Age- and gender-specific distributions of hepatitis B virus (HBV) genotypes in Japanese HBV-positive blood donors

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BACKGROUND: There are an increasing number of reports on the hepatitis B virus (HBV) genotype distribution in acute or chronic HBV-infected patients in Japan; however, reports on the HBV genotype of blood donors are few. To compare the HBV genotypes of hepatitis B surface antigen (HBsAg)-positive blood donors with infected patients, all the HBsAg-positive donors' genotypes were determined.

STUDY DESIGN AND METHODS: Data on Japanese blood donors from October 2006 to September 2007 were obtained from the Japanese Red Cross database. The number of available samples was 1979, and the HBV genotypes were determined in 1887 samples. The six major genotypes of HBV (A-F) were determined by enzyme-linked immunosorbent assay. The presence of the immunoglobulin M (IgM) antibody against the HBV core antigen was determined by enzyme immunoassay among all HBsAg-positive donors.

RESULTS: A significant difference in the HBV genotype distribution between donors and patients was in the C/B genotype ratio. The ratios were low in blood donors (2.0-3.9) and high in patients (5.3-18.2). The genotype B ratio increases from 13.8% in teenage donors to 42.4% in those in their 50s; however; the genotype C ratio decreases from 83.1% in teenage donors to 55.1% in those in their 50s. In both IgM antibody against hepatitis B core antigen and nucleic acid test–positive donors, genotypes A and B were restricted to male donors.

CONCLUSIONS: The age-specific distribution of HBV genotypes in Japanese blood donors was observed in the B/C genotype ratio. The gender-specific distribution of HBV genotype A, which originated from the US or Western countries, was observed in male Japanese donors.

The hepatitis B virus (HBV) genotype is important in epidemiologic studies, analysis of modes of infection, and medical treatment. There are eight HBV genotypes designated as A to H on the basis of greater than 8% nucleotide variation over the entire genome.[1-3] HBV genotypes are distributed in distinct geographical localizations.[1-6] HBV genotype A is detected in America, Northern Europe, and India.[4] Genotypes B and C are prevalent in Asia.[5] Genotype D is detected around the Mediterranean Sea.[4] Genotype E is restricted to Africa,[2] and Genotypes F and H are prevalent in South and Central America.[6] Genotype G has been found in France, Germany, the United States, and Mexico.[3]

The clinical significance of HBV genotype has been reported. The HBV genotype may affect hepatitis B e antigen (HBeAg) seroconversion rates, mutational patterns in the precore and core promoter regions, response to interferon, and the severity of liver disease.[4,5,7,8] Comparisons between Genotypes A and D in Europe and America, and between Genotypes B and C in Asia, have been reported. Genotypes A and B are more sensitive to interferon than Genotypes C and D.[9]

ABBREVIATIONS: HBeAg = hepatitis B e antigen; JRC = Japanese Red Cross; MSM = men who had sex with other men.

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Despite the distinct geographical localization of HBV genotypes, the rate of Genotype A has increased in Japanese blood donors as well as Japanese acute HBV patients.[10] To provide an overview of the present state of HBV infection and the HBV genotype distribution in Japanese blood donors, the HBV genotypes of hepatitis B surface antigen (HBsAg)-positive donors from October 2006 to September 2007 were determined.

MATERIALS AND METHODS

Data of blood donors

Data of Japanese blood donors were obtained from the Japanese Red Cross (JRC) database. The number of total blood donors was 4,974,911, and the number of HBsAg-positive donors was 2043 (0.041%). The number of first-time blood donors was 594,096, and the number of HBsAg-positive first-time blood donors was 1362 (0.23%) from October 2006 to September 2007.

The number of available samples was 1979, and the HBV genotypes could be determined in 1887 samples. The HBV DNA load of the other 92 samples was too low for the determination of the HBV genotypes.

Determination of HBV genotypes and subgenotypes

The six major HBV genotypes (A-F) were determined by enzyme-linked immunosorbent assay (ELISA; HBV Genotype enzyme immunoassay [EIA], Institute of Immunology, Tokyo, Japan). This method involves the use of monoclonal antibodies directed to five epitopes exposed on the pre-S2 region of the HBV genome.[11] When a genotype could not be determined by the ELISA, they were determined by direct sequencing of the surface region using a cycle sequencing kit and a genetic analyzer (BigDye Terminator and ABI PRISM 3100, respectively, PE Applied Biosystems, Foster City, CA). To analyze the sequences, two different computer programs were used (SEQUENCHER MAC, Version 4.1, Hitachi Software Engineering, Tokyo, Japan; or GENETYX MAC, Version 9.0, Software Development Co., Tokyo, Japan). The primers from HBsAg region were used as follows: S1-1 (sense, 5′-TCGTGTTACAGGCGGGGTTT-3′[nts]192-211), S1-2 (antisense, 5′-CGAACCACTGAACA AATGGC-3′[nts]689-5-704), S2-1 (nested sense, 5′-CAAG GTATGTTGCCCGTTTG-3′[nts]455-474), and S2-2 (nested antisense, 5′-GGCACTAGTAAACTGAGCCA-3′[nts]668-687).

The subgenotypes Aa (Asian type) and Ae (European type) were determined on the basis of the α region of 1556 bases (nt 2333-3221 and 1-667) and the subgenotypes Ba (Asian type) and Bj (Japanese type) were determined on the basis of the precore region of nucleotide 1838.[12,13] The primers from α region were as follows. For first polymerase chain reaction (PCR), HB104 (sense, 5′-AGACC ACCAAATGCCCCTATC-3′[nts]2297-2317) and S1-2 (antisense) were used. For nested PCR, HB106 (nested sense, 5′-CCCCTATCYTATCMACACTTCCG-3′[nts]2310-2332) and S2-2 (nested antisense) were used. The primers from precore region were as follows. For first PCR, PC1-1 (sense, 5′-CATAAGAGGACTCTTGGACT-3′[nts]1653-1672) and PC1-2 (antisense, 5′-AAAGAATTCAGAAGGCAAA AAAGA-3′[nts]1949-1972) were used. For nested PCR, PC2-1 (nested sense, 5′-AATGTCAACGACCGACCTTG-3′[nts]1679-1698) and PC2-2 (nested antisense, 5′-TCC ACAGAAGCTCCGAATTC-3′[nts]1922-1941) were used.

Serologic screening and nucleic acid amplification technology

The Japanese screening system was described previously.[14] Briefly, samples were screened for HBsAg by reverse passive hemagglutination and confirmed by EIA (AxSYM, Abbott Laboratories, Abbott Park, IL) and for HBV core antibody (anti-HBc) by hemagglutination inhibition.[14] The sensitivity of reverse passive hemagglutination for HBsAg was approximately 2 ng/mL. The presence of the immunoglobulin M antibody against the HBV core antigen (IgM-HBcAb) was determined by EIA (Abbott Laboratories) among all the HBsAg-positive donors from October 2006 to September 2007.

