図1. アルブミン製剤中のHAVの熱不活化試験

HAV:KRM238 in 25%albumin

細胞:GL37

感染価の測定:免疫染色法

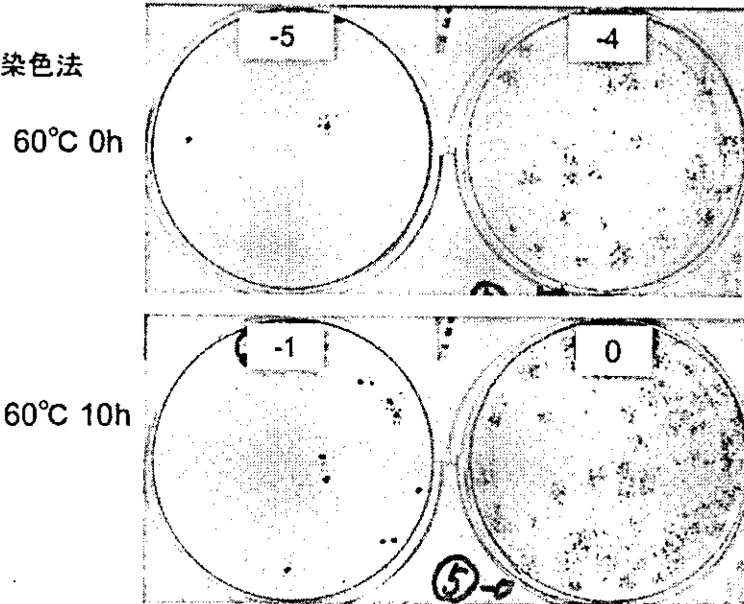
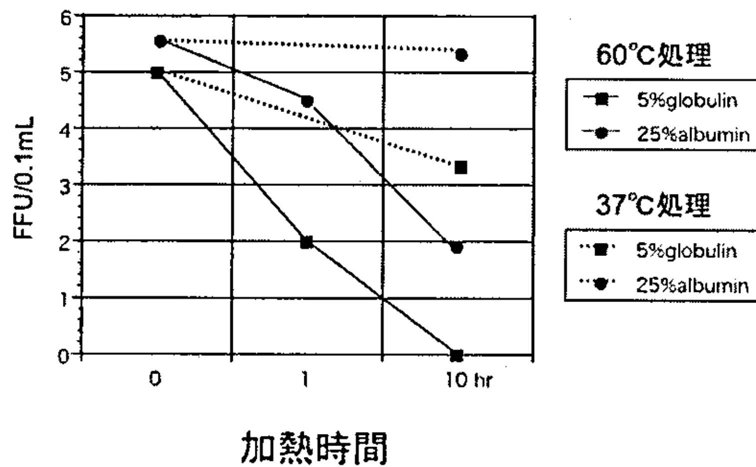


図2. 加熱処理によるHAVの不活化



研究成果の刊行に関する一覧表

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Usuku,S.,Noguchi,Y.,and Takasaki,T.	Newly developed TaqMan assay to detect West Nile Viruses in a wide range of viral strains	Jpn.J. Infect.Dis.	57	129-130	2004
Matsubayashi,K.,Nagaoka,Y.,Sakata,H., et al	Transfusion-transmitted hepatitis E caused by apparently indigenous hepatitis E virus strain in Hokkaido,japan	Transfusion	44	934-940	2004
米山徹夫、清原知子、下池貴志	A型肝炎-我が国の最近の発生動向を中心に	臨床とウイルス	32	149-156	2004

Laboratory and Epidemiology Communications

Newly Developed TaqMan Assay to Detect West Nile Viruses in a Wide Range of Viral Strains

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In the United States during 2003, 9,862 people were reported to be infected with West Nile virus (WNV) to the Centers for Disease Control and Prevention, and 264 of these people died of related encephalitis and meningitis (http://www.cdc.gov/ncidod/dvbid/westnile/surv&control CaseCount03_detailed.htm). There is the possibility of WNV infection even in Japan in the near future.

Genetically, WNV can be divided into two lineages. NY99, prevalent in the United States in a 1999 survey of avian and mosquito samples, belongs to lineage 1 (1). Lanciotti et al. (2) developed a TaqMan Reverse Transcriptase (RT)-PCR assay based on the sequence of the NY99 strain. The 3'NC primers and probe assay detects 0.1 PFU/5 μl of sample of NY99 strain and reacts with other six WNV strains. Two main WNV strains, g2266 (lineage 1) and FCG (lineage 2), are available in local public laboratories in Japan. However, they are genetically distinct from the NY99 strain. The 3'NC primers and probe assay cannot detect the FCG and g2266 strains used as respective control strains in real time polymerase chain reaction (real time PCR) in a Japanese local laboratory. It is thus necessary to develop new primers and probe sets to detect various WNV strains in order to screen avian and mosquito samples.

We developed a new TaqMan RT-PCR assay to detect both

Table 1. Newly developed oligonucleotide primers and probes used in the TaqMan assay

Primer	Genome position[1)]	Sequence (5'-3')
WNV cap-forward	110-127	CAGGAGGGCCCGGYAARA
WNV cap-reverse	179-162	ATCAAGGACAAYMCGCGG
WNV cap-probe	129-154	FAM-CCGGGCTGTCAATATGCTAAAACGCG-TAMRA

[1)]: Genome position according to WNV NY99 complete sequence (GenBank accession number AF196835).

Table 2. Summary of sensitivity and specificity in West Nile viruses using cap primers and probe

Sample		Sensitivity limit[1)]	
		cap primers and probe (PFU/tube)	3'NC primers and probe
WNV	NY99	10~100	0.1
	Eg101	5.4~54	Pos
	Kunjin (OR393)	200	Pos
	FCG	2.8×10^4~2.8×10^5	Neg
	g2266	Pos	Neg
JEV type 1	JEV/sw/Kagawa/24/2002	Neg	
	JEV/sw/Mie/41/2002	Neg	
	JEV/sw/Shizuoka/33/2002	Neg	
JEV type 3	JaGAr01	Neg	
	Nakayama	Neg	
	Beijing	Neg	
Dengue virus	type 1 (Hawaii)	Neg	
Dengue virus	type 2 (New Guinea C)	Neg	
Dengue virus	type 3 (H87)	Neg	
Dengue virus	type 4 (H241)	Neg	

[1)]: Pos shows positivity of TaqMan RT-PCR by qualitative testing using seed virus.

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lineages 1 and 2 WNVs including strains of NY99, Eg101, Kunjin (OR393), g2266, and FCG. To develop the TaqMan primers and probe, we performed multiple alignments using 12 WNV complete sequences (NY99, IS-98 STD, RO97-50, Italy-98, FCG, etc.) submitted to GenBank and used the nucleocapside (cap) coding region, which is highly conserved. Primers and probe are shown in Table 1, and the length of these amplicons was 70 bp. We also confirmed the specificity of this assay by using negative controls of Japanese encephalitis virus and dengue virus, respectively (Table 2).

To estimate the sensitivity of this assay, we performed 10-fold serial dilution samples of NY99, Eg101, Kunjin, and FCG strains using the WNV cap probe and primers. Table 2 shows the sensitivity of our cap primers and probe set. Compared with that of the 3'NC primers and probe, our cap primers and probe has a lower sensitivity to the NY99 strain. Further, our cap primers and probe detected a wider range of strains than did the 3'NC primers and probe, and has sufficient sensitivity for use in WNV-screening of field collected avian and mosquito samples. Our cap primers and probe will be useful for WNV surveillance.

We thank Dr. David W. Smith, Arbovirus Research and Surveillance Group, Department of Microbiology, University of Western Australia for providing the initial stock of Kunjin virus (OR393 strain).

