miniature pigs were negative for anti-HEV IgG, the sera from 20 (13%) of the 152 domestic pigs were positive for anti-HEV IgG. Anti-HEV IgG was not detected in any of the pigs from farms A and the pigs that had been produced in C2, while anti-HEV IgG was detected in 38 and 30% of the serum samples obtained from pigs from farms B and pigs that had been produced in C1, respectively (Table 1).

The serum samples obtained from the 152 domestic pigs and the 38 miniature pigs were also tested for the presence of HEV RNA by RT-PCR (Table 1). Although HEV RNA was not detected in any of the miniature pigs, HEV RNA was detected in the serum samples from 22 (14%) of the 152 domestic pigs, the prevalence differing by swine farm. The prevalence of HEV RNA ranged from 1% among the pigs from farm A to 38% among those from farm C.

Genetic heterogeneity of swine HEV recovered from viremic pigs

The amplification products of ORF2 (412 nucleotides, primer sequences at both ends excluded) from 22 viremic pigs were sequenced and compared. The nucleotide sequence identity among the 22 swine HEV isolates (swJMS1 to 22) obtained from pigs from three farms [A, B, and C (C1 and C2)] ranged between 89.8 and 100%. The 22 swine HEV isolates obtained in the present study were exclusively classifiable into genotype 3, although we used the PCR primers capable of detecting both genotype 3 and genotype 4 swine HEV. Based on pairwise comparison of the 412-nucleotide sequence, the 22 HEV isolates were further classified into three clusters (Fig. 1). Seventeen HEV isolates (swJMS1, 2, 4, 6 to 8, and 12 to 22) from farm C (C1) and the HEV isolate (swJMS5) from farm A were grouped into a cluster. Three HEV isolates (swJMS9 to 11) from farm C (C2) were classifiable into another cluster. A third cluster comprised a single HEV isolate (swJMS3) from farm B.

Prevalence of swine anti-HEV IgG among pigs raised in four swine farms (A, C, D and E)

In an attempt to further investigate the prevalence of HEV infection in four swine farms, serum samples from a total of 160 domestic pigs of 3 to 6 months of age in farms A and C and those from 138 miniature pigs of 4 to 7 months of age in farms D and E were tested for anti-HEV IgG. All domestic pigs in farm A in two surveys conducted 1 yr apart and all miniature pigs in farms D and E were negative for anti-HEV IgG (Table 2). In remarkable contrast, among 112 serum samples obtained from domestic pigs of farm C that had been produced in farms C1, C2, C3 and C4, 96 (86%) tested positive for anti-HEV IgG.

Discussion

The prevalence of HEV RNA among 2-month-old domestic pigs in swine farms in various Asian countries has been reported to be 0% (0 of 180) [23] or 2.7% (two of 73) [22] in Japan, 1.6% (one of 62) [21] in Korea, or 4.5% (three of 67) [24] in Taiwan. In contrast, in the present study, HEV RNA was detected at a higher frequency of 14%

Table 1. Prevalence of anti-HEV IgG and HEV RNA among pigs used for medical experiments at our institute

Farm	No. of pigs tested	Age (months)	No. of pigs positive for[a] (%)		HEV genotype (No. of isolates/isolate name)
			anti-HEV IgG	HEV RNA	
A	84	2	0	1 (1)[b]	3 (1/swJMS5)
B	16	2	6 (38)	1 (6)	3 (1/swJMS3)
C	52	2	14 (27)	20 (38)	
C1	46	2	14 (30)	17 (37)[c]	3 (17/swJMS1, 2, 4, 6 to 8 and 12 to 22)
C2	6	2	0	3 (50)	3 (3/swJMS9 to 11)
Total	152		20 (13)	22 (14)	
D[d]	16	4 to 10	0/0[e]	0/0[e]	
E[d]	22	4 to 10	0/0[e]	0/0[e]	

[a]Serum samples were obtained from 190 pigs that had been brought to our center, at the time when they were used for medical experiments.
[b]The serum sample from this single viremic pig was obtained 1 month after it had been brought to our animal center (i.e. at 3 months of age), while serum samples from the remaining 189 pigs were obtained within 1 week after they had been brought to our center.
[c]Four pigs were positive for both anti-HEV IgG and HEV RNA.
[d]The pigs from farms D and E were all miniature pigs.
[e]Two serum samples were obtained from each miniature pig and each serum sample was tested for anti-HEV IgG and HEV RNA. The first serum sample was obtained at the time when the pig was brought to our center and the second serum sample was obtained 1 month later (just before being used for medical experiments).

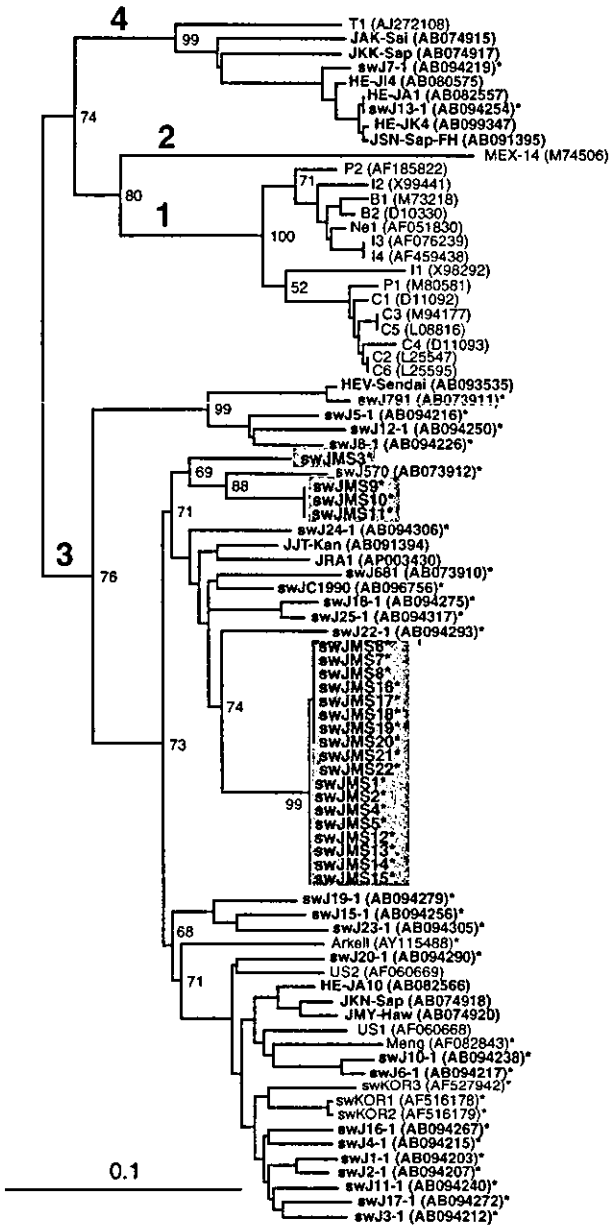

Fig. 1. Phylogenetic tree constructed by the neighbor-joining method based on the partial nucleotide sequence of the ORF2 region (412 nucleotides) of 83 HEV isolates. In addition to the 22 swine HEV isolates found in the present study which are classifiable into three clusters and shaded, 61 reported swine and human HEV isolates of genotypes 1 to 4 whose common 412-nucleotide sequence is known are included for comparison, and their accession nos. are shown in parentheses. The Japanese swine and human HEV isolates are indicated in bold type for visual clarity. The previously reported HEV sequences of genotype 1 are indicated with abbreviations in accordance with the recent review article by Schlauder and Mushahwar (11): B1 and B2 in Burma; C1, C2, C3, C4, C5 and C6 in China; I1, I2, I3 and I4 in India; Ne1 in Nepal; and P1 and P2 in Pakistan. Asterisks denote swine HEV strains. Bootstrap values are indicated for the major nodes as a percentage obtained from 1000 resamplings of the data.

