Full title

Basic reproduction numbers of three strains of mouse hepatitis viruses in mice

Short title

DYNAMICS OF MHV IN MICE

Masataka Nakayama and Shigeru Kyuwa*

Laboratory of Biomedical Science, Department of Veterinary Medical Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

*Corresponding author

e-mail: akyuwa1@g.ecc.u-tokyo.ac.jp

Abstract

Mouse hepatitis virus (MHV) is a murine coronavirus and one of the most important pathogens in laboratory mice. Although various strains of MHV have been isolated, they are generally excreted in the feces and transmitted oronasally via aerosols and contaminated bedding. In this study, we attempted to determine the basic reproduction numbers of three strains of MHV to improve our understanding of MHV infections in mice. Five-week-old female C57BL/6J mice were inoculated intranasally with either the Y, NuU, or JHM variant strain of MHV and housed with two naive mice. After 4 weeks, the presence or absence of anti-MHV antibody in the mice was determined by the enzyme-linked immunosorbent assay. We also examined the distribution of MHV in the organs of Y, NuU, or JHM variant-infected mice. Our data suggest that the transmissibility of MHV is correlated with viral growth in the gastrointestinal tract of infected mice. To the best of our knowledge, this is the first report to address the basic reproduction numbers among pathogens in laboratory animals.

Introduction

The microbiological control of laboratory animals is essential to obtain reproducible and stable experimental results, and mouse hepatitis virus (MHV) is a representative pathogen for microbiological monitoring.[1] MHV is a single-stranded plus-sense RNA virus with petal-like projections that belongs to the family *Coronaviridae*, and its natural host is mice.[2] MHV causes hepatitis, enteritis, and encephalitis in mice, and its pathogenesis varies depending on the viral strain, infectious dose, route of infection, and the genetic background, age, and immune status of the host.[3-5] Experimental infection with MHV has been used to generate hepatitis[6,7] and a demyelinating disease mouse model..[8-11] Studies have also been conducted to elucidate the replication and multiplication mechanisms of coronaviruses using MHV as a model.[12,13] In recent years, the worldwide coronavirus disease 2019 pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to the use of MHV as a surrogate for SARS-CoV-2 in research on disinfectants.[14,15]

In Europe and the United States, MHV has the second highest infection rate in laboratory animals after mouse norovirus and mouse parvovirus.[16] The spread of MHV in animal research facilities causes wasting disease and death in immunocompromised mice such as nude mice and suckling mice,[17,18] and subclinical infection in adult mice

reportedly modifies experimental performance in many experimental models.[19] MHV is generally excreted in the feces of infected mice and is transmitted oronasally, but it is also transmitted via aerosols and contaminated bedding.[20] Depending on the mouse strain and immune status, MHV-infected mice excrete infectious MHV for several days to several weeks.[21] Contamination by MHV in a specific pathogen-free laboratory animal facility requires total culling of the infected mouse colony and disinfection of the facility, and the impact is not small.[22] The basic principle of MHV infection control in facilities housing laboratory animals is to prevent exposure of the animals to MHV. Adequate quarantine is necessary when introducing new animals from other facilities. In addition, wild mice infected with MHV,[23] pet mice,[24,25] and biological materials obtained from infected mice are also sources of infection and should be handled with care.[26,27] It is possible that the virus may be introduced into the facility unknowingly by scientists or caretakers who come into contact with MHV-contaminated animals or breeding equipment.

