

Fig. 1. Phylogenetic tree constructed by the neighbor-joining method based on the entire or nearly entire sequences of HEV genome of 77 isolates using an avian HEV (AY535004) as an outgroup. After the isolate name, the name of the country where the strain was isolated and accession numbers are shown in parentheses. The numbers 1, 2, 3, and 4 in bold indicated HEV genotypes. The 3 isolates HRC-HE14C from the causative donor, JST-Kit-Asa04C from the patient, and JTC-Kit-FH04L from the donor's father are indicated in bold. The letters "s," "b," "d," and "m" after parentheses denote HEV isolates from farmed pig, wild boar, wild deer, and mongoose, respectively. A vertical bar represents a cluster consisting of strains indigenous to Hokkaido, Japan. Bootstrap values are indicated for the major nodes as a percentage of the data set obtained from 1000 resamplings.

September 6 was released because it showed normal ALT and passed all the current blood screening tests. Transfusion was carried out 3 days after the blood donation, and the total amount of HEV in the PLT concentrate was estimated to be approximately 5.4 log copies. He was asymptomatic and did not feel tired or febrile in the periods near the two occasions of blood donation.

A minioutbreak of HEV infection in family members of the causative donor

Besides the causative donor, HEV RNA was detected in the blood of his 69-year-old father, who developed acute hepatitis on September 14, 2004, and finally died of fulminant hepatitis on October 14. Retrospective analysis of the father's blood sample taken on September 24, 41 days after the dining, revealed that the HEV strain, JTC-Kit-FH04L, was genotype 4. HEV RNA sequence analysis of the HEV isolates from the causative donor and his father showed only 9-nucleotide differences of 6588 nucleotides, suggesting that the two strains were extremely close but not identical (Fig. 1).

By retroactive interviewing, it was revealed that the causative donor and his 12 relatives gathered to enjoy grilled meats

including pig liver and intestines at a barbecue restaurant on August 14, 2004.²¹ Blood samples from the relatives were tested for HEV markers with informed consent. Seven of the family members who ate grilled pig liver and/or intestines had IgM- and/or IgG-class anti-HEV in the blood samples taken 37 to 92 days after the barbecue party. Retrospectively, in the previous 6 months or more, dining out at that restaurant was the only occasion all the 13 relatives had eaten together.

Clinical course of the patient

It was confirmed that the PLT concentrate (approx. 200 mL) contaminated with HEV was transfused to a 64-year-old Japanese male patient with non-Hodgkin's lymphoma on September 9, 2004, as shown Day 0 in Fig. 2. The patient had been treated with autologous peripheral blood stem cell transplantation accompanied with heavy chemotherapy since July 30, 2004. In the first 3 weeks after the transfusion, liver function tests sustained to be normal. On Day 22, the ALT level increased transiently at 67 IU per L, and HEV was detected in serum. While the ALT level returned to normal, the viral load in serum showed an exponential increase. Levels of aspartate aminotransferase (AST) and ALT took an upward turn on Day 41. There was no evidence for acute infection of hepatitis A virus, HBV, HCV, cytomegalovirus, or Epstein-Barr virus. He was diagnosed as acute hepatitis E. On Day 45, he was referred to the liver unit of Teine Keijinkai Hospital to treat presumed developing acute hepatitis E. Despite antiviral therapy with interferon (IFN) from Day 45, 2',5'-oligoadenylate synthetase in serum never showed apparent increase and no obvious decrement of viral load had obtained (Fig. 2A). Levels of AST and ALT indicated creeping increase to reach highest levels of 903 and 673 IU per L on Day 59, respectively (Fig. 2C). The treatment was switched from IFN to prednisolone (PSL) in expectation of its anti-inflammatory effect. From Day 59 after induction of PSL treatment, AST and ALT showed rapid decrease and improvement of prothrombin time was observed (data not shown). Dosage of PSL was