The nucleic acid amplification technology (NAT) system has been described elsewhere.[15] The present pool size of JRC is 20. The 95% HBV DNA detection limit of the AMPLINAT MPX test system was found to be 30 (22-60) copies/mL based on a plasma standard quantified in the Amplicor Monitor assay (Roche, Indianapolis, IN)[16] and was found to be 15 IU/mL (60 copies/mL) according to validation studies with the WHO standard by JRC. Serologically positive and alanine aminotransferase (ALT)-elevated (>60 IU/mL) samples were excluded from NAT screening.

Statistical analysis

The proportional distributions of genotypes were compared using Fisher's exact test, chi-square test with Yates' correction, and F-test. A p value of less than 0.05 was considered significant.

RESULTS

The number of total blood donors and that of first-time blood donors of every age group from October 2006 to September 2007 are shown in Fig. 1. The median age of total donors was 37 years and that of first-time donors was 24 years. The male/female ratio of total donors was 1.89 (1.29 for those 20-29 years old and 2.13 for those 30-39 years old) and that of first-time donors was 1.39. The rate

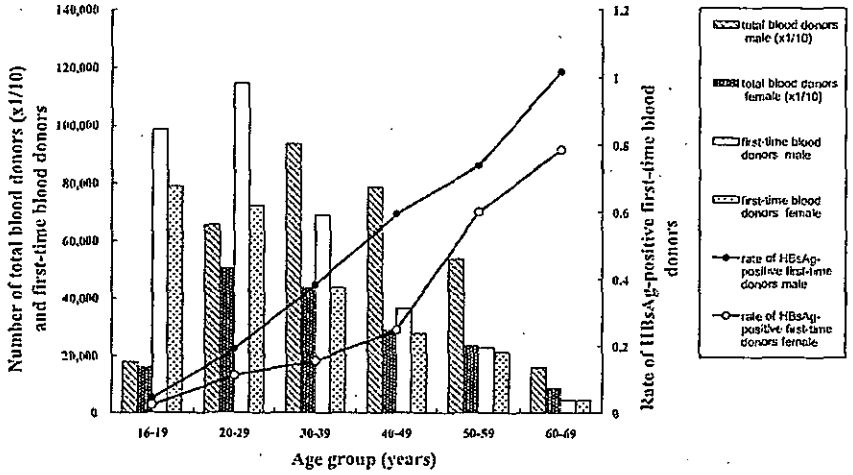

Fig. 1. Number of total and first-time blood donors and rate of HBsAg-positive first-time blood donors. Left axis shows male and female total and first-time blood donors. The number of total blood donors was multiplied by 1/10. Therefore, the number of first-time blood donors was approximately 10% of total blood donors. The median age of total blood donors was 37 years and that of first-time blood donors was 24 years. The right axis shows the rate of HBsAg-positive male and female first-time blood donors. The rate of HBsAg-positive first-time blood donors was found to increase with age. Blood donors aged 16 to 19 years were born after 1986, the year a selective vaccination program to prevent mother-to-infant infection by HBV was started. Blood donors aged 60 to 69 years were born at around World War II.

TABLE 1. Distribution of HBV genotypes in Japanese blood donors and patients

				Genotype (%)			
			Number	A	B	C	D-F and mix
Blood donors	Total HBsAg positive		1887	5.6	30.8	62.6	1.0
	First-time		1349	5.0	31.0	62.3	1.7
	Repeat		538	6.9	30.3	62.3	0.5
	IgM-HBcAb positive		61*	21.7	15.0	63.3	0.0
	NAT-positive†		68	19.1	16.2	63.2	1.5
Patients‡	Chronic	Kobayashi et al.[16]	1077	1.9	9.4	87.7	1.0
		Orito et al.[19]	720	1.7	12.2	84.7	1.4
		Takeda et al.[20]	80	0.0	6.3	93.7	0.0
		Hayashi et al.[21]	123	3.3	15.4	81.3	0.0
	Acute	Takeda et al.[20]	98	18.4	4.1	74.5	3.0
		Hayashi et al.[21]	123	21.1	8.1	67.5	3.3
		Sugauchi et al.[22]	485	19.0	12.0	68.0	1.0

* The genotype of one sample could not be determined.
† The number of NAT-positive donors was not included in the total HBsAg-positive donors. Twenty-two HBcAb-positive cases were excluded.
‡ The representation of patients was modified as a percentage.

of HBV-positive first-time blood donors increased with increasing age (Fig. 1). The rate of HBV-positive first-time blood donors in Japan decreased yearly from 1.07% in 1995 and 0.22% in 2007 (data not shown).

The HBV genotype distribution in HBsAg-positive blood donors from October 2006 to September 2007 is shown in Table 1. The number of first-time donors was 1349 and that of repeat donors was 538. Among repeat donors, HBsAg seroconverted donors were approximately 10%, because JRC accepted the donors' right to refuse to receive the notification of human immunodeficiency virus (HIV), hepatitis C virus (HCV), and HBV infections. Therefore, some HBsAg-positive donors donate repeatedly. All the HBsAg-positive samples were tested for IgM-HBcAb. Sixty-one samples were IgM-HBcAb positive ($1.2 \leq s/n$). Thirty-three of the 61 samples were from first-time donors and 28 samples were from repeat donors. In addition to the 1887 HBsAg-positive donors, 90 HBV NAT-positive (HBsAg-negative and HBV DNA-positive) donors were detected. Twenty-two HBV NAT-positive donors were considered not to be in the serologic window period and were excluded because they were HBcAb and HBV DNA positive but IgM-HBcAb and HBsAg negative. If high-sensitive tests were implemented, some of NAT-positive donors including these 22 donors became HBsAg positive.

Comparison of the genotypes of donors and patients

To compare the genotypes of donors and patients, five references[16-22] are cited in Table 1. The HBV Genotype A distribution in total HBsAg-positive donors (5.6%) was similar to that of chronic patients (0.0%-3.3%) and that of acute patients (18.4%-21.1%) was similar to that of IgM-HBcAb- (21.7%) or NAT-positive (19.1%) donors. A significant difference in HBV genotype distribution between donors and patients was in the C/B genotype ratio. The ratios were low in blood donors (2.0-3.9) and high in patients (5.3-18.2; $p < 0.01$). However, there was no significant difference in the C/B genotype ratio between chronic patients (5.3-14.9) and acute patients (5.7-18.2).

Age-specific distribution of genotypes

The distribution of genotypes among the same age group is shown in Fig. 2. The following calculation was conducted from the data in Table 2. The distribution of Genotype B increases from 13.8% (9/65) in 16- to 19-year-old donors to 42.4% (189/446) in 50- to 59-year-old donors ($p < 0.01$); however, the Genotype C ratio decreases from 83.1% (54/65) in 16- to 19-year-old donors to 54.9% (245/446) in 50- to 59-year-old donors ($p < 0.05$). The genotype distribution among age groups is shown in Table 2. Genotype A was found in approximately 90% (23.6% + 37.7% + 28.3%) of 20- to 49-year-old donors. On the other hand, Genotype C was found in every age group, whereas Genotype B was most prevalent in those 50 to 59 years old (32.5%).