Serological methods such as the enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody methods are commonly used to diagnose MHV in animal testing facilities, and RT-PCR[28] and RT-nested PCR methods have also been used in recent years.[29,30]

Pathogenicity and organ affinity vary among the many strains of MHV.[2,31] In this study, we used the Y, NuU, and JHM variant strains. The Y strain was isolated from suckling mice with symptoms of acute cecal colitis;[32] the NuU strain is a less pathogenic strain that was isolated from nude mice with wasting disease;[33] and the JHM strain was isolated from suckling mice with diarrhea.[34] The JHM strain is used to produce a model of multiple sclerosis because it causes demyelinating encephalitis when inoculated into the brain of mice.[35] However, the JHM strain induces acute fatal encephalitis after intracerebral infection.[3,36] Therefore, we used the JHM variant 2.2-V-1 in this study, which was selected with monoclonal antibody J.2.2, and it loses the ability to cause acute encephalitic illness after intracerebral inoculation.[36] When MHV is inoculated intranasally into mice, it is believed to multiply in nasal epithelial cells and spreads to other organs via the olfactory nerve, lymphatic system, and viremia.[37]

Although there have been reports comparing pathogenicity, physicochemical properties,[38] and gene sequences of MHV viral strains,[39,40] no direct comparison of transmissibility has been conducted. To gain a better understanding of MHV epidemiology, we attempted to determine the basic reproduction numbers of three strains of MHV in mice. The basic reproduction number (R_0) is defined as the average number of secondary infections generated by one infected individual in a population in

which all individuals are susceptible.[41,42] We also examined viral distribution in mice infected with each MHV for a better understanding of the dynamics of MHV infection in mice. The results of this study suggest that viral growth in the gastrointestinal tract plays an important role in the transmissibility of MHV.

Materials and Methods

Mice

Five-week-old, MHV-free, female C57BL/6J (B6) mice were purchased from Japan SLC (Shizuoka, Japan). Some mice were inoculated intranasally with MHV and kept in cages in a negatively pressured isolator (KIS-145; Ishihara Corporation, Osaka, Japan) in the animal room, which was maintained at a temperature of 23 ± 5°C, humidity of 55 ± 5%, and 12-h illumination (light period: 8:00–20:00; dark period: 20:00–8:00). A plastic mouse breeding cage (220 × 160 × 125 mm; Ishihara Corporation) with a stainless-steel wire top was used. Each cage was filled with approximately 1,000 mL bedding (ALPHA-dri; Shepherd Specialty Papers, Watertown, TN, USA). To avoid artificial transmission of MHV, the bedding was not changed during the experiment. Mice were fed γ-ray-sterilized pellets (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water *ad libitum* from a plastic bottle. The animal protocol was reviewed and approved by the Institutional Animal Committee for Use and Care at the Graduate

104 School of Agricultural and Life Sciences (University of Tokyo, Tokyo, Japan), and was
105 conducted in accordance with the Animal Experiment Implementation Regulations and
106 Animal Experiment Implementation Manual of the University of Tokyo.
107
108 **Viruses and cells**
109 Y,[32] NuU,[33] and JHM variant (2.2-V-1)[36] strains were cultured with DBT cells, which
110 are MHV-sensitive.[43] The JHM variant 2.2-V-1 was a kind gift from Dr. John O Fleming
111 (University of Southern California School of Medicine, Los Angeles, CA then). DBT
112 cells were cultured in Eagle's minimal essential medium (E-MEM) containing 5% fetal
113 bovine serum and 1% tryptose phosphate broth (Sigma-Aldrich Co., St. Louis, MO,
114 USA) at 37°C in 5% CO_2 in humidified air.
115
116 **Viral infection experiments**
117 To determine the basic reproduction numbers of three stains of MHV in mice, the
118 concentration of the Y, NuU, and JHM variant strains was adjusted to 1×10^4 PFU/0.02
119 mL saline solution and inoculated intranasally using a micropipette into 5-week-old
120 female B6 mice under isoflurane inhalation anesthesia. Each inoculated mouse was
121 maintained for 2 days in a cage. Then, one inoculated mouse was bred with two naïve

5-week-old, female B6 mice for 4 weeks. Four cages were prepared for the Y strain, and five cages were prepared for the NuU and JHM variant strains. Subsequently, all mice were euthanized. Serum was collected and frozen at −80°C until use. To determine viral growth in each organ in mice, B6 mice were inoculated intranasally with 1×10^4 PFU/0.02 mL of each of the Y, NuU and JHM variant strains. Four mice each were euthanized and necropsied on days 1, 3, and 5 after inoculation, and the brain, liver, jejunum, ileum, and colon were aseptically sampled and frozen at −80□ until use.

