)						
Donors	61-99	100-199	200-299	300-399	400-499	500-	Total
Male	16,809	3,714	226	35	11	29	20,824
Percent*	88.1	85.8	78.7	60.3	52.4	70.7	87.4
Percent†	1.60	0.35	0.02	0.00	0.00	0.00	1.98
Female	2,281	616	61	23	10	12	3,003
Percent*	11.9	14.2	21.3	39.7	47.6	29.3	12.6
Percent†	0.22	0.06	0.01	0.00	0.00	0.00	0.29
Total	19,090	4,330	287	58	21	41	23,827
Percent†	1.82	0.41	0.03	0.01	0.00	0.00	2.27
Percent‡	80.1	18.2	1.2	0.2	0.1	0.2	100.0

* Rate relative to the donors with each ALT level, showing the ratio of sex difference.
† Rate relative to the total donors (1,049,566).
‡ Rate relative to the ALT-disqualified donors (23,827).

ELISA for HEV antibodies

Purified HEV Genotype 1 virus-like particles derived from recombinant baculovirus-infected insect cells were used as antigens for detection of antibodies to HEV.[17,18] HEV RNA–positive samples from 41 donors enrolled in the preliminary study were assayed by commercial HEV antibody ELISA kit (Cosmic Corp., Ltd., Tokyo, Japan) which basically consisted of the recombinant ORF2 protein as the antigen according to the manufacturer's protocol. In the subsequent study of all samples (n = 1389 and 1062) from all areas of

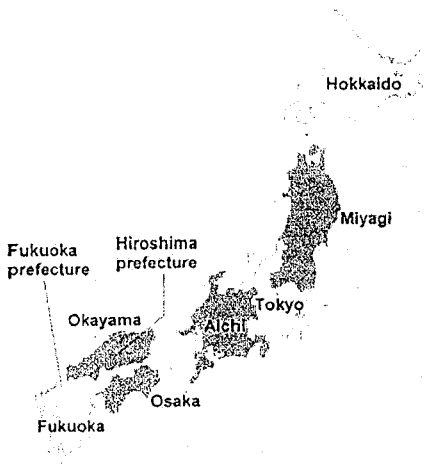

Fig. 1. Map of Japan showing the locations of seven geographic blocks. The 47 blood centers were divided into eastern Japan (three blocks: Hokkaido, Miyagi [six prefectures], and Tokyo [nine prefectures]) and western Japan (four blocks: Aichi [eight prefectures], Osaka [six prefectures], Okayama [nine prefectures] including Hiroshima prefecture, and Fukuoka [eight prefectures] including Fukuoka prefecture).

Japan, ELISA was performed as follows. Wells of microplates (Number 2592, 96-well Stripwell, flat bottom, Corning Life Sciences, Corning, NY) were coated with 50 μL of the recombinant ORF2 protein (3 μg/mL in phosphate-buffered saline [PBS]), and the plates were incubated at room temperature (RT) for 2 hours followed by incubation with 100 μL of blocking buffer containing 40 percent (vol/vol) calf serum (Gibco-BRL, Tokyo, Japan) at RT for 1 hour. The blocking buffer was discarded, and each well was washed five times with 450 μL of washing buffer (0.05% Tween 20 in PBS). To test for anti-HEV immunoglobulin G (IgG), 50 μL of each sample was added to each well at a dilution of 1:100 in saline containing 40 percent calf serum. The microplates were incubated at RT for 1 hour and then washed five times with washing buffer. Fifty microliters of horseradish peroxidase–conjugated goat anti-human IgG (IGB22; Institute of Immunology Co., Ltd., Tokyo, Japan; 1:2000) or immunoglobulin M (IgM; IGM49, Institute of Immunology Co., Ltd.; 1:500) in PBS containing 25 percent (vol/vol) fetal calf serum (PAA Laboratories GmbH, Pasching, Austria) was added to each well and incubated at RT for 1 hour. The wells were washed five times with washing buffer. Fifty microliters of tetramethylbenzidine soluble reagent (Dako Co., Ltd., Carpinteria, CA) as a substrate was added to each well. The plate was incubated at RT for 10 minutes in the dark, and then 50 μL of 1 N sulfuric acid (Kanto Chemical Co., Inc., Tokyo, Japan) as tetramethylbenzidine stop buffer was added to each well. The optical density (OD) of each sample was read at 450 nm. Test samples with OD values equal to or greater than the cutoff value were considered positive for the presence of anti-HEV IgG or anti-HEV IgM in this ELISA. ODs of 0.18 [mean (0.019) + 7 × SD (0.024)] for anti-HEV IgG, and that of 0.19 [mean (0.022) + 6 × SD (0.028)] for anti-HEV IgM were used as the cutoff values. Reactive samples were tested by another HEV antibody ELISA kit (Cosmic) described previously. Samples were determined as positive if they were reactive by both ELISA methods.