Gender-specific distribution of genotypes and subgenotype

The male/female ratio of total donors was 1.89 (3,253,849/1,721,062), that of first-time donors was 1.39 (345,986/248,110; $p < 0.01$), that of Subgenotype Ae was 13.8 (69/5; $p < 0.01$), that of Subgenotype Aa was 4.2 (25/6), that of Subgenotype Ba was 2.45 (98/40), that of Bj was 2.49 (276/111; $p < 0.05$), and that of Genotype C was 2.58 (851/330; $p < 0.01$; Table 3). The significance was compared with the male/female ratio of total donors using the chi-square test. Subgenotype Ae is male-specific.

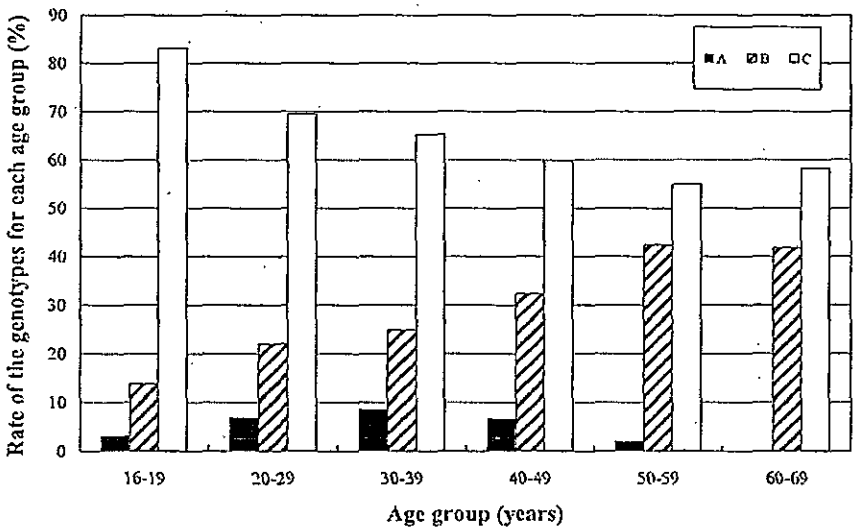

Fig. 2. Ratio of HBV Genotypes A, B, and C within each age group. The numbers of HBsAg-positive donors were 65 (16-19 years), 364 (20-29 years), 469 (30-39 years), 433 (40-49 years), 446 (50-59 years), and 110 (60-69 years). The ratio of Genotype C decreased from 83.1% (54/65) for those aged 16 to 19 years to 54.9% (245/446) for those aged 50 to 59 years; however, that of Genotype B increased from 13.8% (9/65) for those aged 16 to 19 years to 42.4% (189/446) for those aged 50 to 59 years.

TABLE 2. Age-specific distribution of each HBV genotype

Genotypes	Total number (%)	Age (years) 16-19	20-29	30-39	40-49	50-59	60-69
A	106 (100)	2 (1.9)	25 (23.6)	40 (37.7)	30 (28.3)	9 (8.5)	0 (0)
B	581 (100)	9 (1.5)	80 (13.8)	117 (20.1)	140 (24.1)	189 (32.5)	46 (7.9)
C	1181 (100)	54 (4.6)	253 (21.4)	306 (25.9)	259 (21.9)	245 (20.7)	64 (5.4)
D-F and mix	19 (100)	0 (0)	6 (31.6)	6 (31.6)	4 (21.0)	3 (15.8)	0 (0)
Total	1887 (100)	65 (3.4)	364 (19.3)	469 (24.9)	433 (23.0)	446 (23.6)	110 (5.8)

TABLE 3. Age- and gender- specific distributions of HBV genotype or subgenotype

Gender	Age (years)	Total	HBV genotype and subgenotype						
			Aa	Ae	Ba	Bj	B (a/J)*	C	D-F
Male	16-39	687	19	42	54	85	16	463	8
	40-69	684 + 1†	6	27	44	191	22	388	6
Female	16-39	211	3	3	24	24	3	150	4
	40-69	304	3	2	16	87	15	180	1
Total	16-69	1887	31	74	138	387	56	1181	19

* Subgenotype could not be determined.
† Subgenotype could not be determined in one sample Aa or Ae.

TABLE 4. HBV genotype or subgenotype of IgM-HBcAb–positive and NAT-positive donors

Gender	Age (years)	Total	HBV genotype and subgenotype				
			Ae	Ba	Bj	C	E
Male	16-39	78 + 1*	23	9	3	43	0
	40-69	24 + 1†	3	5	2	14	0
Female	16-39	23	0	0	1	21	1
	40-69	2	0	0	0	2	0
Total	16-69	129	26	14	6	80	1

* Genotype of one sample could not determined.
† Subgenotype could not be determined in one sample Aa or Ae.

The trend toward male-specific infection is clear in HBV-positive donors who were infected recently. They are shown as IgM-HBcAb–positive donors (61 donors) and NAT-positive donors (68 donors) excluding HBcAb-positive (22 donors; Table 4): Both IgM-HBcAb– and NAT-positive donors were predominantly male except for those infected with Genotype C.

Although we could not exclude the possibility of reactivations completely in the case of IgM-HBcAb–positive donors, most reactivation cases would be excluded by the interview with donors whether they had medical history or not. The male/female ratio of those infected with Genotype C aged 16 to 39 years is low (2.05: 43/21), and the male/female ratio of those aged 40 to 69 years is high (7.0:14/2; $p < 0.05$).

DISCUSSION

The rate of HBV-positive donors has declined yearly in Japan. However, recently, the distribution of Genotype A has increased in blood donors and acute HBV patients.[10,17-22] The male/female ratio of those infected with Genotype A is different from the ratio of those infected with other genotypes. Particularly, IgM-HBc– or NAT-positive donors are restricted to males.

It is suggested that Subgenotype Ae might have been brought to Japan from the United States by a young male. This might be supported by the finding that the HBV Genotype A was predominant among HBV-HIV dually infected Japanese men who had sex with other men (MSM).[17] The sequences of Genotype A spread by MSM were highly homologous to those of the strains isolated in the United States. Although it has been reported that there is a tendency for Genotype A to spread among men rather than among women,[4] we could not explain whether this phenomenon might be related to MSM.

In addition to Subgenotype Ae, we have recently found Genotype H in a Japanese HBsAg-negative and NAT-positive blood donor.[23] The sequence of Genotype H, which is prevalent only in the United States and Central America, was highly homologous to those of the strains isolated in Los Angeles.

There was no difference in the HBV genotype distribution between first-time donors and repeat donors as shown in Table 1. The only difference between first-time donors and repeat donors was found when the Genotype A distributions of donors aged 16 to 39 and 40 to 69 years were considered (data not shown). The Genotype A ratio in first-time donors aged 16 to 39 years was 5.90% (40/678), whereas that in repeat donors aged 16 to 39 years was 12.27% (27/220; $p < 0.01$). That ratio in first-time donors aged 40 to 69 years was 4.2% (28/671) and in repeat donors aged 40 to 69 years was 3.5% (11/318; not significant).