Serological tests

The anti-MHV antibody titer in mouse serum was determined by ELISA using a commercially available ELISA kit (MONILISA® MHV 96-well; Wakamato Pharmaceutical Co., Ltd., Tokyo, Japan), and absorbance was measured using a plate reader. Transmission from MHV-inoculated mice to naïve mice was judged by seroconversion against MHV in naïve mice housed together with virus-inoculated mice.

Quantification of infectious virus

Quantification of infectious virus in organs was performed by the plaque assay.[44] Briefly, tissue samples from the brain, liver, jejunum, ileus, and colon were homogenized in

chilled E-MEM to generate a 10% solution and then centrifuged at 3,000 rpm for 10 min. Ten-fold serial dilutions were prepared, and each dilution was assayed for infectious viruses in duplicate in DBT cells. For samples from the jejunum, ileus, and colon, 5 mg/mL gentamicin sulfate (Wako Pure Chemical Industries, Ltd., Tokyo, Japan) was added to E-MEM when generating the suspensions.

Calculation of basic reproduction numbers

In the mouse cohabitation experiment, the average number of antibody-positive mice derived from one infected mouse was calculated to be the basic reproduction number.

Statistical analyses

The data obtained in the experiments were subjected to statistical analyses by the Wilcoxon rank-sum test, with $P < 0.05$ considered statistically significant.

Results

Basic reproduction number of three MHV strains

To determine the basic reproduction numbers of three strains of MHV, naïve mice were cohabitated with mice inoculated with either the Y, NuU, or JHM variant strain for 4

158 weeks, and anti-MHV antibody production was examined by ELISA. In all four cages
159 administered the Y strain, all naïve mice cohabitating with the mouse inoculated with
160 the Y strain were seroconverted (Fig. 1). For the NuU strain, one naïve mouse out of
161 two in two cages produced anti-MHV antibodies. All naïve mice in the remaining three
162 cages were negative. Among the five cages administered the JHM variant strain, three
163 inoculated mice showed a positive antibody response while the remaining two did not.
164 Neither naïve mouse cohabitating with JHM variant-infected mice were seroconverted.
165 The basic reproduction number was calculated after excluding the cages in which the
166 inoculated mice did not show a positive antibody response. As shown in Table 1, the
167 basic reproduction numbers of the Y, NuU, and JHM variant strains were determined to
168 be ≥ 2, 0.4, and 0, respectively.
169
170 **Viral growth in organs in Y, NuU, or JHM variant-infected mice**
171 To investigate the relationship between the basic reproduction number and viral growth
172 in various organs in mice, viral growth in the brain, liver, jejunum, ileus, and colon was
173 examined. Five-week-old, MHV-free female B6 mice (n = 4 or 5) were inoculated with
174 1×10^4 PFU of the Y, NuU, or JHM variant strains, and the brain, liver, jejunum, ileus
175 and colon were removed on days 1, 3, and 5 after inoculation. In mice inoculated with

the Y and NuU strains, infectious virus was detected in all organs examined (Table 2, Fig. 2). On the other hand, in mice inoculated with the JHM variant strain, infectious viruses in the jejunum, ileus, and colon were all below the detection limit. The amount of infectious virus detected in the brains of mice inoculated with the Y, NuU, and JHM variant strains tended to increase with each day of inoculation during the experiment (Fig. 2). On the other hand, there was no change in the mean amount of infectious virus detected in the liver of mice on days 3 and 5 after inoculation with the Y, NuU, and JHM variant strains. In mice inoculated with the Y and NuU strains, infectious virus was detected in the jejunum and ileus from day 1 after inoculation. In mice inoculated with the Y and NuU strains, the detection rate of infectious virus up to 3 days after inoculation was higher in the jejunum than in the ileus (Table 2). In mice inoculated with the Y and NuU strains, the detection rate of infectious virus in the colon by day 5 after inoculation was less than 50%, which was the lowest among the organs examined (Table 2).