Table 2. Prevalence of swine anti-HEV IgG among pigs that were raised in farms A, C, D and E

Farm	No. of pigs tested	Age (months)	No. of pigs positive for anti-HEV IgG (%)		
			First survey	Second survey	Total
A[a]	48	3 to 6	0/30	0/18	0/48
C[b]	112		96/112 (86)	–[c]	96/112 (86)
C1	27	5	25/27 (93)	–	25/27 (93)
C2	26	4 and 5	20/26 (77)	–	20/26 (77)
C3	29	4 and 5	22/29 (76)	–	22/29 (76)
C4	30	4 and 5	29/30 (97)	–	29/30 (97)
D	88	4 to 7	0/88	–	0/88
E	50	4 to 7	0/50	–	0/50

[a]Blood samples in the first survey were obtained on November 20, 2002, and blood samples in the second survey were obtained on November 5, 2003.
[b]Pigs that had been produced in farms C1, C2, C3, and C4 were transferred to farm C, where the pigs from each farm, were fattened separately in pig barns in farm C.
[c]A second survey was not conducted.

(22 of 152) among 2-month-old domestic pigs that were used for medical experiments in our institute. Although the reason why a high prevalence of HEV viremia was observed among the 2-month-old domestic pigs in the present study is unclear, the prevalence of HEV RNA differed remarkably according to swine farm, being 1% in farm A, 6% in farm B and 38% in farm C. In previous studies, the prevalence of swine anti-HEV IgG differed by pig herd, ranging from 6 to 30% among 2-month-old domestic pigs [15,21,23]. In support of the high prevalence of HEV viremia in pig farms in Japan, anti-HEV IgG was detected in 27% (14 of 52) of the 2-month-old pigs and 86% (96 of 112) of the 4- and 5-month-old pigs in farm C in the present study. Taken along with previous studies, our results suggest that researchers who use pigs as experimental animals, particularly those from farms B and C, are at risk for exposure to HEV. Therefore, our institute stopped purchasing domestic pigs from these two farms.

Although all pigs that were brought to our center from farm A and all pigs in the two surveys conducted on farm A were negative for swine anti-HEV IgG, one 3-month-old pig that had been brought to our center from farm A at 2 months of age and raised in the pig barn of our animal center for 1 month (from August 4 to September 1, 2003), was positive for HEV RNA. Of note, the swJMS5 isolate recovered from the viremic pig was 99.8 to 100% identical to the 17 HEV isolates (swJMS1, 2, 4, 6 to 8, and 12 to 22) recovered from the pigs that had been produced in farm C1 and brought to our animal center from farm C. Before being used for medical experiments, the swJMS4-infected pig out of the 17 pigs had been reared from May 12 to May 16, 2003 in the same barn of our center as the barn

where the swJMS5-infected pig from farm A was raised, and the swJMS4 and swJMS5 isolates were 100% identical with each other. As other domestic pigs besides the swJMS5-infected pig were used for experiments within 1 week after the infected pig was brought to our center, it is unclear whether these other domestic pigs were infected with HEV in our center. However, it is most likely that only one pig from farm A became HEV-viremic while it was being reared in our center. As periodic testing of pigs in farm A indicated that they were successively negative for anti-HEV IgG, it seemed conceivable to conclude that the domestic pigs on farm A are free from HEV infection. Therefore, our institute has continued to purchase domestic pigs from farm A, but not from farms B and C.

To prevent the transmission of swine HEV, we have instituted hygienic procedures including the use of disposable overalls, washing of boots, use of disinfectant mats at the entrance to the barns and disinfection of the pig barns at our center. Moreover, the pig breeding room was divided with partitions in which one pig was placed in each section, in order to prevent the spread of contagious agents. When a pig is scheduled to be used for experiments after being reared for over 2 weeks in our center, we test paired serum samples, the first of which was obtained on the day that the pig was brought to our center and the second of which is obtained on the day that the pig is used for experiments, for the presence of HEV infection. After instituting these new hygienic procedures, there has been no evidence of transmission of swine HEV at our center.

In our previous study in which we tested serum samples obtained from pigs at 25 commercial swine farms in Japan [23], swine anti-HEV was detected in only 7% of 2-month-old pigs (37 of 500) but in 88% of 4- and 5-month-old pigs (884 of 1000) with the highest absorbance values of 1.869 ± 0.996 in the 4-month-old pigs and 1.637 ± 0.935 in the 5-month-old pigs, suggesting that the prevalence of swine HEV infection in a given farm can be surveyed by testing domestic pigs of 4 to 5 months of age. In the present study, domestic pigs of 4 to 5 months of age were tested for swine anti-HEV IgG in the serosurveys of the farms. The prevalence of swine anti-HEV IgG among the pigs on farms A and C in the serosurveys was 0 and 86%, respectively, showing that this survey can be utilized to screen and monitor whether or not a given farm is free from HEV infection.

Anti-HEV IgG has been detected in many types of animals including monkeys [34], rodents [35,36], dogs [37], cats [38], and chickens [39,40]. In institutions housing experimental animals, there is a risk of cross-species infection of HEV, and other animal species may also be involved in HEV transmission. The possibility of zoonotic HEV infection to humans is supported by the results of a study showing that veterinarians working with swine are at higher risk for HEV infection than are normal blood donors in the United States and other countries [26]. Other researchers have reported that pig handlers had higher rates of anti-HEV IgG than did non-swine workers [25]. Yazaki et al. [9] reported that HEV might be transmitted to humans by ingestion of inadequately cooked pig liver, and Tei et al. [8] showed that HEV infection occurred via zoonotic food-borne transmission of HEV from deer to humans. The potential for cross-species infection of HEV [2–9] raises an important public health concern not only in the community but also in research institutions where many species of animals are raised.

To the best of our knowledge, this is the first report on the prevalence of HEV infection among miniature pigs. Of note, the sera from all 38 miniature pigs of 4 to 10 months of age that had been brought to our center from farms D and E for medical experiments were negative for both HEV RNA and swine anti-HEV IgG, and the sera from 138 additional miniature pigs raised in farms D and E were negative for swine anti-HEV IgG, indicating that the miniature pigs in the two farms are free from HEV. However, we cannot rule out the possibility that a certain population of miniature pigs is infected with HEV. According to our recent data, swine anti-HEV IgG was detectable in one of four miniature pigs raised in a different farm. Therefore, a study on a larger number of miniature pigs including those of 2 and 3 months of age for swine HEV RNA detection is needed to draw a definitive conclusion.

Xenotransplantation is currently being investigated as a possible solution to the worldwide shortage of human organs. Pigs are the most acceptable candidate animals but issues of xenozoonoses remain [5,12,13]. The ubiquitous nature of swine HEV in pigs and the demonstrated ability of cross-species infection raise a potential concern for swine HEV infection in xenotransplantation with pig organs, cells, and tissues [3–6,14–24]. Our present results suggested that the consistent use of barrier precautions and adequate disinfection, combined with screening of pigs admitted to the herd, can exclude HEV infection from purpose-bred pigs intended for use in production of biological therapeutics intended for use in humans even when HEV infection is highly prevalent among other pigs in the country.

In conclusion, it is important to use purpose-bred animals for work directed toward the development of biological therapeutic products intended for use in humans. Breeding and maintenance facilities for such animals should be vigilant in infection control measures intended to prevent introduction of infections into the facilities or transmission within facilities should such introductions occur. Further, sero-prevalence intended to identify such infections when they occur (i.e. in this study, archiving a serum specimen upon admission of the animal to the facility to be used for paired testing in tandem with a second specimen collected at the time of experimentation) is also an important precaution.

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Journal of Medical Virology 73:38–44 (2004)

Prevalence of Antibodies to Hepatitis E Virus Among Apparently Healthy Humans and Pigs in Bali, Indonesia: Identification of a Pig Infected With a Genotype 4 Hepatitis E Virus

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In Indonesia where hepatitis E virus (HEV) is believed to be highly endemic, only three outbreaks of HEV transmission have been documented to date in restricted areas (West Kalimantan and East Java). A total of 1,115 serum samples collected from apparently healthy individuals in Bali, Lombok, and Surabaya in Indonesia in 1996 where epidemic HEV transmissions have never been reported, were tested for IgG class antibodies to HEV (anti-HEV). In Bali, anti-HEV was detected in 20% (54/276) of the tested population, in remarkable contrast with 4% (17/446) in Lombok and 0.5% (2/393) in Surabaya. On the other hand, antibodies to hepatitis A virus were highly prevalent in all three regions (95% in Bali, 90% in Lombok, and 89% in Surabaya). Although the majority of the population in Indonesia is Moslem, Balinese people are mostly Hindu and have a habit of consuming pork. Therefore, serum samples were obtained from the 99 farm pigs in Bali and tested for anti-HEV and HEV RNA. The sera from 71 pigs (72%) were positive for anti-HEV and a 2-month-old pig had detectable HEV RNA. The swine HEV isolate recovered from the viremic pig was named SB66-Bali. The SB66-Bali isolate was most closely related to the genotype 4 isolates from China, India, Japan, and Taiwan, but shared only 82.6–90.0% identity in the common 241–412 nucleotides within open reading frame 2 (ORF2). These results indicate that a presumably indigenous HEV strain(s) is circulating in Bali, Indonesia and that HEV infection may occur via zoonosis even in developing countries.
J. Med. Virol. 73:38–44, 2004.

