Table 1. *Trapping sites, collected rodent species, and seropositivity for* L. interrogans, *SEOV, rat HEV and* Y. pestis

Location	Species	No. tested	No. seropositive against (% positive)			
			*L. interrogans**	SEOV*	Rat HEV†	*Y. pestis*†
Hanoi City	*R. norvegicus*	60	13 (21·7%)	3 (5%)	10 (16·7%)	0 (0%)
	R. tanezumi	4	0 (0%)	0 (0%)	2 (50%)	0 (0%)
Hai Phong Port	*R. norvegicus*	34	9 (26·5%)	11 (32·4%)	11 (32·4%)	0 (0%)
	R. tanezumi	2	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total		100	22	14	23	0

SEOV, Seoul virus; HEV, hepatitis E virus.
* Seroprevalence determined by ELISA and Western blotting.
† Seroprevalence determined by ELISA.

and Hai Phong Port, respectively ($P=0·60$). SEOV antibody-positive *R. norvegicus* were obtained both in Hanoi City and Hai Phong Port, but the positive rate was higher in Hai Phong (32·4%, 11/34) than in Hanoi (5%, 3/60) ($P<0·01$). There were no *R. tanezumi* seropositive to *L. interogans* and SEOV. Prevalence of antibodies against rat HEV were 16·7% (10/60) in *R. norvegius* and 50% (2/4) in *R. tanezumi* captured in Hanoi and 32·4% (11/34) *R. norvegicus* captured in Hai Phong. There was no significant difference between rat HEV prevalence rates of *R. norvegicus* in Hanoi and Hai Phong ($P=0·08$). There were no rats seropositive to *Y. pestis*.

Body weight, geographical origin, sex and co-infection

The mean body weight of *R. norvegicus* in Hai Phong was significantly greater than that of *R. norvegicus* in Hanoi (336·4 g *vs.* 289·4 g, $P<0·05$). However, no significant difference in sex or rate of maturation stage (juvenile and sub-adults *vs.* adults) of *R. norvegicus* was found between rats captured in Hai Phong and rats captured in Hanoi ($P=0·72$ and $P=0·78$, respectively). The mean body weight of *R. norvegicus* infected with SEOV was significantly greater in both males and females than in SEOV-negative rats (Fig. 1). *L. interrogans* antibody-positive females were also significantly heavier than uninfected female rats. However, no significant body-weight difference was found between *L. interrogans* antibody-positive males and antibody-negative males. No significant body-weight difference was found between HEV-infected rats and uninfected rats.

All of the rats with antibodies against *L. interrogans* and SEOV were adult *R. norvegicus* with body weights of >260 g and >340 g, respectively. On the other hand, rat HEV antibody-positive *R. norvegicus* were found in juveniles and sub-adults: one male (84 g) and 4/15 females (100–170 g). There was no infant rat with maternal antibody.

Although no significant difference was found, female *R. norvegicus* tended to be more frequently infected than males with *L. interrogans* (28·3% *vs.* 17·1%, $P=0·20$), SEOV (17·0% *vs.* 12·2%, $P=0·52$) and rat HEV (28·3% *vs.* 14·6%, $P=0·11$).

ORs were calculated in seropositive rats to examine the particular combination of co-infection. ORs of co-infection with *L. interrogans* and SEOV, SEOV and rat HEV, and *L. interrogans* and rat HEV were 2·0 (95% CI 0·56–6·70, $P=0·20$), 1·4 (95% CI 0·35–4·89, $P=0·64$), and 1·4 (95% CI 0·43–4·04, $P=0·64$), respectively. Thus, no significant ORs were obtained in any combination.

Molecular characterization of SEOV

Lung specimens of all *R. norvegicus* and *R. tanezumi* were examined for their virus genome by real-time PCR. All but two of the specimens from seropositive rats were positive by real-time PCR. No real-time PCR-positive specimen was obtained from sero-negative rats.

Based on the real-time PCR results, six of the specimens that showed strong positivity were selected and subjected to reverse transcriptase-PCR. Finally, five S-segment sequences and six M-segment sequences were successfully recovered. The phylogenetic trees were drawn using 1378 nt (194–1571) of the S segment and 1101 nt (1966–3066) of the M segment (Fig. 2). All of the sequences were included in the SEOV clade both in the S-segment and M-segment phylogenetic trees. SEOV from Hai Phong

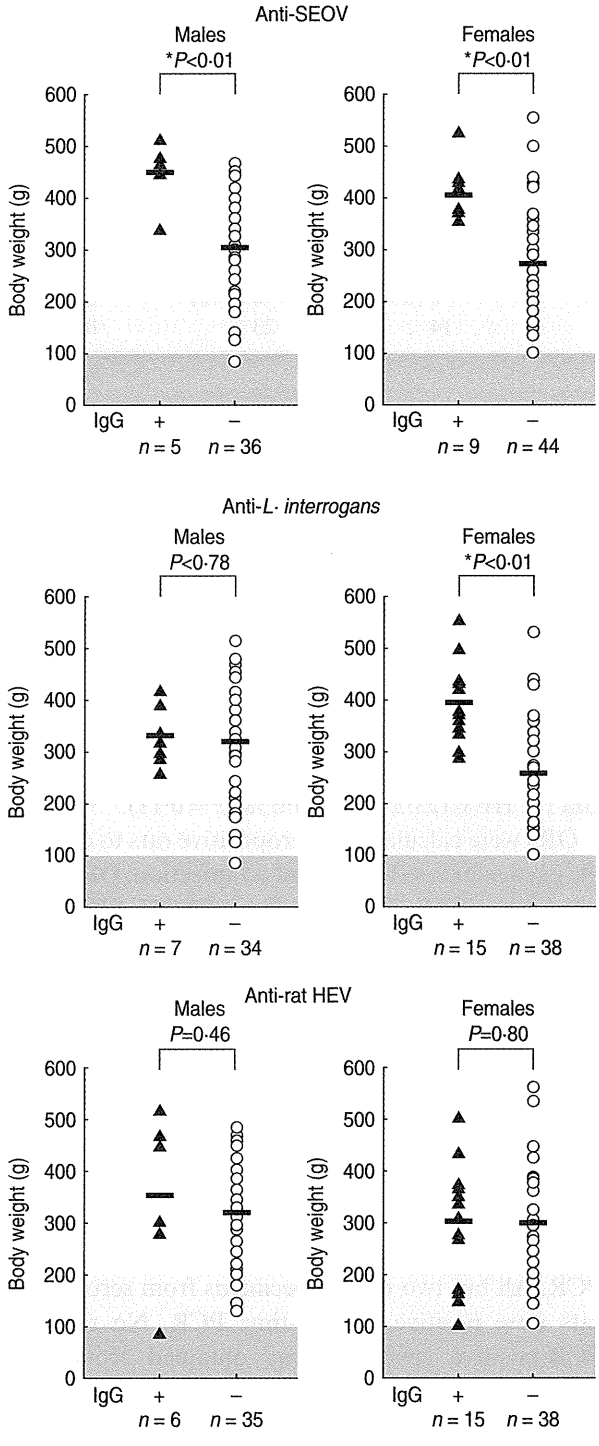

Fig. 1. Relationship between seroprevalence and body weight in *R. norvegicus*. Solid line indicates mean body weight. The grey and light-grey shaded areas indicate juveniles (< 100 g) and sub-adults (100–200 g), respectively. An asterisk indicates statistical significance at $P < 0{\cdot}01$.

(HaiPhong3-11, 17-11, 24-11, 31-11), and SEOV from Southern Vietnam (5CSG and 11CSG), formed one group, which was separated from the other group consisting of SEOV from Hanoi and Singapore.