Discussion

Basic reproduction number is an important epidemiological parameter defined as the average number of secondary infections generated by one infected individual in a

194 population in which all individuals are susceptible.[41,42] Basic reproduction number is
195 not simple and is affected by numerous biological, sociobehavioral, and environmental
196 factors that govern pathogen transmission.[45] In this study, we attempted to determine the
197 basic reproduction number of MHV in mice in laboratory animal facilities. The data
198 suggest that the Y strain is the most transmissible, and the JHM variant is not
199 transmissible by cohabitation. There have been some reports addressing how MHV
200 spreads among mice in laboratory animal facilities,[46,47] but to the best of our knowledge,
201 no study has examined the basic reproduction number of MHV in mice.

202 Infectious virus was detected in all organs of mice inoculated with Y and NuU
203 strains, while infectious virus in the jejunum, ileus, and colon of mice inoculated with
204 JHM variant was below the detection limit. The difference in transmissibility between
205 the Y and JHM variants may be because the JHM variant is less likely to increase in the
206 gastrointestinal tract and be excreted in the feces compared with the Y strain in the case
207 of intranasal inoculation. The quantitative results of infectious virus in organs did not
208 explain the difference in transmissibility between the Y and NuU strains. For both
209 strains, examination of the amount of virus excreted in feces and excretion period using
210 another assay, for example, quantitative PCR is expected to provide evidence of a
211 difference in transmissibility. In addition, considering the infection route of MHV, we

212 could not exclude the possibility of physicochemical stability of each viral strain in the
213 external environment where mouse feces are discharged.

214 Barthold *et al.*[48] detected infectious virus in the gastrointestinal tract at 3 and 5
215 days after intranasal inoculation of BALB/cByJ mice with 10^3 of the median tissue
216 culture infectious dose of the JHM strain. However, in this study, no infectious virus
217 was detected in the gastrointestinal tract of C57BL/6J mice intranasally inoculated with
218 the JHM strain. There are many substrains of JHM with different antigenicities.[49] The
219 JHM strain used in this study is a variant strain resistant to S protein-specific
220 monoclonal antibody[36] and is not identical to the strain used by Barthold *et al.*[48] The
221 reason for the different results from those of Barthold *et al*. may be due to the difference
222 in viral strain and host (BALB/cByJ vs. B6).

223 The cage and rack system have been developed and marketed for the purpose
224 of better microbiological control of laboratory animals. MHV is a representative
225 pathogen for mice and is used for the evaluation of system functions.[50,51] Based on our
226 results, it may be possible to evaluate the protective function of cages and racks against
227 MHV infection in mice by using a strain with relatively high transmissibility, such as
228 the Y strain.

229

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Declaration of Conflicting Interests

The author(s) declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. FELASA working group on revision of guidelines for health monitoring of rodents and rabbits, Mähler Convenor M, Berard M, Feinstein R, et al. FELASA recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units. *Lab Anim* 2014; 48:178-192.
2. Weiss SR and Leibowitz JL. Coronavirus pathogenesis. *Adv Virus Res* 2011; 81:85-164.
3. Hirano N, Murakami T, Taguchi F, et al. Comparison of mouse hepatitis virus strains for pathogenicity in weanling mice infected by various routes. *Arch Virol* 1981; 70:69-73.
4. Barthold SW. Host age and genotypic effects on enterotropic mouse hepatitis virus infection. *Lab Anim Sci* 1987; 37:36-40.
5. Kyuwa S, Tagawa Y, Shibata S, et al. Murine coronavirus-induced subacute fatal peritonitis in C57BL/6 mice deficient in gamma interferon. *J Virol* 1998; 72:9286-9290.
6. Arévalo AP, Pagotto R, Pórfido JL, et al. Ivermectin reduces *in vivo* coronavirus infection in a mouse experimental model. *Sci Rep* 2021; 11:7132.
7. Grabherr S, Ludewig B and Pikor NB. Insights into coronavirus immunity taught

by the murine coronavirus. *Eur J Immunol* 2021; 51:1062-1070.

