A Field Strain of Minute Virus of Mice (MVMm) Exhibits Age and Strain-Specific Pathogenesis

Rachel D. Brownlee[1], Amir Ardeshir[2], Michael D. Becker[3], April M. Wagner[4], David G. Besselsen[4]*

Work was performed at University Animal Care, University of Arizona, Tucson, AZ

Current author affiliations: [1]School of Veterinary Medicine, [2]California National Primate Research Center, University of California, Davis, CA, [3]ACCESS Specialty Animal Hospitals, Los Angeles, CA, [4]University Animal Care, University of Arizona, Tucson, AZ

*Correspondence: David Besselsen, besselsd@email.arizona.edu, 520-621-1564

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Abbreviations: dpi, days post-inoculation; MFI, multiplex fluorescent immunoassay; MVM, minute virus of mice; NS1, nonstructural protein 1; PCA, principal component analysis; qPCR, quantitative PCR; VP2, viral capsid protein 2

Abstract

The influence of mouse strain, immune competency, and age on the pathogenesis of a field strain of minute virus of mice (MVMm) was examined in BALB/c, C3H, C57BL/6, and SCID mice experimentally infected as neonates, weanlings, and adults. Sera, bodily excretions, and tissues were harvested at 7, 14, 28, and 56 days after inoculation and evaluated by serology, quantitative PCR, and histopathology. Seroconversion to recombinant viral capsid protein 2 was consistently observed in all immune competent strains of mice, regardless of age inoculated, while seroconversion to the viral nonstructural protein 1 was consistently detected only in neonate inoculates. Viral DNA was detected by quantitative PCR in multiple tissues of immune competent mice at each time point after inoculation, with the highest levels observed in neonate inoculates at 7 days after inoculation. In contrast, viral DNA levels in tissues and bodily excretions consistently increased over time in immune deficient SCID mice regardless of age inoculated, with mortality observed in neonatal inoculates between 28 and 56 days after inoculation. Overall, productive infection was more frequently observed in immune competent mice inoculated as neonates as compared to those inoculated as weanlings or adults, and immune deficient SCID mice developed persistent, progressive infection, with mortality observed in mice inoculated as neonates. Importantly, the clinical syndrome observed in experimentally infected SCID neonatal mice recapitulates the clinical presentation reported for the naturally infected, immune deficient NOD μ-chain knockout mice from which MVMm was initially isolated.

Introduction

Despite its discovery more than a half century ago, minute virus of mice (MVM) is still detected in contemporary laboratory mouse colonies [1-4]. MVM remains a concern in laboratory mice due to its potential to impact research by inducing clinical disease [5-11], hematopoietic suppression [8-11], *in vitro* and *in vivo* immunomodulatory effects [6, 12-14], tumor suppression [6, 15, 16], and contamination of cell cultures and tissues originating from mice [12, 17-22]. MVM can potentially be transmitted among research facilities due to its environmental stability [23, 24], its potential to induce persistent infection in mice and cell lines [10, 25], and the difficulties associated with eradicating the virus from infected laboratory mouse colonies [3, 26].

During an epidemiologic investigation of mice naturally infected with parvoviruses, our laboratory identified a strain of MVM that is distinct from the previously reported prototype (MVMp), immunosuppressive (MVMi), and Cutter (MVMc) strains of MVM. The novel MVM strain was predominant among the MVM isolates surveyed with 91.3% prevalence, and was named MVMm [27]. Sequence analysis of the viral genome indicates MVMm is 95.5%, 96.0%, and 96.1% homologous to MVMp, MVMi, and MVMc, respectively, with similar organization of the genome. MVMm was subsequently isolated from immune deficient NOD μ-chain knockout mice exhibiting a clinical disease syndrome characterized by growth retardation, reduced fecundity, and premature death [28]. This was the first report of MVM inducing clinical disease in naturally infected mice.