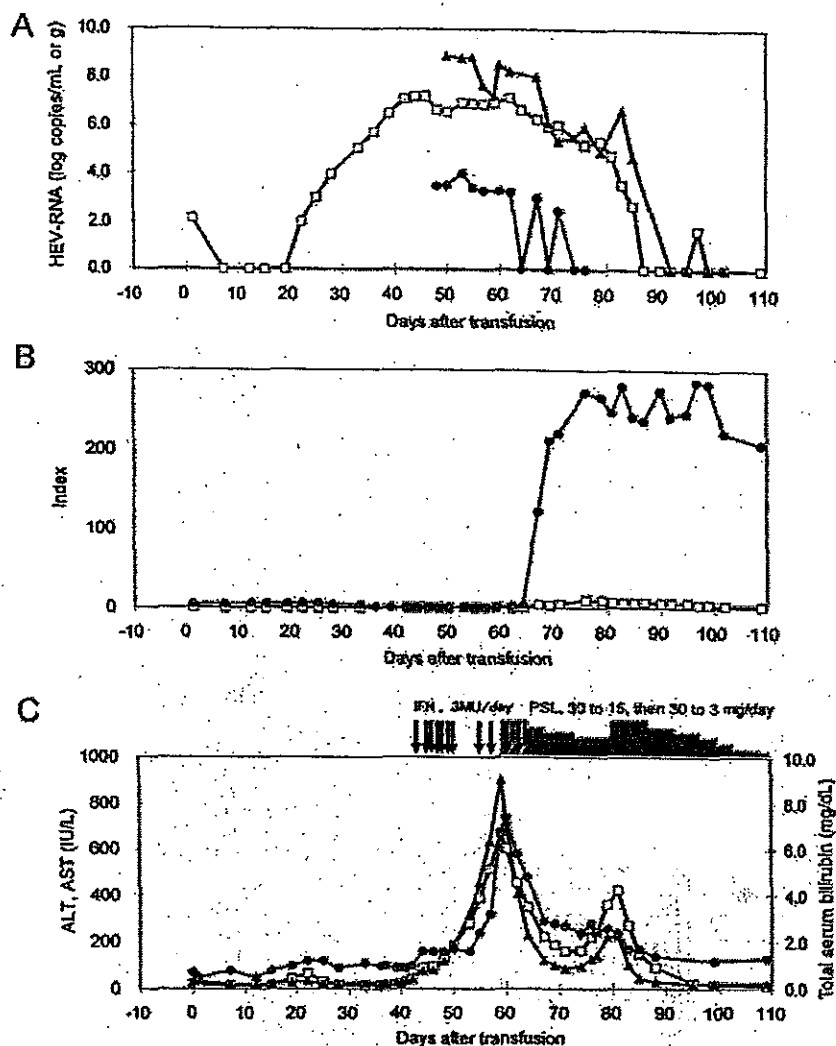


Fig. 2. Clinical course of transfusion-transmitted hepatitis E with kinetics of (A) HEV RNA, (B) serologic, and (C) biochemical markers after transfusion. The patient had transfusion of PLT concentrates contaminated with HEV on Day 0. (A) HEV RNA load was represented as log copies per mL of serum (□) or saliva (●) or per g of feces (▲). There were no data between Day 0 and Day 44 in feces and saliva. (B) Cutoff values of anti-HEV IgM (□) and IgG (●) antibodies are 30 and 13, respectively. (C) Medications were administered with IFN- α from Day 43 through Day 62 and with PSL from Day 59 through Day 112. (□) ALT; (▲) AST; (●) total serum bilirubin.

tapered gradually and discontinued on Day 113. Soon after anti-HEV IgG emerged on Day 67, HEV load in the serum sample had declined rapidly, although anti-HEV IgM in the serum sample remained negative (Figs. 2A and 2B). The levels in aminotransferases were normalized after Day 95 (Fig. 2C). The HEV strain JST-KitAsa04C detected in the patient was genotype 4 and its entire sequence analysis showed only a 1-nucleotide difference of 7255 nucleotides, suggesting the two isolates were identical (Fig. 1).

Serial quantitative changes of HEV load in serum, saliva, and feces of the patient

HEV RNA and anti-HEV were measured for every serum sample before and after the transfusion. In addition, HEV loads were also assessed prospectively for feces and saliva after his transference to the liver unit on Day 45. Any marker for HEV was not detected in serum sampled 37 days before the transfusion. A small amount of HEV RNA was transiently detected in his serum on Day 1, the next day of the transfusion. After the reappearance on Day 22, HEV RNA showed exponential increment with doubling every 29 hours and reached the peak level of 7.2 log copies per mL on Day 44. Beyond its plateau phase lasting 3 weeks, viral load revealed gradual decline over 2 weeks and thereafter decreased promptly. HEV viremia had been finally sustained for 63 days. HEV RNA remained detectable up to Day 97 in serum, Day 71 in saliva, and Day 85 in feces. Peak levels of HEV RNA were found on Day 53 in saliva at 4.0 log copies per mL and on Day 50 in feces at 8.9 log copies per g, respectively. HEV RNA was no longer detectable after Day 99 (Fig. 2A).