DISCUSSION

The present study provides information regarding the prevalence of *L. interrogans*, SEOV and HEV in wild rats in urban areas in Hanoi City and Hai Phong Port in Northern Vietnam. The seroprevalence of *L. interrogans* and rat HEV in rats was high in both cities. Various prevalence rates of *Leptospira* and hantavirus infection in wild *Rattus* spp. have been reported in several countries in Asia: reported prevalence rates of *Leptospira* were 5–30% [19–25] and those of hantavirus were 5–20% [5, 26–30]. Our results regarding the seroprevalence of *L. interrogans* and SEOV are consistent with those of previously reported studies. Therefore, our results confirm the potential hazard to humans. A recent study provided evidence for the presence of anti-rat HEV IgG in forestry workers in Germany [11]. However, the relationship between rat HEV and human disease is still unclear. Therefore, further seroepidemiological studies in cryptogenic hepatitis patients should be conducted.

Although the relationship between each seroprevalence and body weight in *R. norvegicus* was reanalysed with the entry of geographical information to discover the relationship to geographical origin, the mean body weight of male *R. norvegicus* infected with SEOV, *L. interrogans* and rat HEV both in Hanoi and Hai Phong was not significantly different from the mean body weight of uninfected rats ($P > 0{\cdot}09$) (data not shown). On the other hand, the mean body weight of female *R. norvegicus* infected with SEOV and *L. interrogans* both in Hanoi and Hai Phong was significantly greater than the mean body weight of uninfected rats ($P < 0{\cdot}05$) (data not shown). Female *R. norvegicus* infected with rat HEV in Hanoi tended to be heavier than uninfected rats (340·4 g *vs.* 261·7 g, $P = 0{\cdot}08$). Interestingly, rat HEV antibody-positive female *R. norvegicus* in Hai Phong were lighter than uninfected female rats (261·8 g *vs.* 363·6 g, $P < 0{\cdot}05$). However, the reason for the inverse correlation between body weight of rat HEV antibody-positive female *R. norvegicus* in Hai Phong and Hanoi is unclear. Further longitudinal studies are needed to clarify the relationships regarding geographical origin, sex and weight factors.

L. interrogans and SEOV were detected only in adult *R. norvegicus* with body weights of > 260 g and > 340 g, respectively. Furthermore, antibody-positive rates increased with weight (age), suggesting that *L. interrogans* and SEOV are maintained in reservoir

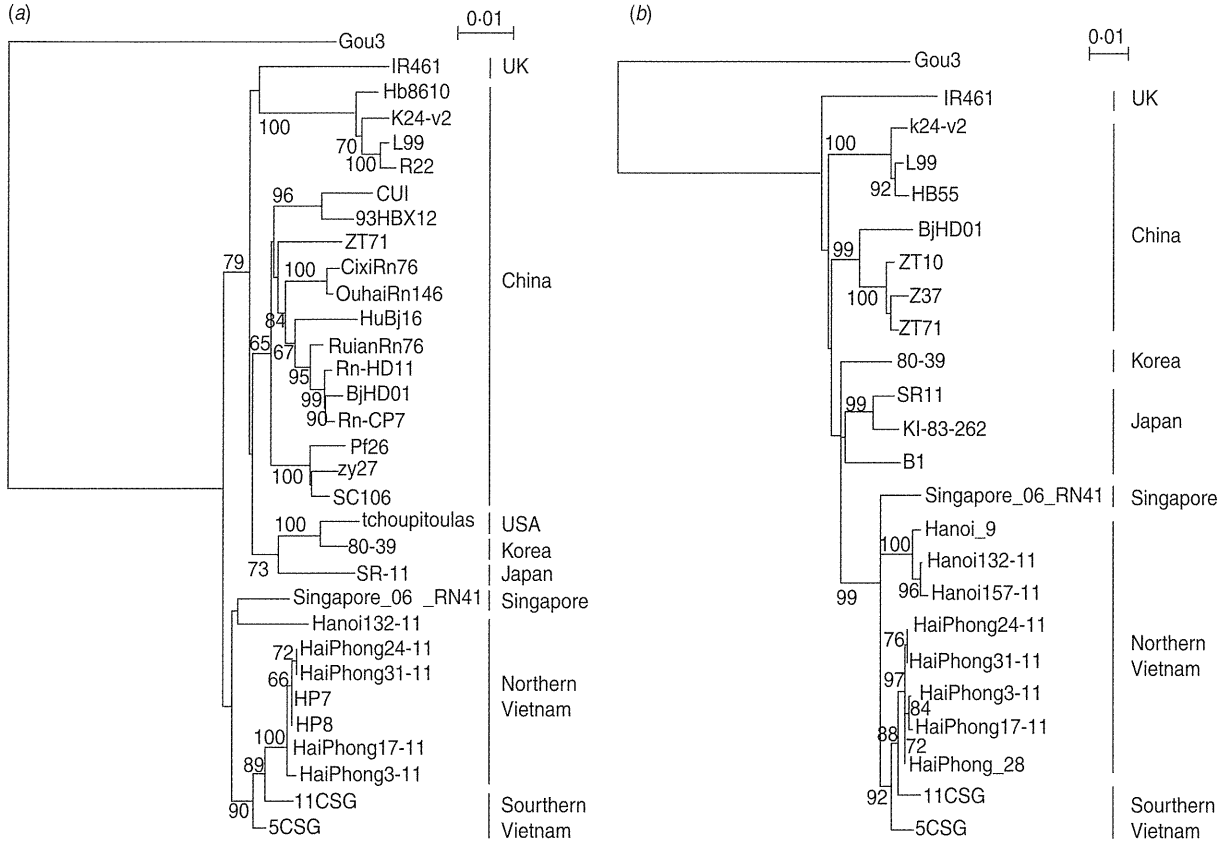

Fig. 2. Phylogenetic analysis of SEOV derived from Hanoi City and the Hai Phong Port area. (*a*) Neighbour Joining (NJ) analysis of hantavirus based on 1378 nt from the S segment. Sequences of SEOV strains Gou3 (AF184988), IR461 (AF329388), Hb8610 (AF288643), K24-v2 (AF288655), L99 (AF288299), R22 (AF288655), CUI (GQ279395), 93HBX12 (EF192308), ZT71 (AY750171), CixiRn76 (FJ803206), OuhaiRn146 (FJ803210), HuBj16 (GQ279380), RuianRn (FJ803216), Rn-HD11 (GQ279392), BjHD01 (AY627049), Rn-CP7 (GQ279383), Pf26 (AY006465), zy27 (AF406965), SC106 (GU361893), tchoupitoulas (AF329389), 80-39 (NC_005236), SR-11 (M34881), Singapore_06_RN41 (GQ274944), 11CSG (AB618113) and 5CSG (AB618112) were used. (*b*) NJ analysis of hantavirus based on 1103 nt from the M segment. Sequences of SEOV strains Gou3 (AF145977), IR461 (AF458104), k24-v2 (AF288654), L99 (AF288298), HB55 (AF035832), BjHD01 (DQ133505), ZT10 (DQ159911), Z37 (AF187081), ZT71 (EF117248), 80-39 (S47716), SR11 (M34882), KI-83-262 (D17594), B1 (AB457794), Singapore_06_RN41 (GQ274942), Hanoi_9 (AB355732), HaiPhong_28 (AB355731), 11CSG (AB618131) and 5CSG (AB618130) were used. Our sequence data for Vietnamese SEOV derived from *R. norvegicus* captured in Hanoi City (S segment; Hanoi132-11) (M segment; Hanoi132-11 and Hanoi157-11) and Hai Phong Port (S segment; HP7, HP8, HaiPhong3-11, HaiPhong17-11, HaiPhong24-11 and HaiPhong31-11) (M segment; HaiPhong3-11, HaiPhong17-11, HaiPhong24-11 and HaiPhong31-11) including our previous data were compared with the published sequence.

populations by horizontal transmission [31, 32]. The lower antibody-positive rates in juvenile *R. norvegicus* might be due to maternal antibodies that prevent vertical transmission [33, 34]. Since the infection rate in sub-adult individuals without maternal antibodies is low, it is speculated that the efficiency of horizontal transmission of the hantavirus is low.

