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Identification of Hepatitis E Virus in Bovine and Porcine Raw Livers

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Several animal species including pigs are directly involved in the zoonotic transmission of the hepatitis E virus (HEV) to humans. This study was conducted to detect HEV in bovine and porcine raw livers by nested reverse transcription-polymerase chain reaction. Zoonotic HEV strains were identified in 1.0 and 3.0% of the tested bovine and porcine livers, respectively. HEV-4 was detected in the bovine livers, but both HEV-3 and HEV-4 were identified in the porcine livers. These results indicate that zoonotic transmission of HEV may occur via consumption of raw or undercooked livers of pigs and cattle.

Keywords: HEV-3, HEV-4, pigs, cattle, liver

Hepatitis E virus (HEV) belonging to the family *Hepeviridae* contains a positive-sense single-stranded RNA genome [1]. Eight genotypes in the species *Orthohepevirus A* have been identified in mammals. HEV generally causes acute hepatitis, and self-limiting resolutions have been observed in most cases. HEV-1 and HEV-2 infect only humans mainly in developing countries, through the consumption of contaminated drinking water [2]. Infection with HEV induces about 20% mortality in pregnant women in developing countries such as India [3, 4]. HEV-3 and HEV-4 infect both humans and several animal species, including pigs [5]. The zoonotic transmission of HEV-3 and HEV-4 frequently occurs via consumption of raw or undercooked animal meats and sausages made from porcine livers [6–8]. Recently, zoonotic infection by HEV-7 was reported in a patient who had consumed camel milk [9]. HEV-3 and HEV-4 have been identified in the fecal and liver samples of pigs in several countries such as Korea [10–14]. Particularly, HEV-3 and HEV-4 were detected in the serum samples of blood donors and patients with acute cryptogenic hepatitis in Korea [15, 16]. The HEV isolated from humans is closely related to swine HEV on the basis of genetic analysis [15, 16]. Therefore, swine HEV may be the possible infection source in those patients. Moreover, although several studies have also indicated HEV infection in cattle [17, 18], detection of HEV in bovine liver samples has not yet been reported. In this study, we detected HEV-3 and HEV-4 in both porcine and bovine raw livers. To our knowledge, this is the first detection of zoonotic HEV-4 in bovine livers. Consumption of raw or undercooked porcine and bovine livers may be a plausible route of zoonotic transmission of HEV to humans.

Raw livers of cattle (n = 100) and pigs (n = 100) were purchased from local grocery markets between February 2017 and July 2018 in Seoul, Korea. Samples of about 1 g of each liver were ground in 3 ml of 4 M guanidine thiocyanate (GTC) buffer (4 M GTC, 0.25 M sodium citrate, and 5% trypsin-EDTA) containing 30 µl of 2-mercaptoethanol as described previously [19]. The homogenates were stored at −80°C until use. Total RNA was extracted from 150 µl of the liver homogenates with TRIzol reagent (Sigma, Germany). A universal primer set (forward primer 5′-AAY TAT GCW CAG TAC CGG GTT G-3′, reverse primer 3′-CCC TTA TCC TGC TGA GCA TTC TC-5′) [20, 21] was used for amplification of a conserved region in ORF2 of HEV-3 and HEV-4. For the 1st round of PCR, the Maxime RT-PCR PreMix Kit (Intron, Korea) was used. The PCR conditions were one cycle of RT reaction at 45°C for 30 min; inactivation of RTase at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 sec, annealing at 51°C for 30 sec,

Table 1. Detection of HEV in bovine and porcine raw liver samples.

Animal	Number of samples	Number of HEV-positive samples (genotype)	Prevalence (%)
Cattle	100	1 (HEV-4)	1.0
Pig	100	3 (1 HEV-3, 2 HEV-4)	3.0

and extension at 72°C for 1 min; and one cycle of extension at 72°C for 5 min. The 2nd round of PCR was conducted with the Maxime PCR PreMix Kit (Intron) and a primer set (forward primer 5′-GTY ATG YTY TGC ATA CAT GGC T-3′, reverse primer 3′-AGC CGA CGA AAT YAA TTC TGT C-5′). The PCR conditions were one cycle of initial denaturation at 94°C for 3 min; 35 cycles of denaturation at 94°C for 30 sec, annealing at 47°C for 30 sec, and extension at 72°C for 30 sec; and one cycle of final extension at 72°C for 5 min. PCR was repeated at least twice for each sample. The HEV-specific 350-bp PCR products were identified using electrophoresis, and their DNA sequences were determined using an automatic DNA sequencer. The 304 bp of HEV sequences without primer sequences were deposited in GenBank. The DNA sequences of HEV identified in this study were phylogenetically analyzed with representative HEV strains acquired from GenBank via the neighbor-joining method using MEGA software (ver. 6.06).