The result was quite different from our expectation, because it was expected that the HBV-positive risk of first-time donors would be higher than that of repeat donors as shown in the case of HCV-positive donors.[10] HBV Genotype A–positive young donors might have a clear understanding of their risks and intend to test whether they would be infected with HBV or HIV. This might be suggested when IgM-HBcAb–positive donors were considered; the rates of repeat donors/first-time donors of Genotypes A, B, and C were 7/6, 4/5, and 17/22, respectively (data not shown). However, we must examine the result precisely, because most repeat donors might refuse to receive the notification of HBV infection and donate repeatedly. It might be interesting to compare the length of seroconversion between HBV- and HCV-positive donors. In any case, to reduce the risk of

posttransfusion HBV infection, we should restrain the right of refusing to receive the notification of HIV, HCV, and HBV infections.

The characteristic difference in HBV genotypes distribution between blood donors and patients is in the B/C genotype ratio. In older blood donors, the ratio of Genotype B is markedly higher ($p < 0.01$).

The HBV genotypes show a relationship to clinical severity as well as a distinctive geographical distribution.[4,7,18] Genotype C is associated with the development of cirrhosis and hepatocellular carcinoma as well as a lower response rate to interferon therapy and with a lower rate of seroconversion from HBeAg to anti-HBe and a higher HBV DNA level compared with Genotype B.[5] ALT levels were significantly lower in patients with HBV Genotype B than in those with HBV Genotype A, C, or D.[24] From these lines of evidence, donors infected with HBV Genotype B would not be aware of the infection and would donate. Although donors infected with HBV Genotype C would donate while they are young and asymptomatic, they would eventually be symptomatic and would not donate when they reach old age.

These facts might be similar to those in the United States and Western Europe where HBV Genotypes A and D are prevalent. Although there are conflicting reports concerning the severity of diseases between those infected with Genotypes A and D, it would be interesting to know whether the distribution of Genotype A and D would change depending on age in these countries. Compared with Genotype D, Genotype A is more prevalent in HBeAg-positive than in anti-HBe–positive patients.[24] Although Genotype A may induce more severe hepatocytic lesions than Genotype D,[4] Genotype A is more sensitive to interferon than Genotype D.[24] HBsAg clearance occurred more often in patients with Genotype A than in those with Genotype D.[25]

In Japan, Genotypes C and B are predominant in HBV-positive donors. There is a distinctive geographical distribution in Japan. In the northern part of Japan, the distribution of Genotype B is 44.7% (350/783) and that of Genotype C is 48.8% (382/783); however, in the southern part of Japan, that of Genotype B is 20.9% (231/1104) and that of Genotype C is 72.4% (799/1104) except for Okinawa. In Okinawa, the southernmost part of Japan, that of Genotype B is 74.2% (23/31) and that of Genotype C is 22.6% (7/31) (data not shown).

Although now, the age-, gender-, and geographic-specific distributions of HBV genotypes have been determined, the specific distribution of the genotypes may change in the near future. Because the Japanese government began a nationwide hepatitis B vaccination program in January 1986 for infants born to HBV-positive mothers to prevent perinatal HBV infection,[26,27] the vertical infection from mother to infants would be reduced and the horizontal infection by sexual contact would be increased. Therefore, the geographical distribution of HBV genotypes would change and the distribution of Genotype A would increase in younger males as shown in Tables 1 through 4. To decrease the risk of posttransfusion HBV infection, we should continue to study the epidemiology of HBV genotype distribution.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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研究報告の概要

○E型肝炎ウイルスgenotype 3の高病性株、日本

本邦において、通常は無症候性のE型肝炎ウイルス(HEV)genotype 3により、患者8名に重症肝炎が引き起こされた。毒性の強いHEVの遺伝的特性を理解するために、HEV genotype 3株に感染した患者8名とブタ5匹から得たウイルスの完全またはほぼ完全なヌクレオチド配列を決定した(swJ19)。系統発生解析では、これらの分離株が、他のgenotype 3分離株と異なるグループに分類され、JIOと称される独自のクラスターを形成することが明らかになった。ヒトのJIO関連ウイルスは、他のHEV genotype 3とは異なる18のアミノ酸をコードした。JIOクラスターのヒトHEV株のほぼすべてに共通する置換は、ヘリカーゼ領域(V239A)に位置し、毒性の増加と関係する可能性が考えられた。ブタ5匹由来の分離株にも特徴的なヘリカーゼのV239A置換が起こっており、JIO関連ウイルスの起源が人獣共通であることが疑われる。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

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
本邦において、通常は無症候性のE型肝炎ウイルスgenotype 3により患者8名に重症肝炎が引き起こされた。分離株が他のgenotype 3分離株と異なるグループに分類され、特徴的なアミノ酸置換が起こっていたとの報告である。 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および液状加熱の2つのウイルス除去・不活化工程が含まれているが、最近HEVの耐熱性を示唆する成績が発表され、液状加熱の有効性に疑念を生じている。しかし疫学的に見て、血漿分画製剤で最も長い歴史を持つアルブミンではHEVの侵淫度が遥かに高い過去においても世界的に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。	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。

(5)

RESEARCH

Virulent Strain of Hepatitis E Virus Genotype 3, Japan

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Hepatitis E virus (HEV) genotype 3, which usually causes asymptomatic infection in Japan, induced severe hepatitis in 8 patients. To better understand genetic features of HEV associated with increased virulence, we determined the complete or near-complete nucleotide sequences of HEV from these 8 patients and from 5 swine infected with genotype 3 strain swJ19. Phylogenetic analysis showed that the isolates from the 8 patients and the 5 swine grouped separately from the other genotype 3 isolates to create a unique cluster, designated JIO. The human JIO-related viruses encoded 18 amino acids different from those of the other HEV genotype 3 strains. One substitution common to almost all human HEV strains in the JIO cluster was located in the helicase domain (V239A) and may be associated with increased virulence. A zoonotic origin of JIO-related viruses is suspected because the isolates from the 5 swine also possessed the signature V239A substitution in helicase.

Hepatitis E virus (HEV) infection is relatively common. Anti-HEV antibodies are found in 10%–20% of the general population in Japan and most Asian countries (*1*,*2*); however, only a small fraction of these infections induce overt hepatitis. Although the mechanisms underlying induction of liver damage by HEV have not been well characterized, HEV genotypes seem to have distinct disease-inducing potential. HEV sequences have been classified into 4 genotypes (*3*). Genotype 1 consists of epidemic strains in developing countries of Asia and Africa. Genotype 2 is represented by the prototype sequences from an epidemic in Mexico, which have also recently been detected in Africa. Genotypes 3 and 4 are distributed worldwide and have been implicated in sporadic cases of acute hepatitis E in humans and domestic pigs. HEV genotypes 3 and 4 are found in Japan, but fulminant or severe acute hepatitis develops more frequently in persons infected with genotype 4 (*4*–*6*). The severity of liver disease may therefore be influenced by the HEV genotype with which the patient is infected as well as host factors such as age, gender, and pregnancy status.