DISCUSSION

In Japan, a nonendemic country for hepatitis E, HEV infection is occurring more frequently than previously recognized. The prevalence of anti-HEV IgG in healthy Japanese persons ranged from 1.9 to 14.1 percent, depending on the geographic area,²⁰ and the prevalence of HEV RNA among Japanese blood donors with ALT level of at least 201 IU per L was 2.8 percent.²¹ The risks of transfusion transmission of HEV might be low; however, five molecularly confirmed cases of transfusion-transmitted HEV infection have been reported in nonendemic countries so far.¹²⁻¹⁶ In none of them, HEV infection routes of the causative donors are known. In this report, we have described the first case that the infection route of donor is clarified as zoonotic food-borne. The conclusion is based mainly on two observations.

First, by the epidemiologic study, the donor was determined to be infected in a minioutbreak of HEV infection in the context of food-borne transmission. Six of the 13 relatives who dined out together were positive for the presence of HEV RNA and/or IgM anti-HEV in their serum samples obtained 37 to 92 days after dining at the restaurant (Appendix 1). As for 4 relatives who were positive for the presence of IgM anti-HEV, HEV viremia might have transiently occurred without any symptom and had subsided by the time when blood samples were taken. Since IgM anti-HEV are regarded as the markers of acute HEV infection besides HEV RNA,¹⁰ these facts strongly suggest that family members had recently become infected with HEV probably at the same time and remained asymptomatic. The party at the barbecue restaurant was the only opportunity all the 13 members had eaten together in the

estimated period of HEV infection, 2 to 10 weeks.^{22,23} Although it was difficult to identify the source of infection because no meat was left, they ingested various kinds of pig meats including liver and intestines, according to the replies to the questionnaire from the family members.²⁴ From this retrospective research, it is strongly suspected that the family members shared the motive of infection with HEV by ingestion of pig liver and intestines. In Japan, HEV has been isolated from farmed pigs,^{9,25} wild deer,^{8,26,27} and wild boar^{10,11,26,27} as well as humans and recent studies also indicated that HEV is moderately resistant to heat inactivation.^{28,29} Some reports suggest that a number of hepatitis E cases in Japan may be via a zoonotic food-borne route.^{8-11,25-27,30}

Second, a single transmission route of HEV in this minioutbreak is corroborated by molecularly confirmed facts. From full-length sequence analysis, HEV RNAs detected in the donor and recipient were identical and closely related to that in his father. Among the strains of genotype 4 indigenous to Hokkaido, Japan, these three strains were segregated into a distinct cluster with a bootstrap value of 99 percent in a phylogenetic tree based on the entire or nearly entire sequences of HEV genome. Moreover, when comparing 412-nucleotide sequences (nucleotides 5985-6396 of HRC-HE14C) of ORF2 region, where many sequences of Japanese swine HEV are retrievable in DDBJ/EMBL/GenBank nucleotide sequence databases, high similarity (409/412 nucleotides, 99.3%) was observed between the HEV sequences derived from the causative donor and his father and strain swJL145 (AB105902),³ which was detected in pig liver sold at a drug store in Hokkaido, Japan.

To date, in acute hepatitis E including transfusion transmission cases, dynamic relationships between infection markers for HEV and disease progression throughout the course from HEV transmission to convalescence of disease have not been demonstrated. This is the first case of acute hepatitis E, in which HEV kinetics in serum as well as in feces and saliva were described by using quantitative RT-PCR for HEV RNA from transfusion up to the end of viremia accompanied by disease progression, and the emergence and increase of anti-HEVs. In the current case, HEV viremia had lasted for 9 weeks or more and viral load reached its peak 15 days before the peak of aminotransferase level and died out promptly right after the appearance of anti-HEV IgG on Day 67. The results led us to understand the chronologic relationship between preceding viremia and after emergence and increase of anti-HEV.

Besides serum, the kinetics of HEV load in feces and saliva were concomitantly observed for the first time in hepatitis E in humans. After the transmission, HEV RNA remained detectable until Day 71 in saliva and Day 85 in feces. Among sera, saliva, and feces, every time point at peak viral loads resembled each other, 50 to 60 days after transmission. These facts may indicate that viral loads in

saliva and feces would also reflect viremia state. In addition, the results for saliva suggest that besides fecal-oral route, oral-oral transmission manner can be another route of human-to-human infection of HEV.