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Transfusion-transmitted hepatitis E caused by apparently indigenous hepatitis E virus strain in Hokkaido, Japan

Keiji Matsubayashi, Yasuhiro Nagaoka, Hidekatsu Sakata, Shinichiro Sato, Kanji Fukai, Toshiaki Kato, Kazuaki Takahashi, Shunji Mishiro, Mitsunobu Imai, Naokazu Takeda, and Hisami Ikeda

BACKGROUND: In industrialized countries, sporadic cases of hepatitis E have been reported in individuals who have never been in an endemic area. Hepatitis E virus (HEV) infection commonly occurs via the fecal-oral route but a potential risk of transfusion transmission route has been suggested.
STUDY DESIGN AND METHODS: A 67-year-old Japanese male patient who had never been abroad received a transfusion of blood from 23 voluntary donors and developed acute hepatitis with unknown etiology after transfusion. His blood samples were tested for viral markers of hepatitis viruses.
RESULTS: HAV, HBV, HCV, CMV, and EBV were ruled out as causative agents in this case. The patient's blood sample in the acute phase contained HEV RNA as well as IgM and IgG anti-HEV. HEV RNA was also detected in one of the FFP units transfused. The donor had no history of traveling abroad and had a normal ALT level at the time of donation. The PCR products from the patient and the donor showed complete identity for two distinct regions of HEV within open reading frame 1.
CONCLUSION: The patient was infected with HEV via transfused blood from a volunteer donor. A potential risk of posttransfusion hepatitis E should be considered even in nonendemic countries.

Hepatitis E virus (HEV) is a major cause of epidemic hepatitis that is usually developed as acute hepatitis in endemic areas in Asia, Africa, Central and South America, and the Middle East.[1] Recent evidence indicates that, in industrialized countries, sporadic acute or fulminant hepatitis E occurs in individuals who have no history of traveling to HEV endemic areas[2-10] and that hepatitis E is a zoonotic disease; pigs and other animals appear to be linked to human infection as reservoirs.[11-18] In Japan, HEV infection has been rarely reported and has been considered as an imported infection from endemic areas for a long time. An epidemiologic study with a sensitive ELISA system, however, revealed that 2 to 14 percent of the healthy population in Japan was seropositive for the presence of IgG anti-HEV.[19] Approximately 13 percent of the non-A, -B, and -C acute hepatitis cases in Japan were caused by HEV.[9] Moreover, after the initial discovery and the characterization of indigenous Japanese strain, JRA1, from a patient with non-A, -B, and -C acute hepatitis, who had never been abroad,[7] several indigenous Japanese HEV strains were recovered from patients with acute or fulminant hepatitis of non-A, -B, and -C etiology.[8-10,20] Although the question of when the first HEV strain made inroad remains unsettled, it is likely that heterogeneous strains of HEV

ABBREVIATIONS: HEV = hepatitis E virus; nt = nucleotide(s).

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have already been circulating and HEV has recently been recognized as an important causative agent of sporadic hepatitis of non-A, non-B, non-C aetiology in Japan. In endemic countries where fecal-oral routes of HEV transmission are common, it is suggested that there is a potential risk of transfusion-transmitted hepatitis E, because HEV viremia is known to appear in the early stage of infection and a significantly higher seroprevalence was observed in transfused hemodialysis patients compared to blood donors.[21] In India, where HEV is endemic, two cases of transfusion-transmitted hepatitis E were reported but they were not confirmed by molecular approaches.[22] Here we report a probable case of transfusion-transmitted hepatitis E in Japan, where HEV had been believed not to be endemic. Sequence analysis showed the isolates of both donor and patient appeared to be identical.

MATERIALS AND METHODS

Characteristics of the patient

In July 2002, a 67-year-old Japanese male patient (S.K.), who had never been abroad, received a transfusion of blood products from 23 voluntary donors during open-heart surgery. Although he was discharged 24 days after the operation, he was hospitalized again for acute hepatitis of unknown etiology with elevated levels of ALT and AST and bilirubinemia. He was followed-up for 134 days after the operation and his blood samples were collected periodically and stored below -20°C until testing.

Transfused blood samples

Twenty-three blood products from the 23 voluntary donors, 14 FFP units, 8 RBC units, and 1 PLT unit, were transfused to the patient during the operation and their stored blood samples were examined virologically including for HEV RNA.

The Japanese Red Cross Blood Centers have implemented a storing system of blood samples for every unit of donated blood since September 1996 to assess adverse effects of transfusion. All of the samples are stored below -20°C until testing.[23]

Blood donor samples with elevated ALT levels

There were 559,545 blood donations in Hokkaido from October 2000 through April 2002. Of these, 15,285 (2.7%) were disqualified because of an elevated ALT of greater than 60 IU per L. Of these, 40 had an ALT level of greater than 500 IU per L and tested negative for the presence of HBV and HCV by NAT. Among them, the samples of 18 donors, 16 men and 2 women, were subjected to RT-PCR testing for the presence of HEV RNA. These samples were stored below -20°C until testing.

RT-PCR for HEV RNA detection

Detection of HEV RNA was performed by nested RT-PCR targeting two distinct regions within ORF1. For 365 nucleotides (nt) within the methyltransferase-coding region, corresponding to nt 105 to 469 of JRA1 strain,[7] RT-PCR was carried out as described previously by Takahashi et al.[8] and a template for direct sequencing was prepared by the second-round PCR with the sense degenerate primer M13/HE5-2 (5′-<u>GTTTTCCCAGTCACGAC</u>GCCYT KGCGAATGCTGTGG-3′) and a mixture of antisense degenerate primers M13/HE5-3 (5′-<u>CAGGAAACAGCTAT GAC</u>TCRAARCAGTARGTGCGGTC-3′) and HE5-6 (5′-<u>CAGGAAACAGCTATGAC</u>TYAAAACAGTAGGTTCGATC-3′). M13 sequences for direct sequencing are underlined.

To amplify sequences within the hypervariable and proline-rich hinge region, corresponding to nt 2127 to 2464 of JRA1 isolate, seminested RT-PCR was performed as described above with the sense primer HE-V1 (5′-ACCTGGGAGTCAGCCAAT-3′) and the antisense primer HE-V2 (5′-AACCAAGTACACTCAGACTCAAAG-3′) for the first-round PCR and internal sense primer HE-V3 (5′-TATACTCGCACCTGGTCGG-3′) and HE-V2 for the second-round PCR.

Sequence analyses of PCR products

The amplification products were sequenced on both strands with a cycle sequencing kit (PRISM BigDye Terminator, Version 2, Applied Biosystems Japan Ltd, Tokyo, Japan) and a genetic analyzer (Prism Model 3100 or 3700, Applied Biosystems Japan Ltd). The PCR product of a 326-nt region was sequenced with M13 primers, M13/RV (5′-CAGGAAACAGCTATGAC-3′) and M13/M4 (5′-GTTTTCCCAGTCACGAC-3′). For the PCR product of the hypervariable region, the same primers for the second-round PCR were used for sequencing. The sequences determined were analyzed with computer software (GENETYX-Win, Version 5.2, Software Development, Tokyo, Japan). The sequences were aligned together with reported HEV strains with a computer program (CLUSTAL W, Version 1.8).[24] A phylogenetic tree based on the 326-nt region within ORF1 was constructed by the neighbor-joining method[25] and the final tree was obtained by a computer program (TreeView, Version 1.6.6).[26] Bootstrap values were determined by resampling 1000 of the data sets.

The nucleotide sequence data reported in this article will appear in DDBJ/EMBL/GenBank nucleotide sequence databases with the accession numbers AB113303 and AB113311 for HRC-SK, AB113304 and AB113312 for HRC-IM, AB113305 for HRC-HE1, AB113306 for HRC-HE2, AB113307 for HRC-HE3, AB113308 for HRC-HE4, AB113309 for HRC-HE5, and AB113310 for HRC-HE6.

