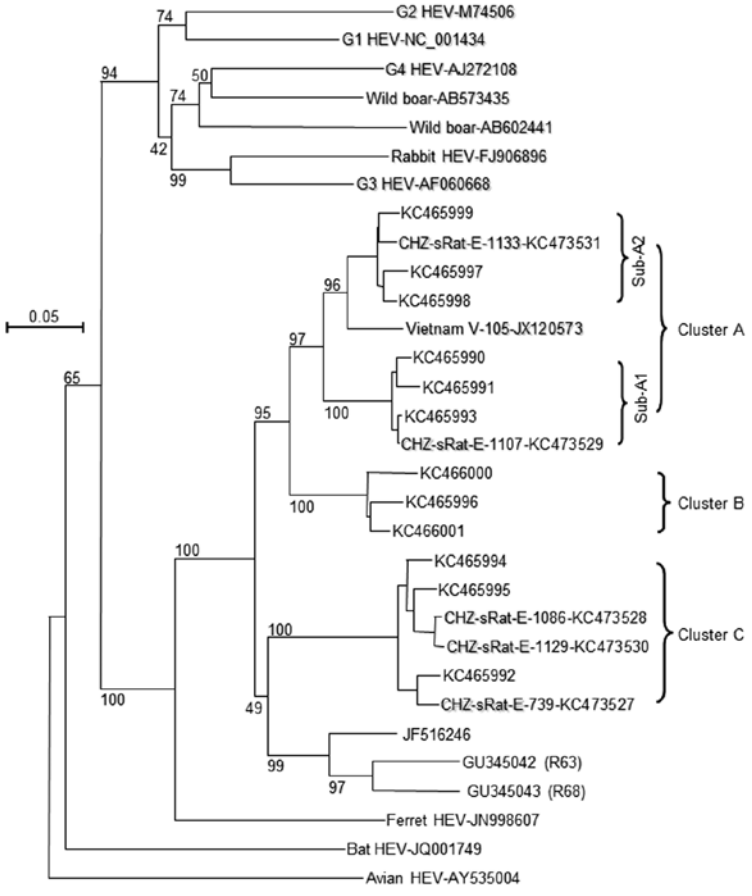

Figure. Phylogenetic analysis of rat hepatitis E virus (HEV) isolated from Asian musk shrews (*Suncus murinus*) in Zhanjiang City, China. Nucleic acid sequence alignment was performed by using ClustalX 1.81 (www.clustal.org). The genetic distance was calculated by using the Kimura 2-parameter method. The phylogenetic tree, with 1,000 bootstrap replicates, was generated by the neighbor-joining method based on the partial sequence (281 nt) of HEV open reading frame 1 of genotype 1–4, wild boar, rabbit, ferret, bat, avian, and rat HEV isolates. The scale bar indicates nucleotide substitutions per site.

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Address for correspondence: Changwen Ke, Institute of Pathogenic Microbiology Center for Disease Control and Prevention of Guangdong Province, 176 Xin Gang West Rd, Guangzhou 510300, China; email: kecw1965@yahoo.com.cn

No Evidence for Hepatitis E Virus Genotype 3 Susceptibility in Rats

To the editor: Hepatitis E virus (HEV) is a positive-sense single-stranded RNA virus (genus *Hepevirus*, family *Hepeviridae*) (*1*). In humans, acute hepatitis infection caused by HEV is a serious public health concern in developing countries. Four HEV genotypes, G1–4, have been isolated from humans (*2*). G3 and G4 HEV have also been isolated from swine, wild boars, wild deer, and mongooses; these animals are thought to be the reservoirs of HEV (*3*). Direct evidence has indicated that HEV is transmitted from pigs or wild boars to humans; therefore, hepatitis E caused by G3 and G4 HEV infection is recognized as a zoonosis (*3*).