Statistical analysis

A two-sided Fisher's exact test was used to compare the percentages of subjects with each HEV marker in the two geographic groups (eastern Japan vs. western Japan) or two age groups (10s-30s vs. 40s-60s).

RESULTS

Prevalence of HEV RNA in donors with elevated ALT levels in Hokkaido

In the primary study, more than 98 percent of those disqualified donors had an ALT level of less than 200 IU per L and more than 87 percent were male (Table 1). The number of donors with elevated ALT levels higher than 500 IU per L was 41 (0.2%). Among the 41 donors, HEV RNAs were detected in 8 (19.5%). Of these, 6 samples were described in our previous study.[9]

Prevalence of HEV RNA in donors with elevated ALT in Japan

Thereafter, we studied a nationwide survey for HEV prevalence in Japanese blood donor samples with elevated ALT levels including levels of less than 500 IU per L, obtained from all Japan. Of 5,621,096 blood donations in 47 blood centers from April 2003 through March 2004, a total of 114,583 (2.0%) were disqualified because of elevated ALT levels of higher than 61 IU per L. Of these, 1389 donors (men vs. women, 5.5 vs. 1; age, 32 ± 11 years [mean ± SD]) showed elevated ALT level of higher than 200 IU per L. A total of 1062 donors with an ALT level of 61 to 199 IU per L were randomly collected from three blood centers as described.

The results are summarized in Table 2 and Fig. 2. Of 1389 donor samples with elevated ALT levels higher than 200 IU per L, 15 (1.1%) were HEV RNA–positive. Although the HEV-positive donor samples were found in any block of Japan, they tended to be more frequent in eastern Japan

TABLE 2. Prevalence of HEV RNA, IgM anti-HEV, and IgG anti-HEV among elevated ALT donors from April 2003 through March 2004 in Japan (total number of donors, 5,621,096)

	ALT levels (61-199 IU/L)						ALT levels (200- IU/L)					
Geographic blocks	Number of donors*	Number RNA-positive (%)		Number IgM-positive (%)		Number IgG-positive (%)	Number of donors†	Number RNA-positive (%)		Number IgM-positive (%)		Number IgG-positive (%)
Hokkaido	364	0	(0.0)	1	(0.3)	21 (5.8)	87	4	(4.6)	3	(3.4)	6 (6.9)
Miyagi	NA	NA	NA	NA	NA	NA	143	3	(2.1)	3	(2.1)	3 (2.1)
Tokyo	NA	NA	NA	NA	NA	NA	335	4	(1.2)	3	(0.9)	19 (5.7)
Aichi	NA	NA	NA	NA	NA	NA	223	1	(0.4)	2	(0.9)	6 (2.7)
Osaka	NA	NA	NA	NA	NA	NA	234	1	(0.4)	1	(0.4)	3 (1.3)
Okayama	345	0	(0.0)	0	(0.0)	7 (2.0)	188	1	(0.5)	1	(0.5)	1 (0.5)
Fukuoka	353	0	(0.0)	0	(0.0)	1 (0.3)	179	1	(0.6)	1	(0.6)	7 (3.9)
Total	1062	0	(0.0)	1	(0.1)	29 (2.7)	1389	15	(1.1)	14	(1.0)	45 (3.2)
(95% CI)				(0.0-0.5)		(1.8-3.9)		(0.6-1.8)		(0.6-1.7)		(2.4-4.3)