ELISA for HEV antibodies

Samples were tested for IgM and IgG antibodies to HEV by ELISA that used virus-like particles as antigen that were produced in baculovirus-infected insect cells.[19]

Assays for viral markers other than HEV

Antibody assays to viruses other than HEV were performed with commercially available kits: anti-HAV IgM (AxSYM HA-M, Version 2.0, Abbott Laboratories, North Chicago, IL), anti-CMV IgM (Celltite SEIKEN Cytomegalo, Denka Seiken Co. Ltd, Tokyo, Japan), anti-EBV IgM and IgG (Diagnostics VCA-Test BML IgG and IgM test, BML, Inc., Tokyo, Japan), anti-HCV (AxSYM HCV, Abbott Laboratories), anti-HBc (AxSYM HBcAb, Abbott Laboratories), and anti-HBs (AxSYM AUSAB, Abbott Laboratories). HBsAg was assayed with AxSYM HBsAg (Abbott Laboratories, North Chicago, IL). HBV DNA and HCV RNA were assayed with NAT probe assays (DNA Probe FR-HBV, REBIOGEN, Inc., Tokyo, Japan; and Amplicor GT HCV Monitor, Roche Diagnostics, Berkeley, CA, respectively), according to the instructions of each company.

RESULTS

Clinical course of the patient

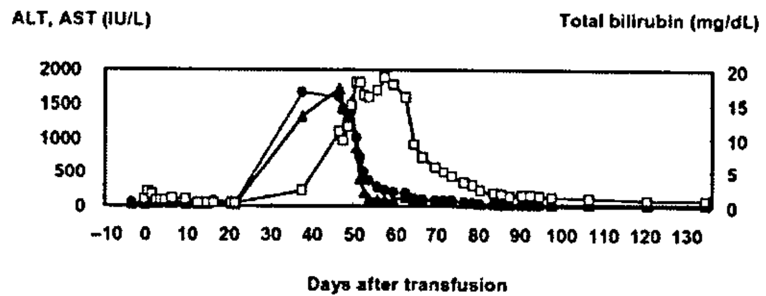
The clinical course of the patient (S.K.) is summarized in Fig. 1. When he was hospitalized again for acute hepatitis, he had an elevated ALT level of 1595 IU per L and an AST level of 1727 IU per L on Day 46 after transfusion; these normalized within 1 month, whereas the total bilirubin level rose to 11.0 mg per dL 2 weeks after the maximum ALT and AST elevation. His clinical state was improved 96 days after the transfusion. The retrospective testing of his blood sample from 4 days before the operation showed that he was negative for the presence of anti-HAV IgM, anti-CMV IgM, anti-EBV IgM, anti-HCV, HCV RNA, HBsAg, and HBV DNA.

In contrast, IgM and IgG class antibodies against HEV were detectable in his plasma sample on Day 37 after transfusion. HEV-RNA was detected from the serum sample of Day 37 and viremia lasted at least until Day 85 post-transfusion. HEV markers were not positive in his blood sample at 4 days before the operation. The IgG anti-HEV continued to be positive for 134 days after transfusion when last tested.

HEV testing of transfused blood

The 23 samples of transfused blood were tested for the presence of HEV RNA to determine whether the HEV was transfusion transmitted. HEV-RNA was detected in one of 23 samples of transfused blood to the patient. The donor I.M. a 24-year-old Japanese woman living in Hokkaido, had a normal ALT level of 10.0 IU per L at the time of donation and IgM- and IgG-class anti-HEV was not detectable (Table 1). FFP from this donor (I.M.) was transfused to the case patient (S.K.). The RBC product from the HEV-positive donation was transfused to another patient, Y.M. A following study revealed that patient Y.M., who had lymphoma showed no sign of hepatitis, clinically, virologically, or serologically of follow-up after transfusion. Neither HEV-RNA nor anti-HEV were detected in his blood 130 days after transfusion.

A blood sample from the HEV-positive donor's (I.M.) previous donation (15 months before the case donation) was available for testing. The sample was negative for the presence of HEV RNA or IgM- and IgG-class anti-HEV and had an ALT level of 8.0 IU per L. Five months after the case donation, the donor had seroconverted with IgM and IgG anti-HEV and HEV RNA was not detectable at that time (Table 1). On interview by telephone, donor I.M. had not been out of Japan during the incubation period and no



Day	-4	37	46	51	66	78	85	93	106	120	134
HEV RNA	-	+	+	+	+	+	+	-	NT	NT	NT
Anti-HEV IgM	-	+	+	+	+	+	+	+	+	+	+
Anti-HEV IgG	-	+	+	+	+	+	+	+	+	+	+

Fig. 1. Clinical course of the patient with hepatitis E and the testing results for HEV. The ALT (●), AST (▲), and total bilirubin levels (□) are also shown.

TABLE 1. Characteristics of the HEV-RNA-positive donor*

	ALT (IU/L)	HEV RNA	Anti-HEV IgM	Anti-HEV IgG
Previous donation (-15 months)	11	-	-	-
Case donation	10	+	-	-
Follow-up exam (+5 months)	8	-	+	+

* + = positive; - = negative.

clinical sign or symptoms of hepatitis during a follow-up of 5 months after the case donation.

HEV sequence study with PCR products from the donor and the patient

The PCR products of donor I.M. and patient S.K. were compared to each other for sequences corresponding to a 326-nt region encoding methyltransferase within the ORF1 of the HEV genome. The sequence (HRC-IM) of the PCR product from the transfused blood that was positive for the presence of HEV RNA showed complete identity with that (HRC-SK) from patient S.K.'s blood at 37 days after transfusion. According to Schlauder and Mushahwar's classification of HEV,[27] these isolates were segregated to genotype IV and were very similar to JKK-Sap and JSY-Sap,[8] which were isolated from hepatitis E patients living in Hokkaido (Fig. 2). JKK-Sap was different by only 1 nt at position nt 261 and JSY-Sap by two nucleotides at the positions nt 261 and nt 330, based on the JKK-Sap sequence, respectively. The amino acid sequences were completely identical for these strains. Furthermore, for the 307-nt proline-rich hinge region of ORF1, the isolates from donor I.M. and patient S.K. showed complete identity.

Detection and analysis of HEV RNA in donors with elevated ALT

Six of the stored samples from 18 donors with elevated ALT levels higher than 500 IU per L, who were all men and aged 29 to 48 year, were positive for the presence of HEV RNA (Table 2). Phylogenetic analysis based on the 326-nt sequence of the ORF1 indicated that isolates of HRC-HE2, HRC-HE4, and HRC-HE5 were segregated to genotype III, and HRC-HE1, HRC-HE3, and HRC-HE6 as well as HRC-IM, to genotype IV (Fig. 2). All four strains of genotype IV were very closely related to each other with 99.4 to 100 percent identity in this region. HRC-HE1 and HRC-HE3 were completely identical with JKK-Sap. The addresses of HEV-positive donors were not concentrated in a particular area but widely distributed over Hokkaido.