KEY WORDS: hepatitis viruses; phylogenetic analysis; zoonosis

INTRODUCTION

Hepatitis E virus (HEV), the causative agent of hepatitis E, has been recognized as a major cause of enterically transmitted non-A, non-B hepatitis in many developing countries where sanitation is suboptimal. The virus is endemic in much of Asia and Africa and one epidemic in Mexico was documented [Purcell and Emerson, 2001]. Transmission of HEV occurs primarily by the fecal–oral route through contaminated water supplies in developing countries.

HEV is an unclassified non-enveloped virus. Its genome is a single-stranded, positive-sense RNA of approximately 7.2 kb. It consists of a short 5′ untranslated region (UTR) followed by three partially overlapping open reading frames (ORFs: ORF1, ORF2, and ORF3), and then a short 3′ UTR terminated by a poly(A) tract [Reyes et al., 1990; Tam et al., 1991]. Although only one serotype has been recognized, extensive genomic

The nucleotide sequence data reported in this study have been assigned DDBJ/EMBL/GenBank accession number AB124818.

Grant sponsor: Ministry of Health, Labour and Welfare of Japan.

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Accepted 2 December 2003
DOI 10.1002/jmv.20059
Published online in Wiley InterScience (www.interscience.wiley.com)

diversity has been noted among HEV isolates, and HEV sequences have tentatively been classified into four genotypes (genotypes 1–4). Recent studies indicate that hepatitis E also occurs among individuals in industrialized countries where hepatitis E had been believed to be non-endemic [Harrison, 1999; Purcell and Emerson, 2001; Smith, 2001]. In addition, increasing evidence has indicated that hepatitis E is a zoonosis [Meng et al., 1997, 1998; Erker et al., 1999; Harrison, 1999; Meng, 2000; Halbur et al., 2001; Okamoto et al., 2001; Smith, 2001; Nishizawa et al., 2003; Takahashi et al., 2003; Tei et al., 2003; Yazaki et al., 2003]. The majority of HEV infections in developing countries are caused by genotype 1, and only isolated cases of infection with HEV of genotype 3 or 4 have been described in industrialized nations [Schlauder and Mushahwar, 2001].

In Indonesia, HEV transmission was recognized in West Kalimantan during a large outbreak involving >2,500 cases that occurred in 1987 and a second outbreak including >1,500 cases that occurred in 1991 [Corwin et al., 1995, 1997, 1999]. Furthermore, an outbreak of HEV transmission has been documented in the Bondowoso District on Java Island in 1998 [Sedyaningsih-Mamahit et al., 2002]. However, no epidemic or sporadic HEV transmission has ever been reported in other parts of Indonesia and the prevalence of HEV infection in this country remains unclear. Therefore, in the present study, the prevalence of HEV antibodies (anti-HEV) was investigated in apparently healthy individuals living in three distinct geographic regions in Indonesia, i.e., Bali Island, Lombok Island, and Surabaya (the capital of East Java), who have different religions and customs, and compared among each other. Balinese people are mostly Hindu and have a habit of ingesting uncooked or undercooked pig meat and/or viscera or blood, whereas in other places in Indonesia the majority of the population are Moslem. Hence, we also investigated the prevalence of HEV infection among 99 pigs raised in various farms in Bali in an attempt to gain insight into the possible zoonotic infection of HEV in this country.

MATERIALS AND METHODS

Serum Samples

Serum samples were collected from 276 apparently healthy individuals (mean ± standard deviation [SD] age, 33 ± 12 years; 119 men and 157 women) who were family members of patients with chronic liver disease seen at Sanglah Hospital of Udayana University, Denpasar on Bali Island of Indonesia in 1996; from 393 voluntary blood donors (age, 36 ± 11 years; all men) at the Blood Transfusion Unit of the Indonesian Red Cross in Surabaya on Java Island in 1996; and from 446 voluntary blood donors (age, 26 ± 11 years; 371 men and 75 women) at Mataram Blood Center on Lombok Island in 1996. Additionally, serum samples were obtained from 797 voluntary blood donors (age, 33 ± 11 years; 709 men and 88 women) at Denpasar Blood Center, Bali, in 2003, to compare the prevalence of hepatitis virus markers including anti-HEV in Bali between 1996 and 2003. Serum samples were also collected from 99 pigs raised in 8 swine farms on Bali Island in 2003.

Human sera from the inhabitants were tested for antibodies against HAV (anti-HAV [total]) by enzyme-linked immunosorbent assay (ELISA) (HAT-EIA, Denka Seiken, Tokyo, Japan). The presence of hepatitis B surface antigen (HBsAg) was determined by passive hemagglutination with a commercial assay kit (Mycell HBsAg [RPHA]; Institute of Immunology Co. Ltd., Tokyo, Japan). Antibodies to HCV (anti-HCV) were assayed by the hemagglutination method (Abbott HCV PHA-II; Dainabot, Tokyo, Japan).

Detection of Human and Swine Antibodies to HEV

To detect human and swine anti-HEV IgG, ELISA was carried out using purified recombinant ORF2 protein of HEV genotype 4 that had been expressed in the pupae of silkworm, as described previously [Mizuo et al., 2002; Takahashi et al., 2003]. The optical density (OD) of each sample was read at 450 nm. The cutoff value used for the human anti-HEV IgG assays was 0.152, and that for the swine anti-HEV IgG assay was 0.366. Test samples with OD values for human anti-HEV IgG or swine anti-HEV IgG equal to or greater than the respective cutoff value were considered to be positive for anti-HEV. The specificity of the human and swine anti-HEV assays was verified by absorption with the same recombinant ORF2 protein that was used as the antigen probe or a mock protein obtained from the pupae of silkworm infected with non-recombinant baculovirus. Briefly, when the OD value of the tested sample decreased to less than 30% of the original value after absorption with the recombinant ORF2 protein and remained greater than 70% of the original value after absorption with a mock protein, the sample was considered to be positive for anti-HEV.

Detection of HEV RNA

Reverse transcription (RT)-polymerase chain reaction (PCR) was performed for detection of HEV RNA in swine serum. Total RNA was extracted from 100 μl of serum, reverse transcribed, and then subjected to nested PCR with the ORF2 primers as described previously [Mizuo et al., 2002]. The size of the amplification product of the first-round PCR was 506 base pairs (bp), and that of the second-round PCR was 457 bp. The nested RT-PCR assay was performed in duplicate, and reproducibility was confirmed. The specificity of the RT-PCR assay was verified by sequence analysis as described below. The sensitivity of the RT-PCR assay was assessed as described previously [Mizuo et al., 2002; Takahashi et al., 2003].

Sequence Analysis of PCR Products

The amplification product was sequenced directly on both strands using the BigDye Terminator Cycle Sequencing Ready Reaction Kit on an ABI PRISM

3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequence analysis was performed using Genetyx-Mac version 12.0.9 (Genetyx Corp., Tokyo, Japan) and ODEN version 1.1.1 from the DNA Data Bank of Japan (DDBJ: National Institute of Genetics, Mishima, Japan) [Ina, 1994]. Sequence alignments were generated by CLUSTAL W (version 1.8) [Thompson et al., 1994]. A phylogenetic tree was constructed by the neighbor-joining method [Saitou and Nei, 1987] based on the partial nucleotide sequence of the ORF2 region (298 nucleotides [nt]). Bootstrap values were determined on 1,000 resamplings of the data sets [Felsenstein, 1985]. The final tree was obtained using the TreeView program (version 1.6.6) [Page, 1996].