Natural infection with MVMp and MVMi with associated clinical disease or pathology in mice has not been reported, and neither of these strains were detected in a prior epidemiologic survey

of parvovirus positive samples obtained from random sources [27]. This lack of detection is not surprising since these two MVM strains were isolated from contaminated cell culture systems over four decades ago [12, 29]. The more common murine parvovirus, mouse parvovirus (MPV), similarly has not been reported to cause clinical disease in naturally or experimentally infected mice [3]. However, differences in susceptibility to infection have been demonstrated between various strains and ages of mice experimentally inoculated with MPV [30-32]. Pathogenesis studies indicate experimental infection with MVMi induces clinical disease and pathology characterized by hematopoietic suppression and cerebellar hypoplasia in BALB/c mice inoculated as neonates, and premature death in severe combined immune deficient (SCID) mice inoculated as neonates, with widespread tissue distribution of the virus [5-11]. Recent studies indicate MVMi infectivity differs by sex [33], and can persist in experimentally infected immune competent mice [34, 35]. On the contrary, mice experimentally infected with MVMp do not display clinical disease or pathology, and virus distribution is limited to the upper respiratory tract [6]. The pathogenesis of the Cutter strain of MVM (MVMc), a cell culture contaminant, has not been assessed.

Given the genetic disparity of MVMm as compared to other MVM strains, its high prevalence in naturally infected mice, its isolation from naturally infected mice afflicted with clinical disease, and the variability in pathogenesis displayed by MVMp and MVMi in experimentally infected mice, the current studies aimed to demonstrate the influence of strain, immunocompetency, and age of inoculation on MVMm infectivity and pathogenesis. We hypothesized that immune deficient animals would exhibit increased susceptibility to experimental MVMm infection compared to immune competent strains, that immune competent strains would differ in their susceptibility, and that age when inoculated would influence tissue and excreted viral DNA loads, clinical disease, and pathogenesis.

Results

MVMm infection in immune competent neonatal mice.

Neonatal mice representing three immune competent strains (BALB/c, C3H, C57BL/6) were oronasally inoculated with MVMm. Serum samples collected at 7, 14, 28, and 56 days post-inoculation (dpi) were monitored for antibody response to MVMp recombinant nonstructural protein 1 (NS1) and viral capsid protein 2 (VP2) antigens by multiplex fluorescent immunoassay (MFI). Seroconversion to the VP2 antigen was consistently observed in all strains, with BALB/c mice seroconverting by 7 dpi, and C3H and C57BL/6 mice seroconverting by 14 dpi. Seroconversion to NS1 antigen was also consistently observed, with BALB/c and C3H mice seroconverting by 14 dpi, and C57BL/6 mice by 28 dpi (Figure 1). DNA was extracted from tissue and bodily excretion samples collected from MVMm-infected neonatal mice at 7, 14, 28, and 56 dpi and evaluated by quantitative PCR (qPCR). Viral DNA was consistently detected in lymphoid tissues in all three immune competent mouse strains at all four time points evaluated, with the highest viral DNA levels detected at 7 and 14 dpi, while viral DNA was consistently detected in intestinal tissues of all mouse strains only at 7 and 14 dpi (Figure 2). Viral DNA was consistently detected in other (extra-intestinal, non-lymphoid) tissues at 7, 14, and 28 dpi in all mouse strains. Viral DNA was inconsistently detected in bodily excretions (feces, nasopharyngeal lavage fluid, urine), with most positive samples detected at 7 dpi and no positive

samples detected at 56 dpi. Overall, seroconversion during the early course of infection (as observed in neonatally-infected BALB/c mice) correlated with less viral DNA detection in tissues at 28 and 56 dpi, while seroconversion later in the course of infection (as observed in neonatally-infected C57BL/6 mice) correlated with more consistent detection of viral DNA at 28 and 56 dpi.

MVMm infection in immune competent weanling mice.

