Short Communication

Ferret Hepatitis E Virus Infection in Japan

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SUMMARY: We examined 85 fecal samples from pet ferrets in 10 animal hospitals in Japan for the detection of ferret hepatitis E virus (HEV) RNA. We found that 6 (7.1%) of the samples were positive for ferret HEV RNA. Phylogenetic analysis based on the partial ORF1 indicated that these ferret HEV strains were clearly separated from the Netherlands strains and were divided into 2 distinct clusters. These results suggest that ferret HEV is genetically diverse, and since ferrets are not indigenous to Japan, ferret HEV has been introduced into Japan through importation.

Hepatitis E virus (HEV) is a single-stranded positive-sense RNA virus that belongs to the genus *Hepevirus* in the family *Hepeviridae* and is the causative agent of hepatitis E (1,2). Four genotypes of HEV (G1–G4) have been detected in humans. Moreover, G3 and G4 HEV have also been isolated from swine, wild boar, and deer and are responsible for zoonotic infections (3–5). In addition to these 4 genotypes, novel HEV or HEV-like viruses have been detected in various animals, including monkeys, rabbits, rats, ferrets, chicken, mink, moose, red fox, and bats (6–13). However, whether the HEV infection is transmitted from these animals to humans is yet unclear.

Ferret HEV was first detected in ferrets in the Netherlands (9). The genome structure of ferret HEV is similar to that of other HEVs and contains 3 open reading frames (ORF1–3). ORF1, ORF2, and ORF3 encode a nonstructural protein of 1589 or 1596 amino acids (aa), a capsid protein of 654 aa, and a functionally unknown protein of 108 aa, respectively (9,14). An earlier study from our group revealed that the expression of the ferret HEV ORF2 protein allowed their assembly into virus-like particles (V-LPs). Enzyme-linked immunosorbent assays (ELISAs) employed for the detection of anti-ferret HEV IgG and IgM antibodies using ferret HEV-LPs as the antigen revealed that the IgG- and IgM-positive rates were 23.3% and 24.4%, respectively, among ferrets in the U.S. (15). The ferret HEV RNA detected in U.S. ferrets was genetically distinct from that of the Netherlands strains (15). Moreover, the antibody against ferret HEV-LPs failed to neutralize G3 HEV, suggesting that the serotypes of these 2 HEVs are different (15). However, the pathogenicity and epidemiology of ferret HEV remain unclear.

In the present study, we collected a total of 85 fecal samples (47 males and 38 females) and 10 serum samples from ferrets in 10 animal hospitals scattered across 5 prefectures in Japan between October 2009 and September 2013. Most of the ferrets were examined for clinical signs of infection such as diarrhea, anorexia, and hypergammaglobulinemia. The age of the ferrets ranged from 4 months to 9 years and 9 months.

The fecal specimens were diluted with 10 mM phosphate-buffered saline to obtain 10% suspensions (16). RNA was extracted from the diluted fecal suspensions using MagNA Pure LC Total Nucleic Acid isolation kit (Roche Applied Science, Mannheim, Germany) according to the manufacturer's recommendations. Reverse transcription (RT) was performed as described previously (17). A nested broad-spectrum RT-polymerase chain reaction (PCR) targeting a portion of ORF1 was performed as described with a slight modification (18). The nested PCR was performed with a forward primer, HEV-cs (5'-TCGCGCATCACMTTYTTCCARAA-3') and an internal reverse primer, HEV-casn (5'-CCAGGCTCACCRGARTGYTTCTTCCA-3').

Six (7.1%) of the 85 fecal samples were positive for ferret HEV RNA. All of the nested PCR products were purified and sequenced. Other than the primer sequences, the nested PCR product contained 388 nucleotides corresponding to the C-terminal ORF1 (nt 3885–4272) in the ferret HEV genome (JN998607). Phylogenetic analysis indicated that these strains (GenBank accession nos. AB898199–AB898204) clustered with ferret HEV, and that they were clearly separated from the Netherlands strains and classified into the 2 clusters A and B (Fig. 1).

Five (YFS2, YFS27, YFS28, YFS47, and YFS80) of these strains belonged to cluster A and shared 92.1%–93.2% and 82.4%–84.0% nucleotide sequence identities with the U.S. and Netherlands strains, respectively. Strain YFS51 belonged to cluster B and was closely related to the U.S. strains, with 99.3%–99.6% nucleotide sequence identities. In contrast, strain YFS51 shared 83.2%–84% nucleotide sequence identities with the Netherlands strains. The complete genome of ORF2 (GenBank accession no. AB898198) amplified from ferret fecal sample (YFS2) shared 91.6%–91.7% and

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Fig. 1. Phylogenetic analysis of ferret HEV isolates. The nucleic acid sequence alignment was performed using Clustal X 1.81. The genetic distance was calculated by Kimura's two-parameter method. A phylogenetic tree with 1,000 bootstrap replicates was generated by the neighbor-joining method (Njplot 2.3) based on the partial ORF1 genome of HEV isolates. P farm, M farm, and C farm: the name of each ferret farm; unknown: ferrets from unknown origins.

83.5%–83.7% nucleotide sequence identities, and 97.4%–97.6% and 93.9%–94.5% amino acid sequence identities, with the U.S. and Netherlands strains, respectively.

These results suggest that ferret HEV is genetically diverse, consisting of at least 3 clusters. The age of the ferrets that were positive for ferret HEV RNA ranged from 5 months to 6 years, suggesting that ferret HEV infection is likely to occur at a young age. Anti-ferret HEV IgG and IgM antibodies were detected by ELISA, as described previously (15). All 10 tested serum samples were negative for ferret HEV RNA, and the anti-ferret HEV IgG- and IgM-positive rates were 20% (2/10) and 10% (1/10), respectively; this result suggests active ferret HEV transmission and prevalence among pet ferrets.

Ferrets are nonindigenous in Japan and have been imported into the country. In the present study, of the 85 ferrets whose fecal samples were examined, 40, 8, 5, and 32 ferrets were imported from the U.S., New Zealand, Canada, or were of unknown origin, respec-

tively. Of cluster A, strains YFS27 and YFS47 were detected in ferrets from different farms (P and M) in the U.S., and the strains YFS2 and YFS28 from ferrets of the same farm (C) in Canada, suggesting that the circulation of genetically similar ferret HEV strains in these farms.

We found that the YFS2 strain-infected ferret exhibited signs of hepatitis such as anorexia and hepatomegaly. Moreover, the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were found to be 605 IU/L and 149 IU/L, respectively, which is higher than the reference values (ALT, 82–289 IU/L; AST, 28–120 IU/L), indicating hepatocellular injury. These results suggest that ferret HEV infection is likely to be associated with hepatitis in ferrets. In addition to human HEV (G1–G4), HEV-like viruses have been detected in a variety of animals, including mongoose, mink, moose, bats, rats, and chicken (3,8,10,11,13); however, there is no evidence that a particular HEV infection results in hepatitis in host animals, except for chickens

infected with avian HEV and humans infected with G1–G4 HEV. Studies that investigate the pathogenicity of ferret HEV in ferrets would be particularly useful for the development of an animal model of hepatitis E for immunological and pathological studies.

In conclusion, we detected ferret HEV RNA in pet ferrets in Japan and confirmed the wide distribution of ferret HEV and its likely introduction into Japan through importation. Further studies on the epidemiology, biology, immunology, and pathology of ferret HEV are required to obtain a better understanding of its pathogenicity in Japan.

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Conflict of interest None to declare.

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