In 1997, we identified a strain of HEV from a patient in Japan who had acute hepatitis (designated JIO) that clustered with genotype 3 sequences. From 2004 through 2006, JIO-related viruses were isolated from 7 additional patients who had acute or severe hepatitis. To better understand genetic features of HEV associated with severe hepatitis, we compared the complete or near-complete sequence of JIO isolates from these 8 patients with other well-characterized genotype 3 and 4 isolates. To determine whether these human genotype 3 sequences were zoonotic in origin, we sequenced full-length viral genomes from 5 swine infected with the swJ19 strain of HEV. These 5 animals were part of a larger outbreak of HEV infection that occurred in swine at a single farm in southern Japan during 2000–2002. The GenBank/EMBL/DDBJ accession numbers for nucleotide sequences of HEV isolates are AB291951–7/AB291960 (for the human isolates) and AB443623-7 (for the swine isolates).

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Methods

We enrolled 8 patients who were infected with HEV genotype 3 and had clinical features of hepatitis (Table 1). A zoonotic source of HEV infection was identified for 3 of these patients: pig liver for patient 4, pig meat for patient 6, and wild boar meat for patient 7. Prothrombin time, a surrogate marker of hepatic insufficiency, averaged 63.9% (± 29.1%) of the reference range among the 8 HEV genotype 3–infected patients. Hepatitis was particularly severe in patients 3, 5, 7, and 8; at the peak of disease, prothrombin times for these patients ranged from 27% to 46% of the reference range. These sporadic HEV cases were not clustered geographically; they were distributed across several regions of Japan, including southern (Okinawa) and northern (Saitama) prefectures (Figure 1). Informed consent was obtained from all patients after the nature and purpose of the study was explained to them.

To assess possible zoonotic origins of these human infections, we sequenced HEV strain swJ19 isolates from 5 of 11 swine with previously documented infections (*7*). These animals had been raised commercially at a farm in the southern part of Miyazaki Prefecture where HEV infections were detected during 2000–2002. All animals received humane care, and the study was approved by the institutional review committee of Toshiba General Hospital, Tokyo, Japan.

To determine whether infections could be linked to a common genotype 3 virus, we compared the genetic structure and sequence homology of 8 human and 5 swine HEV strains. The entire or near-complete nucleotide sequences of the 8 JIO strain isolates from the human patients and the swJ19 strain isolates from the 5 swine were determined by a method reported previously (*8,9*), with some modifications. In brief, nucleic acids were extracted from serum with the QIAamp MinElute Virus Spin Kit (QIAGEN GmbH, Hilden, Germany). HEV RNA genomes were reverse transcribed, and cDNA was amplified by PCR with primers specific for 23 overlapping regions of the HEV genome. Reverse transcription and first-round PCR were conducted by using the SuperScript III One-Step RT-PCR System (Invitrogen Corporation, Carlsbad, CA, USA); second-round PCR was conducted with the Platinum Taq DNA polymerase (Invitrogen). The 5′- and 3′-terminal sequences were amplified by using the SMART RACE cDNA Amplification Kit (Clontech Laboratories Inc., Mountain View, CA, USA) and Oligo (dt)20 Primer (Invitrogen), respectively. The sequences enriched in G-C were amplified with the TaKaRa LA Taq in GC Buffer (TaKaRa Shuzo Co. Ltd., Shiga, Japan). The sequences not amplifiable by the above PCR methods were subjected to PCR with primers deduced from adjacent 5′ and 3′ sequences. The final products were sequenced in the 377 DNA Sequencer with use of the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Genetic analyses of HEV sequences were conducted by the unweighted pair-grouping method with arithmetic means by using computer software GENTYX-MAC Version 13 (Genetyx Corporation, Tokyo, Japan).

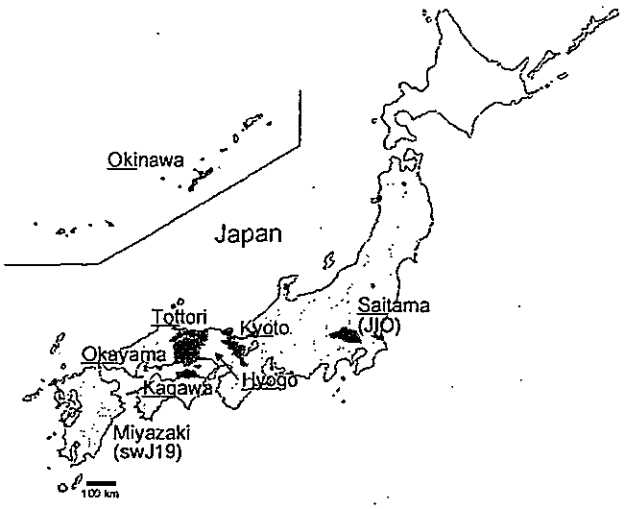

Figure 1. Map of Japan showing prefectures where human cases of hepatitis E virus have been found. Underlining indicates part of prefecture name included in isolate name; yellow indicates cases in swine; red indicates cases in humans.

Table 1. Profiles of 8 patients infected with hepatitis E virus, JIO strain, Japan*

Patient no.	Age, y/sex	Residence	Month of disease onset	Diagnosis	Nadir PT, %	Presumed route of transmission	Isolate name
1	50/M	Saitama	1997 Apr	Acute hepatitis	100	Unknown	JIO-Sai97L
2	76/M	Tottori	2004 Jan	Acute hepatitis	92	Unknown	JYM-Tot04L
3	63/M	Okinawa	2004 May	Acute hepatitis	46	Unknown	JYU-Oki04L
4	71/F	Okayama	2004 Dec	Acute hepatitis	75	Pig liver	JSS-Oka04L
5	65/M	Tottori	2005 Jun	Acute severe hepatitis	34	Unknown	JIY-Tot05L
6	78/M	Okinawa	2005 Jul	Acute hepatitis	92	Pig meat	JSO-Oki05L
7	63/M	Kagawa	2006 Mar	Acute hepatitis	45†	Wild boar meat	JTK-Kag06C
8	79/M	Kyoto	2006 Sep	Fulminant hepatitis	27	Unknown	JSW-Kyo-FH06L

*PT, prothrombin time.
†Only 1 determination was made.

Results

The prototypical isolate, JIO-Sai97L, had a genome length of 7,215 nt that contained a 5′ untranslated region (UTR), 3 open reading frames (ORFs), a 3′ UTR, and a poly-A tail. Lengths of HEV genomes from 6 other patients (JYM-Tot04L, JYU-Oki04L, JSS-Oka04L, JIY-Tot05L, JSO-Oki05L and JSW-Kyo-FH05L) were identical to that of JIO-Sai97L. An exception was the HEV isolate JTK-Kag06C from patient 7, which was slightly longer (7,236 nt). The 5 HEV isolates from swine (swJ19-1, swJ19-2, swJ19-5, swJ19-7, and swJ19-8) had genomes of 7,210 nt. The 3 ORFs of all swine and human HEV genomes had identical protein coding capacity. HEV isolates from all human patients had 97.9%–98.6% sequence homology with the prototypical JIO-Sai97L strain from patient 1. The 5 swine swJ19 isolates had 98.3%–99.9% sequence homology when compared with each other and 98.0%–99.8% homology when compared with the JIO strain from human patients.