Serologic and qPCR analysis of weanling immune competent mouse inoculates was performed as for neonate inoculates (Figures 1 and 2). Seroconversion to recombinant VP2 antigen was observed at 14, 28, and 56 dpi in all immune competent mouse strains. Seroconversion to recombinant NS1 antigen was observed, though less consistently, in C3H and C57BL/6 mice at these same time points. Seroconversion to recombinant NS1 antigen was not observed in any BALB/c mice at any time point after inoculation. The majority of C57BL/6 animals had detectable viral DNA in lymphoid and other (extra-intestinal, non-lymphoid) tissues collected at all four time points after inoculation, while detection was less reproducible in intestine and bodily excretions in this mouse strain. Viral DNA was inconsistently detected in tissues and bodily excretions collected from BALB/c and C3H mice. Viral DNA levels detected in immune competent weanling mice were comparable to the levels observed at 28 and 56 dpi of neonatal mice.

MVMm infection in immune competent adult mice.

Serologic and qPCR analysis of adult immune competent mouse inoculates was performed as for neonate and weanling inoculates (Figures 1 and 2). Seroconversion to recombinant VP2 antigen was consistently observed in all strains, with BALB/c mice and C3H mice seroconverting by 14 dpi, and C57BL/6 mice by 28 dpi. Seroconversion to recombinant NS1 was less consistently observed in all three mouse strains, and only at 28 and 56 dpi. An Adaptive Lasso model revealed age of inoculation significantly ($p<0.0001$) affected seroconversion to NS1, while dpi significantly ($p<0.0001$) predicted seroconversion to VP2 in all immune competent strains. The majority of C57BL/6 animals had detectable viral DNA in lymphoid and other (extra-intestinal, non-lymphoid) tissues, while detection was less reproducible in intestine and bodily excretions in this mouse strain. Viral DNA was inconsistently detected in tissues and bodily excretions collected from BALB/c and C3H mice. Viral DNA levels detected in adult mice were comparable to the levels observed at 28 and 56 dpi of neonatal mice.

MVMm infection in SCID mice.

Neonatal, weanling and adult SCID mice were inoculated oronasally with MVMm. Consistently increasing levels of viral DNA were detected in all tissues and excretions of infected mice in each age group at 7, 14, 28, and 56 dpi, except for infected neonates which were found dead (7 mice) or became moribund and were euthanized (1 mouse) prior to 56 dpi. Principal component analysis (PCA), which transforms the data to highlight any variance, demonstrated complete separation between neonatally-infected BALB/c, C3H, C57BL/6 and SCID mice at 7 dpi, when plotted along the axes of greatest variation (Figure 3). PCA determined that serology and viral DNA load in excretions separated data the most significantly. PCA did not find consistent separation of data between BALB/c, C3H, C57BL/6 and SCID weanling and adult mice at any time point (Figure 1 and 2). CBC results from blood collected at 28 dpi revealed neonates inoculated with MVMm developed statistically significant anemia, leukopenia, and

thrombocytosis ($p<0.05$) as compared to mock inoculates (Figure 4). Histopathology was performed on SCID mice inoculated as neonates. Rare intranuclear inclusion bodies were detected in spleen and mesenteric lymph node of SCID mice inoculated with MVMm as neonates, while these inclusions were not observed in mock-inoculated SCID neonates. No histologic lesions were observed in peripheral lymph node, thymus, heart, lung, salivary gland, jejunum, colon, liver, pancreas, kidney, gonad, bone marrow or brain.

Discussion

MVMm is the most recently isolated strain of minute virus of mice and the only strain to be directly isolated from naturally infected mice displaying clinical disease. Previous reports have determined that MVMm is the most prevalent strain in contemporary laboratory mouse colonies [27] and can cause death in immune deficient mice [28]. The present studies aimed to differentiate strain susceptibility, tissue tropism, and pathogenesis amongst three immune competent and one immune deficient mouse strains infected with MVMm at multiple ages to better understand progression and detection of infections in contemporary laboratory mouse colonies.