Statistical Analysis

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

RESULTS

Prevalence of Anti-HEV and Anti-HAV Among Healthy Individuals in Three Distinct Geographic Regions in Indonesia in 1996

A total of 1,115 serum samples obtained from apparently healthy individuals in Bali, Lombok, and Surabaya in 1996 were tested for the presence of anti-HEV IgG. In Bali, anti-HEV IgG was detected in 20% (54/276) of the tested population, in remarkable contrast with the detection rate of 4% (17/446, $P < 0.0001$) in Lombok and 0.5% (2/393, $P < 0.0001$) in Surabaya (Table I). On the other hand, anti-HAV was highly prevalent in all three regions with similar detection rates (95% in Bali, 90% in Lombok, and 89% in Surabaya).

Comparison of the Prevalence of Hepatitis Virus Markers Among Healthy Individuals in Bali Between 1996 and 2003

As the prevalence of anti-HEV IgG in 1996 was the highest in Bali among the three regions studied, the prevalence of various hepatitis virus markers was compared among individuals in Bali between 1996 and 2003 (Table II). Between 1996 and 2003, there were no significant differences in the prevalence of anti-HEV IgG (20% vs. 18%, $P = 0.5805$), anti-HAV (95% vs. 87%, $P = 0.0608$), HBsAg (7% vs. 5%, $P = 0.2589$), nor anti-HCV (2% vs. 0.8%, $P = 0.1323$). The prevalence of anti-HEV IgG was >10% in the age groups of 16–19, 20–29, 30–39, 40–49, and 50–59 years both in 1996 and 2003, indicating that HEV is circulating and is highly endemic in Bali.

Prevalence of Anti-HEV Among Farm Pigs in Bali

Serum samples obtained from 99 farm pigs in Bali were tested for anti-HEV IgG. The sera from 71 pigs (72%) were positive for anti-HEV, with the prevalence differing by swine farm, ranging from 29 to 100% (Table III). The prevalence of swine anti-HEV differed remarkably by age, being 38% among the 2-month-old pigs, 46% among the 3-month-old pigs, 88% among the 4-month-old pigs, 89% among the 5-month-old pigs, and 82% among the 6-month-old pigs (Table IV). Upon considering pigs of 4–6 months of age, swine anti-HEV was detectable in 87% of the pigs (55/63).

Table IV shows the mean OD values of swine anti-HEV according to the age (months after birth) of the pigs. Swine anti-HEV was detected at the highest OD value (2.056 ± 0.753) in 2-month-old pigs, and then the titer decreased gradually, detectable at a mean OD value of 0.877 in 5-month-old pigs and 0.682 in 6-month-old pigs.

Genetic Analysis of Swine HEV Recovered From a Viremic Pig in Bali

HEV RNA was detectable in one 2-month-old pig out of the 99 pigs tested: the pig was raised in a swine farm in Tabanan (Tables III and IV). The swine HEV isolate recovered from the viremic pig was named SB66-Bali. The 412-nt sequence of ORF2 of SB66-Bali was determined and compared with that of known human and swine HEV isolates of genotypes 1–4. The SB66-Bali isolate was related most closely to the prototype genotype 4 isolate of T1 (accession no. AJ272108) with

TABLE I. Prevalence of Anti-HEV IgG and Anti-HAV Among Apparently Healthy Individuals in Bali, Lombok, and Surabaya in Indonesia in 1996

	Bali			Lombok			Surabaya		
Age (yr)	No.	Anti-HEV	Anti-HAV[a]	No.	Anti-HEV	Anti-HAV[a]	No.	Anti-HEV	Anti-HAV[a]
16–19	39	7 (18%)	17/20 (85%)	179	6 (3%)	15/20 (75%)	12	0	9/12 (75%)
20–29	86	11 (13%)	18/20 (90%)	130	5 (4%)	18/20 (90%)	121	1 (0.8%)	15/20 (75%)
30–39	67	17 (25%)	20/20 (100%)	63	1 (2%)	20/20 (100%)	114	0	17/20 (85%)
40–49	45	6 (13%)	20/20 (100%)	65	5 (8%)	19/20 (95%)	97	0	20/20 (100%)
50–59	27	11 (41%)	20/20 (100%)	8	0	7/8 (88%)	42	1 (2%)	20/20 (100%)
60–64	12	2 (17%)	11/12 (92%)	1	0	1/1 (100%)	7	0	7/7 (100%)
Total	276	54 (20%)	106/112 (95%)	446	17 (4%)	80/89 (90%)	393	2 (0.5%)	88/99 (89%)

[a]When more than 20 samples were available for an age group, 20 randomly selected samples were tested for anti-HAV.

TABLE II. Comparison of the Prevalence of Anti-HEV IgG, Anti-HAV, HBsAg, and Anti-HCV Among Apparently Healthy Individuals Living in Bali Between 1996 and 2003

Age (yr)	Anti-HEV		Anti-HAV[a]		HBsAg		Anti-HCV	
	1996	2003	1996	2003	1996	2003	1996	2003
16–19	7/39 (18%)	10/99 (10%)	17/20 (85%)	14/20 (70%)	2/39 (5%)	5/99 (5%)	0/39	0/99
20–29	11/86 (13%)	27/193 (14%)	18/20 (90%)	17/20 (85%)	9/86 (10%)	9/193 (5%)	1/86 (1%)	1/193 (0.5%)
30–39	17/67 (25%)	64/291 (22%)	20/20 (100%)	18/20 (90%)	1/67 (1%)	13/291 (4%)	2/67 (3%)	3/291 (1%)
40–49	6/45 (13%)	29/133 (22%)	20/20 (100%)	19/20 (95%)	2/45 (4%)	5/133 (4%)	1/45 (2%)	2/133 (2%)
50–59	11/27 (41%)	14/78 (18%)	20/20 (100%)	19/20 (95%)	3/27 (11%)	6/78 (8%)	1/27 (4%)	0/78
60–64	2/12 (17%)	0/3	11/12 (92%)	3/3 (100%)	1/12 (8%)	0/3	0/12	0/3
Total	54/276 (20%)	144/797 (18%)	106/112 (95%)	90/103 (87%)	18/276 (7%)	38/797 (5%)	5/276 (2%)	6/797 (0.8%)

[a]When more than 20 samples were available for an age group, 20 randomly selected samples were tested for anti-HAV.

nucleotide sequence identity of 86.9%, and was only 81.5, 77.6, and 77.8% similar to the B1 isolate (M73218) of genotype 1, MEX-14 isolate (M74506) of genotype 2, US1 isolate (AF060668) of genotype 3, respectively. The phylogenetic tree constructed based on the common 298 nt within the ORF2 sequence confirmed that the SB66-Bali isolate obtained in the present study belonged to genotype 4, but that it was separate from the clusters consisting of Chinese, Japanese, and Taiwanese strains of the same genotype that had been recovered from humans or swine (Fig. 1). Although the SB66-Bali isolate bifurcated from the common trunk which consisted of the three Taiwanese strains, it differed from the TW5483E isolate of human origin by 10.0% and the TW32SW and TW74SW isolates of swine origin by 10.0–10.7% in the common 299-nt ORF2 sequence. In addition, the SB66-Bali isolate had merely 82.6–89.0% identity with Chinese HEV strains of human and swine origin, and 86.2–88.8% identity with Japanese HEV strains of human and swine origin (Table V). Recently, Arankalle et al. [2002] reported 12 swine HEV isolates of genotype 4 in Western India, which shared only 84.0–85.9% nucleotide identity with the SB66-Bali isolate in the partial ORF2 sequence of 241–263 nt (Table V).