* Random sampling of donors with elevated ALT (61-199 IU/L) from three prefectures (Hokkaido, Hiroshima, and Fukuoka).
† All donor samples with elevated ALT levels of higher than 200 IU per L during this period.
CI = confidence interval; NA = not available.

(Hokkaido, Miyagi, and Tokyo; p = 0.015). No HEV RNA–positive sample was detected in 1062 donors with elevated ALT levels of 61 to 199 IU per L. The results indicate that HEV RNA–positive donors with elevated ALT levels higher than 200 IU per L were widely distributed over Japan and the prevalence was the highest in Hokkaido.

Antibodies against HEV in donors with elevated ALT levels in Japan

Of 1389 donor samples with elevated ALT levels higher than 200 IU per L, 14 samples (1.0%) were positive for the presence of IgM antibodies to HEV. Donors with IgM anti-HEV were also frequently found in eastern Japan (p = 0.099) and associated with positive HEV RNA (Table 2). Of 1062 donor samples with elevated ALT levels of 61 to 199 IU per L, only 1 sample was positive for the presence of IgM anti-HEV.

Of 1389 donor samples with elevated ALT levels higher than 200 IU per L, 45 samples (3.2%) were positive for the presence of IgG anti-HEV. Again, donors with IgG anti-HEV were more frequent in eastern Japan (p = 0.003) and not associated with HEV RNA–positive donors (Table 2). The frequency of IgG anti-HEV–positive donors appeared to be age-dependent, that is, from 0 percent of donors in their 10s to 12.5 percent of donors in their 60s (10s-30s vs. 40s-60s; p < 0.0001; Fig. 2). Of 1062 donor samples with elevated ALT levels of 61 to 199 IU per L, 29 samples (2.7%) were positive for the presence of IgG anti-HEV (Table 2). Again, the IgG anti-HEV–positive donors were more frequent in eastern Japan (p < 0.0001) and it appeared to be age-dependent (10s-30s vs. 40s-60s; p = 0.001, data not shown).

Analysis for HEV RNA–positive donors

We verified in detail the HEV RNA–positive samples obtained from two studies. Results of analyses for 8 (ALT ≥ 500 IU/L from Hokkaido) and 15 (ALT ≥ 200 IU/L from Japan) HEV RNA–positive donors are summarized in Table 3. The ensuing investigation revealed that all had no history of recent travel in HEV-endemic areas and remained asymptomatic despite of their elevated ALT levels. The concentration of HEV RNA varied from 1.9 to 7.5 log copies per mL. Of the 23 samples, 3 were seronegative, 2 were IgM anti-HEV–positive, 17 were IgM/IgG anti-HEV–positive, and 1 were IgG anti-HEV–positive samples. Twenty-three HEV RNA–positive samples were segregated into Genotype 3 (n = 19) and Genotype 4 (n = 4). These constituted 21 males and 2 females ages 25 to 62 years. Some of the 23 HEV RNA–positive donors were repeat donors. The results of the tests with samples from their other donations revealed that HEV RNA was detected in the previous donation in Donor 12 (HRC-HE12). The sample was negative for the presence of both IgM and IgG

