**NOTE** *Virology*

# Mice are Not Susceptible to Hepatitis E Virus Infection

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ABSTRACT. To determine whether or not mice are susceptible to hepatitis E virus (HEV) infection, C57BL/6 mice were experimentally infected with genotypes 1, 3 and 4 HEV by intravenous injection. Serum and stool samples were collected and used to detect HEV RNA and anti-HEV antibodies by RT-PCR and ELISA. The virus infection was monitored up to two months after inoculation; however, none of the serum or stool samples was positive for virus replication, demonstrating that C57BL/6 mice were not susceptible to HEV.
KEY WORDS: C57BL/6, hepatitis E, hepatitis E virus, HEV, mouse.

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Hepatitis E is a serious public health concern in many developing countries, and recognized as sporadic and endemic acute hepatitis in many industrialized countries. Pregnant women have a high risk associated with hepatitis E, with a high mortality rate (up to 20%) [5, 25]. The causative agent of hepatitis E is hepatitis E virus (HEV), and this virus transmits primarily via the fecal-oral route through contaminated drinking water [1, 6]. HEV is the sole member of the genus *Hepevirus* in the family *Hepeviridae*. HEV is a small round non-enveloped virus, 27–34 nm in diameter, containing an RNA genome approximately 7.2 kb in length [2, 3]. The RNA consists of a single-strand RNA molecule containing three discontinuous and partially overlapping open reading frames (ORFs). The 3' terminus of the RNA is polyadenylated. HEV isolates were grouped into at least four major genotypes, genotypes 1, 2, 3 and 4 (G1, G2, G3 and G4) on the basis of nucleotide and deduced amino acid sequences [3, 6, 24]. Because G3 and G4 HEV were isolated from pigs and wild boars in addition to humans, and much direct and indirect evidence has indicated that HEV transmits from pigs or wild boars to humans, hepatitis E is recognized as a zoonotic disease [8, 18, 23]. Many studies have reported the detection of HEV RNA and the HEV-specific antigen (HEV-Ag) in pig and wild boar stool and serum specimens, and suggested the active circulation of this virus among these animals [18, 20, 26]. HEV-specific antibodies have been detected in many animals including sheep, cows, dogs, cats, wild rats, wild deer and mongoose, in addition to pigs and wild boars [9, 12, 14, 15, 19]. However, it is obscure whether or not HEV substantially replicates in these animals. In this study we infected C57BL/6 mice with G1, G3 and G4 HEV, and monitored the virus growth to determine the susceptibility of mice to HEV infection.

G1 HEV strain was derived from stool specimens from a cynomolgus monkey (*Macaca fascicularis*), born and grown in the Tsukuba Primate Center for Medical Science, National Institute of Infectious Diseases (NIID), which had been experimentally infected with an Indian strain [10]. The G3 HEV strain (DQ079632) was derived from stool specimens collected on a pig farm in Japan. The G4 HEV strain (DQ079628) was from a stool specimen collected from a wild boar caught in Aichi prefecture, Japan. The stool specimens were used to prepare 10% (w/v) suspensions as described [10]. These suspensions were positive for HEV RNA by reverse-transcription polymerase chain reaction (RT-PCR). The concentrations of the G1, G3, and G4 HEV were $5 \times 10^4$, $2 \times 10^4$ and $1 \times 10^5$ copies per one m*l* of suspension, respectively, by real time RT-PCR (unpublished).

To confirm the infectivity of these stool specimens, 3 cynomolgus monkeys (4 year-old males) were inoculated intravenously with 2 m*l* of one of the suspensions, and the stools were collected daily, and used to detect HEV RNA and HEV-Ag. Sera were collected weekly before and after the inoculation to detect HEV RNA, HEV-Ag, and HEV-specific IgG antibodies. The sera were also used to determine ALT values. All monkey experiments were reviewed by the Institute's ethical committee and carried out according to "Guides for animal experiments performed at NIID" under codes 990058, 000019 and 504006. The primates were individually housed in BSL-2 facilities. Detection of HEV RNA, HEV-Ag, and IgG has been described previously [8, 10, 11]. The ALT value was measured as described [10]. As shown in the figure, HEV RNA and HEV-Ag were detected within one week in the sera (A) and stools (B) of all three monkeys after inoculation, and ALT values increased more than three-fold compared with that of pre-inoculation in infected monkeys, though the increase was slow and the values were low in G1 HEV- and G3 HEV-infected animals (C), indicating that all three HEV strains, G1, G3 and G4, were infectious. Furthermore, drastic increases of IgG antibody titers, probably due to extensive replication of the virus, were demonstrated in these animals (D). These results confirmed that the HEVs used in these experiments were indeed infectious.