Multiplex serology was used to evaluate antibody induction following MVMm inoculation, and utilized purified MVMp VP2 and NS1 antigens, which share high amino acid homology with their MVMm counterparts [27]. Seroconversion to VP2 was consistently observed by 28 dpi in immune competent mice inoculated as neonates, weanlings, and adults. The majority of BALB/c and C3H mice became seropositive at 7 and 14 dpi, with C57BL/6 mice seroconverting at 14 and 28 dpi. Antibody response to NS1 was less consistent, but was observed from 14 to 56 dpi, with C57BL/6 mice again seroconverting at a delayed rate. These data indicate mouse strain-specific host factors play a role in antibody response to MVMm. The pattern observed is similar to previous studies that suggested mouse strain differences in seroconversion to another murine parvovirus (MPV-1) may be due to differences in the predominant T-helper cell phenotype (Th1 vs Th2) exhibited by that mouse strain [30, 32]. Interestingly, principal component analysis indicates mouse strain related differences in seroconversion to MVMm are present at an extremely early age, with clear separation observed among the 4 strains of neonate inoculates at 7 dpi. While it is possible that maternal antibodies to VP2 were detected, the amount of MVMm inoculum from inoculated neonates to which naïve dams were exposed would have been far less than received by the adult mice inoculated directly with MVMm, and the latter cohort did not seroconvert to VP2 by 7 dpi. The NS1 protein is not present in infectious parvovirus virions, so antibody response to this antigen implies viral entry and transcription, thereby indicating productive infection of mice by MVMm [32]. NS1 seroconversion was more prevalent among neonatal inoculates than in mice inoculated as weanlings or adults, regardless of animal strain. Collectively these results are similar to findings from prior studies that documented high rates of NS1 seroconversion among neonatal inoculates following inoculation with MPV1 or hamster parvovirus [36, 37].The Adaptive Lasso statistical model was able to predict decreasing seroconversion to NS1 with increasing inoculation age across all strains, and supports the hypothesis that age at infection plays a pivotal role in the ability of MVMm to actively reproduce within host cells.

Detection of MVM DNA by qPCR in immune competent mice was observed, albeit inconsistently. Viral DNA was detected in intestinal tissue primarily during the early stages of infection, while detection in lymphoid tissues was observed through all time points. This temporal pattern of tissue tropism for MVMm infected mice is similar to that observed in experimentally induced MVMi infections [6, 34]. Interestingly, PCA highlighted that the variation seen between mouse strains inoculated as neonates was most influenced by viral loads in excretions, while data collected from weanlings and adults did not show distinct separation between strains (Figures 1 and 2, respectively). These data support the hypothesis that there is strain-related variation in response to MVMm infection. Overall, viral DNA was more likely to be detected in mice inoculated as neonates as compared to weanling and adults. There was also an increased rate of detection in weanling C3H and C57BL/6 mice as compared to weanling BALB/c. These findings are similar to previous reports focused on MVM and MPV pathogenesis that demonstrated mouse age- and strain-related susceptibilities [6, 30-32, 34]. Notably, the viral DNA levels detected in some tissue samples were significantly higher than were present in inocula, which definitively indicates viral genome replication consistent with productive infection.

Increasing levels of viral DNA in lymphoid and non-intestinal tissues of SCID mice inoculated as neonates, weanlings, or adults were detected throughout the 56-day course of infection. These findings suggest persistent, productive infection by MVMm. Histology was performed on tissues from SCID mice inoculated as neonates as this cohort displayed the most consistently positive viral DNA results, and because immune deficient mice have been shown to be more susceptible to MVMi infection [3, 34, 35]. Amphophilic intranuclear inclusion bodies were detected in the spleen and mesenteric lymph node of multiple neonatal SCID mice, providing morphologic evidence of viral replication. In addition, a statistically significant anemia, leukopenia, neutropenia, lymphopenia, and thrombocytosis was observed at 28 dpi, the final time point for which tissues and blood could be sampled from neonatal inoculates. These data indicate that MVMm persistently and productively infects immune deficient SCID mice with eventual mortality, likely due to hematopoietic suppression of both red and white blood cell lineages.