Comparison of nucleotide sequences of the 13 human and swine HEV isolates in this study with those of published HEV genotype 3 sequences showed that the 13 complete and near-complete sequences described in this study closely matched those of 2 well-characterized genotype 3 viruses: JRA1 (89.4%–89.7% nucleotide identity) and swJ570 (88.9%–89.0% nucleotide identity). The 13 human and swine genotype 3 isolates displayed weak homology with other HEV genotypes. The B1 isolate of genotype 1 (GenBank accession no. M73218) was only 74.1%–74.7% similar to these genotype 3 viruses, the M1 isolate of genotype 2 (accession no. M74506) was only 73.6%–74.0% similar, and the T1 isolate of genotype 4 (accession no. AJ272108) was only 75.6%–76.0% similar.

Using the 13 complete or near-complete genomic sequences of HEV genotype 3 isolates described in this study (Figure 2), we constructed a phylogenetic tree. HEV sequences from the 8 patients (JTK-Kag06C, JYU-Oki04L, JSS-Oka04L, JIO-Sai97L, JSO-Oki05L, JSW-Kyo-FH06L, JIY-Tot05L, JYM-Tot04L) clustered on a branch separate from the other genotype 3 sequences, forming a distinct grouping related to the prototypical JIO strain. The swJ19 HEV sequences from the 5 swine (swJ19-1, swJ19-2, swJ19-7, swJ19-5, and swJ19-8) clustered closely with the JIO-related viruses from the human patients, indicating that the human and swine HEV isolates were highly similar (Figure 2, panel A). Another 18 swine isolates, from farms other than the 1 involved in the swJ19 outbreak, were phylogenetically distinct from those of the outbreak farms (Figure 2, panel B).

Another genotype 3 cluster was formed by 6 isolates from Hyogo Prefecture in western Japan (Figure 2, panel A). In this cluster were 5 HEV isolates from persons in whom hepatitis developed after they ate uncooked deer meat (*10*) and from serum from a local boar and a deer (*11*). Unlike the JIO-related viruses, which were broadly distributed from the most southern to northern Japanese prefectures, HEV strains responsible for the infections in Hyogo Prefecture were not commonly found in other parts

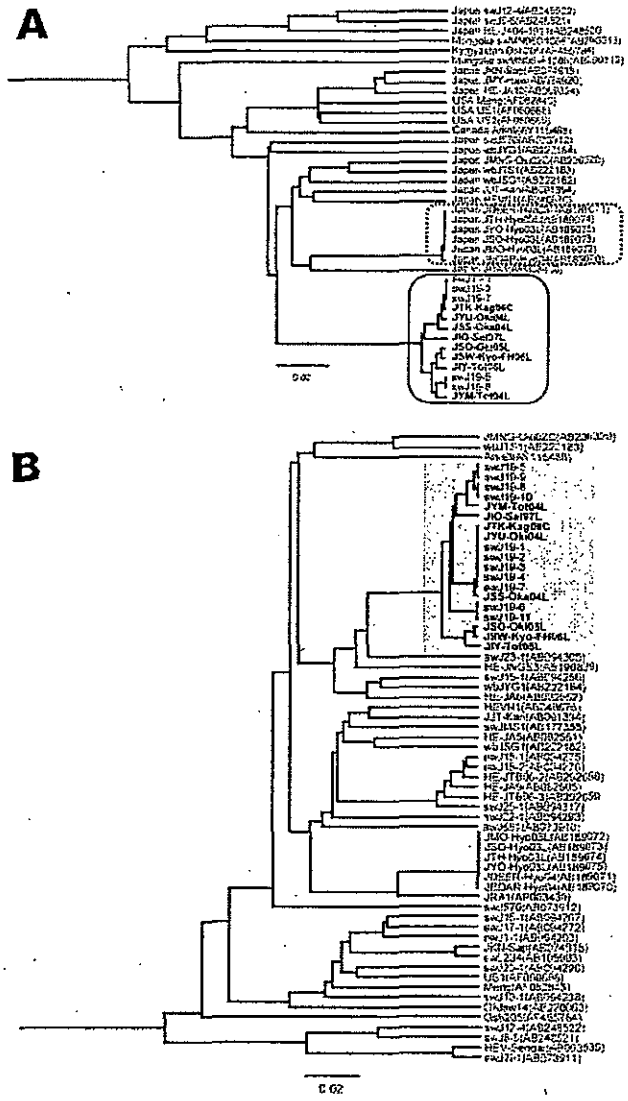

Figure 2. A) Phylogenetic tree (unweighted pair-grouping method with arithmetic means) constructed on the complete or near-complete nucleotide sequences of hepatitis E virus (HEV) genotype 3 isolates. Clustering of nucleotide sequences of 8 human patients infected with JIO strain of HEV and 5 swine infected with swJ19 strain of HEV is boxed by a solid line. Another clustering of local genotype 3 isolates from Hyogo Prefecture, Japan, is boxed by a dotted line. B) Phylogenetic tree (unweighted pair-grouping method with arithmetic means) constructed on a partial sequence of 412 nt in open reading frame (ORF) 2 (nt 5994-6405 of the US2 genome) of HEV genotype 3. Partial nucleotide sequences of 8 human JIO and 11 swine HEV swJI9 isolates (accession nos. AB094279–AB094289) are shown (shading). Analyses of full sequences of 5 of these 11 swine isolates were performed (swJ19-1, swJ19-2, swJ19-5, swJ19-7, and swJ19-8). Scale bars indicate nucleotide substitutions per site; boldface indicates isolates from humans.

of the country. Broad distribution of the JIO-related viruses seems to be unique in HEV epidemiology. In 2 (25%) of these 8 patients, pig liver or meat had been implicated in HEV infection.

Comparison of the 13 JIO-related viruses (Figure 2, panel A) with the other genotype 3 strains also showed 18 aa differences: 12 in ORF1, 3 in ORF2, and 3 in ORF3 (Table 2). Three mutations in the JIO strain were characteristic of genotype 4 viruses, which are typically more pathogenic than other HEV genotypes. ORF1 differences were found at amino acids 605 (serine to proline, S605P), 978 (isoleucine to valine, I978V), and 1213 (valine to alanine, V1213A). The V1213A substitution is potentially most relevant because it was not found in the prototypical isolate from patient 1 (JIO), who had mild clinical disease when infected in 1997, but was present in highly related isolates from the other 7 patients who had more severe hepatitis during 2004–2006. V1213A in ORF1 corresponds to V239A of the helicase domain, and its surrounding sequences were well conserved in HEV isolates of genotypes 3 and 4 (online Appendix Figure, available from www.cdc.gov/EID/content/15/5/704-appF.htm). Because V239A is common in genotype 4 isolates, we analyzed genomes of the genotype 3 JIO-related viruses for evidence of intergenotypic recombination. Comparison of 28 genotype 4 sequences with those of the JIO-related isolates showed no obvious signs of recombination (data not shown), which suggests that the V293A substitution arose independently in this genetically unique cluster of genotype 3 viruses. Notably, all 5 isolates recovered from swine on the Miyazaki Prefecture farm during the outbreak of 2000–2002 possessed the V239A substitution.