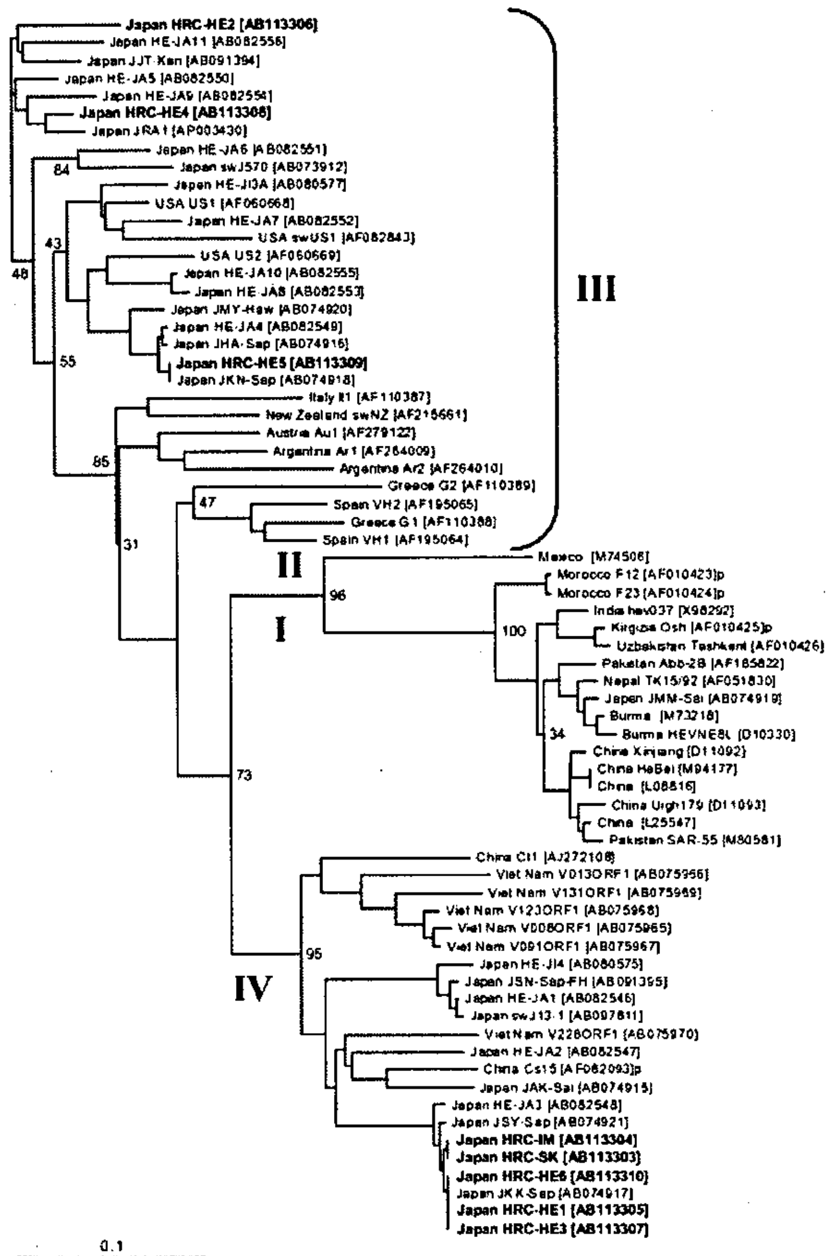


Fig. 2. Phylogenetic tree based on a 326-nt region of ORF1 for HEV strains derived from the case donor HRC-IM and patient HRC-SK and six donors with elevated ALT of greater than 500 IU per L. Accession numbers for the reference sequences are indicated parentheses. The isolated strains in this study are shown in boldface. Bootstrap values are indicated for the major nodes as a percentage of the data obtained from 1000 resamplings.

Although HRC-HE1 and HRC-HE3 showed identical sequences, the two donors lived in different cities, and there was a time lag of 6 months between their donation dates.

Because some of the donors with a high ALT level were repeat donors, samples from different donations could be

TABLE 2. Characteristics of disqualified donors with elevated ALT of greater 500 IU per L*

Donor	Date of donation	Age (years)	Sex	ALT level (IU/L)	Anti-HEV IgM	Anti-HEV IgG	HEV RNA	HEV strain (genotype)
1	October 2000	45	Male	878	–	–	–	
2	August 2000	29	Male	11	–	–	–	
	December 2000	29		767	+	+	+	HRC-HE1 (IV)
	August 2001	30		12	+	+	–	
	February 2002	31		19	+	+	–	
	May 2002	31		16	–	+	–	
3	December 2000	42	Male	558	–	–	–	
4	January 2001	32	Female	670	–	+	–	
5	March 2001	30	Male	506	+	+	+	HRC-HE2 (III)
6	April 2001	35	Male	1008	–	–	–	
7	April 2001	40	Male	1470	+	+	+	HRC-HE3 (IV)
8	June 2001	33	Female	545	–	–	–	
9	June 2001	36	Male	675	–	–	–	
10	July 2000	46	Male	21	–	–	–	
	July 2001	47		713	+	+	+	HRC-HE4 (III)
11	July 2001	31	Female	748	–	–	–	
12	August 2001	36	Male	1458	–	–	–	
13	August 2001	49	Male	647	–	–	–	
14	October 2001	39	Male	641	–	+	+	HRC-HE5 (III)
15	April 2000	47	Male	17	–	–	–	
	November 2001	48		740	+	+	+	HRC-HE6 (IV)
16	November 2001	40	Male	771	–	–	–	
17	December 2001	56	Male	531	–	–	–	
18	April 2002	33	Female	948	–	–	–	

* + = positive; – = negative.

studied. For all HEV-RNA-positive donors except for Donor 14, anti-HEV IgM was detected in the HEV-RNA-positive donations (Table 2). In the three HEV-infected donors, Donors 2, 10, and 15, ALT levels were normal and neither HEV RNA nor anti-HEV was detected in the previous donations. For Donor 2, HEV RNA was negative and ALT not elevated except for the HEV-RNA-positive donation; the HEV-positive donation had both IgG and IgM antibody to HEV and his anti-HEV IgM was still detectable 13 months after the HEV-positive donation.

DISCUSSION

We report the first case of transfusion-transmitted acute hepatitis E fully investigated by molecular approaches. The HEV-positive blood donor was asymptomatic and resident in Japan, where hepatitis E has been considered not endemic, and her donation was made in an early stage of HEV infection. Fecal-oral transmission is the common route of HEV infection in the outbreaks in endemic areas,[1] whereas little is known about the transmission routes for sporadic hepatitis E cases in industrialized countries. Vertical transmission as well as transfusion transmission has been suggested in endemic areas.[28]

In India, where hepatitis E is endemic, Arankalle and Chobe[22] reported two cases of transfusion-transmitted hepatitis E by means of retrospective analyses. Nevertheless, they were not successful in demonstrating the association of blood transfusion with hepatitis E infection by molecular approaches probably because of degradation of HEV RNA in the specimens during storage.

In our study, specimens from both the donors and the patient before and after the transfusion were available in good condition, which made it possible to determine that the blood transfusion was associated with HEV infection with molecular approaches.

The case patient was positive for both anti-HBc and HBsAg and negative for the presence of HBV DNA before and after transfusion, suggesting that he was not in an active HBV carrier state. Testing results for other five viruses regarding to hepatitis except for HEV showed that they were ruled out as a causative agent to this case. Based on the clinical data, he was diagnosed with acute hepatitis E. The case donor had a seroconversion of anti-HEV and appeared to be asymptomatic for HEV infection. The amplification products of two distinct regions of HEV corresponding to the methyltransferase gene and the hypervariable and proline-rich hinge domain of ORF1 from both the patient and the donor were sequenced, showing complete identity. Therefore, it is highly probable that the transfusion was responsible for the current hepatitis E case. In addition, the onset of the hepatitis was closely associated with the timing of the blood transfusion. The onset of the hepatitis was somewhere between 24 and 46 days after transfusion, which corresponded to the incubation period of 32 days in a case of transmission of HEV to a human volunteer.[29] Nevertheless, the possibility cannot be ruled out that the infection

occurred via other route and the sequence identity of the HEV in the donor and the patient was coincidental. RBCs, derived from the HEV-positive donation, did not appear to cause hepatitis E by transfusion to another patient. The patient had no clinical sign of hepatitis during follow-up after transfusion; neither HEV RNA nor anti-HEV were detected on the 130 days after transfusion but any other sample from the patient was not available for testing. The viral load of HEV in the RBC product could be too low to cause infection.