In summary, several strains and ages of mice were infected with a clinically-relevant field strain of MVM. High viral DNA loads in tissues and excretions, with consistent NS1 antibody response in animals infected as neonates, demonstrates an age-related difference in susceptibility to MVMm. The variation in the amount of viral DNA found in tissues and excretions over the time course of infection, along with the differing seroconversion rates among immune competent strains, indicates strain-related variability in MVMm pathogenesis. SCID mice uniquely exhibited persistent, productive infection at all ages, with morbidity and mortality seen in mice infected as neonates. Hematopoietic suppression, as evidenced by anemia and leucopenia noted in neonates at 28 dpi, likely contributed to lethality. Increasing tissue viral DNA loads among SCID mice inoculated as weanlings and adults suggests mortality may have been observed in those animals if the time course after infection would have been prolonged beyond the 56 dpi end point for the study. These data and conclusions support the hypotheses that age, strain, and immunocompetency factors convey differences in susceptibility, clinical disease, and pathogenesis of MVMm infection in mice. Importantly, the clinical syndrome observed in experimentally infected SCID neonatal mice recapitulates the clinical presentation reported for

the naturally infected, immune deficient NOD μ-chain knockout mice from which MVMm was isolated.

Methods

Mice.

Four- and nine-week-old male and female BALB/cAnNHsd (BALB/c), C3H/HeNHsd (C3H) and C57BL/6NHsd (C57BL/6) mice were obtained from Envigo (Indianapolis, IN). Four- and nine-week-old male and female C.B-17/IcrHsd-*Prkdc*^{scid} (SCID) mice were obtained from an intramural breeding colony. Mice of each age were placed on experiment, with additional breeding age mice used to produce the neonatal mice needed. Mouse strains were chosen based on previous reports of susceptibility to mouse parvovirus infections [5, 6, 10, 32, 36]. All mice were specified to be free of murine viruses (mouse hepatitis virus, minute virus of mice, mouse parvovirus, mouse rotavirus, encephalomyelitis virus, pneumonia virus of mice, Sendai virus, lymphocytic choriomeningitis virus, murine norovirus, ectromelia virus, Hantaan virus, mouse adenovirus, mouse cytomegalovirus, respiratory enteric virus III, K virus, lactic dehydrogenase elevating virus, polyoma virus, and mouse thymic virus), pathogenic bacteria, and endo and ectoparasites by intramural and vendor-supplied health surveillance reports.

Each experimental group was housed separately in microisolater caging. All manipulations of mice were performed in a class-II biological safety cabinet using standard microisolation technique. Animals were housed in sterilized static microisolation caging on aspen chip bedding (changed weekly) in a biocontainment facility at a temperature of 22-24ºC, humidity of 30-70%, 12-15 air exchanges per hour, and a 14/10 hour light/dark cycle. Irradiated Teklad Global 19% Protein diet (breeders) or Teklad NIH-31 diet (weanlings and adults) (Envigo, Madison, WI) and hyperchlorinated water were provided ad libitum. The University of Arizona Institutional Animal Care and Use Committee approved all animal procedures which were performed in accordance with the *Guide for the Care and Use of Laboratory* Animals [38]. The animal care and use program of the University of Arizona is fully accredited by AAALAC International.

Viral infections.

MVMm was isolated and propagated as described previously [27]. Multiple strains of mice (BALB/c, C3H, C57BL/6, SCID) were inoculated with MVMm as neonates (1 day old), weanlings (4 weeks of age), or adults (9 weeks of age). Neonates were inoculated oronasally with 10 μL of viral inoculum, a dosage of $\log_{10}$ $TCID_{50}$ 5.6. Weanling and adult mice were inoculated orally with a dosage of $\log_{10}TCID_{50}$ 5.9 of viral inoculum. Mock-infected mice (Tris-EDTA buffer [pH 8.7]) were included for studies in SCID mice. Clinical observations of MVMm-infected mice were performed daily throughout the course of the study. Mice were euthanized at 7, 14, 28 or 56 dpi, or when observed to be moribund, by carbon dioxide inhalation. Blood was collected by cardiocentesis from each mouse. Serum obtained from immune competent mice was diluted 1:5 (vol/vol) in phosphate buffered saline and stored at -80ºC until evaluated by MFI. Whole blood from SCID mice was collected in an EDTA microtainer and a complete blood count (CBC) was performed with a Hemavet 850 Multispecies Hematology Analyzer (Drew Scientific, Miami Lakes, FL). Various tissues and bodily excretions were collected from each mouse and included mesenteric lymph node, spleen, peripheral lymph