On the other hand, there were juvenile and sub-adult *R. norvegicus* infected with rat HEV. The manner in which rat HEV is transmitted in rats is still unknown. Our data indicated that rat HEV might have vertical transmission in addition to horizontal transmission in rodents. In human cases, vertical transmission of HEV has been reported [35, 36]. In fact, HEV RNA was detected by PCR in cord or birth blood samples of infants born from acute HEV-infected mothers, indicating that HEV is commonly transmitted from infected mothers to their babies [36, 37]. Further experiments on wild rats or laboratory rats are required to demonstrate vertical transmission of rat HEV in rats.

Nevertheless, the density of *R. norvegicus* in Hanoi was higher than that in Hai Phong as indicated by the trapping rate, and the seroprevalence of *L. interrogans*, SEOV and rat HEV in Hanoi was lower than in Hai Phong. It has been reported that the

prevalence of hantavirus in *Peromyscus maniculatus* in North America and that of hantavirus in *Myodes glareolus* in Europe, in which rodents have a seasonal fluctuation of population density, were higher just after the seasonal high population density [38, 39]. However, seasonal patterns in the prevalence of SEOV and *L. interrogans* were not observed in *R. norvegicus* in previous studies [40–42]. Therefore, further longitudinal studies are needed to clarify the relationship between density and *R. norvegicus* seroprevalence in Vietnam.

Our results show that female *R. norvegicus* were more frequently infected with SEOV, *L. interrogans* and rat HEV than males. On the other hand, field studies on SEOV infection in rodents have shown that a higher percentage of infected individuals is commonly observed to be males [43]. Nuttall and Krojgaard *et al.* found no sexual difference in rates of infection with *L. interrogans* in rats [44, 45], whereas Easterbrook *et al.* suggested that female rats are more prone to infection [42]. The reasons for the female-biased SEOV, *L. interrogans* and rat HEV infections are unknown.

Easterbrook *et al.* previously reported that there was a correlation between prevalence of *L. interrogans* infection and HEV infection in *R. norvegicus* but not between SEOV infection and *L. interrogans* or HEV infection [42]. In the present study, no significant correlation was found in any of the pathogens tested. The reasons for no correlation in the pathogens are unknown.

All hantavirus genome-positive specimens were also positive by serological assay in this study. This result provides convincing evidence that animals chronically infected with hantavirus have specific antibodies as reported previously [46].

In our previous phylogenetic study, the M segment of SEOV from Hai Phong formed a distinct clade from those of SEOV from Hanoi [5]. Phylogenetic analyses of the S- and M-segment nucleotide sequences indicated that SEOVs from Hai Phong and Hanoi form different clades. Furthermore, the SEOV from Hai Phong was placed more closely to SEOV from Saigon Port in Ho Chi Minh City (11CSG and 5CSG) compared to SEOV from Hanoi. The cytochrome *b* sequences of *R. norvegicus* in Saigon and some *R. norvegicus* in Hai Phong were identical, but there were small differences between cytochrome *b* sequences of *R. norvegicus* captured in Saigon and Hanoi and between cytochrome *b* sequences of *R. norvegicus* captured in Hanoi and Hai Phong (data not shown). These results indicate that *R. norvegicus* has recently moved between Saigon and Hai Phong. Together with the phylogenetic tree of SEOV, these results suggest that SEOV in Hai Phong might have been transported from Saigon Port with *R. norvegicus*. However, since the distance between Hanoi and Hai Phong is only about 90 km, it is also speculated that variable SEOVs were able to be separately maintained.

Taken together, serological evidence of human pathogens, *L. interrogans*, SEOV and rat HEV, was obtained in *Rattus* spp. captured in urban areas of Northern Vietnam, Hanoi and Hai Phong. Further differential diagnosis of AFI in humans is needed to determine the number of cases of each infection, and continued rodent surveillance is important to estimate the emergence of rodent-borne diseases.

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DECLARATION OF INTEREST

None.

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Original Article

Three cases of acute or fulminant hepatitis E caused by ingestion of pork meat and entrails in Hokkaido, Japan: Zoonotic food-borne transmission of hepatitis E virus and public health concerns

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Aim: In developed countries including Japan, the transmission route of indigenous hepatitis E virus (HEV) infection is obscure. Accordingly, public health implications of indigenous HEV infection have not been well addressed. The aim of this study was to clarify the route of transmission of a small outbreak of acute hepatitis E and assess the public health implications of indigenous zoonotic HEV transmission.

Methods: Three patients with non-A, B and C acute hepatitis, two of whom presented in a critical condition, were assessed for HEV infection using polymerase chain reaction and their route of infection; the genome sequences of the infecting HEV were also analyzed. A phylogenetic tree based on the full, or near full, HEV RNA sequences were constructed by neighbor-joining method.

Results: All three patients ingested grilled pork meat and entrails at the same barbecue restaurant in Abashiri, Hokkaido, Japan. When comparing partial to entire, or nearly entire, nucleotide sequences of HEV detected in these patients, they were 99.9–100% identical to each other. These genotype 4 isolates had great resemblance to the genome sequences of the isolates from the mini-outbreak in 2004 in Kitami, a city adjacent to Abashiri. These Kitami/Abashiri strains were segregated into a single cluster on the phylogenetic tree of HEV genotype 4 indigenous to Japan.

Conclusion: Indigenous HEV transmission via a zoonotic food-borne route has been demonstrated in Kitami and Abashiri via pork meat and entrails contaminated with virulent HEV strains. Because a similar outbreak can recur in the future, infection sources and distribution routes should be clarified rapidly for public health.

Key words: fulminant hepatitis, genotype 4, hepatitis E, Kitami/Abashiri strains, zoonosis

INTRODUCTION

EVIDENCE NOW SHOWS that the hepatitis E virus (HEV) infection is no longer confined to developing countries. HEV transmission routes specific to industrialized societies have been eagerly investigated.[1–5] These studies have shown a zoonotic transmission of HEV from ingestion of the meat of deer, wild boars and pigs in industrialized countries.[3,6–9] It is unclear, however, whether these instances of zoonotic HEV transmission have occurred randomly. Mini-outbreaks of HEV infection may inevitably repeat themselves even in developed countries, especially if the society concerned has a history of zoonotic transmission specific to the regional livestock cultivation and distribution industries.

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A mini-outbreak of acute HEV infection in Abashiri, a city in the northern Japanese prefecture of Hokkaido, has occurred. In the course of the investigation for the transmission route of indigenous HEV, attention has been given to a similar mini-outbreak of hepatitis E occurring 2 years previously at Kitami, another city situated 50 km from Abashiri.[8,9] This finding has provided an opportunity to address the public health implications of sporadic community-acquired cases of HEV infection in Japan by comparing viral sequences of Abashiri's acute HEV patients with those of Kitami's patients.

In the present study, the route of transmission in a mini-outbreak of acute hepatitis E is clarified, and the public health implications of indigenous zoonotic HEV transmission in Japan is also investigated.

METHODS

Patients

THREE PATIENTS EXHIBITING the features of non-A, B and C acute hepatitis presented in Hokkaido, Japan. Although different physicians treated the patients at different institutions, all of them ate pork meat and entrails together at a barbecue party in a restaurant at Abashiri on 1 February 2006. The ingested pork meat and entrails were grilled by gas cookers during concurrent alcohol consumption. It was unclear whether they were thoroughly cooked or not.