DISCUSSION

In Indonesia, only three outbreaks of HEV transmission that occurred in 1987, 1991, and 1998 have been documented thus far [Corwin et al., 1995, 1997, 1999; Sedyaningsih-Mamahit et al., 2002], and water with fecal contamination is presumed to be the cause of epidemics of hepatitis E in Indonesia as in other countries in tropical and subtropical areas including Central and Southeast Asia, northern and western Africa, and a small part of Mexico, which have impoverished social and economic conditions as well as inadequate hygiene and sanitation [Purcell and Emerson, 2001; Smith, 2001]. Unlike in the HEV outbreaks in West Kalimantan in 1987 and 1991, where the practice of boiling water reduced significantly the risk of epidemic HEV infection [Corwin et al., 1997], there was no significant influence attributed to "boiling water" on epidemic HEV infection in Bondowoso District, East Java Province where an outbreak of HEV transmission occurred in 1998 [Sedyaningsih-Mamahit et al., 2002]. The communities in outbreak areas used river water as the primary source of water for bathing, human-waste disposal, and drinking purposes, while those in non-outbreak areas did not. Although the antibody to HAV, which is another water-borne hepatitis virus, was highly prevalent in the three geographic regions in the present study, with comparable positivity (95% in Bali, 90% in Lombok, and 89% in Surabaya), people living in Bali, Lombok, and Surabaya boil their water before drinking it. In the three regions that were studied, it is most likely that HAV infection is acquired through ice that is added to a glass of water or crushed together with fruits. Ice blocks are produced in large quantities from unboiled tap water by ice factories. Another possibility is

TABLE III. Prevalence of Anti-HEV and HEV RNA Among Pigs From Various Farms in Bali

Farm	No. of pigs	Age (month)	Anti-HEV IgG No. (%)	Anti-HEV IgG Optical density (OD) (mean ± SD)	HEV RNA
Bangli	19	4.3 ± 1.0	12 (63)	0.943 ± 0.478	0
Damasaba	13	5.4 ± 0.7	12 (92)	0.690 ± 0.316	0
Denpasar	11	3.6 ± 1.0	8 (73)	1.112 ± 0.735	0
Jagapati	19	4.6 ± 0.6	18 (95)	0.908 ± 0.415	0
Kapal	9	2.8 ± 0.4	4 (44)	1.785 ± 0.802	0
Kelambitan	14	3.0 ± 0.5	4 (29)	1.032 ± 0.409	0
Sibang	4	5.3 ± 1.0	4 (100)	0.586 ± 0.132	0
Tabanan	10	4.6 ± 1.1	9 (90)	1.536 ± 0.693	1 (10%)
Total	99	4.2 ± 1.1	71 (72)	1.018 ± 0.583	1 (1%)

the method of washing tableware. After eating or drinking, plates and glasses are washed by soap (sometimes without it) using tap water or sometimes using water taken from a well.

Despite the common situation with regard to the prevalence of anti-HAV in the three regions studied, anti-HEV IgG was detected in 20% of the tested population in Bali, in contrast with the detection rate of 4% in Lombok and 0.5% in Surabaya on Java Island. It is probable that several forms of HEV transmission in addition to fecal–oral transmission occur in Bali: (a) zoonotic infection, (b) transmission by food, and (c) transmission via blood transfusion. Viremic blood donors are potentially able to cause transfusion-associated hepatitis E not only in areas of high endemicity [Arankalle and Chobe, 2000], but also in non-endemic countries like Japan. A Japanese man in his 60s who contracted hepatitis E from a blood transfusion that he had received during heart surgery at a city hospital in Hokkaido in 2002 was identified (ABC Newsletter January 24, 2003; accessible at http://www.americasblood.org).

Evidence is accumulating that hepatitis E is a zoonosis [Balayan, 1997; Harrison, 1999; Meng, 2000; Smith, 2001; Tei et al., 2003; Yazaki et al., 2003], and cross-species infection of HEV has been documented [Meng et al., 1998; Erker et al., 1999; Halbur et al., 2001]. Anti-HEV IgG has been detected among pigs in high HEV-endemic countries such as China, Nepal, and Thailand as well as among pigs in low- or non-endemic countries such as Australia, Canada, Germany, Japan, New Zealand, Taiwan, and the United States [Clayson et al., 1995; Chandler et al., 1999; Hsieh et al., 1999; Meng et al., 1999; Garkavenko et al., 2001; Takahashi et al., 2003]. In the present study, we found a high prevalence of swine anti-HEV IgG among Balinese pigs of 2–6 months of age (72% or 71/99). Thus, HEV is enzootic in pigs whether or not hepatitis E is common in the resident human population. Several recent reports from China, Japan, Spain, Taiwan, and the United States have indicated that in particular geographical regions, HEVs isolated from pigs and humans are closely related genetically [Meng et al., 1997, 1998; Hsieh et al., 1999; Pina et al., 2000; Huang et al., 2002; Wang et al., 2002; Wu et al., 2002; Nishizawa et al., 2003]. Therefore, it needs to be elucidated in future studies whether or not HEVs isolated from patients with sporadic acute hepatitis E in Bali are closely related to the SB66-Bali isolate (genotype 4 HEV strain) obtained in the present study.

TABLE IV. Age-Dependent Prevalence of Anti-HEV and HEV RNA Among Pigs in Bali

Age (month)	No. of pigs	Anti-HEV IgG No. (%)	Anti-HEV IgG OD (mean ± SD)	HEV RNA
2	8	3 (38)	2.056 ± 0.753	1 (13%)
3	28	13 (46)	1.440 ± 0.671	0
4	17	15 (88)	0.938 ± 0.524	0
5	35	31 (89)	0.877 ± 0.417	0
6	11	9 (82)	0.682 ± 0.345	0

The custom in Bali is quite different from that in other places in Indonesia, although regions with a similar custom as that in Bali may be found in Manado of North Celebes Island, which is mostly inhabited by people with a Christian tradition, or in a part of North Sumatra surrounding the Lake of Toba ("Batak-Toba" ethnic group having a religion other than Islam). The majority of Balinese people are Hindu, and have a custom of raising domestic animals such as pigs within their housing sites and of using pigs in the Balinese Hinduism ceremony, which is held frequently year-round. They used to ingest uncooked or undercooked pig meat and viscera during the ceremony, and some Balinese people like to consume certain vegetables mixed with fresh blood from pigs (called "KOMOH" in Bali). As described in recent reports [Tei et al., 2003; Yazaki et al., 2003], it is likely that foods can act as vehicles for transmission of HEV. On the other hand, Surabaya is inhabited by Javanese people, and the majority of people in Surabaya are Moslem. Most of the blood donors who donated at the Blood Transfusion Unit of the Red Cross in Surabaya, do not consume pork (because the Chinese people in Surabaya usually do not go to the Blood Transfusion Unit of the Indonesian Red Cross to donate blood, but directly donate blood at the blood transfusion unit at

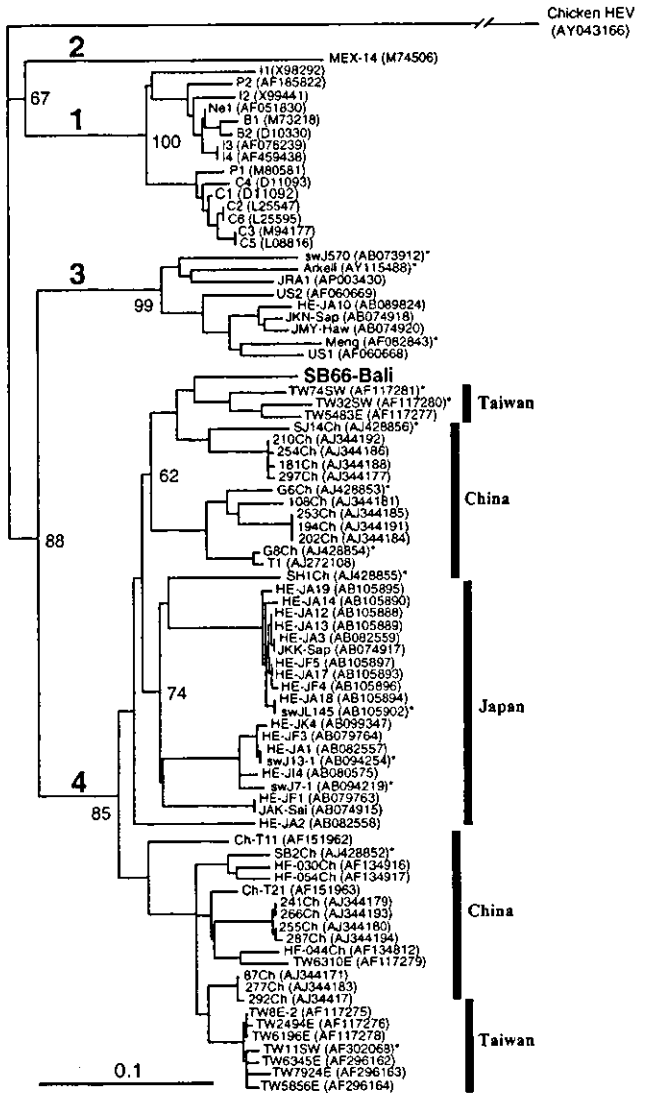

Fig. 1. Phylogenetic tree constructed by the neighbor-joining method based on the partial nucleotide sequence of the open reading frame 2 (ORF2) region (298 nt) of 83 hepatitis E virus (HEV) isolates, using a chicken HEV (AY043166) as an outgroup. In addition to the SB66-Bali Indonesian isolate found in the present study which is indicated in bold type for visual clarity, 82 reported HEV isolates of genotypes 1–4 whose common 298 nt sequence is known are included for comparison and their accession nos. are shown in parentheses. The previously reported HEV sequences of genotype 1 are indicated with abbreviations in accordance with the recent review article by Schlauder and Mushahwar [2001]: B1 and B2 in Burma; C1, C2, C3, C4, C5, and C6 in China; I1, I2, I3, and I4 in India; Ne1 in Nepal; and P1 and P2 in Pakistan. Asterisks denote swine HEV strains. Bootstrap values are indicated for the major nodes as a percentage obtained from 1,000 resamplings of the data.