8. Savarin C and Bergmann CC. Fine Tuning the Cytokine Storm by IFN and IL-10 Following Neurotropic Coronavirus Encephalomyelitis. *Front Immunol* 2018; 9:3022.
9. Sariol A, Mackin S, Allred MG, et al. Microglia depletion exacerbates demyelination and impairs remyelination in a neurotropic coronavirus infection. *Proc Natl Acad Sci USA* 2020; 117:24464-24474.
10. Pan R, Zhang Q, Anthony SM, et al. Oligodendrocytes that survive acute coronavirus infection induce prolonged inflammatory responses in the CNS. *Proc Natl Acad Sci USA* 2020; 117:15902-15910.
11. Chakravarty D, Saadi F, Kundu S, et al. CD4 deficiency causes poliomyelitis and axonal blebbing in murine coronavirus-induced neuroinflammation. *J Virol* 2020; 94:e00548-20.
12. Gribble J, Stevens LJ, Agostini ML, et al. The coronavirus proofreading exoribonuclease mediates extensive viral recombination. *PLoS Pathog* 2021; 17:e1009226.
13. Cong Y, Ulasli M, Schepers H, et al. Nucleocapsid protein recruitment to replication-transcription complexes plays a crucial role in coronaviral life cycle. *J*

Virol 2020; 94:e01925-19.

14. Ma B, Gundy PM, Gerba CP, et al. UV Inactivation of SARS-CoV-2 across the UVC spectrum: KrCl* excimer, mercury-vapor, and LED sources. *Appl Environ Microbiol* 2021 :AEM0153221.
15. Pereira Oliveira G and Kroon EG. Mouse hepatitis virus: A betacoronavirus model to study the virucidal activity of air disinfection equipment on surface contamination. *J Virol Methods* 2021; 297:114274.
16. Pritchett-Corning KR, Cosentino J and Clifford CB. Contemporary prevalence of infectious agents in laboratory mice and rats. *Lab Anim* 2009; 43:165-173.
17. Ishida T and Fujiwara K. Pathology of diarrhea due to mouse hepatitis virus in the infant mouse. *Jpn J Exp Med* 1979; 49:33-41.
18. Sebesteny A and Hill AC. Hepatitis and brain lesions due to mouse hepatitis virus accompanied by wasting in nude mice. *Lab Anim* 1974; 8:317-326.
19. Report of the Working Group on Hygiene of the Gesellschaft für Versuchstierkunde-Society for Laboratory Animal Science (GV-SOLAS). Implications of infectious agents on results of animal experiments. *Lab Anim* 1999; 33 Suppl 1:S39-87.
20. de Bruin WC, van de Ven EM and Hooijmans CR. Efficacy of soiled bedding

291 transfer for transmission of mouse and rat infections to sentinels: A systematic
292 review. *PLoS One* 2016; 11:e0158410.
293 21. Compton SR, Ball-Goodrich LJ, Paturzo FX, et al. Transmission of enterotropic
294 mouse hepatitis virus from immunocompetent and immunodeficient mice. *Comp*
295 *Med* 2004; 54:29-35.
296 22. Shek WR, Smith Al and Pritchett-Corning TR. Microbiological Quality Control for
297 Laboratory Rodents and Lagomorphs. In: Fox JG, Anderson LC, Otto GM et al.
298 (eds) *Laboratory Animal Medicine*. 3rd ed. Academic Press 2015, pp463-510.
299 23. Becker SD, Bennett M, Stewart JP, et al. Serological survey of virus infection
300 among wild mouse mice (*Mus domesticus*) in the UK. *Lab Anim* 2007; 41:229-238.
301 24. Dammann P, Hilken G, Hueber B, et al. Infectious microorganisms in mice (*Mus*
302 *musculus*) purchased from commercial pet shops in Germany. *Lab Anim* 2011;
303 45:271-275.
304 25. Hayashimoto N, Morita H, Ishida T, et al. Microbiological survey of mice (*Mus*
305 *musculus*) purchased from commercial pet shops in Kanagawa and Tokyo, Japan.
306 *Exp Anim* 2015; 64:155-160.
307 26. Kyuwa S. Replication of murine coronaviruses in mouse embryonic stem cell lines
308 *in vitro*. *Exp Anim* 1997; 46:311-313.