The clinical profiles of the patients are shown in Table 1. Serum anti-hepatitis B core antigen immunoglobulin (Ig)M, hepatitis C virus RNA, and anti-hepatitis A IgM were not detected in any patient although patient

Table 1 Characteristics and clinical features in patients with acute HEV infection

Patient characteristics, laboratory data and outcome	Patient number		
	1	2	3
Age (years)/sex	53/male	58/male	56/male
Symptoms	Jaundice, malaise	Appetite loss	Fever
Estimated time of infection	1 February	1 February	1 February
Time of onset	Fourth week of March	First week of March	First week of March
Underlying liver disease†	None	None	Inactive HBV carrier
Alcohol intake	30 g/day, 33 years	30 g/day, 20 years	30 g/day, 30 years
Peak AST (IU/L)	297	9045	3266
Peak ALT (IU/L)	929	5297	4468
Peak total bilirubin (mg/dL)	12.6	2.6	10.3
Lowest prothrombin time (%), INR	74.0, 1.25	38.0, 2.04	16.6, 4.82
HEV RNA/genotype	+/4	+/4	+/4
Anti-HEV IgG/OD‡ value	+/2.170	+/2.878	+/1.761
Anti-HEV IgM/OD value	+/3.118	+/2.878	+/1.761
Anti-HAV IgM	<0.8	<0.8	<0.8
HBsAg (IU/mL)	0.01	0.05	10.74
Anti-HBc IgM	1.7	<0.09	<0.09
HBV DNA (log copies/mL)	<2.6	n.t.§	<2.6
Anti-HCV (U)	<1.0	<1.0	<1.0
HCV RNA (IU/mL)	<50	<50	<50
Disease progression	Self-limited hepatitis	Acute severe hepatitis¶	Fulminant hepatitis††
Outcome	Survived	Survived	Survived

Normal range: AST, aspartate aminotransferase (10–40 U/L); ALT, alanine aminotransferase (5–45 U/L); total bilirubin (0.2–1.0 mg/dL); prothrombin time (80–100%, 0.84–1.14); anti-HEV IgG, hepatitis E virus immunoglobulin G (<0.191); anti-HEV IgM (<0.447); anti-HAV, hepatitis A virus IgM (<0.8); HBsAg, hepatitis B surface antigen (<0.06); anti-HBc, hepatitis B core IgM (<0.9); HBV, hepatitis B virus DNA (<2.6 log copies/mL); anti-HCV, hepatitis C virus (<1.0); HCV RNA (<50 IU/mL).
†Underlying liver disease defined as disease which had been diagnosed before onset of hepatitis E.
‡"OD", optical density.
§"n.t.", not tested.
¶"acute severe hepatitis" defined as hepatitis with ≤40% in lowest prothrombin time without hepatic coma.
††"fulminant hepatitis" defined as hepatitis with hepatic coma within 8 weeks after onset.

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3 had hepatitis B surface antigen. The clinical courses of these patients differed considerably with patient 1's showing a self-resolving course, patient 2's presenting with serious coagulopathy due to severe hepatitis and patient 3's developing fulminant hepatitis with recovery within 3 months after hospital admission.

Among the nearly 40 customers having eaten the grilled pork meat and entrails at the same restaurant in Abashiri on the same day, 11 of them (nine men and two women) voluntarily agreed to be checked for HEV. The biochemistry, anti-HEV IgM and IgG, and HEV RNA were checked using blood sample collections at 25–29 weeks after the dinner on 1 February 2006.

Informed consent was obtained from all patients and volunteers after explaining the nature and purpose of the study; approval for this study was obtained from the hospital's institutional review board. The study protocol conformed to guidelines provided in the Declaration of Helsinki for clinical trials.

Detection of HEV-related antibodies

Serum anti-HEV IgG and IgM were determined by using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Viragent HEV-Ab; Cosmic, Tokyo, Japan).

Detection of the full-length sequences of HEV isolates

Detection and nucleotide sequencing of the serum HEV RNA were performed by methods described previously.[10,11] Briefly, the nucleic acids were extracted from the serum with commercial kits (Smitest EX-R&D; Genome Science, Fukushima, Japan). The nucleotide sequences of HEV were reverse transcribed to cDNA and amplified by polymerase chain reaction (PCR) in 17 overlapping regions with 20-mer primers deduced from the nucleotide sequences of HEV deposited in the international DNA Data Bank of Japan (DDBJ)/GenBank/European Molecular Biology Laboratory (EMBL) database. Reverse transcription was performed, and the first and second round of PCR was carried out in the presence of Platinum *Taq* DNA Polymerase High Fidelity (Invitrogen, Carlsbad, CA, USA). The final products were sequenced in a 377 DNA sequencer. The sequences rich in G-C were amplified, and those not amplifiable by the above PCR methods were subjected to PCR with primers deduced from adjacent 5′- and 3′-sequences. The 5′- and 3′-terminal sequences were amplified with 5′-Full RACE Core Set (TaKaRa Bio, Shiga, Japan) and Oligo (dt) 20 primer (Invitrogen), respectively.

Phylogenetic analyses of HEV isolates

A phylogenetic tree based on the full, or nearly full, HEV RNA sequence was constructed by the neighbor-joining method. Analyses were performed with the use of computer software (GENETYX-MAC ver. 13.0; Genetyx, Tokyo, Japan).

RESULTS

Diagnosis of acute HEV infection in 4 patients

SERUM HEV RNA was detected in all three patients in the early phases of hepatitis. Given the clinical presentations, absence of markers of acute hepatitis A, B and C, and the presence of HEV RNA and anti-HEV, these patients were diagnosed with acute hepatitis E (Table 1) infection. In addition, among the 11 volunteers who attended the same barbecue party, a 51-year-old man who was a colleague of patient 3 showed an elevation of both serum anti-HEV IgG and IgM; he, however, had normal alanine aminotransferase levels and no HEV RNA by PCR for which he was, therefore, diagnosed with an asymptomatic HEV infection. None of the four patients above dined together again after 1 February 2006.

Similarity of nucleotide sequences of the HEV in three patients with acute hepatitis E in Abashiri

Full-length sequences in 7255 nucleotides were determined for genotype 4 HEV from patient 2 (JMM-Aba06C) and patient 3 (JKO-Aba-FH06C). Also, partial nucleotide sequences containing 432 nucleotides in the replicase region and 1136 nucleotides in the ORF2 region of HEV were analyzed for patient 1 (JKU-Aba06). Comparison of the full-length sequence between JMM-Aba06C and JKO-Aba-FH06C revealed only six nucleotide differences out of 7255 nucleotides with a sequence homology of 99.92% (Table 2). In addition, when these three isolates were compared among each other for 1568 nucleotide sequences, JKU-Aba06 (patient 1) showed 100% identicalness with JKO-Aba-FH06C (patient 3) and 99.94% with JMM-Aba06C (patient 2). The GenBank/EMBL/DDBJ accession numbers for the nucleotide sequences of HEV isolates were AB291967–70.

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Table 2 Nucleotide sequence analyses for the hepatitis E virus strains from the mini-outbreaks in Kitami and Abashiri

Isolate names/regions	Overlap match (%) where mini-outbreak occurred				
	JKU-Aba06	**JMM-ba06C**	**JKO-Aba-FH06**	HRC-HE14C	JTC-Kit-FH04L
JKU-Aba06/Abashiri					
JMM-Aba06C/Abashiri	99.94				
JKO-Aba-FH06C/Abashiri	**100**	**99.92**			
HRC-HE14C/Kitami	99.11	99.38	99.43		
JTC-Kit-FH04L/Kitami	98.92	99.43	99.49	**99.86**	
JST-Kit04C/Kitami	99.11	99.38	99.43	**99.99**	**99.99**

Full, or nearly full, length nucleotide sequences were determined and compared to each other except for JKU-Aba06.
Among JKU-Aba06 and 5 other strains, 1568 nucleotide sequences containing 432 nucleotides in the replicase region and 1136 nucleotides in ORF2 were compared.
Bold letters represents the strains separated in Kitami and Abashiri regions in Hokkaido Prefecture, Japan.