hospitals). Approximately 10–15% of the people residing in Mataram city on Lombok Island are Hindu or Christian. Thus, the number of blood donors in Mataram enrolled in the present study who consume pork is not negligible, and this may be the cause of the observed higher prevalence of anti-HEV in Lombok than in Surabaya (3.8% [17/446] vs. 0.5% [2/393], $P = 0.0013$).

Our present study indicates that the prevalence of HEV infection differs according to the religion and custom of the resident human population in different regions in Indonesia and that HEV antibodies are highly prevalent in Bali (20%), although epidemic HEV infection has not been documented thus far in regions other than West Kalimantan and the Bondowoso District of East Java in this country. We would like to speculate that HEV infection in Bali is transmitted by ingestion of uncooked or undercooked pig meat and viscera contaminated with HEV, based on Balinese people's dietary habits, the finding of a high prevalence of swine anti-HEV IgG among Balinese pigs of 2–6 months of age and the identification of a pig infected with a genotype 4 HEV that may be indigenous to Indonesia. Based on the results obtained in the present study, it is concluded that a presumably indigenous HEV strain(s) is circulating in Bali, Indonesia and that HEV infection may occur via zoonosis even in developing countries. Extended studies must be undertaken to determine to what extent the pig meat and viscera ingested by Balinese Hindus are contaminated with HEV; to determine the prevalence of hepatitis E among patients with acute hepatitis in Indonesia; and to elucidate whether HEV strains recovered from patients with clinical HEV infection are closely related to swine HEV strains in Indonesia.

TABLE V. Comparison of a Swine HEV Isolate in Bali, Indonesia (SB66-Bali) With 77 Swine and Human HEV Isolates of Genotype 4 Whose Common 241–412 Nucleotide Sequence Is Known

Country/ host	No. of isolates compared	Nucleotide length compared (nt)	Identity %
China			
Swine	5	300	85.7–88.0
Human	21	299–412	82.6–89.0
India			
Swine	12	241–263	84.0–85.9
Japan			
Swine	10	412	87.8–88.8
Human	18	412	86.2–88.8
Taiwan			
Swine	3	299	83.6–90.0
Human	8	299	83.6–90.0

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総説

肝臓　45巻4号　177 185 (2004)

＜総　説＞

E型肝炎研究これからの課題

三代　俊治

はじめに

E型肝炎に関しては，既に2001年の本誌に田中栄司等による優れた総説[1]があるから，ここに敢えて屋上架屋の愚を冒すことをば避け，本稿では，主に2001年以降の研究展開に鑑みて今後に残された課題は奈辺にあるかを考察する．以て肝臓学会諸兄姉の研究のヒントとなれば幸いである．本稿全体をではなく，関心のある項目だけを拾い読みせられることを御薦めする．

1．分子生物学

E型肝炎ウイルス(HEV)を巡る諸問題に対処する最も有効な武器の一つは分子生物学的手法である．予防と治療を可能にする為にはワクチンと抗ウイルス剤を開発せねばならない．その為には，HEVの分子生物学的性質を熟知することが必要である．

既に周知の如く，HEVのゲノムは約7kb長の一本鎖positive strand RNAであり，5'側にはRNA polymerase等の非構造蛋白をコードするORF1，3'側にキャプシド蛋白をコードするORF2と機能不明蛋白をコードするORF3が存在し，3'端にはpoly-A tailが存在する[2]．

5'端にはキャップ構造が存在し[3,4]，キャップ附加酵素(capping enzyme)はHEVゲノムによってコードされており[5]，且つこのキャップ構造の存在は感染性の維持の為に必須である[6]．即ち，HEV RNAはpositive strandでありcapもpoly-Aもあるから，全くmRNAそのものである．mRNAは翻訳されなければ只の無用の長物である．そしてその翻訳はキャップ依存性に行われる．従って，ゲノム複製を阻害する物質をHEVに対する抗ウイルス剤として考慮する(例えばRNA polymerase blockerの開発)という正攻法の傍らに，翻訳開始を阻害する薬剤(例えばcapping enzyme blocker)の開発を試みるという脇道もある．

機能不明だったORF3蛋白は，宿主細胞内でシグナル伝達系蛋白のSH3ドメインに結合してMAPKを活性化する性質などが報告されたが[7,8]，その後，燐酸化された形のORF3蛋白がORF2によってコードされるキャプシド蛋白と結合し，以てHEVのアセンブリに寄与している可能性も指摘された[9]．ゲノム上でもORF3の翻訳開始コドンはORF2のそれと殆ど同じ場所に配置されているから，前者も後者と同様にstructural proteinであっても全然おかしくない．もしORF3蛋白がHEVのアセンブリに必須であるなら，ORF3も抗ウイルス剤開発のターゲットの一つになり得る．

細胞培養系未確立であるが故に，HEVのワクチン開発も分子生物学者の仕事の一つである．我が国では国立感染研のグループがORF2蛋白を加工して中空ウイルス粒子を作成する技術を夙に開発し，edible vaccine(「食べるワクチン」)を現実化する道をひたすら歩んで来た[10～12]が，残念ながら実用化のスピードに関しては米国のrecombinant vaccine[13,14]に先行されている．後者は現在ネパールでfield trialが進行中である．DNA vaccineも開発が進められている[15,16]．

扨て，抗ウイルス剤や有効なワクチンの開発など今後の課題を解決する為に，培養系のない状況下では実験動物を用いた分子生物学的アプローチが殊更重要である．これまでは，ヒトHEVに感受性のあるサルやブタを用いてそれが行われて来たが，2001年に発見されたトリHEV(avian hepatitis E virus)[17～20]が今後の実験手法を大きく変える可能性がある．このウイルスはhepatitis-splenomegaly syndromeと呼ばれて来たニワトリの病気の原因ウイルスであったが，そのキャプシド蛋白をコードする遺伝子領域をシークエンスしてみると，ヒトやブタのHEVと一脈通じる(しかし一寸見ただけでは気付かぬ程度の)相同性を持っていたし，抗原エピトープにも或る程度の共通性がみられた．丁度，嘗てB型肝炎ウイルスの研究の為にウッドチャックやアヒルのヘパドゥナウイルスが用いられたように，このavian HEVもヒトやブタのHEV研究の為に大きく貢献すると思われる．また，avian HEVとhuman or swine HEVとの間の遺伝子

東芝病院研究部

距離は相当に遠いので，その中間に来るウイルスが新たに見つかる可能性も出て来た．

2．疫　学

感染症は，一般に，治療剤（抗生物質や抗ウイルス剤）と予防ワクチンの両方が揃った瞬間に『制御の段階に入った』とされる．しかし，ワクチンがなくても予防は可能である．疫学的な実態が充分に解明されれば，それに基づく『衛生知識』が我々を感染から回避させてくれる．

1）Waterborne transmission

嘗てE型肝炎は“waterborne hepatitis”として名を馳せていた．アジア・アフリカ諸国で，主として洪水の後に下水が飲料水に混入することによる集団発生が頻発したからである[21~24]．しかしこのルートによる感染は，必ずしも，井戸水や川の水を飲料水として使用する地域にのみ発生したのではない．水道水から感染したという報告もある[25]．そのケースでは，浄化プロセスにトラブルのあった浄水場から水道をひいている地域住民に集団発生が見られたのである．