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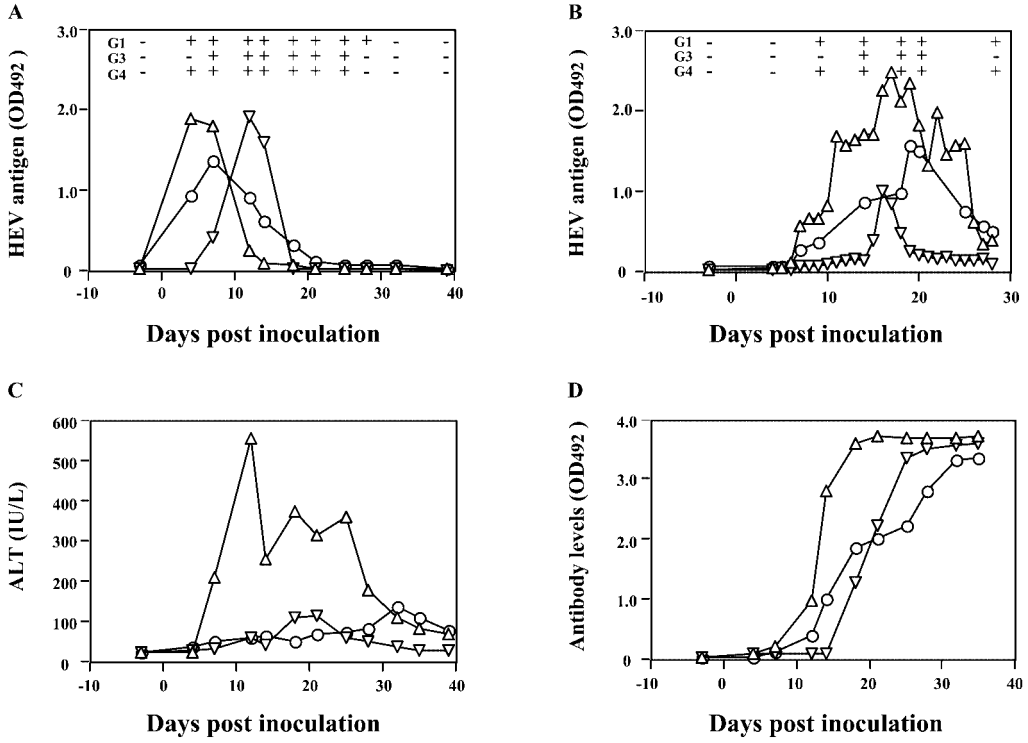


Fig. 1. Kinetics of biochemical, serological, and virological markers in monkeys after inoculation. HEV-Ag and HEV RNA in sera (A) and stools (B), was measured by an antigen ELISA and RT-PCR. ALT was indicated (C), and IgG antibody was measured by an antibody-ELISA (D). ○, monkey inoculated with G1 HEV; ▽, monkey inoculated with G3 HEV; △, monkey inoculated with G4. HEV RNA was monitored by RT-PCR . + , Positive; – , Negative.

Six 4-week-old and five 9-month-old C57BL/6 mice, negative for anti-HEV antibody and HEV RNA, were inoculated intravenously with 100 $\mu l$ of 10% fecal suspensions. Serum and fecal samples were obtained at 1 week before, and at 1, 2, 3, 4, 6, 8, and 10 weeks after inoculation, and HEV RNA and anti-HEV IgG antibodies were measured. However, neither serum nor fecal specimens were positive for HEV infection, clearly indicating that HEV did not replicate in C57BL/6 mice (data not shown). In other words, the C57BL/6 mice were not susceptible to hepatitis E virus.

Anti-HEV IgM and/or IgG antibody and HEV RNA are frequently detected in pigs and wild boars in various countries, and these 2 animals are recognized as the main reservoirs of HEV. Although the infection is asymptomatic when G3 and G4 HEVs are used to inoculate pigs, it is obvious that pigs are susceptible to HEV infection [17]. Interestingly, pigs were resistant to experimental infection with G1 and G2 HEVs [16]. Although experimental data is not available for wild boars, these animals are genetically close to pigs, and wild boars are likely to be susceptible to HEV. Direct and indirect evidence of HEV transmission from wild boars and pigs to humans has been reported in Japan, suggesting that these animals are the main zoonotic reservoirs in this country [8, 27]. Chimpanzees, rhesus monkeys, cynomolgus monkeys, and marmosets have been used for experimental infection and to evaluate the efficacy of HEV vaccines, and HEV has been used as a challenge virus, indicating that these monkeys are susceptible to HEV infection [13, 22, 29, 30]. In addition to these animals, anti-HEV IgG antibody has been detected in dogs, cats, cows, goats, sheep, and rodents including rats [4, 7, 14, 19], and anti-HEV IgG antibody and HEV RNA were detected from mongoose and wild deer [9, 21, 28]. However, the susceptibility of these animals to HEV infection has not been fully evaluated, and whether or not HEV replicates *in vivo* in these animals is unknown.

We evaluated the susceptibility of B57C/6 mice by directly inoculating infectious HEV through intravenous injection. Although two different age groups, at 4 weeks and 9 months, were used, none of the mice was successful in producing *in vivo* HEV replication. Our study clearly demonstrated that C57BL/6 mice are resistant to HEV infection. By contrast, our preliminary results indicated that HEV is capable of replicating in chimeric mice harboring replaced human hepatocyte cells when exactly the same amount of the G1, G3 and G4 HEV suspension is used (manuscript in preparation). These results indicate that the human hepatocyte is a major target cell for HEV infection, and HEV is not capable of replicating in mice.

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