Soon after the transference to liver unit in the hospital, IFN- α therapy was started against HEV infection, indicating the exponential increase of viral load in sera. The levels in 2',5'-oligoadenylate synthetase, however, induced by IFN and regarded as a predictive marker for favorable IFN efficacy,³¹ did not show sufficient increase in serum (data not shown), and HEV load monitored concomitantly indicated no actual decrement during treatment. Thereafter, single-nucleotide polymorphisms in markers predicting the therapeutic efficacy of IFN, such as mannose-binding lectin,³² MxA,³³ LMP7,³⁴ and osteopontin,³⁵ were examined, and all of them did not show the phenotype associated with favorable efficacy of IFN (data not shown).

Throughout his clinical course, no distinct positive result for IgM anti-HEV was observed. It is possible that the concentration of IgM anti-HEV was too low to be detected by the method we used. In fact, some of his samples showed equivocal reaction. Furthermore, underlying disease and the preceding treatment including autologous peripheral blood stem cell transplantation and large dosage chemotherapy might have led the patient to an immunocompromised state that responds inadequately for HEV infection. In fact, both serum levels in IgG and IgM had been indicated consistently less than lower limitation of normal ranges in the entire course (data not shown).

We should note that the present case was not revealed if the two practices had not been introduced, which are not widespread outside Japan. They are ALT screening and donor blood sample repository system. As a safety measure, the Japanese Red Cross Blood Center introduced ALT testing for a surrogate marker for non-A, non-B hepatitis virus infection. Because ALT testing contributes little for HCV infection after HCV antibody testing started, ALT screening has been discontinued in the United States and some other countries. Although the cutoff value may need to be reevaluated, the current case suggests that ALT testing may contribute to excluding blood with the presence of HEV. On the other hand, the Japanese Red Cross has established storing repository samples of all donations since 1996. Blood samples are collected from each donation and stored for 10 years at -30°C to investigate for lookback study such as the suspected cases of transfusion-transmitted infection and alloantibodies for TRALI. This system plays a very important role in the hemovigilance system in Japan.^{36,37}

In the present case of transfusion-transmitted acute hepatitis E, the infection route in the blood donor was, for the first time, clarified to be zoonotic food-borne manner. In addition, the entire course including incubation period

and disease progression in acute HEV infection was followed by serologic and virologic markers, and the patient was treated by monitoring them. To our knowledge, this is the first report for acute HEV infection in humans, in which various infection markers were prospectively monitored simultaneously with disease progression, excepting experimental hepatitis E in a volunteer.³⁸

Our data suggest that hepatitis E is likely caused by consumption of contaminated pig meat, and there is a risk of transfusion transmission of HEV in Japan. The most effective preventive measure to reduce the risk of blood-borne transmission is to screen the blood supply for HEV or to implement pathogen inactivation. The epidemiology and the transfusion-related risks for HEV infection have not been fully understood in industrialized countries including Japan. We are undertaking epidemiologic studies of HEV infection in Japanese blood donors and a feasibility study of NAT screening for HEV in Hokkaido, Japan.

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APPENDIX 1

HEV infection markers in the 13 family members who participated in the dinner on August 14, 2004							
Number*	Age (years)	Sex	Days after Aug 14, 2004	ALT (IU/L)	HEV markers		
					RNA (10 ³ /mL)	IgM† (index)	IgG‡ (index)
1	39	Male	23	27	+(3.1)	-(3.4)	-(2.0)
			37	236	+(4.8)	+(60.4)	+(14.2)
			49	70	+(2.1)	+(269.5)	+(154.7)
			53	44	-	+(257.8)	+(150.5)
			77	20	-	+(174.6)	+(163.0)
2	69	Male	41	1511	+(2.6)	+(187.2)	+(271.4)
3	43	Male	92	34	-	+(174.7)	+(297.7)
4	68	Male	79	15	-	+(51.7)	+(283.3)
5	37	Female	79	13	-	+(110.9)	+(90.3)
6	15	Male	90	17	-	+(63.3)	+(250.6)
7	58	Female	79	25	-	-(4.0)	+(25.9)
8	67	Female	79	15	-	-(1.4)	-(12.9)
9	38	Female	89	12	-	-(6.1)	-(1.1)
10	15	Male	77	19	-	-(0.3)	-(0.5)
11	14	Male	77	19	-	-(7.5)	-(0.3)
12	46	Male	90	15	-	-(2.2)	-(0.4)
13	6	Female	90	15	-	-(26.6)	-(1.1)

* Data shown were originally reported by Kato et al.²⁴ without describing quantitative test results of antibodies and viral RNA and follow-up data of the causative donor.

