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.

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-
In the present study, 6 new HEV RNAs were detected 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 very homologous to 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.

REFERENCES

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.