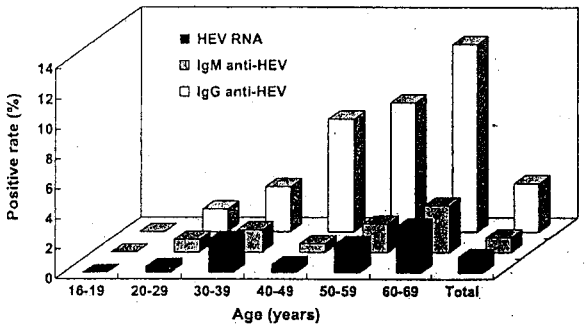

Fig. 2. Age-specific prevalence rates of HEV RNA (■), IgM anti-HEV (▨), and IgG anti-HEV (□) in Japanese donors with elevated ALT levels of 200 IU per L and greater from April 2003 through March 2004. The total number of tested donors was 1389.

anti-HEV with normal ALT. The donated blood (whole blood) was not used for transfusion, because of the low volume of red cells. The plasma was in quarantine. Except for Donor 12, neither HEV RNA nor anti-HEV was detected in other donations.

When the 412-nucleotide ORF2 partial sequences of the HEV-positive 23 isolates were compared with those of reported HEV isolates from pigs or pig livers of Japan, all had a high nucleotide sequence identity of higher than 92.2 percent. More specifically, HRC-HE8 and JRC-HE5 had the highest nucleotide sequence identity, of 99.8 percent, with swJ11-4 and swJ19-1, respectively. Also, JRC-HE1, HRC-HE12, and HRC-HE3 had 99.3, 99.3, and 98.8 percent identities with swJ18-3, swJ13-1, and swJL145, respectively (Table 3).

DISCUSSION

The aim of this study was to investigate the prevalence of HEV among elevated ALT blood donors in Japan. The results of the primary study suggest that HEV was a major causative agent among blood donors with ALT levels higher than 500 IU per L in Hokkaido, since we demonstrated that HEV RNA was detected in 8 of 41 (19.5%) of the high ALT donor samples. Subsequently, a nationwide survey for HEV prevalence in blood donor samples with elevated ALT from all JRC revealed that 1.1 percent (n = 15) of donor samples with elevated ALT levels higher than 200 IU per L were positive for the presence of HEV RNA. No HEV RNA–positive samples were detected in donor samples with elevated ALT levels of 61 to 199 IU per L. Although the 15 HEV RNA–positive donors were widely distributed over Japan, they were frequently found in eastern Japan, especially in Hokkaido (4/15), Miyagi (3/15), and Tokyo (4/15).

It should be noted that in Hokkaido, 8 of the 41 donors with ALT levels of 500 IU per L or greater were positive for the presence of HEV, which is known to be transmitted by transfusion. Thus, as a result of performing HEV tests as the following study among 124 blood donors with ALT levels of 200 to 499 IU per L in Hokkaido, 1 donor (0.8%) was HEV RNA–positive (data not shown). Based on these results, in the subsequent study we expanded the area of investigation to nationwide and studied HEV prevalence in Japanese blood donor samples with elevated ALT including levels of less than 500 IU per L, obtained from all Japan. As for the geographical distribution of hepatitis E in Japan, it was reported that there was a higher prevalence of HEV-infected donors in the eastern part of Japan (Hokkaido, Miyagi, and Tokyo blocks).[15] We cannot clearly explain the reason why blood donors with HEV markers were more frequent in eastern than western Japan. Further studies with a larger number of donors including normal ALT levels will be necessary to draw a definitive conclusion.

Twenty-three HEV RNA–positive samples were divided into Genotype 3 (n = 19) and Genotype 4 (n = 4). Because it is commonly assumed that blood donors are healthy adults, most of those HEV-positive donors appeared to be asymptomatic. Since the isolates of acute hepatitis E patient samples were predominantly Genotype 4 in Japan,[19] the genotypes may play an important role in clinical progression of HEV infection. HEV-positive donors with ALT levels higher than 500 IU per L appeared to be asymptomatic and their ALT elevation was transient (unpublished observation).