Discussion

Circumstantial evidence indicates that HEV genotype influences the severity of liver disease. Remarkably, HEV seroprevalence studies in Egypt found no clinical illness in any person, including pregnant women, although most (67.7%–84.3%) had been exposed to HEV genotype 3 (*13,14*). In contrast, results of a survey of 254 patients with HEV infection in Japan showed hepatitis associated with genotype 4 to be more severe than that associated with genotype 3 (*4*). Our results showed a clustering of 8 HEV isolates of JIO strain, genotype 3, recovered from patients with clinical hepatitis.

Despite the high disease-inducing activity of the HEV JIO strain, the 8 patients infected with this strain were distributed widely over Japan. This distribution is at odds with a local cluster of genotype 3 infections restricted to persons with hepatitis and to wild animals living in Hyogo Prefecture, Japan (Figure 2, panel A) (*11*). Wide regional distribution has also been reported for some HEV genotype 4 isolates (*15*). Why JIO strains caused more severe hepatitis than might be expected for a genotype 3 virus is

Table 2. Amino acid residues in 8 human and 5 swine hepatitis E virus isolates compared with those in the other genotype 3 isolates*

Amino acid position†	Conserved in genotype 3	Human no.								Swine no.					Conserved in genotype 4
		1	2	3	4	5	6	7	8	1	2	3	4	5	
ORF1															
154	A	A	T	A	A	T	T	A	T	A	A	T	A	T	T
547	R	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	R
598	R	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	K
605	S	P	P	P	P	P	P	P	P	P	P	P	P	P	P
721	A	T	T	T	T	T	T	T	T	T	T	T	T	T	A
807	A	S	S	S	S	S	S	S	S	S	S	S	S	S	A
978	I	V	V	V	V	V	V	V	V	V	V	V	V	V	V
979	S	K	K	K	K	K	K	K	K	K	K	K	K	K	E
1135	I	T	T	T	T	T	T	T	T	T	T	T	T	T	V
1213‡	V	V	A	A	A	A	A	A	A	A	A	A	A	A	A
1246	Q	H	H	H	H	H	H	H	H	H	H	H	H	H	D
1469	C	S	S	S	S	S	S	S	S	S	S	S	S	S	C
ORF2															
98	P	S	S	P	P	S	S	P	P	P	P	S	P	S	A
113	V/I	T	T	T	T	T	T	T	T	T	T	T	T	T	V
660	S	S	S	S	F	F	F	S	F	S	S	S	S	S	Y
ORF3															
91	S	N	N	N	N	N	N	N	N	N	N	N	N	N	S
97	A	A	V	V	V	V	V	V	V	V	V	V	V	V	A
98	P	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	P

*Eighteen amino acids of 8 human isolates (JIO strain) and 5 swine isolates (swJ19 strain) not shared by other genotype 3 isolates. The 3 at positions 605, 978, and 1213 (boldface) were the same as the corresponding residues in genotype 4 isolates.
†Corresponds to the position in hepatitis E virus (HEV)-US2 (GenBank/EMBL/DDBJ accession no. AF060669) (*12*).
‡V1213A in the open reading frame (ORF) 1 polyprotein corresponds to V239A in the HEV-US2 genotype 3 isolate helicase domain within ORF1 (online Appendix Figure, available from www.cdc.gov/EID/content/15/5/704-appF.htm).

not clear, but the reason may depend on the magnitude of virus replication. Alternatively, recombination between divergent HEV strains (*16*) may have played a role. This possibility prompted us to look for any recombination of JIO strains with genotype 4 strains that cause severe hepatitis in Japan. However, we found no evidence of recombination between the JIO strain of genotype 3 HEV with which the 8 persons were infected and 28 isolates of genotype 4 retrieved from the public and our own databases. The 18 aa substitutions were unique to the 8 human JIO and 5 swine sw19 isolates and not present in other genotype 3 viruses. Three differences in ORF1 (S605P, I978V, and V1213A) were common in wild type genotype 4 but not in genotype 3 isolates (Table 2). Because S605P and I978V are located in an ORF1 region that has high sequence divergence, they are unlikely to be responsible for an enhanced disease-inducing capacity. In contrast, V1213A changes at amino acid 239 of helicase, an enzyme capable of enhancing the efficiency of viral replication (*17*), were detected in 7 of the 8 patients (online Appendix Figure). Indeed, the helicase region of the prototypical JIO-Sai97L isolated in 1997 did not contain this amino acid polymorphism. Remarkably, all 5 swine isolates recovered in Miyazaki Prefecture during 2000–2002 belonged to the JIO strain and possessed V1213A (*hel*V239A). Taken together, the evidence strongly suggests a zoonotic origin for the 8 human HEV infections with JIO-related viruses.

Experimental and circumstantial evidence suggests that *hel*V239A may have enhanced the helicase activity of the genotype 3 JIO strain to levels comparable with those of the more pathogenic genotype 4 viruses. However, the role of *hel*V239A in enhancing helicase activity should be evaluated in vitro in future studies; its role in inducing hepatitis is yet to be confirmed. In addition, the effect of other mutations of JIO strains need to be fully explored before a conclusion can be drawn regarding the hepatitis-inducing capacities of this strain of HEV.

Findings from this study have public health implications. Because farm swine constitute a melting pot for generating various HEV mutants, at least in Japan where virtually all swine become infected with HEV within 4 months of birth, it is conceivable that virulent HEV mutant(s) arise on pig farms. Such occurrence has been described for influenza, for which point mutations are associated with increased virulence (*18,19*); for example, mutant influenza viruses that arose on chicken farms in Hong Kong in 1997 were transmitted to humans and had fatal consequences (*20,21*). In addition, although a vaccine against HEV has recently been developed (*22*), a vaccination strategy for humans and animals has yet to be defined. The results of our study indicate that selective vaccination of farm swine bearing HEV isolates of high virulence, such as those of the JIO strain in Miyazaki Prefecture, should be recommended to decrease the incidence of fulminant or severe acute hepatitis E in Japan and elsewhere in the world.

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Dr Takahashi is principal investigator in the Department of Medical Sciences at Toshiba General Hospital. His research interest is hepatitis viruses, most recently hepatitis E virus.

