Short Communication

No Evidence of Rat Hepatitis E Virus Excretion in Urine Samples of Rats

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SUMMARY: To investigate whether rat hepatitis E virus (rat HEV) is excreted in the urine of HEV-infected rats, we infected 3 Wistar and 6 nude rats with rat HEV and examined the rat-HEV RNA in serum, fecal, and urine samples. We detected rat-HEV RNA in the serum and fecal samples of all 9 rats but not in any of the urine samples. Our results suggest that in rats, rat HEV is not transmitted via urine.

Acute hepatitis E caused by hepatitis E virus (HEV) infection is a serious public health concern in both developing and developed countries (1). HEV is a positive-sense single-strand RNA virus that belongs to the family *Hepeviridae*, which is divided into the genera *Orthohepevirus* and *Piscihepevirus*, and the *Orthohepevirus* genus comprise the species *Orthohepevirus A* to *D* (2, 3). The human-pathogenic HEV genotypes 1 to 4 (G1 to G4) belong to *Orthohepevirus A*, whereas other HEV-like viruses found in different animal species are grouped into other species. The rat HEV is a representative of *Orthohepevirus C* and has been detected in a variety of rat and shrew species (4–8). The reverse genetics system for rat HEV has been established (9), which may enable the establishment of a rodent model for human HEV in the future.

In general, HEV is transmitted by the fecal-oral route or blood transfusion (10). However, a recent study reported the detection of HEV in the urine of humans and monkeys, indicating that urine could be another source of HEV transmission (11). In the present study, to determine whether virus excretion in urine is a common feature of HEV infection, we experimentally infected Wistar rats and nude rats with rat HEV and analyzed the rat HEV shedding into the urine. All animal experiments were reviewed by the Ethical Committee of Japan's National Institute of Infectious Diseases (NIID) and carried out according to the "Guides for Animal Experiments Performed at NIID," under codes 113029 and 114012. Rats were individually housed in Biosafety Level 2 (BSL-2) facilities.

Because rat HEV inoculation leads to persistent infection in nude rats (12), these rats may serve as a good model for investigation of the virus distribution in tissue and excretions including urine. Therefore, we collected rat fecal, serum and urine samples from 6 nude rats (LR1, LR2, RR1, RR2, CR1, and CR2; Long-Evans *rnu/rnu*, Japan SLC, Hamamatsu, Japan), which had been experimentally infected with rat-HEV strain R63/DEU/2009 in a previous study (9) and further housed in the BSL-2 facilities after its completion.

In the previous study, rats LR1 and LR2 were intra-hepatically inoculated with 1.3×10^{14} copies of capped rat-HEV RNA, and rat HEV was recovered, without any sequence changes, from their fecal specimens. Rats RR1 and RR2 were intravenously inoculated with 0.3 ml of stool suspension from rats LR1 and LR2 containing of 3.24×10^9 copies and 5.82×10^9 copies of rat HEV, respectively. Rats CR1 and CR2 were intravenously inoculated with 1.21×10^9 copies of rat HEV, which had been isolated from PLC/PRF/5 cell culture supernatant that was infected with rat HEV using a fecal specimen from rat LR1 (9).

Urine, fecal, and serum samples were collected from rats LR1 and LR2 starting at 15-weeks post-inoculation (pi), from rats RR1 and RR2 at 8-weeks pi, and from rats CR1 and CR2 at 5-weeks pi, and each nude rat was monitored for 4 weeks. For the detection of rat-HEV RNA, the urine samples were collected daily and fecal and serum samples were collected weekly. To reduce the risk of contamination, fecal and urine samples were collected separately by using metabolic cage (Lab Products, New Brunswick, NJ, USA).

For RNA extraction, the fecal specimens were diluted with 10 mM phosphate-buffered saline to prepare a 10% suspension, then shaken at 4°C for 1 h. Both the urine samples and the 10% fecal suspension were clarified by centrifugation at 10,000 *g* for 30 min, and the supernatant was then passed through a 0.45- μ m membrane filter (Millipore, Bedford, MA, USA). The RNA extraction from 200- μ l fecal suspension, urine and serum samples was conducted using the MagNA Pure LC system with a

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MagNA Pure LC Total Nucleic Acid isolation kit (Roche Applied Science, Mannheim, Germany). For the detection and quantification of rat-HEV RNA, a quantitative reverse transcription-polymerase chain reaction (RT-qPCR) assay was performed using the 7500 FAST Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) as described (9). This assay has a sensitivity to detect 10 copies of template in a 20 µl reaction.