In this study, the routes of HEV transmission of infected donors are not clear. The HEV RNA–positive donors had no history of recent travel abroad in areas where HEV is hyperendemic. Yazaki and his colleagues[4] reported that of the 363 packages of raw pig liver sold in grocery stores as food in Hokkaido, 7 (1.9%) packages had detectable HEV RNA. In this study, some isolates from the HEV RNA–positive donor samples showed close sequence homology with the isolates from pigs in Japan, suggesting that HEV transmission may be associated with the consumption of undercooked or inadequately cooked pig meat. Emerson and colleagues[20] reported that some HEV would most likely survive the internal temperatures of rare-cooked meat. When the 412-nucleotide ORF2 partial sequences of the 23 HEV RNA–positive donor isolates were compared with those of reported HEV isolates from pigs or pig livers of Japan, at least 9 isolates (39%) showed close sequence homology (98.5%-99.8%) with the

TABLE 3. Profile of HEV RNA–positive donors

Donor*	Geographic blocks	Date of donation	Age (years)	Sex	ALT (IU/L)	HEV RNA (log copies/mL)	Anti-HEV IgM	Anti-HEV IgG	HEV genotype	Strain	HEV strain with the highest homology among the known swine isolates [Accession No.] (%)†
1	Hokkaido	Dec. 2000	29	M	767	5.6	+	+	4	HRC-HE1	swJL145‡ [AB105902] (98.5)§
2	Hokkaido	Mar. 2001	30	M	506	5.0	+	+	3	HRC-HE2	swJHR1-1 [AB194528] (93.9)
3	Hokkaido	Apr. 2001	40	M	1,470	6.9	+	+	4	HRC-HE3	swJL145‡ [AB105902] (98.8)§
4	Hokkaido	Jul. 2001	47	M	713	5.1	+	+	3	HRC-HE4	swJTT1-1 [AB194526] (93.4)
5	Hokkaido	Oct. 2001	62	M	2,080	6.3	+	+	3	HRC-HE9	swJL234‡ [AB105903] (98.5)§
6	Hokkaido	Oct. 2001	39	M	641	5.1	+	+	3	HRC-HE5	swJL234‡ [AB105903] (98.5)§
7	Hokkaido	Nov. 2001	48	M	740	3.6	+	+	4	HRC-HE6	swJL145‡ [AB105902] (98.5)§
8	Hokkaido	Feb. 2003	39	F	578	6.2	−	+	3	HRC-HE7	swJL234‡ [AB105903] (96.1)
9	Hokkaido	Jul. 2003	35	M	575	5.0	+	+	3	HRC-HE8	swJ11-4‡ [AB094243] (99.8)§
10	Hokkaido	Oct. 2003	38	M	244	3.4	−	−	3	HRC-HE10	swJHK5-1‡ [AB194486] (95.4)
11	Hokkaido	Nov. 2003	52	M	576	3.9	+	+	3	HRC-HE11	swJL234‡ [AB105903] (96.1)
12	Hokkaido	Jan. 2004	38	M	793	5.9	+	+	4	HRC-HE12	swJ13-1‡ [AB094254] (99.3)§
13	Miyagi	Dec. 2003	39	M	470	5.4	+	+	3	JRC-HE4	swJ24-1 [AB094306] (92.5)
14	Miyagi	May 2003	25	M	222	4.2	+	+	3	JRC-HE6	swJL234‡ [AB105903] (95.1)
15	Miyagi	Jan. 2004	34	M	273	3.8	+	+	3	JRC-HE7	swJ2-1‡ [AB094207] (92.7)
16	Tokyo	Mar. 2004	41	F	216	1.9	+	+	3	JRC-HE9	swJAK6-2 [AB194512] (93.7)
17	Tokyo	Jun. 2003	34	M	211	3.1	+	+	3	JRC-HE5	swJ19-1 [AB094279] (99.8)§
18	Tokyo	Nov. 2003	34	M	447	6.8	−	−	3	JRC-HE1	swJ18-3 [AB094277] (99.3)§
19	Tokyo	Feb. 2004	36	M	328	5.2	+	−	3	JRC-HE10	swJC1990 [AB096756] (92.7)
20	Aichi	Feb. 2004	62	M	281	3.9	+	+	3	JRC-HE11	swJS21-1 [AB194524] (92.2)
21	Osaka	Mar. 2004	37	M	793	5.9	−	+	3	JRC-HE8	swJHR1-1 [AB194528] (95.9)
22	Okayama	May 2003	29	M	554	5.3	+	+	3	JRC-HE2	swJIW4-1 [AB194496] (92.7)
23	Fukuoka	Aug. 2003	57	M	398	7.5	+	−	3	JRC-HE3	swJHR1-1 [AB194528] (93.4)