node, thymus, jejunum, colon, heart, lung, salivary gland, liver, pancreas, kidney, gonad, bone marrow, brain, feces, urine and nasopharyngeal lavage fluid. Representative samples of each tissue from SCID mice were fixed in 4% paraformaledehyde for histopathology or stored frozen at -80ºC for DNA extraction. Routine histotechnology was performed on fixed tissues with paraffin-embedding, 5 µM sections, and hematoxylin and eosin staining. DNA was extracted from fresh frozen specimens (approximately 20 mg tissue or 50 µL of fluid) using a MagneSil KF Genomic DNA extraction kit (Promega Corp., Madison, WI) and a KingFisher robotic extraction station (Thermo Fisher Scientific, Waltham, MA) per manufacturer's recommendations. Extracted DNA was stored at -20ºC until evaluated by quantitative PCR.

Quantitative polymerase chain reaction (qPCR).

Extracted DNA was screened by an MVM-specific qPCR assay as previously described [27, 36, 39]. Reactions were performed with a Stratagene Mx3000P qPCR System (Agilent Technologies, Santa Clara, CA) and products were analyzed by the accompanying software. Each 20 µL reaction consisted of 1X TaqMan buffer (50 mM KCl, 10 µM EDTA, 10 mM Tris-HCl [pH 8.3] and 60 nM Passive Reference), 5.5 mM MgCl2, 200 µM (each) dATP, dCTP, dGTP, and 400 µM dUTP, 300 nM primers, 100 nM probe, 0.2 U of AmpErase uracil-N-glycosylase (UNG), 0.5 U AmpliTaq Gold Polymerase (Thermo Fisher Scientific, Carlsbad, CA) and 2 µL template DNA. Thermal cycling conditions consisted of 50ºC for 2 min for UNG incubation, polymerase activation at 95ºC for 10 min, and then 45 cycles of 95ºC for 15 sec followed by 60ºC for 1 min. Samples were considered positive if they exhibited a cycle threshold (Ct) < 35. Ct values were reciprocally transformed using an assay-derived logarithmic scale based on the standard curve.

Serology.

The MFI format was used to evaluate sera for the presence of virus-specific antibodies. Baculovirus-expressed recombinant MVMp NS1 and recombinant MVMp VP2 were prepared as previously described [40, 41]. Purified, recombinant NS1 and VP2 antigens were covalently coupled to carboxylated polystyrene microspheres (Luminex Corporation, Austin, TX) at a coupling concentration of 25 µg of protein per 5x106 microspheres according to manufacturer's recommended protocols. Ovalbumin, A92L mouse fibroblast cell lysate, and Hi-Five insect cell lysate were similarly coated to microspheres to serve as control antigens, with cell lysates prepared by three freeze-thaw cycles. Microspheres were stored at 4ºC in the dark until use. Evaluation of mouse sera for recombinant NS1- and VP2-specific antibodies was performed automatically (LiquiChip Workstation, Qiagen, Valencia, CA) as described previously [27]. Briefly, antigen-coated microspheres were incubated for 60 min with dilute sera at a final dilution of 1:500 in 100 µL diluent, washed twice, incubated with phycoerythrin-conjugated F(ab')2 fragment goat antimouse IgG (H+L) secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA), washed twice, and resuspended in stop solution containing formalin. The microplate was then shaken for 5 min and analyzed. Baseline values of 100 (VP2) and 250 (NS1) were used to discriminate negative and positive samples. Baselines were determined previously as the mean plus 5 standard deviations of results obtained for 50 serum samples from mice known to be negative for murine parvovirus infections.

