Epidemiological Screening for Hepatitis E Virus in Bile Specimens from Livestock in Northwest China

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Hepatitis E has been hypothesized as a zoonosis. However, there is no definite conclusion about which animal species contribute to hepatitis E virus (HEV) infection in humans. In this study, HEV RNA was detected only in swine bile specimens and not in bile specimens collected from cattle, goats, or dogs. We postulate that swine are the main animal reservoir for HEV.

Hepatitis E is a serious public health problem in many countries. Hepatitis E virus (HEV) is transmitted via a fecal-oral route commonly through contaminated water (1, 3). Recent studies have also shown that zoonotic food-borne transmission of HEV from domestic pigs, wild boar, or wild deer to humans may occur, as seen by autochthonous infection in Japan among people who ingest uncooked or undercooked meat or viscera from pigs, wild boar, and deer (6, 9). Although anti-HEV antibodies have been found in many livestock species, there are no reports about the detection of HEV in animals other than domestic pigs. The involvement of other animals in human HEV infection needs to be evaluated. In this study, we attempted to identify the potential animal reservoir for human HEV.

From April to May 2007, bile samples (n = 1,295) were collected from swine (n = 603; aged 4 to 6 months), cattle (n = 127; aged 2 to 4 years), goats (n = 390; aged 8 to 12 months), and dogs (n = 178; aged 1 to 2 years) in northwest China (Xi’an, Kashi, and Datong). Samples were diluted in a 10% RNA solution, 1/6H9262 for 10 min. Aliquots (100 ml each) of the clarified material were used for viral RNA extraction with TRIzol reagent (Invitrogen) according to the manufacturer’s protocol. The extracted RNA was dissolved in 80 µl RNase-free pure water. The extracted viral RNA was immediately used for reverse transcription and cDNA synthesis with the SuperScript III first-strand synthesis system (catalog number 18080-051; Invitrogen). Briefly, 8 µl RNA solution, 1 µl 10 mM E5 reverse primer (5’-WGARAG CCAAAGCACATC-3’), and 1 µl 10 mM deoxynucleoside triphosphates were incubated at 75°C for 5 min and then at −3°C for 3 min. A total of 10 µl of cDNA synthesis mix was added and incubated at 42°C for 50 min. Reactions were terminated by heating the mixtures at 75°C for 5 min. Reaction mixtures were stored at −20°C for PCR amplification. Primers for nested PCR, amplification conditions, and nucleotide sequencing were similar with those from our previous report (11).

HEV RNA was detected and sequenced from 11 of the 603 swine bile samples tested. None of the bile samples from cattle, goats, or dogs were positive for HEV RNA. A phylogenetic tree constructed using the neighbor-joining method with MEGA software indicated that all 11 sequences were located on the same branch as AB197673 and AB197674, indicating that they belonged to genotype IV of HEV (Fig. 1).

After the first characterization of swine HEV in 1997 (8), there was growing evidence indicating that hepatitis E is a zoonosis. Large-scale investigation indicated that transmission of the virus was most likely directly from swine to humans (11). Although a high percentage of goats, cattle, and dogs were seropositive for anti-HEV (4), there has been no large-scale screening for HEV natural animal reservoirs in these species prior to this study. In the present study, the only bile samples positive for HEV RNA were from swine, and therefore, we postulate that swine are the main animal reservoir for HEV in nature.

It was proven that bile was the sample most frequently positive for HEV RNA in experimentally infected animals, followed by mesenteric lymph nodes, livers, feces, and sera (2). Consequently, the positive rate for HEV RNA in this study may more accurately reflect the true infection rates than those in other studies which have tested feces. In this study, 2% of swine bile samples were positive for HEV RNA. The 2% positive rate is much lower than those found in other studies in east China because of drier weather, which may not facilitate the transmission of HEV (11).

The genome of HEV is an ~7.2-kb positive-sense, single-stranded RNA. It contains a short, 5’ untranslated region, three open reading frames (ORF1, ORF2, and ORF3), and a
short, 3’ untranslated region terminated by a poly(A) tail (8). HEV isolates worldwide have been classified into four genotypes based on the phylogenetic analysis of their full-length genomes. ORF2 encodes capsid protein, and phylogenetic analysis based on the alignment of the ORF2 sequence is expected to yield results similar to those based on the analysis of the entire viral genome (8). The primers used in this study were based on part of the ORF2 sequence of four HEV genotypes and could amplify all the subtypes prevailing in China; therefore, the positive rate of HEV in the present study is credible (5, 7, 10). The HEV strains detected in the present study were located on the same branch as strains detected in humans that provide further evidence for the zoonotic characters of HEV.

**Nucleotide sequence accession numbers.** The 11 nucleotide sequences reported to GenBank were given the following accession numbers: ZJXS45 (EU489506), ZJXS42 (EU489507), ZJXS131 (EU489508), ZJXS47 (EU489509), ZJXS3 (EU489510), ZJXS9 (EU489511), ZJXS13 (EU489512), ZJXS24 (EU489513), ZJKS56 (EU489514), ZJKS12 (EU489515), and ZJKS28 (EU489516).

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