* HEV RNA–positive donors; samples from Donors 1 through 8 were obtained from the primary study (ALT ≥ 500 IU/L from Hokkaido) and Donors 9 through 23 from the secondary study (ALT ≥ 200 IU/L from all Japan).
† Nucleotide sequences were compared to the GenBank databases utilizing the BLAST program available at http://www.ncbi.nlm.nih.gov as of March 2008.
‡ Isolates from Hokkaido.
§ Identities of 412-nucleotide ORF2 sequences over 98.5 percent are indicated.
+ = positive; − = negative; M = male; F = female.

isolates from pigs or liver of pigs.[2,4] It should be noted that among 12 HEV RNA–positive donors from Hokkaido, 10 isolates (83%) showed high nucleotide homology (>95%) of 412-nucleotide sequences with the isolates from pigs or pig livers from Hokkaido. The results are consistent with the possibility that at least some of the HEV RNA–positive donors were infected through the zoonotic food-borne route. Similarly, Feagins and colleagues[21] recently reported that of the 127 packages of commercial pig livers purchased from local grocery stores in the United States, 14 (11.0%) tested positive for the presence of HEV RNA. The widespread distribution of HEV is being clarified in developed countries other than Japan.[22,23]

In this study, IgM anti-HEV–positive as well as HEV RNA–positive samples were also frequently found in eastern Japan. IgM anti-HEV is known as a marker of the early seroconversion period. ALT elevation is observed in the early/middle stage of the infection; that is, ALT elevation follows viremia and accompanies/precedes seroconversion.[24] Most (12/15) of the HEV RNA–positive donor samples were positive for the presence of IgM anti-HEV. Of the 15 IgM anti-HEV–positive samples, 14 showed elevated ALT levels higher than 200 IU per L.

Although there were no HEV RNA–positive samples and only one IgM anti-HEV–positive sample detected in donors with elevated ALT levels of 61 to 199 IU per L, 2.7 percent of them were positive for the presence of IgG anti-HEV, which was comparable to the positive rate (3.2%) of IgG anti-HEV–positive donors with elevated ALT levels higher than 200 IU per L. In contrast to IgM anti-HEV–positive donors, IgG anti-HEV–positive donors were not associated with positive HEV RNA. There are several reports from Japan that IgG anti-HEV–positive samples are not rare (1.9%-14.1%) in blood donors with normal ALT levels who are mostly HEV RNA–negative.[13,25,26] In the present report we observed that the number of IgG anti-HEV–positive samples increased with advancing age in both groups, that is, one with an ALT level higher than 200 IU per L and the other with ALT levels of 61 to 199 IU per L. The IgG anti-HEV appears to be present for a prolonged period after infection. Ijaz and his colleagues[27] reported HEV-infected patients with non–travel-associated disease were more likely to be older and tended to be male in England. They estimated that male sex is a risk factor for acquiring the non–travel-associated disease. Most (14/15) of our HEV RNA–positive donors were also male. Because high-ALT-level donors were male-dominant, it will be necessary to investigate whether HEV RNA–positive donors were also male-dominant in ALT-normal donors. We also observed in this report that the number of IgG anti-HEV–positive donors increased with advancing age. This suggests that high prevalence of IgG anti-HEV in older Japanese persons is the consequence of their increased exposure to HEV with time. Among donors with ALT levels of higher than 200 IU per L, positive rates of IgG anti-HEV and HEV RNA were dissociated in Fukuoka (IgG anti-HEV vs. HEV RNA, 3.9% vs. 0.6%) and Tokyo (5.7% vs. 1.2%), in contrast to those (6.9% vs. 4.6%) in Hokkaido. These observations suggest that HEV infection was once prevalent in Fukuoka and Tokyo, while it is now prevalent in Hokkaido. It will be essential to investigate HEV prevalence among blood donors with normal ALT levels in each area of Japan to clarify these points.