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No. 6

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2009. 6. 15	該当なし	

一般的名称	人血清アルブミン	研究報告の公表状況		公表国
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) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)		松林圭二, 坂田秀勝, 阿部生馬, 佐藤進一郎, 加藤俊明, 池田久實. 第57回日本輸血・細胞治療学会総会; 2009 May 28-30; 大宮.	日本

研究報告の概要

○北海道内献血者におけるHEV感染の動向—4年間のまとめ—

【背景】北海道はHEV浸淫地区と考えられ、献血者におけるHEV感染の実態を解明するため、2005年から道内献血者のHEV RNAスクリーニング調査(HEV NAT)を実施してきた。

【方法】2005年1月から2008年11月にかけて、北海道内で献血した献血者、総数1,075,793名(男性663,155名、女性412,638名)について、20本プールによるHEV NATを行った。核酸抽出を行い、RT-PCRによりHEV RNAを検査した。また、陽性献血者について追跡調査および遡及調査を行い、喫食歴や自覚症状に関するアンケート調査、HEV抗体測定、HEV RNA定量、生化学検査、分子系統樹解析等を行なった。

【結果】HEV NAT陽性者総数は140名(男性103名、女性37名)で、2005年30名(男性17名、女性13名)、2006年39名(男性27名、女性12名)、2007年31名(男性28名、女性3名)、2008年40名(男性31名、女性9名)であった。またHEV NAT陽性頻度(献血者延べ1万人当りの陽性者数)は、平均1.3人(男性1.6人、女性0.9人)、2005年1.0人(男性1.0人、女性1.1人)、2006年1.4人(男性1.6人、女性1.1人)、2007年1.2人(男性1.7人、女性0.3人)、2008年1.7人(男性2.0人、女性1.0人)であった。献血時のHEV抗体保有率は3割以下で、感染初期の献血が多かった。陽性者のHEV genotypeは3型と4型で、9割以上を3型が占めた。3型はさらに複数のクラスターに分類され、一部はブタ由来HEV株と高い相同性を示した。陽性者の約7割は献血前に動物内臓肉の喫食歴があり、また、陽性者の約半数は、その後ALT値の上昇が見られた。

【結論】北海道内の献血者集団におけるHEV RNA陽性頻度は高く、zoonotic infectionが起きていると考えられる。とくに男性におけるHEV陽性頻度は上昇傾向にあり、HEVは今後も十分な注意を要する肝炎ウイルスの一つである。

報告企業の意見	今後の対応
北海道内の献血者集団におけるHEV RNA陽性頻度は高く、特に男性においては上昇傾向にあり、zoonotic infectionが考えられるとの報告である。 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および液状加熱の2つのウイルス除去・不活化工程が含まれているが、最近HEVの耐熱性を示唆する成績が発表され、液状加熱の有効性に疑念を生じている。しかし疫学的に見て、血漿分画製剤で最も長い歴史を持つアルブミンではHEVの侵淫度が遥かに高い過去においても世界的に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。	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。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

6

O-051 北海道内献血者における HEV 感染の動向—4 年間のまとめ—

北海道赤十字血液センター検査部[1]，日本赤十字社血漿分画センター品質管理部 検査課[2]
松林圭二[1]，坂田秀勝[1]，阿部生馬[2]，佐藤進一郎[1]，加藤俊明[1]，池田久實[1]

【背景】北海道は HEV 浸淫地区と考えられ，献血者における HEV 感染の実態を解明するため，2005 年から道内献血者の HEV RNA スクリーニング調査（HEV NAT）を実施してきた．
【方法】2005 年 1 月から 2008 年 11 月にかけて，北海道内で献血した献血者，総数 1,075,793 名（男性 663,155 名，女性 412,638 名）について，20 本プールによる HEV NAT を行った．Qiagen BioRobot 9604/MDx で核酸抽出を行い，TaqMan RT-PCR 法により HEV RNA を検査した．また，陽性献血者について追跡調査および遡及調査を行い，喫食歴や自覚症状に関するアンケート調査，HEV 抗体測定（HEV Ab IgM，IgG，特殊免疫研究所），HEV-RNA 定量，生化学検査，分子系統樹解析等を行なった．
【結果】HEV NAT 陽性者総数は 140 名（男性 103 名，女性 37 名）で，2005 年 30 名（男性 17 名，女性 13 名），2006 年 39 名（男性 27 名，女性 12 名），2007 年 31 名（男性 28 名，女性 3 名），2008 年 40 名（男性 31 名，女性 9 名）であった．また HEV NAT 陽性頻度（献血者延べ 1 万人当りの陽性者数）は，平均 1.7 人（男性 1.6 人，女性 0.9 人）で，2005 年 1.0 人（男性 1.0 人，女性 1.1 人），2006 年 1.4 人（男性 1.6 人，女性 1.1 人），2007 年 1.2 人（男性 1.7 人，女性 0.3 人），2008 年 1.7 人（男性 2.0 人，女性 1.0 人）であった．献血時の HEV 抗体保有率は 3 割以下で，感染初期の献血が多かった．陽性者の HEV genotype は 3 型と 4 型で，9 割以上を 3 型が占めた．3 型はさらに複数のクラスターに分類され，一部はブタ由来 HEV 株と高い相同性を示した．陽性者の約 7 割は献血前に動物内臓肉の喫食歴があり，また，陽性者の約半数は，その後 ALT 値の上昇が見られた．
【結論】北海道内の献血者集団における HEV RNA 陽性頻度は高く，zoonotic infection が起きていると考えられる．とくに男性における HEV 陽性頻度は上昇傾向にあり、HEV は今後も十分な注意を要する肝炎ウイルスの一つである．

O-052 輸血前後感染症検査の実施状況と検査を契機に見出された C 型肝炎の 1 症例

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【はじめに】当院では 2005 年 3 月より，輸血前後感染症検査を実施している．今回，2008 年 11 月までの検査状況と，検査を契機に見出された C 型肝炎の 1 症例を報告する．
【方法】1）輸血前検査は，初回輸血または前回輸血から 3 ヵ月を経過した患者を対象とし，輸血施行を確認した時点で実施した．2）輸血後検査は，最終輸血後 3 ヶ月を経過した時点で，輸血歴リストを提示し，主治医が必要と判断した患者について実施した．
【結果】1）輸血前検査実施件数は 1270 件（内科系 61.4%，外科系 38.6%），平均年齢は 70.6 歳であった．2）輸血後検査実施件数は 640 件（50.4%），未実施件数は 630 件（49.6%）であり，未実施の内訳は，死亡 468 件（74.3%），ターミナル 26 件（4.1%），連絡不能 87 件（13.8%），他院入院中 36 件（5.7%），その他 13 件（2.1%）であった．3）輸血前検査実施の際，HCV コア抗原のみ陽性となる症例を経験した．
【症例】87 歳女性．1996 年，心臓カテーテル施行．2004 年，乳癌手術．2008 年 7 月，認知症が進行し，食欲不振・脱水にて入院．同年 8 月，胃ろう造設術後，出血性ショックにて RCC6 単位，FFP10 単位の輸血を実施．輸血前検査により，HCV 抗体陰性，HCV コア抗原陽性であることが判明．輸血後，コア抗原量が上昇し，重度の肝機能異常が認められた後，HCV 抗体が陽性化したが，1 週間後には陰性化した．免疫抑制状態・免疫寛容状態などが想定されたが，確定することはできなかった．
【まとめ】今回の症例では，輸血前検査を実施していたことで，輸血による感染ではなく，輸血前からの感染であったことを把握できた．感染症は自覚症状がないこともあり，早期に発見し，必要な治療を開始することが重要である．その点からも輸血前感染症検査は意義があると思われた．輸血後検査実施率が 50% に留まっている現状は，死亡率が高いことに起因し，輸血を施行する患者は高齢者が多く，予後が悪いことが考えられた．