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Experimental Infection of Mice with Hamster Parvovirus: Evidence for Interspecies Transmission of Mouse Parvovirus 3

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Hamster parvovirus (HaPV) was isolated 2 decades ago from hamsters with clinical signs similar to those induced in hamsters experimentally infected with other rodent parvoviruses. Genetically, HaPV is most closely related to mouse parvovirus (MPV), which induces subclinical infection in mice. A novel MPV strain, MPV3, was detected recently in naturally infected mice, and genomic sequence analysis indicates that MPV3 is almost identical to HaPV. The goal of the present studies was to examine the infectivity of HaPV in mice. Neonatal and weanling mice of several mouse strains were inoculated with HaPV. Tissues, excretions, and sera were harvested at 1, 2, 4, and 8 wk after inoculation and evaluated by quantitative PCR and serologic assays specific for HaPV. Quantitative PCR detected viral DNA quantities that greatly exceeded the quantity of virus in inocula in multiple tissues of infected mice. Seroconversion to both nonstructural and structural viral proteins was detected in most immunocompetent mice 2 or more weeks after inoculation with HaPV. In neonatal SCID mice, viral transcripts were detected in lymphoid tissues by RT-PCR and viral DNA was detected in feces by quantitative PCR at 8 wk after inoculation. No clinical signs, gross, or histologic lesions were observed. These findings are similar to those observed in mice infected with MPV. These data support the hypothesis that HaPV and MPV3 are likely variants of the same viral species, for which the mouse is the natural rodent host with rare interspecies transmission to the hamster.

Abbreviations: HaPV, hamster parvovirus; mfi, median fluorescent intensity; MFI, multiplex fluorescent immunoassay; MPV, mouse parvovirus; MVM, minute virus of mice; NS1, nonstructural protein 1; qPCR, quantitative PCR; VP2, viral capsid protein 2.

Murine parvoviruses are among the most prevalent infectious agents detected in contemporary laboratory mouse colonies.[27,35] Various disease syndromes in mice have been associated with minute virus of mice (MVM) infection,[10,24,25,39-41] whereas mouse parvovirus (MPV) infection has not been associated with clinical disease or pathologic lesions to date. Initial MPV infection occurs in the intestinal tract, followed by persistence in lymphoid tissues.[3,19] Neonatal SCID mice inoculated with MPV or MVM develop chronic productive infection with persistent fecal shedding of virus.[3,40] Both minute virus of mice and MPV can have deleterious effects on research due to in vitro and in vivo immunomodulatory effects,[9,13,24,28-31] tumor suppression,[17,18,24,28,30] and contamination of cell cultures and tissues originating from mice.[9,11,12,15,16,28,32]

Hamster parvovirus (HaPV) initially was isolated 2 decades ago from a large commercial colony of Syrian hamsters that experienced morbidity and mortality approaching 100% among suckling and weanling hamsters.[4] Affected hamsters exhibit domed craniums, a potbellied appearance, abnormally small testicles, and discolored, malformed, or missing incisors. Neonatal hamsters inoculated with a low dose of HaPV survive to at least 6 wk of age and display incisor and testicular lesions identical to those observed during the initial HaPV epizootic. Neonatal hamsters inoculated with a high dose of HaPV uniformly succumb to a lethal hemorrhagic disease affecting the kidneys, gastrointestinal tract, testicle or uterus, and brain, generally by 7 to 8 d after inoculation. Histologic lesions of thrombosis in blood vessels and basophilic intranuclear inclusion bodies in endothelial cells suggest the pathogenesis for the hemorrhagic disease is thrombogenesis due to viral infection of endothelial cells with subsequent ischemic necrosis.

Although HaPV was isolated initially from Syrian hamsters, whether this species is the natural rodent host for this virus is questionable. Clinical disease is observed in fetal or neonatal Syrian hamsters experimentally infected with the rodent parvoviruses minute virus of mice, H1, and Kilham rat virus, which typically cause subclinical infection in their natural mouse and rat hosts.[14,20-23,44] The clinical signs (tooth loss or discoloration, facial bone deformities, diarrhea, stunted growth, ataxia, and death) and gross and histologic lesions observed in these experimental infections are similar to those exhibited by HaPV-infected hamsters. These findings suggest the hamster is likely an aberrant host for HaPV, and another subclinically affected species may serve as a reservoir of HaPV. The lack of similar disease outbreaks or parvovirus isolations in Syrian hamsters supports the concept that this species is an aberrant host for HaPV.