We also found six HEV-RNA-positive samples among donors with an ALT level of greater 500 IU per L in the same area, Hokkaido, and some of them had strains quite similar to the case strain, HRC-IM and HRC-SK.

Phylogenetic analyses of HEV isolates indicated a cluster of genotype IV indigenous to Hokkaido. The cluster includes highly homogeneous strains of genotype IV with 99.4 to 100 percent nucleotide sequence identities: JKK-Sap and JSY-Sap from hepatitis E patients; HRC-HE1, HRC-HE3, and HRC-HE6 from voluntary blood donors with an ALT level of greater than 500 IU per L; and HRC-IM from the case donor. Of three HEV strains of genotype III isolated in this study, HRC-HE5 showed very similar nucleotide sequence to JKN-Sap and JHA-Sap[8] isolated from acute hepatitis E patients. They all were derived from individuals living in Hokkaido. These results suggest that multiple HEV strains of genotype III and IV indigenous to Hokkaido may exist and are circulating there. It is interesting to note that a recently isolated swine HEV strain in Hokkaido showed 99 percent nucleotides homology over the entire genome with a human HEV strain of genotype IV.[16,17] Moreover, a direct evidence of HEV transmission from animal to human via uncooked deer meat was provided in Japan.[18] These support the idea that hepatitis E is a zoonotic disease and swine and deer are as reservoirs for human infection.

By implementation of sensitive HBV and HCV tests including NAT for donor screening, the residual risk of posttransfusion hepatitis B and C has become minimal.[30-32] Regarding other viruses associated with hepatitis such as HAV, HEV, CMV, and EBV, a specific test for each virus is not performed as routine donor screening in Japan. Although it may not be very effective in the early stage of infection or as a surrogate test for HBV or HCV infection,[33] ALT testing may be helpful in preventing posttransfusion hepatitis caused by other viruses associated with hepatitis. Approximately 8000 (2.3%) units of donated blood are disqualified yearly owing to an elevated ALT level of higher than 60 IU per L in Hokkaido. Forty donors showed ALT levels of higher than 500 IU per L in 1.5 years, of which at least six samples without HBV DNA nor HCV RNA were HEV-positive. It should be noted that the 6 donors were disqualified not by donor interviewing but by ALT testing. This suggests the possibility that asymptomatic HEV infection was present among other blood donors. Although the appropriate cutoff value might be reconsidered, ALT screening should not be discontinued because information about HEV infection is still poor and there is no other screening test to eliminate such asymptomatic HEV-positive donors in Japan.

In conclusion, although transfusion-transmitted HEV is probably much too rare to sustain HEV transmission in industrialized countries, where HEV infection is believed to be nonendemic, it should be taken into account that HEV is spread through uncertain routes, and the potential risk of transfusion-transmitted HEV infection should be considered. Further epidemiologic study is required to understand the current transmission routes of HEV infection.

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■ 肝炎

A型肝炎 ―我が国の最近の発生動向を中心に―

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1．はじめに

A型肝炎はピコルナウイルス科のA型肝炎ウイルス（HAV）によって引き起こされる急性疾患で，他のウイルス性肝炎（B型肝炎やC型肝炎）と違って，慢性化することはない．感染を仲立ちするベクターもなく，安全で有効なワクチンが開発され，人類が制圧可能な病気のひとつである．病原ウイルスは直径27nmの正二十面体でヒトエンテロウイルスと同じく腸管感染でヒトからヒトへ伝播する．ウイルスゲノムの塩基配列の相同性がエンテロウイルスのそれとかなり異なることなどから，ヘパトウイルス属として分類上明確に区別されるようになった．ヒトから分離されるHAVは4種，2亜型（IA, IB, II, IIIA, IIIB, VII）の遺伝子型であるが，抗原的には高度に保存されており，単一の血清型しか知られていない．我が国で分離される株の大部分は遺伝子型IAまたはIIIB型に属する（図1）[1]．他に3種の遺伝子型（IV,V,VI）のサル

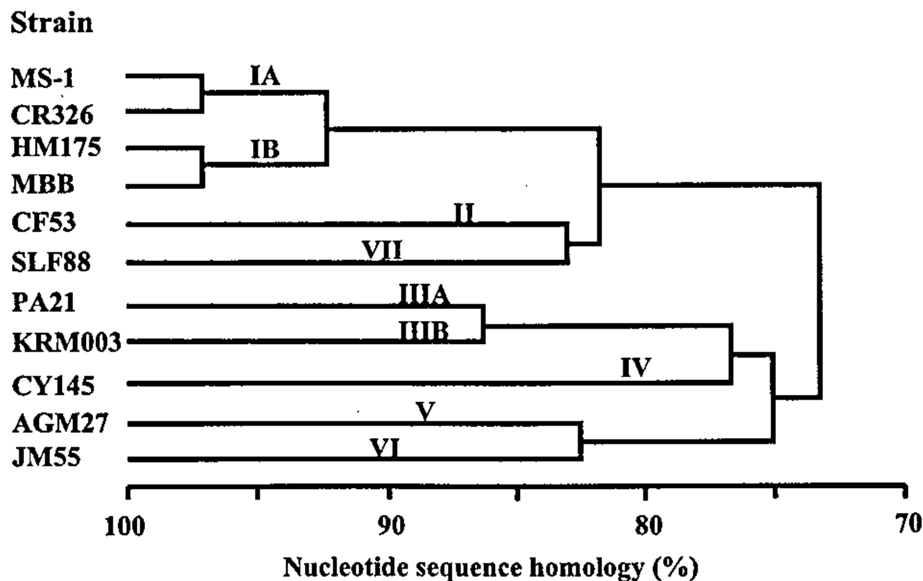

図1　HAV株の分子系統樹　VP1/2A領域，168塩基配列の比較

Hepatitis-A ―Review of hepatitis A virus infection in Japan―

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HAVが分離されているが，伝播はサルに限局されている．ウイルスの遺伝子解析は分子疫学必須の手法として実験室で広汎に使われるようになった．A型肝炎の場合は後述するように汚染食材を介して集団感染をひきおこすことが社会的な問題になっている．個々のウイルスRNAの塩基配列を調べて，感染源が明らかになれば，新たな発生に対処することも可能である．

2．感染の疫学的特徴

HAVは糞便中に排泄され，口から体内に入り感染する．糞口感染するウイルス病の特徴にたがわず，アジア，アフリカなどの途上国のように，人口過密，衛生環境の悪いところの乳幼児に蔓延している．こうした地域では成人する前に殆どすべてのひとがHAVに対する抗体を持つ．子供の頃感染すれば，不顕性の割合も多く，病気の症状も軽く非黄疸性のことが多い．日本や欧米のように衛生環境の整った地域では患者の年齢層もあがり，症状も重くなる．北欧やカナダでは散発症例の大部分は途上国から帰国する旅行者にみられる．人口10万人当たりの罹患率は1998年米国で8.6，オランダで6である．日本では0.33（2002年）[2]であるから，世界でも罹患率の最も低いグループに属する．図2に示すように日本の年齢別抗体保有率のパターンは，年々高年齢側に移動している．1994年の40歳未満の抗体保有率は1％以下であり，40歳以上では年齢に伴い上昇し，65歳以上では90％である[3]．このことは高齢になって急に感染が増加するのではなく，日本でも終戦直後までHAVが蔓延していたが，その後衛生環境が整備されるにつれて，蔓延状態は終息したことを物語っている．1960年以降生まれた人は殆ど抗体を持っていない．従って若年層は無論のこと，中高年層も感受性者が多くなり，国内での小流行の原因となっている．免疫のない人がHAVの常在している国々に旅行し，感染して帰国，発病することがしばしば見受けられる．