As to the donors with ALT levels higher than 500 IU per L, our preliminary study indicated that, besides HEV, other viruses (hepatitis A virus [HAV], Epstein-Barr virus [EBV], cytomegalovirus [CMV], and human parvovirus B19 [B19] were detectable in some of the 41 donors (data not shown). Among hepatitis-associated viruses, screening tests including nucleic acid testing (NAT) for HCV and HBV have been implemented in Japan. Although ALT testing may not be very effective in the early stage of infection or as a surrogate test for HBV or HCV infection, it may be an effective method for eliminating the other hepatitis viruses in transfusion blood, especially HEV, HAV, EBV, CMV, and B19, which could be eliminated from blood for transfusion by ALT testing. Although the distinct populations collected during different periods, HEV RNA was detected in 8 of 41 (19.5%), 1 of 124 (0.8%), and 0 of 364 (0.0%) among donors with high ALT levels of 500 or greater, 200 to 499, and 61 to 199 IU per L in Hokkaido, respectively. Therefore, it is assumed that HEV RNA–positive rate may be lower among the ALT-normal donors (ALT < 61 IU/L) and that elimination of blood with high ALT levels may be effective in reducing the risk of infection caused by HEV. HEV NAT screening has been implemented as a trial in Hokkaido, the highest HEV-prevalent area in Japan.

Further, elimination of blood donors with ALT levels of 500 IU per L or greater would be an effective tool to reduce the infection risks of not only HEV but also HAV, EBV, CMV, and B19. Although ALT testing appears effective in decreasing the risk for infection of HEV, there are some problems. First, ALT testing resulted in the loss of much of the donor blood, which might have been appropriate for transfusion. Approximately 2 percent of donated blood is disqualified owing to an elevated ALT level of greater than 60 IU per L in Japan. Ninety-eight percent of these donors had an ALT level of less than 200 IU per L. Furthermore, studies in the United States and Europe have confirmed that values of ALT in normal males are considerably higher than those in normal females so that a single cutoff value for ALT rejects a higher proportion of men than women.[28,29] Second, hepatitis viruses including HEV RNA were detected in ALT-normal donors. It has been reported that HEV RNA–positive samples were detected in volunteer donors with ALT levels of 61 IU per L.[13] In the near future, it is necessary to compare the virus-positive rates both in normal and in high-ALT donors and to reevaluate a cutoff value of ALT after considering the balance of the benefits and costs.

Besides ALT testing, IgM anti-HEV screening may be effective to eliminate asymptomatic HEV RNA–positive donors in the middle stage of infection. Most of the HEV-positive samples with high ALT levels were also positive for the presence of IgM anti-HEV, although neither ALT test nor IgM anti-HEV will be effective to eliminate HEV-positive donors in the window period. Since the zoonotic food-borne route appears to be a major cause of HEV infection in Japan,[1-8] it is most important to halt the potential spread of HEV by disseminating information on the risk of eating viscera or vaccination of animals as reservoirs.

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