27. Maherbir E, Bauer B and Schmidt J. Rodent and Germplasm Trafficking: Risks of microbial contamination in a high-tech biomedical world. *ILAR J* 2008; 49:347-355.

28. Casebolt DB, Qian B and Stephensen CB. Detection of enterotropic mouse hepatitis virus fecal excretion by polymerase chain reaction. *Lab Anim Sci* 1997; 47:6-10.

29. Yamada YK, Yabe M, Takimoto K, et al. Application of nested polymerase chain reaction to detection of mouse hepatitis virus in fecal specimens during a natural outbreak in an immunodeficient mouse colony. *Exp Anim* 1998; 47:261-264.

30. Hanaki K, Ike F, Hatakeyama R, et al. Reverse transcription-loop-mediated isothermal amplification for the detection of rodent coronaviruses. *J Virol Methods* 2013; 187:222-227.

31. Barthold SW and Smith AL. Mouse hepatitis virus strain-related patterns of tissue tropism in suckling mice. *Arch Virol* 1984; 81:103-112.

32. Barthold SW, Smith AL, Lord PF, et al. Epizootic coronaviral typhlocolitis in suckling mice. *Lab Anim Sci* 1982; 32:376-383.

33. Hirano N, Tamura T, Taguchi F, et al. Isolation of low-virulent mouse hepatitis virus from nude mice with wasting syndrome and hepatitis. *Jpn J Exp Med* 1975; 45:429-432.

34. Cheever FS and Mueller JH. Epidemic diarrheal disease of suckling mice: I. manifestations, epidemiology, and attempts to transmit the disease. *J Exp Med* 1947; 85:405-416.

35. Cheever FS, Daniels JB, Pappenheimer AM, et al. A murine virus (JHM) causing disseminated encephalomyelitis with extensive destruction of myelin. *J Exp Med* 1949; 90:181-210.

36. Fleming JO, Trousdale MD, el-Zaatari, et al. Pathogenicity of antigenic variants of murine coronavirus JHM selected with monoclonal antibodies. *J Virol* 1986; 58: 869–875.

37. Perlman S, Sun N and Barnett EM. Spread of MHV-JHM from nasal cavity to white matter of spinal cord. Transneuronal movement and involvement of astrocytes. *Adv Exp Med Biol* 1995; 380:73-78.

38. Hirano N, Ono K and Matumoto M. Comparison of physicochemical properties of mouse hepatitis virus strains. *Nihon Juigaku Zasshi* 1989; 51:665-667.

39. Homberger FR. Sequence analysis of the nucleoprotein genes of three enterotropic strains of murine coronavirus. *Arch Virol* 1995; 140:571-579.

40. Yamada YK and Yabe M. Sequence analysis of major structural proteins of newly isolated mouse hepatitis virus. *Exp Anim* 2000; 49:61-66.

345 41. Diekmann O., Heesterbeek JAP and Metz JAJ. On the definition and the
346 computation of the basic reproduction ratio R_0 in models for infectious diseases in
347 heterogeneous populations. *J Math Biol* 1990; 28:365–382.
348 42. Fraser C, Donnelly CA, Cauchemez S, et al. WHO Rapid Pandemic Assessment
349 Collaboration. Pandemic potential of a strain of influenza A (H1N1): early findings.
350 *Science* 2009; 324:1557-1561.
351 43. Hirano N, Fujiwara K, Matumoto M. Mouse hepatitis virus (MHV-2). Plaque assay
352 and propagation in mouse cell line DBT cells. *Jpn J Microbiol* 1976; 20:219-225.
353 44. Hirano N, Fujiwara F, Hino S, et al. Replication and plaque formation of mouse
354 hepatitis virus (MHV-2) in mouse cell line DBT culture. *Arch Gesamte Virusforsch*
355 1974; 44:298-302.
356 45. Delamater PL, Street EJ, Leslie TF, et al. Complexity of the Basic Reproduction
357 Number (R_0). *Emerg Infect Dis* 2019; 25:1-4.
358 46. Rehg JE and Toth LA. Rodent quarantine programs: purpose, principles, and
359 practice. *Lab Anim Sci* 1998; 48:438-447.
360 47. Compton SR, Ball-Goodrich LJ, Paturzo FX, et al. Transmission of enterotropic
361 mouse hepatitis virus from immunocompetent and immunodeficient mice. *Comp*
362 *Med* 2004; 54:29-35.