3．感染の経過

臨床経過から他のウイルス性肝炎と区別するのは難しい．診断にはウイルス学的あるいは血清学的な検査が必要である．定型的には発熱，筋肉痛，倦怠感，悪心，食欲不振，嘔吐といった前駆症状に続いて，肝障害が起こる．血清トランスアミナーゼ（ALT）などの肝臓由来の酵素やビリルビンの血中濃度が上昇し，黄疸は通常約4週間続く．これらの症状はウイルスによる直接の肝細胞障害ではなく，感染に対する宿主の免疫反応によるもので，一定の臨床経過を経て，ゆっくりではあるが完全治癒にむかう．感染後終生免疫が得られ，慢性のキャリアーも存在しない．感染年齢の高齢化に伴い，稀に劇症化して致死的になることが危惧されている．劇症化A型肝炎の報告は，日本では年に1～2

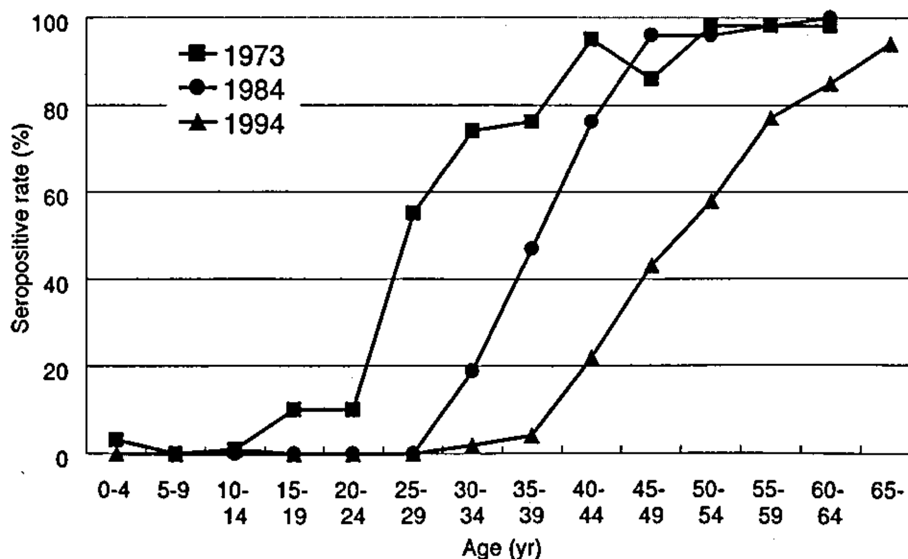

図2　日本人の抗HAV抗体保有状況の推移

例である．米国の統計ではA型肝炎の致死率は0.3％でその70％が50歳以上の高齢者である．妊婦が感染しても特に重い症状を呈することはなく，また胎児に感染して影響がでることも知られていない[4]．

図3に典型的なA型肝炎の経過を示した．A型肝炎の潜伏期は約4週であるが，黄疸の出現する2～3週前の期間から，1週後まで感染性のウイルスは糞便中に検出される．血液中のウイルスは糞便中のそれよりはるかに少ない．最近のPCR法を使った研究では，HAV RNAは血液中でも3ヶ月にわたり検出し得る例が示されている．HAVのウイルス血症は考えられていた以上に長いようである[5]．

発症とともに血清トランスアミナーゼが上昇し，HAV特異的IgM抗体とIgG抗体が血清中にほぼ同じ頃出現する．IgM抗体価は発症1ヶ月後がピークで，徐々に減衰し，3～6ヶ月後には検出限界以下となる．IgG抗体は終生持続する．

4. 診　　断

臨床的には他のウイルス性肝炎と区別しがたいので，疫学的にA型肝炎が疑われても最終診断は血清中のウイルス特異的IgM抗体か，糞便か血清中のHAV遺伝子の検出による．培養細胞によるウイルス分離には長期間を要するので診断目的には適さない．一般的な血清疫学調査などにはIgG抗体を測定する．

