Prevalence of Hepatitis E Virus (HEV) Infection in Wild Boars and Deer and Genetic Identification of a Genotype 3 HEV from a Boar in Japan

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Zoonotic transmission of hepatitis E virus (HEV) from captured wild deer or boars to humans has been suggested. Antibody to HEV was detected in 9% of 35 wild boars and 2% of 117 wild deer tested, and a presumably indigenous HEV of genotype 3 was isolated from a boar in Japan.

Hepatitis E virus (HEV), the causative agent of hepatitis E, is an important human pathogen (4, 18, 21). The genome of HEV is approximately 7.2 kb in size and contains three open reading frames (ORF1, ORF2, and ORF3) (18). Although only one serotype has been recognized, extensive genomic diversity has been noted among HEV isolates, and HEV sequences have tentatively been classified into four genotypes (genotypes 1 to 4) (20). Transmission of HEV occurs primarily by the fecal-oral route through contaminated water supplies in developing countries (18). In addition, increasing evidence has indicated that hepatitis E is a zoonosis (4, 8, 10–13, 15, 16, 21, 24, 29). It has recently been suggested that zoonotic foodborne transmission of HEV from domestic pigs, wild boars, or wild deer to humans plays an important role in the occurrence of cryptic hepatitis E in Japan, where people have distinctive habits of ingesting raw fish (sushi or sashimi) and, less frequently, uncooked or undercooked meat (including the liver and colon or intestine of animals) (9, 23, 24, 29). The first animal strain of HEV to be isolated and characterized was a swine HEV from a pig in the United States in 1997 (10). Since then, many swine HEV isolates, which exhibit extensive genetic heterogeneity, have been identified worldwide and shown to be genetically closely related to strains of human HEV (1, 3, 5, 6, 16, 17, 25–27, 30). In previous studies, a high prevalence of the swine immunoglobulin G (IgG) class of antibody to HEV (anti-HEV) was found among 2- to 6-month-old Japanese pigs (58% or 1,448 of 2,500) (22), and a pair of Japanese swine and human HEV isolates of genotype 4 with 99% identity over the entire genome were identified (15). In addition, a certain proportion of packaged raw pig livers for sale in stores as food (1.9% or 7 of 363) were contaminated with HEV, which had high nucleotide sequence identity, up to 100%, with the HEV isolates recovered from Japanese patients with hepatitis E who had ingested undercooked pig liver before disease onset (29). As for HEV from wild boars, although Chandler et al. (1)
reported the prevalence of HEV antibody among captured wild pigs (boars) in Australia (25% or 15 of 59), boar HEV strains have not yet been identified. Furthermore, although HEV RNA was detected in a leftover portion of deer meat that was implicated in the development of food-borne hepatitis E (24), the prevalence of HEV infection among wild deer remains unknown.

Therefore, in the present study, we obtained and analyzed paired serum and liver specimens, serum only, or liver tissues only from 41 wild boars (Sus scrofa leucomystax) that had been captured in Tochigi, Toyama, Nagano, Kanagawa, and Okayama Prefectures on mainland Honshu, Tokushima Prefecture on Shikoku Island, and Saga, Oita, Kumamoto, and Miyazaki Prefectures on Kyushu Island (listed by location from north to south in Japan) between December 2002 and February 2003 and between December 2003 and March 2004. We tested 132 wild deer (Sika deer; Cervus nippon) that had been caught on Hokkaido Island (C. nippon yesoensis), in Tochigi and Nagano Prefectures on mainland Honshu (C. nippon centralis), and in Oita and Kumamoto Prefectures on Kyushu Island (C. nippon nippon) between October 2003 and March 2004 (Fig. 1). A total of 35 serum samples and 33 liver tissues, including 27 paired serum and liver specimens, were available from the 41 boars, and 117 serum samples and 132 liver tissues, including 117 paired serum and liver specimens, were available from the 132 deer. The serum samples were tested for the presence of anti-HEV IgG by in-house enzyme-linked immunosorbent assay using purified recombinant ORF2 protein that had been expressed in the pupae of silkworms (14) as the antigen probe as described previously (22), with slight modifications. For the boar anti-HEV IgG assay, the peroxidase-conjugated rabbit IgG fraction to swine IgG (whole molecule) (ICN/Cappel, Aurora, Ohio) was used as described previously for the swine anti-HEV IgG assay (22), and for the deer anti-HEV IgG assay, peroxidase-labeled affinity-purified antibody to deer IgG (H11001; Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.) was used instead of the enzyme-labeled anti-swine IgG antibodies. The serum samples and liver tissues were tested for the presence of HEV RNA by reverse transcription (RT)-PCR by the method described previously, with primers targeting the ORF2 region (14). To confirm the presence of HEV RNA, a part of the ORF1 region was amplified by nested RT-PCR (14). The two RT-PCR assays used had the capability to amplify all four known genotypes of HEV (14, 22, 29). The amplified products were sequenced directly on both strands.