HEV was detected from one of the 100 bovine and three of the 100 porcine liver samples showing 1.0 and 3.0% prevalence, respectively (Table 1). The one HEV isolate

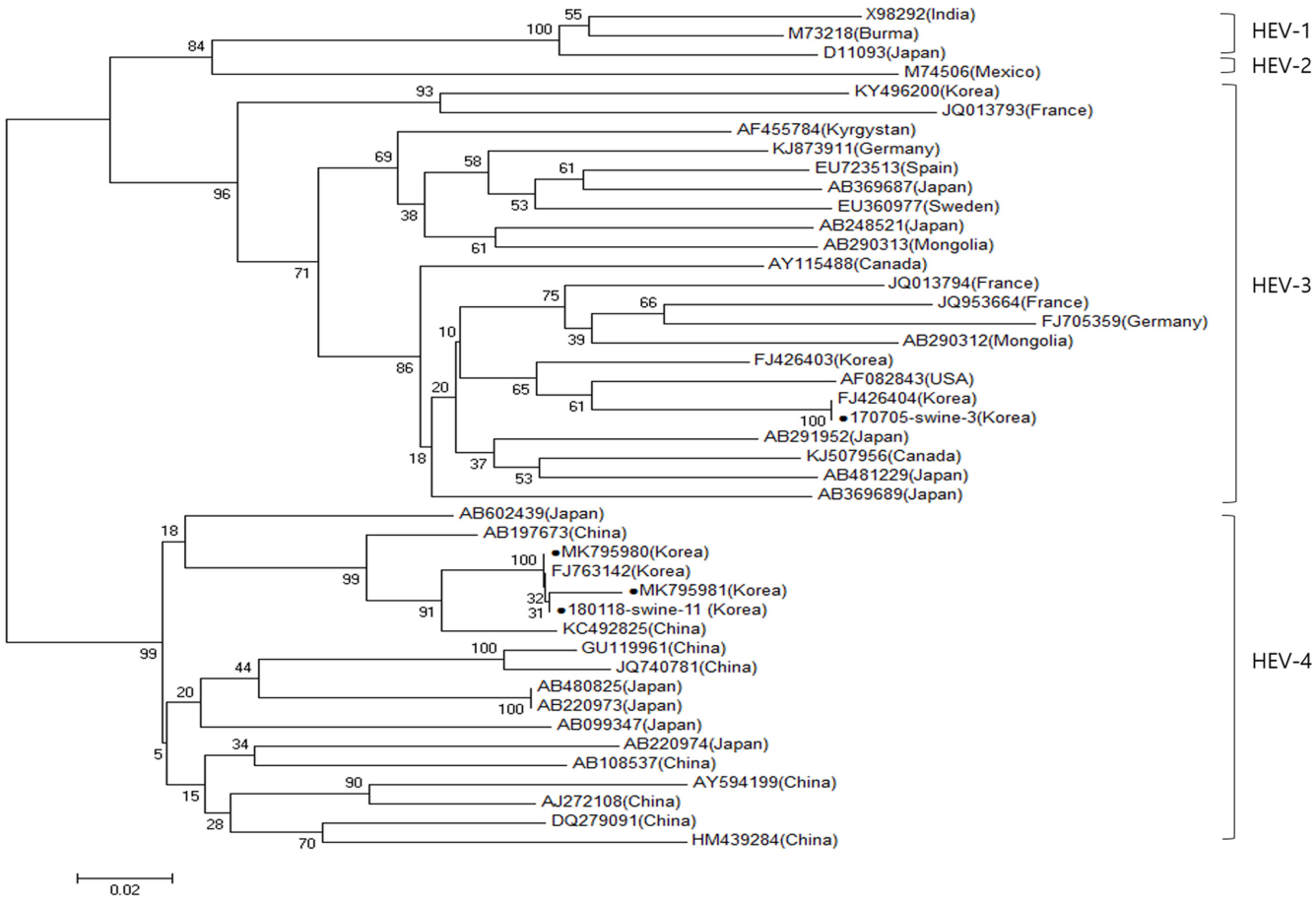

Fig. 1. Phylogenetic analysis of HEV ORF2 sequences.