5. 日本での発生動向

a) 国内感染例

日本のA型急性肝炎の最近の特徴として罹患年齢の上昇化，春をピークとする季節性がある．年間500例前後の発生があり，国内の感染が主である．

2002年は全体で500例が報告された．感染地域の内訳は，国内感染例422例（85％），海外感染例69例（14％）であった（図4）．国

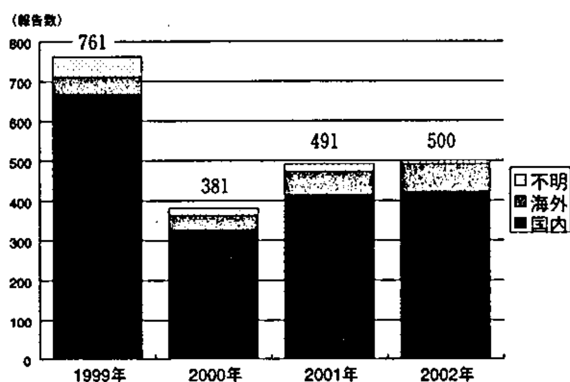

図4　年次別，地域別A型肝炎患者数

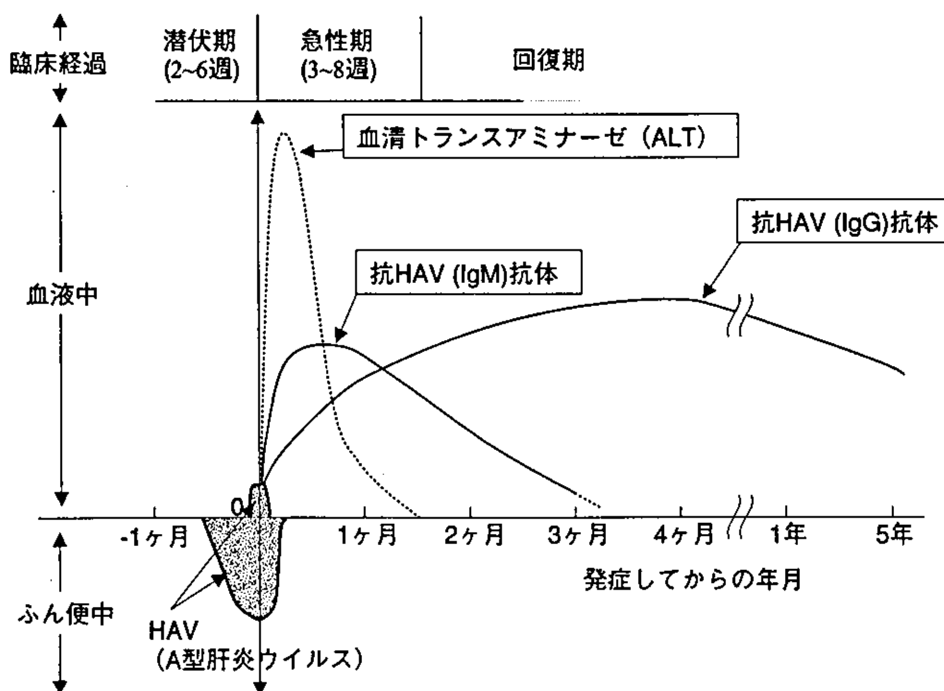

図3　A型肝炎ウイルス感染症の臨床ウイルス学的経過

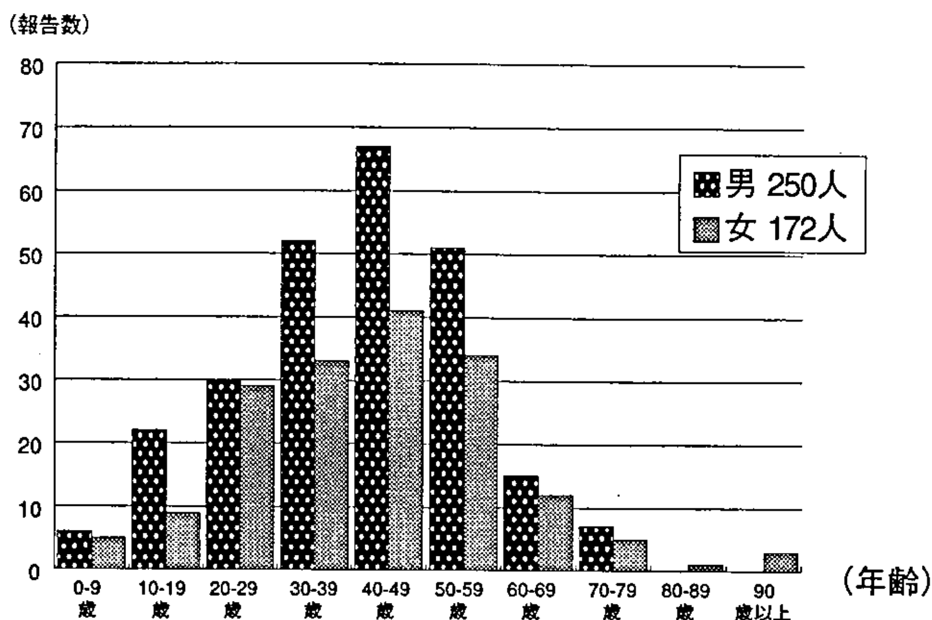

図5 2002年，国内で感染したA型肝炎患者の年齢分布

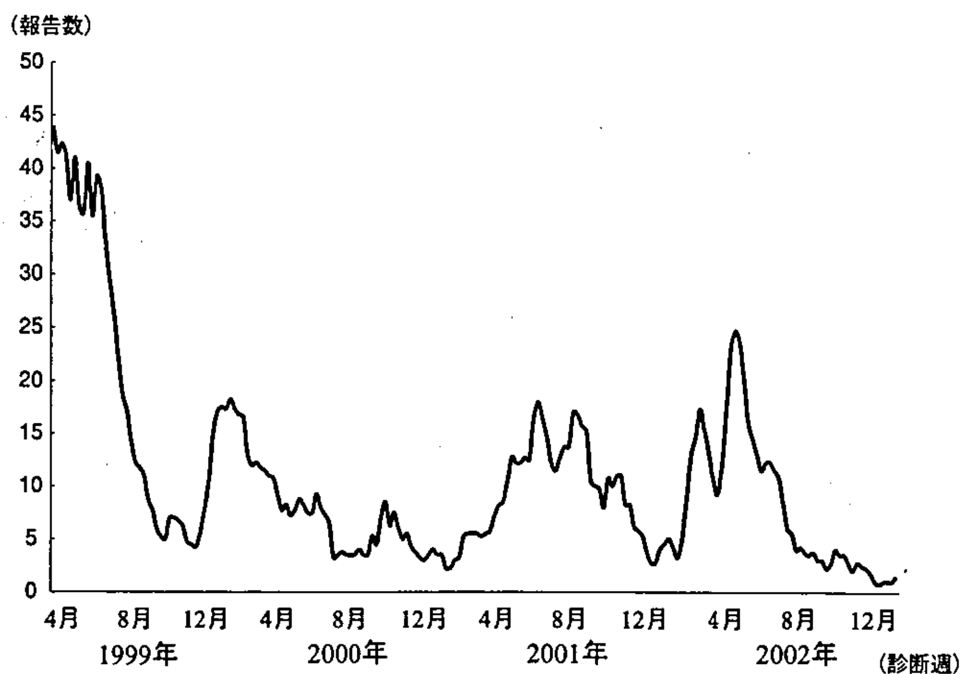

図6 A型肝炎の流行パターン，1999～2002年

内感染者の年齢分布は，40歳代をピークにしてその前後の成人層に多く見られる（図5）．小児の報告は非常に少ない．20例を超えて報告された都道府県は，東京，千葉，神奈川，愛知の4県であった．人口10万人当りの罹患率では山形（1.05）と山口（1.8）の両県が1.0を越えた[2]．報告の多い地域の中でも，集団発生が確認されている地域と集団発生が確認されていない地域があった．1999年からの地域分布では，報告の多い地域は一定せず，毎年異なる．日本では上水道の改善によりHAVに汚染された環境から感染する機会が低くなったため，地域分布が一定していないと考えられる．

春をピークに冬から夏にかけて毎年流行が見られる（図6）．汚染された生牡蠣などの

喫食の影響が考えられている．オランダではひとの移動にともない，夏から秋に流行がみられ，秋に低い日本の流行様式とは異なる．季節性は米国にはみられない．

感染源を調べると，352人（83%）に飲食物が疑われ，牡蠣を含む海産物が原因と思われたものは121人（30%）になる．性行為で感染したと思われる報告例も1例あった（図7）．後述のように東南アジア，中国からの輸入海産物が感染の原因と考えられる報告もある．

家族内や，職場，学校などで周囲に感染が広がったのは120人（30%）で，272人（65%）の症例では周囲に同疾患の罹患者は認められなかった[2]．福祉施設内感染は1998年以降報告されていないが，ひとたび汚染されれば，施設内では感染が広がりやすい状況に変わりはなく，従業員へのワクチン接種の徹底や衛生環境の整備にはより注意を払わなければならない．

b)　海外感染例

海外感染例は東南アジアの旅行者が多いとされている．2002年は中国での感染が疑われた例が多く例年とはやや異なる（図8）．2002年の中国での感染者は，長期出張中あるいは帰国後に発症し，7月に報告された例が多