48. Barthold SW and Smith AL. Response of genetically susceptible and resistant mice to intranasal inoculation with mouse hepatitis virus JHM. *Virus Res* 1987; 7:225-239.

49. Taguchi F and Fleming JO. Comparison of six different murine coronavirus JHM variants by monoclonal antibodies against the E2 glycoprotein. *Virology* 1989; 169:233-235.

50. Lipman NS, Corning BF and Saifuddin M. Evaluation of isolator caging systems for protection of mice against challenge with mouse hepatitis virus. *Lab Anim* 1993; 27:134-140.

51. Macy JD, Cameron GA, Ellis SL, et al. Assessment of static isolator cages with automatic watering when used with conventional husbandry techniques as a factor in the transmission of mouse hepatitis virus. *Contemp Top Lab Anim Sci* 2002; 41:30-35.

377 **Figure Legends**

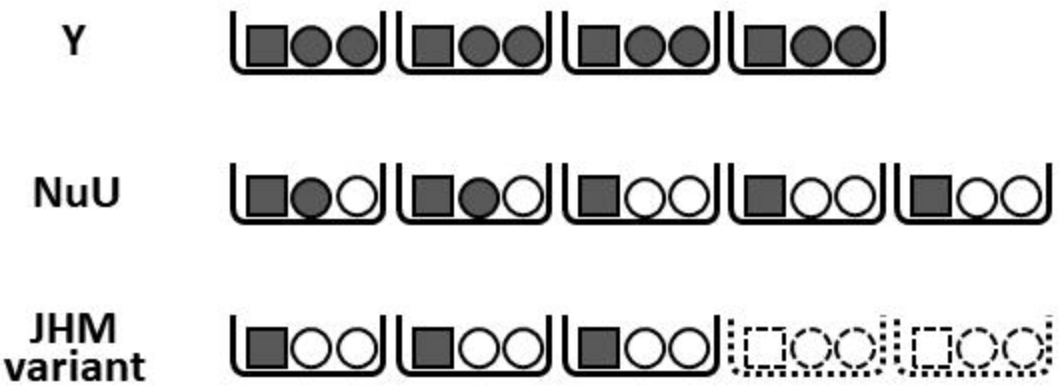
378 **Figure 1.** Transmission of three strains of MHV in mice by cohabitation in a cage. Four,
379 five, and five B6 mice were inoculated intranasally with either the Y, NuU, or JHM
380 variant of MHV, respectively. Two days later, each mouse was cohabitated with two
381 naïve B6 mice in a cage and bred for 4 weeks. Sera were removed, and the anti-MHV
382 antibody response was examined by ELISA. Grey and white colors indicate a positive
383 and negative response, respectively. The square and circle indicate the inoculated and
384 naïve mice, respectively. Since two mice inoculated with the JHM variant had a
385 negative antibody response (shown by dotted line), data from these cages were excluded
386 in the calculation of the basic reproduction number.