Although rats have long been suspected to be a potential reservoir for human HEV, no direct evidence has been found. The susceptibility of rats to human HEV genotypes is

controversial. For example, anti-HEV IgG has been detected in various rat species, including Norway (*Rattus norvegicus*), black (*Rattus rattus*), and cotton (*Sigmodon hispidus*) rats, by using ELISA with antigens derived from G1 HEV. These results suggest that HEV or HEV-like virus infections occur in wild rats. However, the virus genome has not been detected, and the source of the infection was confirmed in few cases; thus far, it is not clear whether the anti-HEV IgG was induced by HEV or other HEV-like viruses. The detection of a partial genome of G1 HEV from wild rats in Nepal was reported in 2002 (*4*); however, this report was retracted in 2006 because the isolated strain was determined to be a result of laboratory contamination. Recently, Lack et al. isolated strains of G3 HEV from a variety species of wild rats in the United States (*5*); this finding suggests that wild rats are hosts for G3 HEV. Maneerat et al. also reported that human HEV (presumably G1) was transmissible to Wistar laboratory rats (*6*). However, Purcell et al. recently reported that G1, G2, and G3 do not infect laboratory rats (*7*), and we found in a previous study that laboratory rats are not susceptible to G1, G3, or G4 HEV (*8*).

To further investigate the potential susceptibility of rats to infection with human HEV, we experimentally injected nude rats with G3 HEV and monitored virus growth. We used 2 samples of G3 HEV for the infection experiments, 1 derived from fecal specimens collected from a pig farm in Japan (GenBank accession no. DQ079632) and 1 derived from the supernatant of a hepatocarcinoma cell line, PLC/PRF/5, that was injected with the pig specimen. The infectivity of these samples was confirmed by experimental infections of cynomolgus monkeys (*9*; data not shown).

Six 15-week-old female nude rats (athymic rats, Long-Evans-run/run; Japan SLC, Inc., Hamamatsu, Japan) were used in this study. These rats, which are bred to be immunodeficient, are known to be susceptible to rat HEV, but it is unknown if they are susceptible to other types of HEV. All rats were negative for G3 HEV RNA and anti-HEV antibodies, as determined by nested reverse transcription PCR (*10*) and ELISA (*8*), respectively. Rats were housed individually in biosafety level 2 facilities. Experiments were reviewed by the ethics committee of the National Institute of Infectious Diseases (NIID) Japan and carried out according to the "Guidelines for animal experiments performed at NIID" under code 113060.

The 6 rats were randomly assigned to 2 groups, injected intravenously with 500 μL of an HEV sample suspension through the tail vein, and monitored for 3 months. The 3 rats in group 1 were injected with the sample derived from pig feces, which contained 5×10^4 copies of G3 HEV; the 3 rats in group 2 were injected with the cell culture supernatant sample, which contained 4×10^6 copies of G3 HEV. Serum samples were collected weekly for examination of HEV RNA and anti-HEV IgG and IgM and were also used to determine alanine aminotransferase values. Fecal samples were collected every 3 days to detect HEV RNA. The animals were humanly killed by exsanguination 91 days postinjection, liver tissues were collected, and a 10% tissue suspension was prepared as described (*8*).

For groups 1 and 2, all serum samples collected 1–13 weeks postinjection were negative for HEV RNA and anti-HEV IgG and IgM. HEV RNA also was not detected in fecal samples or liver tissues (Table). Alanine aminotransferase elevation was not observed in any serum samples.

In conclusion, even by using samples with high titers of HEV RNA in injection experiments, we were unable to cause infection with G3 HEV in immunodeficient nude rats. We found no evidence that rats are susceptible to infection with G3 HEV.

This study was supported in part by grants for Research on Emerging and Re-emerging Infectious Diseases, Research on Hepatitis, and Research on Food Safety from the Ministry of Health, Labour and Welfare, Japan.

Tian-Cheng Li, Yasushi Ami, Yuriko Suzaki, Naokazu Takeda, and Wakita Takaji

Author affiliations: National Institute of Infectious Diseases, Tokyo, Japan (T.-C. Li, Y. Ami, Y. Suzaki, W. Takaji); and Osaka University Research Institute for Microbial Diseases, Osaka, Japan (N. Takeda)

DOI: http://dx.doi.org/10.3201/eid1908.130200

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Address for correspondence: Tian-Cheng Li, Department of Virology II, National Institute of Infectious Diseases, 4-7-1 Gakuen, Musashimurayama, Tokyo 208-0011, Japan; email: litc@nih.go.jp

Fatal Case of Enterovirus 71 Infection and Rituximab Therapy, France, 2012

To the Editor: Enterovirus 71 (EV-71) causes primarily asymptomatic or benign infections in children <5 years of age. However, it may cause severe and sometimes fatal neurologic complications, such as brainstem encephalitis and polio-like paralysis (*1*). Over the last 15 years, large outbreaks of EV-71 infection have been described in the Asia–Pacific region, associated with the regular emergence of new genetic lineages (*2*). Since the 1978 outbreak in Hungary, rare sporadic cases have been reported in Europe (*1*). In France, during 2000–2009, a total of 81 hospitalized patients with EV–17 infection were reported by the sentinel surveillance system, including 2 child deaths, 1 due to proven rhombencephalitis (*3*,*4*).