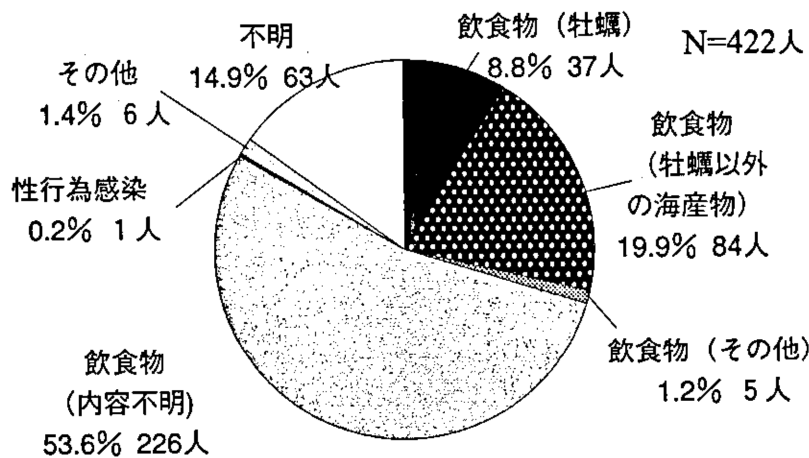

図7　2002年，国内で感染したA型肝炎の感染源，感染経路

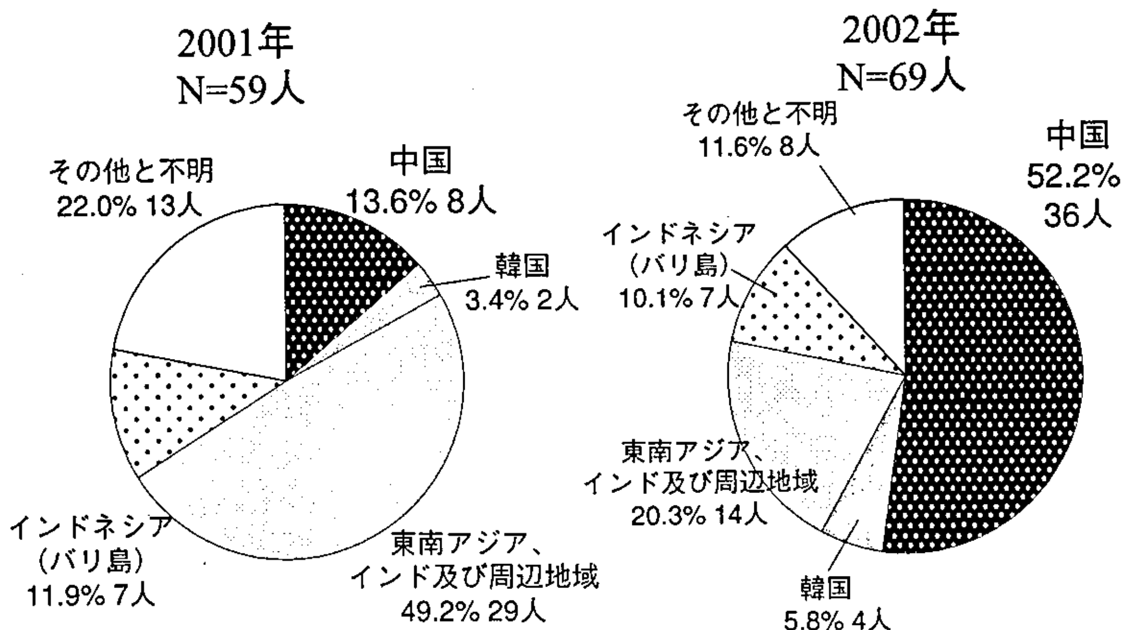

図8　2001～2002年，海外感染者の旅行先

かった．現地では飲食店が感染場所として疑われているが，明確な因果関係は確認されなかった．今後，海外進出している企業でもワクチン接種などのA型肝炎の感染対策が必要であり，同伴する家族にも病気の理解とワクチンの勧奨が重要となるであろう．海外で感染する主な患者層は，女性の患者は20歳代に，男性は30歳に患者数のピークがみられた（図9）．数は少ないが毎年同じような傾向が認められる．海外旅行に出かける年齢構成比と似ていて，国内で感染した患者の年齢分布（図5）と比べると興味深い．

6．最近の国内外の流行例

汚染食材を介した集団発生は社会にあたえる影響も大きく，患者の糞便や血清，汚染が疑われた食材などから得られたHAV RNAの塩基配列を調べて，原因を究明する努力が関係者の間でなされている．A型肝炎の潜伏期は1ヶ月と長く，原因となった食材も保存されていることが稀なので，感染源や感染経路の特定は一般に困難である．今後の予防対策の一助に，感染源や感染経路が調査された最近の国内外の報告例をあげてみた．

(1) 2003年10月～11月，米国ペンシルベニア州にあるレストランAを利用した人の間で集団感染が報告された．調理従事者を含め患者数は555名，3名が死亡している．充分に加熱していない青ネギを含んだ食材が感染源とみられた．メキシコの農場から出荷され，ウイルスの遺伝子配列から同一の感染源と確認された[6]．

(2) 2002年3月～4月，東京都江東区の寿司店で調理従事者を含む喫食者24名がA型肝炎に感染した．調べたHAVの遺伝子型はIA型でかつ同一の塩基配列であった．調理従事者から寿司食材または調理器具を介した単一暴露が主原因とされた[7]．

(3) 2002年1月 浜松市や，2002年4月 江戸川区の飲食店でウチムラサキ（通称，大アサリ）の喫食者にHAVとノロウイルス（NV）の2種のウイルスによる重感染と思われる食中毒が発生した．腹痛下痢，嘔吐等の食中毒症状を呈した後，A型肝炎を発病したものである．中国産のウチムラサキが原因と推定され，IA型のHAVが同定された．A型肝炎の患者数は4～5名であった[7]．

7．その他の感染経路

社会的に大きな問題となるのは血液製剤を介しての医療感染事故である．1990年代からイタリー，ドイツ，ベルギー，米国などで第8因子製剤による感染例が報告されたが，日本では確

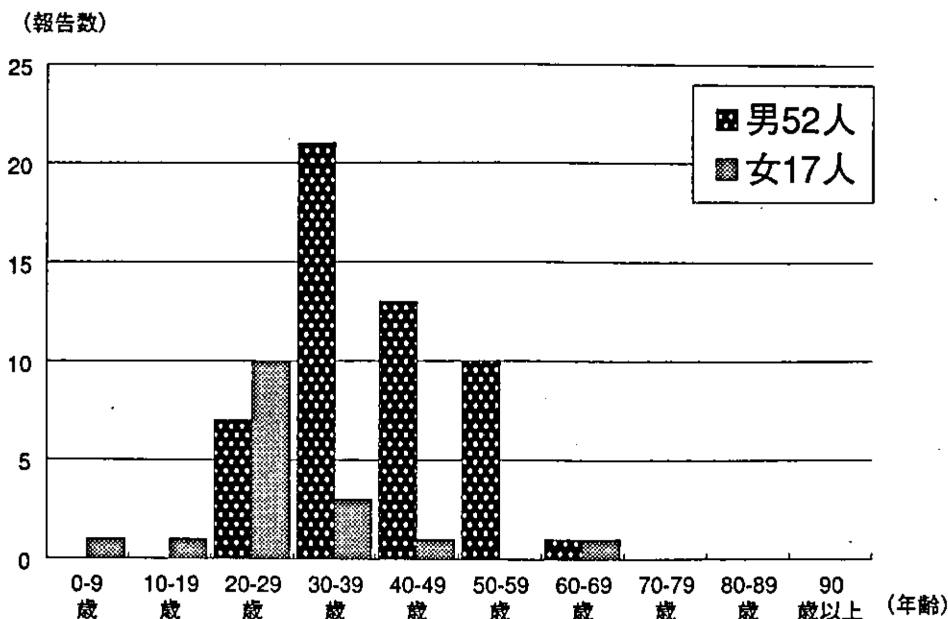

図9 2002年，海外感染者の年齢分布

認されていない[8]．HAVは非経口的に体内に侵入すると通常の経口感染よりはるかに感染効率が良いことがチンパンジーで確かめられている[9]．前述のようにウイルス検査の精度が上り，少数例とはいえ血清中にHAVが長期間存在する可能性が明らかになってきた．輸血，血液製剤，注射針等を介した非経口的感染にも注意を払わねばならない．血液製剤の材料となる血液の一層厳密な検査体制が求められている．

8. おわりに

日本でのA型肝炎は主に散発的な発生である．小規模な集団発生によるものが時々話題になるが，年間発生数は，減ることもなく，横ばい状態が続いている．抗体調査から推定されるように，日本ではHAV感受性者の割合が非常に多くなってきているが，近隣の東南アジア諸国は衛生環境の悪いHAV汚染地域である．東南アジア方面などのHAV汚染国への渡航者，医療関係者，調理従事者，福祉施設従業員等ハイリスク該当者にワクチンを徹底接種することが現時点での有効な予防策である．実際の患者数は報告された数の10倍になるという調査報告[10]もある．質の高いサーベイランスのデータを蓄積することや，血清疫学的，ウイルス学的調査研究がますます重要になってくるであろう．完全治癒するとはいえ，黄疸になれば，その症状は軽いものではなく，長期間の療養を要する．良いワクチンがあることと考えあわせ，B型肝炎ワクチンとともに全国的な規模での予防体制を導入する議論が必要であろう．

謝　辞

図4から図9は感染症発生動向調査IDWR（厚生労働省・国立感染症研究所，感染症情報センター）2003年5月までの暫定集計によるものである．関係者に深謝いたします．

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