387

388 **Figure 2.** Viral growth in the organs of mice inoculated with the Y, NuU, and JHM
389 variants. Viral titers of the brain (a), liver (b), jejunum (c), ileus (d), and colon (e) in
390 mice inoculated intranasally with the Y (■), NuU (▲), or JHM variant (□) of MHV was
391 measured on days 1, 3, and 5 after infection. The horizontal black bar indicates the
392 average of four samples. The detection limit of MHV in the plaque assay is indicated by
393 the orange line. □: $P < 0.05$

394

Figure 1



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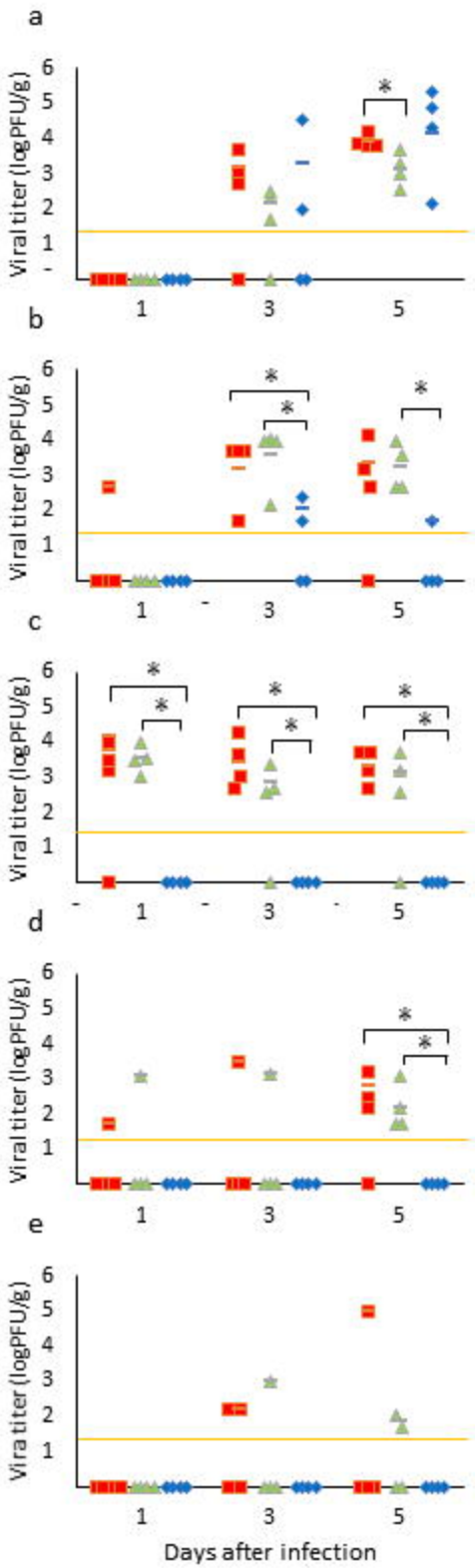

Table 1. Basic reproduction number of three strains of MHV

Viral strain	Basic reproduction number
Y	≥2
NuU	0.4
JHM variant	0

Table 2. Detection rates of infectious viruses in various organs in mice inoculated intranasally with three strains of MHV

Viral strain	Days after infection	Brain	Liver	Jejunum	Ileus	Colon
Y	1	0/5(0%)*	1/5(20%)	4/5(80%)	1/5(20%)	0/5(0%)
	3	3/4(75%)	4/4(100%)	4/4(100%)	1/4(25%)	2/4(50%)
	5	4/4(100%)	3/4(75%)	4/4(100%)	3/4(75%)	1/4(25%)
NuU	1	0/4(0%)	0/4(0%)	4/4(100%)	1/4(25%)	0/4(0%)
	3	3/4(75%)	4/4(100%)	3/4(75%)	1/4(25%)	1/4(25%)
	5	4/4(100%)	4/4(100%)	3/4(75%)	4/4(100%)	2/4(50%)
JHM variant	1	0/4(0%)	0/4(0%)	0/4(0%)	0/4(0%)	0/4(0%)
	3	2/4(50%)	2/4(50%)	0/4(0%)	0/4(0%)	0/4(0%)
	5	4/4(100%)	1/4(25%)	0/4(0%)	0/4(0%)	0/4(0%)

*Number of samples with infectious virus detected in plaque assay/total number of samples