Close relationship of HEV genome between mini-outbreaks in Abashiri in 2006 and Kitami in 2004

To assess any relationship between HEV isolates of the present mini-outbreak in Abashiri in 2006 with those in Kitami in 2004, partial or entire genome sequences of Abashiri isolates such as JKU-Aba06, JMM-Aba06C and JKO-Aba-FH06C were compared with Kitami isolates HRC-HE14C, JTC-Kit-FH04 and JST-Kit04C.[8,9] JMM-Aba06C and JKO-Aba-FH06C showed 99.38–99.49% identicalness with HRC-HE14C, JTC-Kit-FH04L and JST-Kit04C (Table 2). These Abashiri isolates, JMM-Aba06C and JKO-Aba-FH06C, revealed merely 37 or 41 nucleotide differences from JTC-Kit-FH04L and 41 or 45 nucleotide differences with JST-Kit04C. The chronological intervals of blood sampling were 513–538 days in these two mini-outbreaks.

Persistence of similar HEV genome at Kitami and Abashiri

In addition to the mini-outbreak of acute hepatitis E at Kitami in 2004, sporadic cases of the same disease were also reported around Kitami and Abashiri. A comparison of the entire, or nearly entire, nucleotide sequences of the HEV genomes of the seven isolates separated in Kitami and Abashiri and one isolate identified in Monbetsu (70 km from Kitami and 90 km from Abashiri) was made (Table 3). Two HEV strains, HE-JA36 and HE-JA41, were isolated from the patients in Kitami, and HE-JA28 was separated from the patient in Monbetsu (H. Okamoto, Jichi Medical School, Tochigi, Japan, pers. comm.). The data demonstrated 99.3–99.9% homology among these eight isolates. The constructed phylogenetic tree based on the full-length genome analysis confirmed that strains obtained in Kitami and Abashiri belonged to genotype 4 indigenous to Japan. A single cluster segregated from adjacent strains separated in other parts of Hokkaido, Japan (Fig. 1b) was gathered. The Kitami/Abashiri strains showed only 84–88.3% sequence homology with HEV genotype 4 isolates detected from other regions in Japan (Fig. 1b, Table 3). The genome sequence of case 1 was not included in this phylogenetic tree because only partial sequences of the HEV genome were done.

Absence of nucleotide substitution at nucleotide 1816 and 3148 in Kitami/Abashiri strains responsible for fulminant hepatitis

Because nucleotide substitution at nucleotide 1816 and 3148 in genotype 4 HEV was reported to be significantly associated with fulminant hepatitis,[12] nucleotide sequences were compared in JKO-Aba-FH06C and JTC-Kit-FH04L. By analyzing full-length genome sequences, both T at nt 1816 and C at nt 3148 were observed in JKO-Aba-FH06C and JTC-Kit-FH04L.

DISCUSSION

TO DATE, SPORADIC indigenous hepatitis E infections have been reported throughout the industrialized world.[20–24] The transmission routes of HEV in these countries remain obscure although a zoonotic foodborne route has been shown in several instances ascertained molecularly.[3,7,9] Autochthonous HEV infection, therefore, seems to be a growing public health concern even in developed countries.

Hepatitis E virus isolates identified from the three symptomatic acute hepatitis E patients who had eaten

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Table 3 Comparison of entire-length nucleotide sequences in Kitami/Abashiri strains with those in other HEV isolates

Genotype	Isolate name	Accession no.	Host	Diagnosis	Collection date (year/month/day)	Habitat (city, prefecture, country)	Nucleotide length	Nucleotide JKO-Aba-FH06C	Identity (%) JMM-Aba06C	Reference
4	**JKO-Aba-FH06C**	**Current study**	**Human**	**FH**	**2006/3/10**	**Abashiri, Hokkaido, Japan**	**7255**		**99.9**	**Current study**
4	**JMM-Aba06C**	**Current study**	**Human**	**AH**	**2006/3/9**	**Abashiri, Hokkaido, Japan**	**7255**	**99.9**		**Current study**
4	**HE-JA36**	**AB220977**	**Human**	**AH**	**2004/1/6**	**Kitami, Hokkaido, Japan**	**7266**	**99.5**	**99.4**	**Inoue *et al.*[12]**
4	**HRC-HE14C**	**AB291965**	**Human**	**Blood Donor**	**2004/9/20**	**Kitami, Hokkaido, Japan**	**7255**	**99.4**	**99.4**	**Matsubayashi *et al.*[9]**
4	**JST-KitAas04C.**	**AB291966**	**Human**	**AH**	**2004/10/12**	**Kitami, Hokkaido, Japan**	**7255**	**99.4**	**99.3**	**Matsubayashi *et al.*[9]**
4	**JTC-Kit-FH04L**	**AB291959**	**Human**	**FH**	**2004/9/24**	**Kitami, Hokkaido, Japan**	**7209**	**99.4**	**99.3**	**Matsubayashi *et al.*[9]**
4	**HE-JA28**	**AB220976**	**Human**	**AH**	**2002/12/13**	**Monbetsu, Hokkaido, Japan**	**7266**	**99.4**	**99.4**	**Inoue *et al.*[12]**
4	**HE-JA41**	**AB220979**	**Human**	**AH**	**2004/8/17**	**Kitami, Hokkaido, Japan**	**7265**	**99.4**	**99.3**	**Inoue *et al.*[12]**
4	JSM-Sap95	AB161717	Human	AH	1995/3/28	Sapporo, Hokkaido, Japan	7202	98.3	98.2	Takahashi *et al.*[13]
4	HE-JF4	AB220972	Human	FH	2002/10/2	Sapporo, Hokkaido, Japan	7271	97.6	97.5	Inoue *et al.*[12]
4	JKK-Sap	AB074917	Human	AHS	2000/11/10	Sapporo, Hokkaido, Japan	7235	97.6	97.5	Takahashi *et al.*[11]
4	JTS-Sap02	AB161718	Human	AH	2002/9/14	Sapporo, Hokkaido, Japan	7202	97.5	97.5	Takahashi *et al.*[11]
4	JYW-Sap02	AB161719	Human	AH	2002/8/30	Sapporo, Hokkaido, Japan	7202	97.5	97.5	Takahashi *et al.*[11]
4	HE-JF5	AB220973	Human	FH	2002/12/2	Sapporo, Hokkaido, Japan	7270	97.5	97.5	Inoue *et al.*[12]
4	HE-JA19	AB220975	Human	AH	2002/12/24	Sapporo, Hokkaido, Japan	7262	97.3	97.3	Inoue *et al.*[12]
4	HE-JA37	AB220978	Human	AH	2004/1/30	Sapporo, Hokkaido, Japan	7281	97.1	97	Inoue *et al.*[12]
4	HE-JA1	AB097812	Human	AH	1997/12/6	Sapporo, Hokkaido, Japan	7258	88.4	88.4	Nishizawa *et al.*[14]
4	HE-JF3	AB220971	Human	FH	1998/6/19	Mito, Ibaraki, Japan	7262	88.3	88.2	Inoue *et al.*[12]
4	swJ13-1	AB097811	Swine			Hokkaido, Japan	7258	88.3	88.3	Nishizawa *et al.*[14]
4	JSN-Sap-FH02C	AB20239	Human	FH	2002/3/21	Sapporo, Hokkaido, Japan	7251	88.1	88.1	Takahashi *et al.*[15]
4	JYN-Sap01C	AB193177	Human	AH	2001/12/28	Sapporo, Hokkaido, Japan	7256	88.1	88.1	Takahashi *et al.*[15]
4	HE-JK4	AB099347	Human	AH	2002/4/25	Tochigi, Japan	7250	88.1	88	Kuno *et al.*[16]
4	JYN-Nii02L	AB193178	Human	AH	2002/4/30	Niigata, Japan	7154	88.1	88	Takahashi *et al.*[11]
4	JAK-Sai	AB074915	Human	AH		Saitama, Japan	7236	88.1	88	Takahashi *et al.*[13]