The A450 value of boar anti-HEV antibodies ranged from 0.003 to 0.866, and 3 (9%) of the 35 serum samples had an A450 value of >0.300. The A450 values of these three samples (0.335, 0.655, and 0.866) decreased to less than 30% of the original values after absorption with the same recombinant ORF2 protein that was used as the antigen probe, but they remained greater than 70% of the original values after absorption with a mock protein obtained from the pupae of silkworms infected present study and 18 human and swine HEV strains isolated in Japan is indicated by a vertical bar. Bootstrap values of >70% are indicated for the major nodes as a percentage of the data obtained from 1,000 resamplings (2).
with nonrecombinant baculovirus, confirming the specificity of the assay. Therefore, these three serum samples from boars in Nagano Prefecture (two samples) and Miyazaki Prefecture (one sample) were conservatively regarded as being positive for boar anti-HEV IgG in the present study. The \( A_{450} \) value of deer anti-HEV IgG ranged from 0.012 to 1.442, and 2 (2%) of the 117 serum samples had \( A_{450} \) values of \( \geq 0.300 \) (0.591 and 1.442); the specificity was confirmed by the absorption assay. Deer anti-HEV IgG was detectable in 1 (3%) of the 32 serum samples from deer in Hokkaido and 1 (2%) of the 53 samples from deer in Tochigi Prefecture.

Among all of the serum and liver specimens from the boars and deer, HEV RNA was reproducibly detected in paired serum and liver specimens obtained from a male boar with a body weight of 60 kg that had been caught in Saga Prefecture on Kyushu Island on 19 December 2003, although the viremic boar was negative for anti-HEV IgG and had no clinical manifestations. The HEV sequences amplified from the serum and liver tissue of the infected boar were 100% identical in both a 412-nucleotide (nt) sequence of the ORF2 region and another 412-nt sequence of the ORF1 region (accession no. AB180052 to AB180055). The HEV isolate (wbJSG1) obtained in the present study was close to known human and swine genotype 3 isolates, with 82.9 to 93.9% identity in the 412-nt ORF2 sequence, and was most closely related to the HE-JA5 isolate of genotype 3 which is presumed to be indigenous to Japan (14). The phylogenetic tree constructed by the neighbor-joining method (19) based on the partial ORF2 sequence of 298 nt confirmed that the wbJSG1 isolate belonged to genotype 3, and it segregated into a cluster consisting of 18 HEV strains that had been isolated from 10 Japanese patients with no history of travel to countries where the virus is endemic who developed sporadic acute or fulminant hepatitis (14, 29) and from eight Japanese farm pigs (16, 22) (Fig. 2). Among HEV strains recovered from patients who developed hepatitis E after ingestion of uncooked or undercooked meat or liver from wild boars or deer for which the ORF2 sequences were not available, the wbJSG1 isolate obtained in the present study was 90.2 to 90.9% identical in the 317-nt ORF1 sequence to the HEV isolates of genotype 3 (AY427956 and AY427957) recovered from four HEV-infected boars in Nagasaki Prefecture (23) and only 87.4 to 87.7% identical in the 326-nt ORF1 sequence to the HEV isolates of genotype 3 (AB111479 to AB111483) recovered from four patients with hepatitis E who had consumed raw meat from a wild deer in Kyogo Prefecture and from a leftover portion of the deer meat that had been kept frozen to be eaten in the future (24). In addition, the wbJSG1 isolate was merely 77.5% similar in the 326-nt ORF1 sequence to the JSF-Tot03 isolate of genotype 4 (AB111478) that had been recovered from a patient who had eaten uncooked liver from a wild boar in Tottori Prefecture (9), suggesting that heterogeneous strains of HEV of genotype 3 or 4 are circulating among wild boars and deer in Japan. Based on the finding that one of the two wild boars caught in Saga Prefecture in the present study was HEV viremic, meat and liver from the wbJSG1-infected boar were not ingested, thereby possibly preventing food-borne transmission of HEV.

Prevalence of anti-HEV IgG in pigs is usually age dependent; swine HEV RNA can often be detected in pigs 2 to 4 months of age but is less commonly detected in older pigs (16, 22, 28). The HEV-infected boar identified in the present study weighed 60 kg, suggesting that the viremic boar was approximately 2 years of age and that wild boars can acquire de novo HEV infection at older ages than domestic pigs. However, it is difficult to estimate ages of wild boars and deer living under natural conditions based on their body weights. It has been reported that periodic growth incremental lines found universally in dental hard tissues allow for reliable estimation of age in wild animals (7). Therefore, extended studies must be undertaken to investigate the seroprevalence of HEV and frequency of viremia in wild deer and boars in relation to their ages estimated by use of tooth increments.

The results obtained in the present study indicate that wild boars and deer in Japan are infected with HEV, although at much lower rates than domestic pigs in Japan, and that a certain proportion of wild boars in Japan are HEV viremic and may act as sources of HEV infection in humans. The isolation of a domestic HEV strain from a Japanese wild boar with high nucleotide sequence identity to human HEV in Japan provides further evidence for zoonotic food-borne transmission of HEV from wild boars to humans. Further extended studies are required to fully elucidate the epidemiology of HEV infection in animals and possible zoonotic transmission in an attempt to prevent cryptic hepatitis E occurring not only in industrialized countries but also in developing countries.

**Nucleotide sequence accession numbers.** The sequences determined in the present study have been deposited in the DDBJ, GenBank, and EMBL nucleotide databases under the following accession numbers: AB180052 for the ORF1 sequence of the wbJSG1 isolate recovered from the liver of the HEV-infected boar (ORF1, liver), AB180053 (ORF2, liver), AB180054 (ORF1, serum), and AB180055 (ORF2, serum).

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