HEVは糞便中に排出されるから，生活排水やそれに汚染された飲み水の中にHEVが検出される[26]のは当然である．しかし，必ずしも『水を飲む』という行為だけがHEV感染を媒介するのではないらしい．生活排水を灌漑用水として使用する農家では34.8%の人がHEV抗体陽性（コントロールは4.4%）だったという報告もある[27]．

2）Imported and domestic infection

先進国でのE型肝炎は専ら，endemic areasへの旅行者が旅先で感染して帰国後に発病する『輸入感染症』として知られていた[28~30]．数多ある輸入感染の報告の中で，一つ，筆者の興味を惹いた論文がある．スウェーデン語で書かれた論文だからMedlineの英文タイトルからしかその中身を推察出来ないが，17例の輸入E型肝炎例のうち僅かに1例のみに二次感染の発生が見られた，という論文である[31]．序でだから此処で二次感染について述べるが，最近日本で経験されるようになったE型肝炎の事例に於いても，筆者の知る限り，E型肝炎の二次感染は極めて稀である（というより，筆者が掌握している数十例では二次感染発生が皆無！）．これはA型肝炎で二次感染が頻発する[32]のと好対照である．その差の理由は不明である．

後述するHEVの土着化の話に絡めて云えば，ヒトからヒトへの二次感染の起こり難さに鑑みて，endemic areaへの旅行者が持ち帰ったHEV株が土着化したのではない可能性を考えたい．即ち，ヒト以外のナニモノかがHEVを持ち込んだと．

閑話休題，先進国のE型肝炎は必ずしも輸入感染のみではないことが次第に判明して来た．Endemic areasへの渡航歴のない症例から，地域毎にユニークな配列を持つHEV株が続々と見つかって来たからである．最初は米国から[33]，引き続き欧州から[34~38]，そして日本からも[39]．

HEVは本当に『先進国にも土着した』のであろうか？この問いに端的に答えるには先進国の下水を調べるのが最も手っ取り早い．そして，スペインのバルセロナ，米国のワシントンD.C.，フランスのナンシーの下水から，HAVと共にHEVが採れた[40]．下水道は地域社会の消化管である．その消化管からHEVが採れたのだから，HEVはその地域に内在していると云える．

3）Genotype distribution

現代疫学の最強の武器は分子疫学的手法である．病原体ゲノムの塩基配列を調べることにより，その病原体が辿った足跡をトレース出来る．

Fig. 1の分子系統樹に示す如く，HEVのgenotypeは大別して四つに分かれ，その分布には明確な地域差がある．おしなべて，Afro-Asian諸国のHEV株はgenotype Iの中でコンパクトなクラスターを形成し，先進諸国のそれはgenotype IIIの中で多様性に富む集団を作る．図中にはないが，最近韓国から報告されたブタ由来株はgenotype IIIの米国株と日本株の近くに来るという[41]．また，genotype IIはメキシコからの一本の株だけで代表されて来たが，最近の報告によればナミビアの株がこのグループに新たに加わった[42]．

扨て，分子系統樹は静止画像であるかに見えて実は動画である．配列決定された夫々のウイルス株には，その株が存在した『時』がある．時を巻き戻して行くと，次第に枝の長さも枝分かれの数も減少して行き，最期には一点に収束する．それが最初の先祖ウイルスである．そこまで辿り着かなくても，せめてgenotype IIIの中の米国株と日本株と韓国株が何時枝分かれしたのかぐらいは知りたいところである．何故なら，それが解かれば，HEVのグローバライゼーションの背景に如何なる社会的歴史的事実が存在したのかを推察出来るからである．その推察が可能になった暁には，感染症一般の国際化を未然に防ぐ為の方策が立

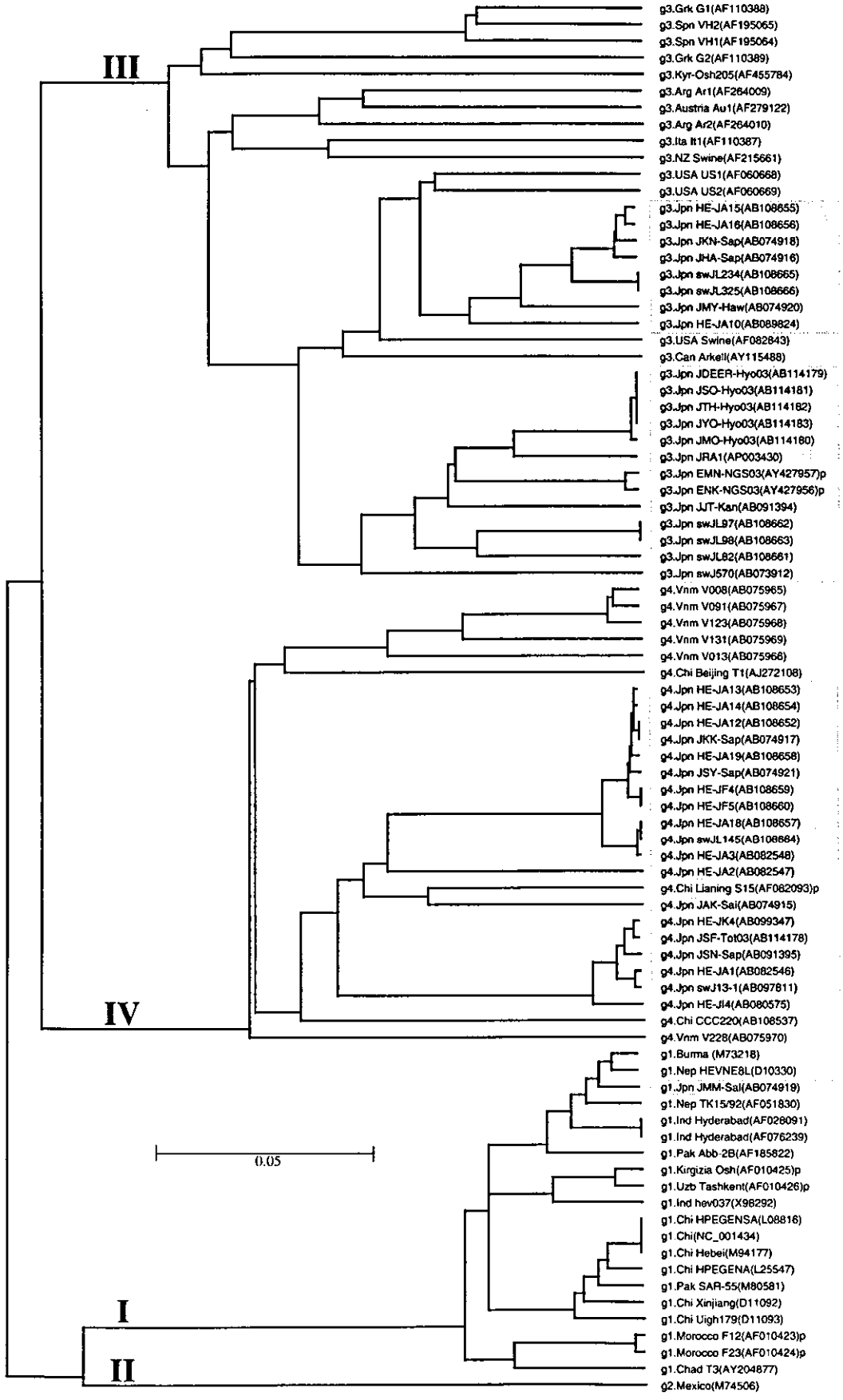

Fig. 1 分子系統樹(日本の株を網掛けで示す)

てられる．その為には，系統樹動画の巻き戻しをせねばならず，その為にはHEV genomeの進化速度を知らねばならない．筆者等の試算によれば，それは約1.4×10^{-3} base substitutions per site per yearである（論文未出）．