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4	JSF-Tot03C	AB193176	Human	FH	2003/3/12	Tottori, Japan	7251	88	88	Takahashi *et al.*[15]
4	swJB-H7	AB481227	Swine			Japan	7253	87.8	87.8	
4	HE-JI4	AB080575	Human	AH	1905/6/22	Tochigi, Japan	7186	87.4	87.3	Takahashi *et al.*[17]
4	HE-JA2	AB220974	Human	AH	1998/9/4	Sapporo, Hokkaido, Japan	7268	86.7	86.6	Inoue *et al.*[12]
4	CCC220	AB108537	Human	AH	2000/6	Changchun, Jilin, China	7193	85.4	85.4	Liu *et al.*[18]
4	E087-SAP04C	AB369688	Human	AH		Sapporo, Hokkaido, Japan	7227	85.2	85.2	
4	IND-SW-00–01	AY723745	Swine			India	7262	84.6	84.6	
4	E067-SIJ05C	AB369690	Human	AH		Shinjuku, Tokyo, Japan	7236	84.5	84.4	
4	swCH31	DQ450072	Swine			China	7248	84.3	84.3	
4	SH-SW-zs1	EF570133	Swine			China	7293	84.3	84.2	
4	swCH25	AY594199	Swine			Xinjian, China	7270	84.2	84.2	
4	T1	AJ272108	Human			Beijing, China	7232	84.2	84.2	
4	KNIH-hHEV4	FJ763142	Human	AH		South Korea	7260	84.1	84.1	
4	swCH189	FJ610232	Swine			Gansu, China	7284	84.1	84.1	
4	swGX40	EU676178	Swine			China	7267	84	84	
4	JYI-ChiSai01C	AB197674	Human	AH	2001/4/12	Shanghai, China	7260	84	84.4	Koike *et al.*[19]
4	JKO-ChiSai98C	AB197673	Human	AH	1998/10/27	Xian, Shaanxi, China	7257	84	83.9	Koike *et al.*[19]
4	HEVN2	AB253420	Human			Okinawa, Japan	7253	84	84	
4	Ch-S-1	EF077630	Human			China	7261	83.9	83.8	
4	swGX32	EU366959	Swine			China	7281	83.8	83.8	
4	DQ1	DQ279091	Swine			China	7234	83.8	83.7	
1	B1	M73218	Human			Burma	7207	75.4	75.3	
3	US1	AF060668	Human			USA	7202	75.3	75.3	Schlauder *et al.*[20]
2	M1	M74506	Human			Mexico	7180	73.9	73.9	

Bold letters represents the strains separated in Kitami and Abashiri regions in Hokkaido Prefecture, Japan.
AH, acute hepatitis; ASH, acute severe hepatitis; FH, fulminant hepatitis.

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Figure 1 (a) Three symptomatic and one asymptomatic patient with acute infection of hepatitis E virus (HEV) attended the same barbecue restaurant in Abashiri, Hokkaido, Japan. Among them, two were admitted in Sapporo and one in Abashiri. (b) Phylogenetic tree based on the full, or nearly full, length nucleotide sequence of HEV genotype 4 by neighbor-joining method. Two of the three isolates, JKO-Aba-FH06 and JMM-Aba06C, analyzed in this study were segregated into a unique cluster ("Kitami/Abashiri strains") distinct from others in genotype 4 indigenous to Japan.

grilled pork meat and entrails at the same party had extremely high sequence homology among the HEV isolates in genomic analyses (Table 2), suggesting a zoonotic transmission of indigenous Japanese HEV strains from pigs to humans in Japan.

In addition, this study demonstrates the persistent presence of virulent strains of HEV in the Kitami/Abashiri region of Hokkaido, Japan. The HEV strain in the Kitami cases of 2004 shows high sequence homology with the Abashiri cases of 2006, as described above. Among the strains, JKO-Aba-FH06C, isolated from patient 3 in Abashiri, and JTC-Kit-FH04L, isolated from Kitami in 2004, demonstrate the strongest resemblance in full-length sequence of nucleotides (Table 2).

The isolates identified from the Kitami and Abashiri patients have also been segregated into a single cluster on the phylogenetic tree of HEV genotype 4 (Fig. 1). They show subtle, but distinct, differences in nucleotide identity from other strains in Hokkaido Prefecture, such as JSM-Sap95 and JKK-Sap[11] isolated at Sapporo (Fig. 1, Table 3). Given that the causative strains in the above Abashiri cases have a close resemblance to those in Kitami in 2004 and that the two areas are contiguous to each other, these HEV instances may share the same channels of distribution and production of pork meat.

The public health impact of the above findings demonstrates significance because the HEV strains of Kitami/Abashiri belong to genotype 4, which is associated with development of fulminant acute hepatitis E.[24] In fact, one of the three HEV patients during the mini-outbreak at Kitami in 2004 has died of fulminant hepatitis.[8,9] Among the six total hepatitis E patients in Kitami and Abashiri, two patients have developed fulminant hepatitis while one has presented with severe hepatitis. Given these facts, suspicion arises that the Kitami/Abashiri strains may be associated with disease progression. In the meantime, another case of fulminant hepatitis due to infection with HEV genotype 4 has been reported in Hakodate, Hokkaido, approximately 400 km from Abashiri and Kitami;[25] the nucleotide sequence of the HEV isolate has shown extreme homology with the Kitami/Abashiri strains. These facts indicate that the Kitami/Abashiri strains of HEV may be spreading to different parts of Hokkaido Prefecture, and further expansion of this strain to other islands of the Japanese archipelago may be possible.

The association between the genomic distinction and fulminant hepatitis in genotype 4 of HEV remains unclear. Because the T at nucleotide 1816 and C at nucleotide 3148 have remained in the JTC-Kit-FH04L and JKO-Aba-FH06C responsible for fulminant hepatitis, no apparent correlation exists between the nucleotide substitutions C1816 and U3148[12] and disease progression in patients infected with the Kitami/Abashiri strains.

In order to prevent HEV infection caused by ingestion of pork meat and entrails, the regional community may need further instruction on proper food handling and cooking techniques. Individual indigenous sources and routes of infection should be clarified. Moreover, administrative precautionary measures must be taken for protecting the livestock and distribution industries as well as regional consumers' health.

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NOTE *Public Health*

Detection and Phylogenetic Analysis of Hepatitis E Viruses from Mongooses in Okinawa, Japan

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ABSTRACT. Hepatitis E virus (HEV) infection has previously been reported in wild mongooses on Okinawa Island; to date however, only one HEV RNA sequence has been identified in a mongoose. Hence, this study was performed to detect HEV RNA in 209 wild mongooses on Okinawa Island. Six (2.9%) samples tested positive for HEV RNA. Phylogenetic analysis revealed that 6 HEV RNAs belonged to genotype 3 and were classified into groups A and B. In group B, mongoose-derived HEV sequences were very similar to mongoose HEV previously detected on Okinawa Island, as well as to those of a pig. This investigation emphasized the possibility that the mongoose is a reservoir animal for HEV on Okinawa Island.