Statistical analyses.

Statistical analyses were performed using R Statistical Software (R Foundation for Statistical Computing, Vienna, Austria) or JMP®, Version 13 (SAS Institute Inc., Cary, NC). Specifically, Principal Component Analysis (PCA) was used to identify those principal components which accounted for the variation within data [42]. Generalized regression (Adaptive Lasso) was used with validation (Leave-One-Out) to predict NS1 and VP2 using the predictors [43]. Log_{10} or Johnson Sb distributional transformation were used to transform covariates and dependent variables. Complete blood count parameters obtained from mock and MVMm-infected SCID mice were compared with a Student's t-test (95% confidence intervals, two-tails) to determine statistical significance. A p-value of 0.05 was considered statistically significant for all analyses. The MFI and viral copies were plotted using heatmap.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Figure Legends

Figure 1. Heat map demonstrating seroconversion to MVM recombinant nonstructural protein 1 (NS1) and viral capsid protein 2 (VP2) at 7, 14, 28, and 56 days post-inoculation (dpi) by immune competent mice (BALB/c, C3H, C57BL/6) infected as neonates (A), weanlings (B), or adults (C). Darker colors correlate with higher serum antibody levels, and lighter colors correlate with lower serum antibody levels, measured in median fluorescent intensity (mfi).