4) Zoonotic transmission

1997年に米国のブタからHEVが採れ，それがヒトのHEVに非常に近縁のウイルスである[43]と解って以来，zoonosis（人畜共通感染症）の可能性を示唆する報告が相次いだ[37,44~47]が，2000年の時点では未だ，zoonosisの最も積極的な支持者であるMengですら“Is hepatitis E a zoonosis?”と敢えて『疑問符』を付した論文タイトル[48]を余儀なくさせられるような状況が続いていた．状況証拠は有るものの直接証拠が無かったから，孤軍奮闘の彼は，迂闊なことを書いてPork Statesの有力議員等から圧力が掛かったりするのを恐れたのである．

しかし2001年以降，zoonosis仮説を支持する報告が米国以外の国からも続々と出される[49~55]に及んで，Mengも「米国の飼育ブタの80%以上はHEVに感染した証拠を有つ」と堂々と発言出来るようになったのである（因みに日本のブタは100%）．

剰え，2003年には，日本から出た一連の仕事によってzoonosis仮説が殆ど決定付けられた．先ず，Tei, et al[56]は，日本の野生ジカのサシミを食べて4人の日本人男性がHEVに感染した事例に於いて，食べ残しの保存シカ肉から採れたHEVの塩基配列と患者からのそれとが一致することを証明し，以て世界初の直接証拠を提供した．また，以下は間接証拠であるが，Matsuda, et al[57]は，野生イノシシのナマギモを食べた後で重症のE型肝炎に罹患した2人の日本人男性（1人は劇症肝炎で死亡）の事例を報告したし，Yano, et al（論文未出）は，イノシシBBQパーティー後に発生した集団HEV感染を報告し，Yazaki, et al[58]は，感染患者にはブタレバーを食べたものが多く且つ市販ブタレバーの一部にHEV RNAを検出したと報告した．ブタHEV株とヒトHEV株が全長塩基配列で99%も一致する例があると報告したのも日本からである[59]．

かくて，HEVの感染ルートの一つとしてzoonotic transmissionが存在することは最早疑いがない．しかし，かかるハイリスク食品を食べたアナムネのないE型肝炎患者も多数存在するから，その他の感染ルートの解明も急がれる．例えば，これは未だ1例報告でしかないが，ペット猫からの感染を疑わせた事例も報告されている[60]．

5) Blood-borne transmission

HEV spreadの基本感染経路はfecal-oral transmissionであるが，感染初期にはviremiaが出現する（意外に長期に持続する[39]）ので，輸血やdrug abuse等によるblood-borne transmissionも発生し得る．Endemic areasではこの感染ルートの存在が夙に知られていた[61,62]が，non-endemic countriesに於いても，例えば日本では2002年にMatsubayashi, et al（論文未出）が輸血後E型肝炎例を報告した．外国からは，献血者血漿をサルに接種してE型肝炎を再現せしめたという報告もある[63]．

日本赤十字社の現在進行形の調査によれば，ALT検査でスクリーンアウトされたドナー血の中には無視し得ぬ頻度でHEV RNAが含まれている．上述した輸血後E型肝炎例のドナーは，不顕性感染のウインドウ期献血であったが，そのようなドナーが如何程存在するかは，ALT normalドナーの調査結果を待たねばならない．ドナースクリーニングの必要性を占う意味でも，今後の重要課題の一つである．

6) Differences by regions

世界レベルで見てendemic areasとnon-endemic areasが存在するのと同様に，国レベルで見てもその中に多発地域と寡発地域が存在する．しかも，地域毎にユニークなHEV strainsが存在する．日本では，おしなべて『東高西低』のパターンであり，特に北海道に多発する．この地域差の一部は食習慣の差異で説明し得るかもしれないが，真相は未だ不明の墟である．宿題の多い研究領域である．

3．臨　　床

E型肝炎はA型肝炎と臨床的観点のみからは識別し得ないとされており，通常はacute self-limited hepatitisを起こすだけである．臨床面で問題となるのは，劇症肝炎と診断である．

1) Fulminant hepatitis

HEVが劇症肝炎の原因の一つである可能性は早くから指摘されていた[64~68]．特に，妊婦がHEVに感染すると20%強が劇症肝炎で死亡するとのインドからの報告（筆者註：原著論文が見当たらない！）が非常に有名であるが，これには疑問符を投ずる研究者が多い．例えばFagan, et al[69]は，HEV感染の診断が不確かであると指摘するし，Meng, et al[70]は，妊娠した若いブタにHEVを接種したところ感染は成立した

が劇症肝炎は再現出来なかった，と報告している．(この Meng, et al の論文には，vertical transmission は起らなかったことも記載してある)．よって，妊娠が HEV 感染の増悪因子であることを示すポジティブな証拠は未だ得られないでいる．

日本の劇症肝炎に於ける HEV の関与の度合いについては，厚生労働省研究班による調査が進行中であるが，HBV と HAV に較べては明らかにマイナーな原因である．但し，北海道や岩手に於いてはその限りでない[71,72]．これまでに確認された日本のE型劇症肝炎は未だ20例に満たないが，その少数の症例に基づいて総括すれば，明らかに中年以上の男性に多く発生している．妊婦は1例もない．

2) Diagnosis

HEV 感染の血清学的診断は，HAV や HBV や HCV のそれのレベルまでには未だ到達していない．

1998年に，米国の CDC と NIH の主導する Hepatitis E Virus Antibody Serum Panel Evaluation Group が，164本の暗号化血清検体を用いて12種類の抗体アッセイキットの性能を検定したことがある[73]．その結果は『惨憺たるもの』だった．肝炎を実際に起こした患者に由来する検体については或る程度の正答率を示したが，normal population 由来の検体についてはキット間のバラツキが著明に見られたので，その論文の著者等は "anti-HEV seroprevalence data in non-HEV-endemic countries may be unreliable and should be interpreted with caution" と結論したのである．

その後，抗体検出系は随分と改良されては来たが，上記の結論は今でも大筋に於いて不変である．即ち，false negative は減少して来たものの false positive (特に IgM 抗体) は今猶散見されるのである．従って，臨床例のより確かな診断の為には抗体検出に加えて HEV RNA genome 検出をも行う必要がある．HEV は diversity が高いウイルスだから，primer を選択するに際しては，様々な genotypes や variants を数多く配列決定し報告した実績のあるラボからの論文[75,76]を参照するのが安全である．

4．おわりに

我が国はE型肝炎研究に関しては明らかに後進国であった．その証拠に，Entrez PubMed を『title に hepatitis E を含む』で検索すると，1990年から2000年までの間には我が国からの論文が13本(全体が541本だから僅か2.4%)しかない．ところが，2001年にはその数字が6.3%へと上昇し，2002年には9.9%，そして2003年には吃驚仰天35%へと跳ね上がっている(一寸これはヤリスギかもしれない)．

この調子で我が国の研究者達がE型肝炎の謎の全解明へ向けて邁進し続けてくれるだろうと信じつつ念じつつ，擱筆．

(後　註)

本稿を本誌編集委員長の清澤研道先生に見て頂いたところ，「"原著論文が見当たらない" というのはこれのことでせう」とて Khuroo, et al[76] をファックスして頂いた．これこれ，確かにこの論文である．HEV が分子クローニングされる10年も前に書かれたこの論文から『インド/妊婦/劇症肝炎/HEV ストーリー』は始まったのである．そしてそのストーリーの語り部は殆ど Khuroo 只一人であり続けて来た．2003年に発表された彼の最近の論文[77]によれば，*Forty-seven (61.8%) of the 76 pregnant women developed fulminant hepatic failure (FHF), …… Thirty-four (10.1%) nonpregnant women developed FHF, …… FHF had occurred in four (40%) of 10 patients with HE in first trimester as against 41 (74.5%) of 55 patients in second trimester and beyond (P＝0.015)* という調子で，この物語は益々エスカレートしているように見える．ただ，一寸奇妙なことに，その2カ月後に同じジャーナルに発表された彼の論文[78]には，*Early predictors of a poor outcome are non-E aetiology, prothrombin time ＞30 s, grade of coma ＞2 and age ＞40 years*……(下線筆者)と書かれている．ひょっとして，インドの妊婦は病原体の如何に拘わらず劇症肝炎を起こしやすいのだろうか？

彼はまた，HEV は非常に高率に垂直感染するというデータ(感染妊婦8名から生まれた6名の児に HEV 感染を認めたと)も報告している[79]．しかし，幸か不幸か，本文でも述べたように動物実験の成績は彼のデータをサポートしない[70,80]．

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