Rat-HEV RNA was consistently detected in the fecal specimens of the infected nude rats in amounts ranging from 8.74×10^{10} to 4.18×10^{11} copies/g (Fig. 1). Rat-HEV RNA was also detected in all serum samples of these rats in amounts ranging from 6.28×10^6 to 1.11×10^8 copies/ml. These results indicate that all the 6 nude rats presented with persistent rat-HEV infection. However, rat-HEV RNA was not detected in any of the urine samples of the nude rats suggesting that rat HEV is not excreted in the urine of rat-HEV-infected nude rats.

Considering that the severe immunosuppression of nude rats may affect the rat-HEV excretion, we subsequently infected Wistar rats with rat HEV and tested the

urine for rat-HEV RNA. Three 15-week-old Wistar rats (female, W1, W2, and W3; Japan SLC) were used in this study. All rats were negative for rat-HEV RNA and anti-rat-HEV antibodies as determined by nested broad-spectrum reverse transcription-polymerase chain reaction (RT-PCR) (13) and enzyme-linked immunosorbent assay (ELISA) (14).

Each Wistar rat was intravenously inoculated through the tail vein with 0.5 ml of 10% stool suspension from nude rat LR1 containing 1.0×10^7 copies/ml of rat-HEV RNA (R63/DEU/2009). For the next 3 weeks, fecal and urine samples were collected daily and serum samples were collected weekly for the detection of rat-HEV RNA by RT-qPCR. Sera (1:200 dilution) were further analyzed for the presence of anti-rat-HEV IgG antibodies by an ELISA (14).

In all 3 Wistar rats, rat-HEV RNA was first detected in the fecal specimens on day 5 or day 6 pi and reached a peak on days 8 to 10 pi, in amounts between 1.39×10^8 copies/g and 2.02×10^8 copies/g (Fig. 2). In the sera, rat-HEV RNA was detected only at 1-week pi in amounts

Fig. 1. Detection of rat HEV RNA in experimentally infected nude rats. Fecal ($\triangle$), serum (∇), and urine ($\diamond$) samples were collected from 6 nude rats (LR1, LR2, RR1, RR2, CR1, CR2), that had been persistently infected with rat HEV after a previous experimental inoculation. The amount of rat HEV-RNA in fecal (copies/g), serum, and urine (copies/ml) samples was determined by RT-qPCR.

Fig. 2. Detection of rat HEV RNA in experimentally infected Wistar rats. Three Wistar rats (W1, W2, and W3) were intravenously inoculated with rat HEV. Fecal ($\triangle$) and urine ($\diamond$) samples were collected daily and serum samples (∇) were collected weekly for the detection of rat HEV RNA by RT-qPCR. Anti-rat HEV IgG antibody ($\circ$) in rat serum samples was detected by ELISA.

between 1.01×10^3 copies/ml and 2.59×10^5 copies/ml. Anti-rat-HEV IgG antibodies were detected in all rats beginning at 2-weeks pi and reaching a peak at 3-weeks pi, with OD values higher than 3.0. Rat-HEV RNA was not detected in any of the rat urine samples indicating that rat HEV is not excreted in the urine of infected Wistar rats.

To confirm whether a trace amount of rat HEV was present in urine and because the sensitivity of the RT-qPCR for the detection of rat-HEV RNA was 10 copies, we concentrated the urine samples 15 times by ultracentrifugation (100,000 g, 2 h) and used the concentrated samples for rat-HEV detection. No rat HEV was detected in the concentrated samples. In a previous study, HEV antigen (HEV Ag) was detected in the urine of HEV-infected monkeys and the ratio of HEV Ag to HEV RNA in the urine was significantly higher than in sera and feces (11). We therefore also performed an ELISA for the detection of rat HEV antigen (9) in the urine samples from our experiments, but no rat-HEV antigen was detected (data not shown). In conclusion, our results indicate that rat HEV is not transmitted via the urine of rats.

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Conflict of interest None to declare.

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