KEY WORDS: hepatitis E virus, mongoose, Okinawa, phylogenetic analysis.

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Hepatitis E virus (HEV) is a causative agent of acute hepatitis in humans, and is the only member of the family *Hepeviridae*, and consequently genus *Hepevirus* [1]. The HEV genome is a single stranded, positive-sense RNA of ~7.2 kb that contains three open reading frames (ORFs) [1]. HEV strains have been classified into 4 major genotypes: genotypes 1–4. Genotypes 1 and 2 are the cause of the epidemic hepatitis E, which is spread mainly via the fecal-oral route among humans in developing countries [1, 18]. Genotype 3 is distributed worldwide including developed countries, while genotype 4 is found in Taiwan, China, Japan, and India [18]. In addition, zoonotic transmission has been reported for genotypes 3 and 4 [18]. HEV RNA has been detected in humans, pigs, wild boars, and wild deer [9, 10, 13, 17], and HEV antibodies have been detected in many animals [2, 10, 13]. Recently, newly reported rabbit HEV from China [20] and wild boar HEV from Japan were proposed to belong to a new genetic group [12, 14]. In addition, a novel HEV was detected in wild rats from Germany and the United States [3, 8]. However, sequence identity and phylogenetic analysis showed that rat HEV is clearly distinguished from known major genotype 1–4 HEV [3].

Okinawa Prefecture is a group of islands located in the southernmost part of Japan, and wild mongooses (Small Asian mongoose; *Herpestes javanicus*) are found in this region. The mongoose was first imported into Okinawa Island from India in 1910 in the hope that they would fight and kill the venomous *Habu* snake found on the island. Subsequently, the mongoose's habitat on Okinawa Island has widened rapidly because there are no animals that can fight and kill them, and they inhabit the same region as the wild boar, which is an important reservoir of HEV, and they can also access pig farms easily. On the basis of these facts, it is expected that mongooses may be exposed to HEV in the feces of pigs and wild boars. The positive rate of HEV antibodies in mongooses on Okinawa Island was reported as 8.3% (7/84) and 21% (21/100) [5, 7], and HEV RNA was detected in 1.0% (1/100) of mongooses' serum in 2002 [7]. These findings suggest that mongooses might act as a reservoir of HEV; however, HEV RNA has not been detected in any other mongooses since the above mentioned study. Therefore, we carried out a further investigation to determine whether HEV RNA was present in mongooses captured on Okinawa Island. Here, we report the detection of HEV RNA in mongooses, and present their phylogenetic analysis.

We collected 209 bile samples from 100 mongooses that had been captured on Okinawa Island between December 2004 and November 2005 and from 109 mongooses captured between October 2007 and September 2008. Viral RNA was extracted from the samples using the QIAamp Viral RNA Mini Kit (Qiagen, Tokyo, Japan). The extracted RNA was subjected to reverse transcription (RT) and polymerase chain reaction (PCR) amplification using the One-Step RT-PCR Kit (Qiagen) with primers for the ORF2 region of the HEV genome, namely HE044 and HE040 [6]. The resultant PCR products were subjected to nested-PCR amplification using TaKaRa Ex Taq (Takara Bio Inc., Otsu, Japan) with primers HE110-2 and HE041 [6]. The ampli-

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fication products were purified using the QIAquick PCR Purification Kit (Qiagen), and nucleotide sequences of the partial ORF2 region (412 nucleotides (nt)) were determined by direct sequencing. Multiple sequence alignment and phylogenetic analysis were conducted using Molecular Evolutionary Genetics Analysis (MEGA) software (version 5.0) [16]. The full genome sequences of the HEV RNA obtained in this study were determined using previously described methods [7, 11]. Briefly, HEV RNA was subjected to RT with SuperScript II RNase H (−) Reverse Transcriptase (Invitrogen Corporation, Tokyo, Japan), and PCR amplification of several overlapping regions of HEV RNA was carried out using TaKaRa Ex Taq (TaKaRa). The 5' and 3' terminal sequences were amplified using the 5'-Full RACE Core Set (TaKaRa) and 3'-RACE System for Rapid Amplification of cDNA Ends (Invitrogen), respectively. Multiple sequence alignment and phylogenetic analysis were conducted as described above.

HEV RNA was detected in 6 (2.9%) of 209 mongooses from Okinawa Island. Figure 1 shows the phylogenetic tree constructed from the nucleotide sequences of the partial ORF2 region of HEV. All 6 strains detected in this study belonged to genotype 3 and were classified into 2 groups, groups A and B, which also contain the pig-, wild boar-, and mongoose-derived HEV previously detected on Okinawa Island. Of the 6 HEV RNAs, 4 (JMNG1-Oki05, JMNG2-Oki05, JMNG36-Oki08 and JMNG142-Oki08) belonged to group A and were detected in mongooses captured in Oogimi village, which is located in the northern region of Okinawa Island, and 2 (JMNG26-Oki08 and JMNG43-Oki08) belonged to group B and were detected in mongooses captured in Higashi village, which is next to Oogimi village. JMNG-Oki02C, which was previously isolated [7] and also belongs to group B, was detected in a mongoose captured in Higashi village. The nucleotide sequence similarity of the HEV detected in this study ranged from 86.9–89.6% between groups A and B. In group A, the mongoose-derived HEVs showed 91.7–92.2% sequence similarity to each other (excluding the 100% similarity between JMNG1-Oki05 and JMNG2-Oki05; these HEV RNAs were detected in mongooses captured in the same year and month). In group B, 3 mongoose-derived HEVs showed 97.8–99.3% sequence similarity to each other, and JMNG26-Oki08 and JMNG43-Oki08 showed 98.5–98.8% similarity to swJOK1-1, which was detected in a pig on Okinawa Island [15].

Of the 6 HEV RNAs, 2 (JMNG26-Oki08 and JMNG36-Oki08) were determined for full genome sequences. The phylogenetic tree based on the full or nearly full nucleotide sequences of HEV revealed that JMNG26-Oki08 and JMNG36-Oki08 were located in distinct clusters within genotype 3 (data not shown). They displayed 86.6% nucleotide sequence similarity. Conversely, JMNG26-Oki08 formed a compact cluster with JMNG-Oki02C, which was obtained from a mongoose in 2002, with a nucleotide sequence similarity of 97.9%. In addition, they displayed amino acid identity levels of 99.2–99.5% for ORF1, ORF2, and ORF3.

In the present study, 6 new HEV RNAs were detected in mongoose from Okinawa Island. The sequence similarity of the partial ORF2 ranged from 86.6–89.6% between groups A and B. Given this sequence similarity, the existence of genetically various mongoose HEV strains is predicted on Okinawa Island. These mongoose-derived HEV RNAs were detected in adjoining villages, but the mongoose HEV RNAs of group A were identified only in Oogimi village, while those of group B were identified only in Higashi village. However, because a mountain separates these villages, mongooses might not pass from one village to the other.

In group A, mongoose-derived HEV sequences were genetically diverse, but in group B, the 3 mongoose-derived HEV sequences (JMNG26-Oki08, JMNG43-Oki08 and JMNG-Oki02C) were very homologous to each other. In addition, JMNG26-Oki08 and JMNG-Oki02C were also high homologous when their full genome sequences were compared. We previously reported that JMNG-Oki02C was closely related to a pig HEV (swJOK1-1) [7]. JMNG26-Oki08 and JMNG43-Oki08 were also very homologous to swJOK1-1. There is the possibility that the HEV transmitted in pig farms is the direct ancestor of the HEV detected in these mongooses. Because, the mongooses access pig farms, probably to eat the feed of the mongoose, and then mongooses may have been infected with HEV through contact with pig feces in there. Adversely, mongooses infected with HEV may transmit it to pigs.