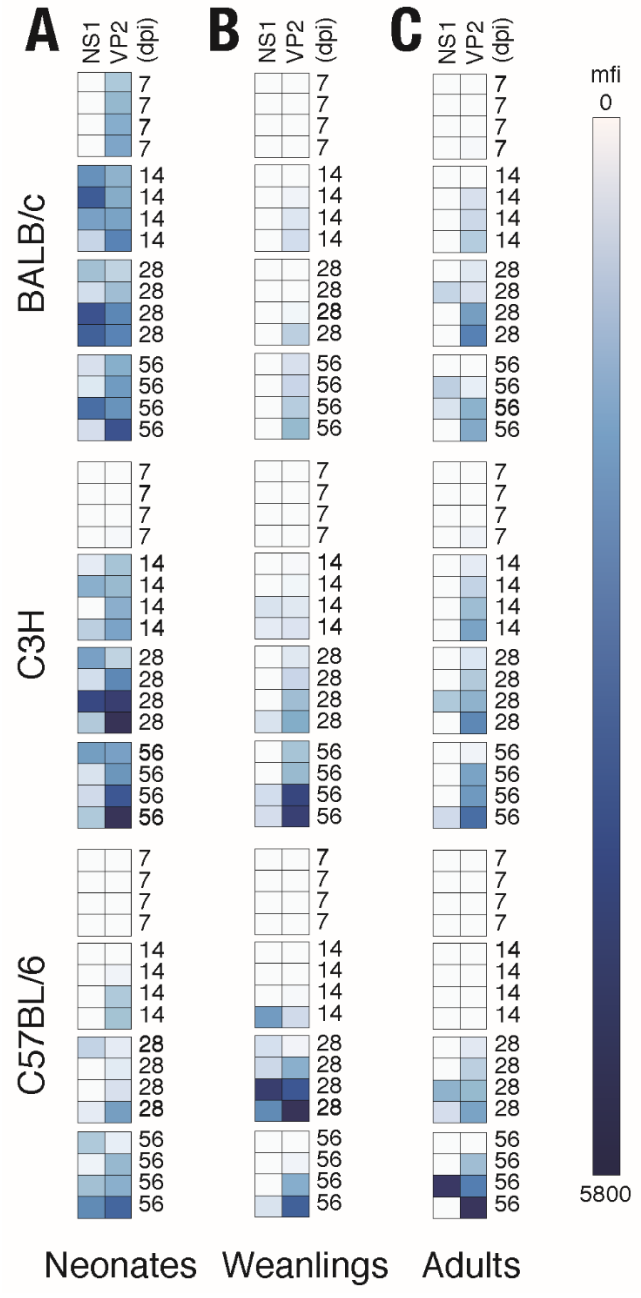

Figure 2. Heat map demonstrating viral DNA loads (reciprocally transformed Ct values) of lymphoid tissues (mesenteric and peripheral lymph nodes, spleen, thymus), gastrointestinal tissues (jejunum, colon), other tissues (bone marrow, pancreas, liver, kidney, gonad, salivary gland, heart, lung, brain), and bodily excretions (feces, nasopharyngeal lavage fluid, urine) measured at 7, 14, 28, and 56 days post-inoculation (dpi) in BALB/c, C3H, C57BL/6, and SCID mice infected as neonates (A), weanlings (B), or adults (C). SCID mice inoculated as neonates did not survive to 56 dpi. Darker colors correlate with higher viral DNA loads, and lighter colors correlate with lower viral DNA loads.

Figure 3. Principal component analysis (PCA) of viral DNA loads and serum antibody levels to NS1 and VP2, explains 52.1% (PC 1) and 36% (PC 2) of the variation between neonatally-infected BALB/c, C3H, C57BL/6 and SCID mice at 7 dpi along axes of greatest data variation (A). A loading plot showing the respective variables that determined the principal components, with those that cluster within the same quadrant exhibiting co-variability (B). PCA contribution scores revealed serology (NS1 and VP2), and viral DNA loads in excretions provided the most significant contributions to the principal components (C).

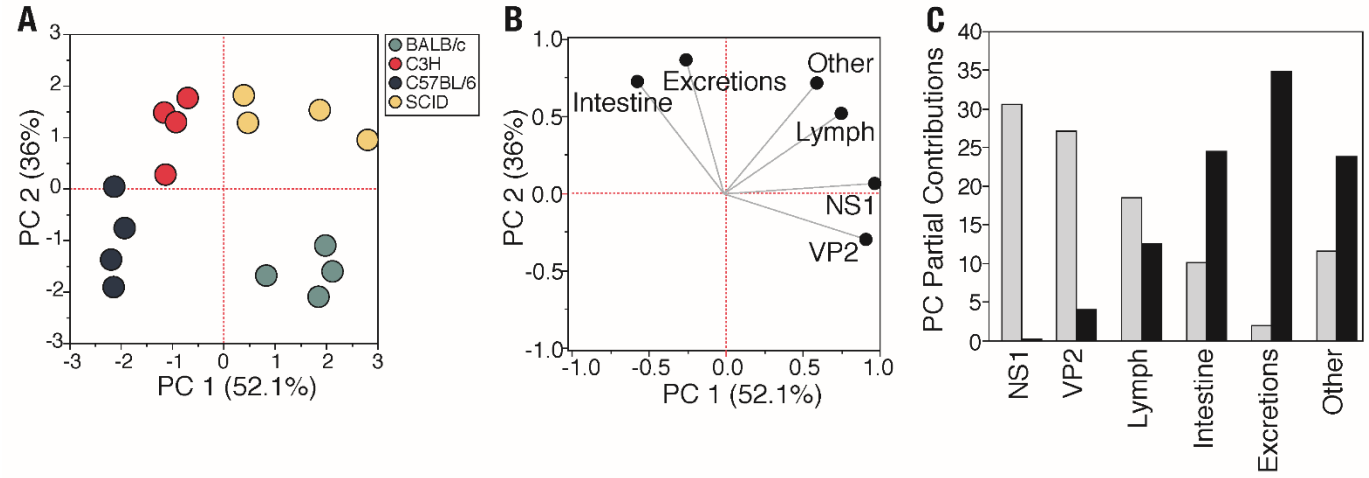

Figure 4. White blood cell (WBC), neutrophil (NEU), lymphocyte (LYM), red blood cell (RBC), and platelet (PLT) counts from SCID neonates inoculated with MVMm or a mock inoculate and evaluated at 28 dpi. Bars are standard error bars and p-values were calculated from a t-test (two tailed).