* Number 1 is the causative donor; Number 2 is the donor's father and died of hepatitis E; others are their relatives.

† Positive ≥ 30 index.

‡ Positive ≥ 13 index.

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研究報告 調査報告書

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一般的名称	①乾燥抗 HBs 人免疫グロブリン ②ポリエチレングリコール処理抗 HBs 人免疫グロブリン		研究報告の 公表状況	Vox Sanguinis 2008; 95 (SUPPL. 1): 282-283	公表国 中国	
販売名 (企業名)	①ヘブスプリン (ベネシス) ②静注用ヘブスプリン-IH (ベネシス)					
研究報告の概要	<p><目的> 中国の4つの都市における血液ドナー中の HEV 陽性率を評価し、HEV 感染を排除するための ALT 測定の価値を評価すること。</p> <p><方法> ルーチンのスクリーニング検査 (HCV 抗体、HIV 1/2 抗体、HBsAg、梅毒および ALT) で陰性と判定されたドナー検体と ALT 値が高いだけの検体を、中国の4つの都市 (北京、ウルムチ、昆明、広州) の4つの血液センターから 2005 年に収集し、-40℃で冷凍した。全部で 6,665 の血液ドナーの検体について、2007 年に HEV IgG 抗体、HEV IgM 抗体、HEV Ag の測定を行った。</p> <p><結果> 検査を実施した 6,665 の血液ドナーのうち、HEV IgG 抗体、HEV IgM 抗体、HEV Ag の各々の陽性率は、24.23% (1,615/6,665)、1.08% (72/6,665)、0.03% (2/6,665) であった。ALT のみが高かった 487 のドナーの HEV IgG 抗体、HEV IgM 抗体、HEV Ag の陽性率 (30.80%、2.05%、0.21%) はすべて、ルーチンスクリーニングで陰性であった 6,178 のドナーの陽性率 (23.71%、1.00%、0.02%) よりも高かった ($P < 0.05$)。2 名の HEV Ag 陽性ドナーのうち、1 名はルーチンのスクリーニングで陰性で、HEV Ag ELISA S/CO の平均値が 3.4、HEV IgG 抗体が陰性、HEV IgM 抗体が陰性であった。他方の 1 名は ALT のみが高く、HEV Ag ELISA S/CO の平均値が 18.0、HEV IgG 抗体が陽性で S/CO の平均値が 10.8、HEV IgM 抗体が陰性であった。</p> <p><結論> HEV は中国における風土病である。中国におけるルーチンのスクリーニングで陰性と判定された血液ドナーの中で、1%が HEV IgM 抗体陽性または HEV Ag 陽性であり、HEV に感染性がある可能性がある。ALT スクリーニングは、中国では HEV 感染血液の排除に一定の役割を有している可能性がある。</p>					<p>使用上の注意記載状況・ その他参考事項等</p> <p>代表として静注用ヘブスプリン-IH の記載を示す。</p> <p>2. 重要な基本的注意</p> <p>(1) 本剤の原材料となる血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体陰性で、かつ ALT (GPT) 値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査 (NAT) を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した高力価の抗 HBs 抗体を含有する血漿を原料として、Cohn の低温エタノール分画で得た画分からポリエチレングリコール 4000 処理、DEAE セファデックス処理等により抗 HBs 人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及びろ過膜処理 (ナノフィルトレーション) を施しているが、投与に際しては、次の点に十分注意すること。</p>
	報告企業の意見				今後の対応	
<p>中国における血液ドナーの約 1%は、抗 HEV IgM 陽性又は HEV 抗原陽性であり、HEV 感染の可能性があるとの報告である。</p> <p>静注用ヘブスプリン-IH については、万一、原料血漿に HEV が混入したとしても、EMC および CPV をモデルウイルスとしたウイルスバリデーション試験成績から、本剤の製造工程において十分に不活化・除去されると考えている。</p> <p>ヘブスプリンについては、EMC および CPV をモデルウイルスとしたウイルスバリデーション試験成績では本剤の製造工程において十分な LRV が得られないため、製造工程における不活化・除去が十分であるとは説明困難である。そのため、ヘブスプリン用の原料血漿については、弊社にて HEV についてのミニプール NAT を試行的に導入した。</p>				<p>本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。</p>		