Pig, wild boar and wild deer meat has been suspected or proved to be directly responsible for cases of HEV infection in humans in Japan [4, 17, 19]. Although mongooses are not generally eaten by humans, they may indirectly contribute to human HEV infections by infecting pigs that are subsequently consumed by humans.

The present findings raise the possibility that the mongoose is a reservoir of HEV on Okinawa Island. However, further studies are necessary to explain the role played by mongooses in the spread of HEV on Okinawa Island.

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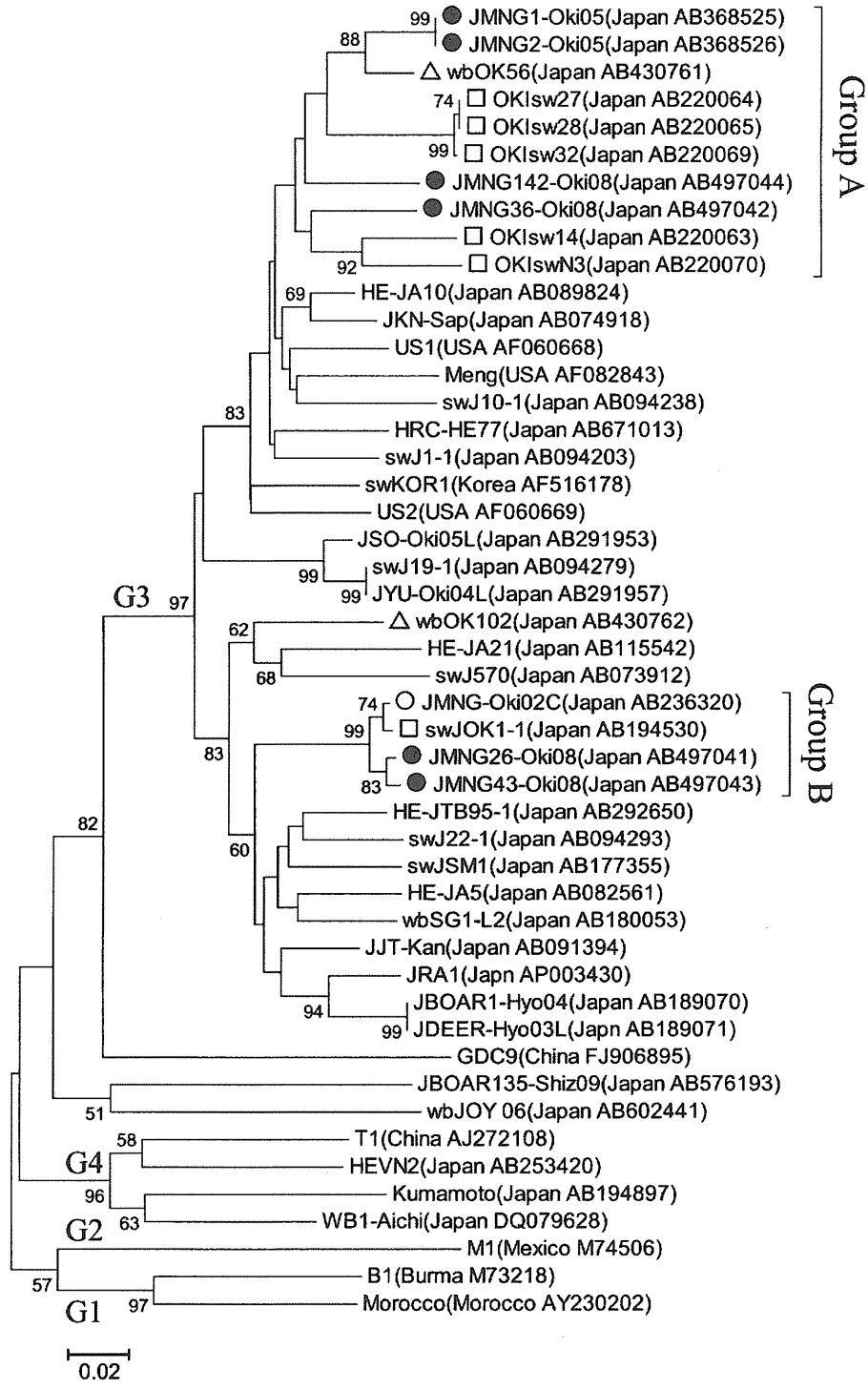

Fig. 1. Phylogenetic tree of 48 HEV isolates constructed using the neighbor-joining method on the nucleotide sequence of the partial ORF2 region (412 nt). The location where each isolate was detected and the DDBJ/EMBL/GenBank accession number of each isolate are shown in parentheses. G1–4 indicate the genotype. Bootstrap support values >50%, which are given as a percentage of 1,000 replicates, are indicated at each node. The symbols indicate the mongoose HEV isolates obtained in this study (●), and mongoose HEV (○), pig HEV (□), and wild boar HEV (△) isolates previously detected on Okinawa Island. GDC9, JBOAR135-Shiz09, and wbJOY06 are suggested to represent novel genotypes.

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BRIEF REPORT

Detection and molecular characterization of hepatitis E virus in clinical, environmental and putative animal sources

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Abstract Putative animal reservoirs and environmental samples were studied to investigate potential routes of transmission for indigenous hepatitis E virus (HEV) infection in Hokkaido, Japan. A total of 468 liver samples and 954 environmental samples were collected from 2003 to 2011 for this study. Four swine livers (1 %) were positive for HEV RNA; two strains belonged to genotype 3 and the other two strains were genotype 4. Genotype 3 HEV was detected in a sewage sample and a seawater sample. HEV strains derived from swine liver, seawater and raw sewage samples shared 93-100 % sequence similarity with human HEV strains.

Keywords HEV · Hepatitis · Genotype

Hepatitis E virus (HEV) is an enterically transmitted pathogen that causes acute hepatitis [5]. HEV is a single-stranded, positive-sense RNA virus without an envelope, and it belongs to the genus *Hepevirus* of the family *Hepeviridae* [14]. HEV sequences have been classified into four major genotypes [22]. Genotypes 1 and 2 HEVs are so far restricted to humans and are associated with large waterborne epidemics in developing countries. Genotypes 3 and 4 HEVs are zoonotic agents that have been detected in humans as well as animal species including swine, wild boars, deer, rabbits and mongooses [15, 17, 24, 31]. Recently, additional putative new genotypes of HEV were identified from rats in Germany [6] and wild boars in Japan [28].

Swine HEV infection is widespread worldwide [7, 23, 30]. In Japan, sporadic human cases of acute HEV infection that are linked to the consumption of raw or insufficiently cooked boar or deer meat have been reported [12, 27, 29]. The HEVs found in animals are genetically closely related to those from humans in the same geographic regions [30]. Because HEV is excreted in feces [2, 7], there is a risk of HEV contamination of environmental water. HEV has been detected in sewage and river water from industrialized countries [11, 19].

To assess possible risk factors and transmission routes for indigenous HEV infections, the prevalence of HEVs in possible animal reservoirs and environmental samples was tested in Hokkaido, the region of Japan where hepatitis E is the most prevalent and where blood-donor screening for HEV RNA was started as a research study in 2005. Additionally, the genetic diversity of HEVs isolated from clinical, environmental and putative animal sources during the same period was investigated.

The serum samples of persons who were reported to the local health centers as hepatitis E or carriers of HEV were analyzed. The sites where health centers were located in Hokkaido were grouped into four regions to investigate the relationship between genetic diversity of HEVs and their geographical distribution: E, S, C and N (Figure 1). Because the patients and carriers did not report that they had traveled to a high-endemic country in the three months prior to onset of illness or notification, it was assumed that they had been exposed to indigenous HEV. HEV carriers

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