Partial ORF2 sequences (304 bp) of HEV isolates obtained from bovine and porcine liver samples were compared with those of HEV-1, HEV-2, HEV-3, and HEV-4 strains identified from human and animals. Three HEV-4 (GenBank Nos. MK795980, MK795981, and ID 180118-swine-11) and one HEV-3 (ID 170705-swine-3) determined in this study are indicated as black dots. The scale bar indicates the branch length corresponding to 0.02 substitutions per site.

identified in the bovine liver samples was HEV-4. Among the three HEV isolates detected in the porcine liver samples, one was HEV-3 and two were HEV-4. The partial genomic sequence of the one HEV-4 isolate obtained from the bovine liver sample was submitted to GenBank (accession number MK795980). The nucleotide sequence of the bovine HEV-4 showed a very close relationship with those of the human strains of HEV-4 reported from Korea (FJ763142) and China (KC492825) (Fig. 1), that is, 95.4–99.6% nucleotide identity with the two representative human HEV-4 strains (data not shown). Of the nucleotide sequences of the two HEV-4 isolates identified in the porcine liver samples, the sequence of one was deposited in GenBank (accession number MK795981). The swine HEV-4 isolate (MK795981) was also very closely related to the representative Korean and Chinese HEV-4 strains (Fig. 1). It shared 94.1–98.3% nucleotide identity with the two human HEV-4 strains (data not shown). The nucleotide sequence of the other swine HEV-4 isolate (identification number 180118-swine-11) was 100% identical with that of the human strain of HEV-4 which was previously reported from Korea (FJ763142). The HEV-3 (identification number 170705-swine-3) obtained from the one porcine liver sample showed 100% nucleotide sequence identity with the Korean swine strain (FJ426404) previously reported by our laboratory. When the sequence of the HEV-3 was compared in the phylogenetic tree analysis, it was closely related to the swine HEV-3 strain reported from the USA (Fig. 1).

In this study, we found that small numbers of porcine and bovine livers sold in local grocery stores contained the zoonotic viruses HEV-3 and HEV-4. These results imply that raw or undercooked porcine and bovine livers may be directly involved in the zoonotic transmission of HEV to humans. Several studies have indicated that livestock products could be the source of foodborne HEV transmission to people. For example, HEV was detected in 1.9%, 6.4%, and 11% of porcine livers in Japan, the Netherlands, and the USA, respectively [7, 22, 23]. In Japan, more than a few cases of fulminant hepatitis might have been caused by porcine liver consumption [7]. The zoonotic transmissions of HEV were further confirmed in people who consumed deer and wild boar meat [6, 8, 24]. Sausages made from porcine livers might be another source of HEV transmission to people in Europe [22, 25]. Of the eight HEV genotypes, HEV-3 has been mainly identified in the developed countries worldwide. Both HEV-3 and HEV-4 have been found in the raw porcine livers sold at grocery stores in Japan and European countries [7, 22, 23, 25]. In this study, we found both HEV-3 and HEV-4 in porcine livers sold at grocery markets in Korea. In a previous study, we detected both HEV-3 and HEV-4 in the fecal samples of growing pigs [14]. These results indicate that many pig populations reared in Korea may be infected with zoonotic HEV-3 and HEV-4. Interestingly, HEV-4 was identified from a bovine liver sample. To our knowledge, this is the first report of HEV-4 detection in bovine livers. Detection of HEV in bovine livers has been attempted in Hungary, but it was unsuccessful [26]. In contrast, HEV-4 was found in 8.79–37.14, 37.14, and 3.14% of bovine feces, milk, and sera, respectively, in China [17, 18, 27]. Collectively, these results imply that cattle are susceptible to HEV infection. Consuming raw or under cooked bovine and porcine livers should be considered a risk factor in the zoonotic transmission of HEV to people.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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