We report here a fatal case of EV-71 rhombencephalitis in an immunocompromised adult who was receiving rituximab therapy. Rituximab is a chimeric anti-CD20 monoclonal antibody that is widely used for treating B-cell lymphoma and an increasing number of autoimmune diseases. Since rituximab became commercially available, several infectious side-effects for the drug have been reported, including hepatitis B reactivation, progressive multifocal leukoencephalopathy, and enteroviral meningoencephalitis (*5*). The first 2 cases of rituximab-associated enteroviral meningoencephalitis were reported in 2003 (*6*), and 5 additional cases have been reported to date (*7,8*).

In May 2012, a 66-year-old woman was hospitalized in the neurology unit of Bordeaux University Hospital with a 10-day history of fever, asthenia, and psychomotor retardation. She had no history of travel and had not been in close contact with sick persons. She had received a diagnosis of grade I follicular lymphoma 3 years earlier, and it had been treated with 6 cycles of R-CHOP (rituximab, cyclophosphamide, hydroxydaunorubicin, oncovin, prednisolone). Since July 2010, the lymphoma had been in remission, and she had been receiving maintenance therapy with rituximab since that time. The most recent rituximab infusion had been administered in March 2012. Her condition was treated initially with broad-spectrum antibiotics and acyclovir. Still, aphasia, facial paralysis, spastic movements, and consciousness disorders rapidly developed. On day 6, she was transferred to the intensive care unit for ventilatory support.

On patient's admission, blood samples showed lymphopenia (0.64×10^3 cells/mm^3) and low immunoglobulin levels, i.e., IgG 4.5 g/L (reference range 6.75–12.8 g/L) and IgM 0.33 g/L (reference range 0.56–1.9 g/L). Three cerebrospinal fluid (CSF) samples were collected on days 1, 4, and 6. CSF leukocyte count rose from 5 to 89 cells/mm^3, with lymphocytes from 24% to 95%, and protein levels rose from 0.68 to 1.03 g/L (reference range 0.15–0.45 g/L). CSF glucose level varied from 3.5 to 4.5 mmol/L (reference range 2.7–3.9 mmol/L). Enterovirus RNA was detected in the patient's first 3 CSF samples and in CSF, stool specimens, and blood until 4 weeks after admission (online Technical Appendix Table 1, wwwnc.cdc.gov/EID/article/19/8/13-0202-Techapp1.pdf). PCR assays of the first 3 CSF samples were negative for JC polyomavirus, herpes simplex virus, varicella-zoster virus, cytomegalovirus, Epstein-Barr virus, human herpesvirus 6, adenovirus, and *Toxoplasma gondii*. Serologic tests for parvovirus B19, mumps virus, and measles virus were IgM negative. Samples were also negative for antibodies against Hu, Ri, Yo, and voltage-gated potassium channel antigens. All bacterial cultures were negative. No evidence for central nervous system infiltration by lymphoma cells was found, on the basis of CSF cytology.

Results of brain magnetic resonance imaging (MRI) scans performed on days 2 and 6 were normal, despite the patient's consciousness disorders (Figure, panel A). However, on day 13, MRI scans showed bilateral and symmetric T2 and FLAIR hypersignals in the medulla, the pons, and the mesencephalon, compatible with rhomboencephalitis (Figure, panel B). On day 24, the MRI scan showed a supratentorial extension involving white matter, the insular cortex, and basal ganglia (Figure, panel C). The patient's neurologic condition deteriorated progressively, and she died of enteroviral rhomboencephalitis 32 days after admission.

The EV associated with the rhomboencephalitis was identified as an EV-71 genogroup C2 isolate by 1D gene complete sequencing and phylogenetic analysis (online Technical Appendix Figure; online Technical Appendix Table 2). The 1D gene sequences determined from cerebrospinal fluid and fecal specimens from the patient showed 95%–97% nucleotide