During a molecular epidemiologic investigation of mice naturally infected with parvoviruses, our laboratory identified 3 genetically distinct variants of MPV.[7] MPV1 was detected most frequently (78% of samples evaluated), whereas 2 novel MPV

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genotypes, designated 2 and 3, were detected in 21% and 1% of the samples evaluated, respectively. Genetically, MPV3 is almost identical to HaPV with 98.1% nucleotide sequence homology. Likewise, the amino acid sequences for the predicted viral proteins are highly conserved, with conservation of a 3-codon insertion in the VP1 amino terminus that is absent in other rodent parvoviruses. Unfortunately, the mouse colony in which MPV3 was detected was depopulated before virus isolation could be attempted to enable further characterization of this novel variant. However, the genetic similarity between HaPV and MPV3 suggests that HaPV might be useful as a surrogate isolate to evaluate the pathogenesis of MPV3 in mice. In addition, the infectivity of HaPV in mice needs to be assessed to determine the potential for interspecies transmission between mice and hamsters. The objective of the current studies was to evaluate the infectivity of HaPV in potentially susceptible strains and ages of mice.

Materials and Methods

Virus propagation. HaPV was obtained from an intramural stock and propagated in baby hamster kidney (BHK21) cells as described previously.[5] Cell lysates were prepared and the 50% tissue culture infectious dose was determined. In addition, HaPV was propagated by inoculation of newborn hamsters to obtain viral stocks with higher titers than those obtained by cell culture propagation. Untimed pregnant female Golden Syrian hamsters were obtained from Charles River Laboratories (Wilmington, MA). Hamsters were specified to be free of pathogenic bacteria, endo- and ectoparasites, and antibodies to Sendai virus, pneumonia virus of mice, lymphocytic choriomeningitis virus, and reovirus by the supplier. Each pregnant hamster was housed individually in microisolation caging during gestation and lactation. At 3 d of age, hamster pups were inoculated orally with cell-culture–propagated HaPV. Hamsters were euthanized 10-d after inoculation by carbon dioxide inhalation, and the spleen, kidneys, and liver of each animal were collected aseptically and stored frozen at –80 °C. Tissues then were homogenized at a 1:5 dilution (v/v) in Tris–EDTA buffer (pH 8.7), frozen and thawed 3 times, twice subjected to centrifugation (1,000 × g for 5 min) to remove cellular debris, and the resulting supernatant was passed through a 0.45-µm then a 0.2-µm filter. Ten-fold serial dilutions of this filtrate were administered by gavage to 6-wk-old Swiss Webster mice (n = 3 mice per dilution group), and the 50% infectious dose was determined based on seroconversion to MPV rVP2, HaPV rVP2, and rNS1 as measured by multiplex fluorescent immunoassay.

All manipulations of mice and hamsters were performed in a class II biological safety cabinet by 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 to 24 °C, humidity of 30% to 70%, 12 to 15 air exchanges hourly, and a 14:10-h light:dark cycle. A Teklad Global 19% Protein diet (breeders) or a Teklad NIH31 diet (weanlings; Harlan Teklad, Madison, WI) and hyperchlorinated water were provided ad libitum. The University of Arizona Institutional Animal Care and Use Committee approved all animal procedures.

Mouse infection. Breeding age male and female C3H/HeNUac (C3H) and C.B-17/IcrAcc-scid (SCID) mice were obtained from intramural breeding colonies and bred to produce the neonatal and weanling mice of these strains needed for experimental infection studies. Breeding-age male and female BALB/cAnNHsd (BALB/c) mice were obtained from Harlan (Indianapolis, IN), and 8-wk-old male and female NTac:NihBlackSwiss (BSW) mice were obtained from Taconic (Hudson, NY). Mouse strains were selected on the basis of their relative susceptibility to MPV1 infection.[3,8] 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 orphan virus 3, 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 control or experimental group was housed separately in microisolation caging.

Multiple strains of mice (C3H, BALB/c, BSW, SCID) were inoculated with HaPV as neonates (3 d of age or younger) or weanlings (3 to 4 wk of age). Experiments were performed at different times over the course of several years, with different routes of inoculation (oronasal or gavage) and doses of virus inoculum ($\log_{10}$ $TCID_{50}$ 3.7 to 5.0 or $\log_{10}$ ID_{50} 4.0). Mock-infected mice (Tris–EDTA buffer [pH 8.7]) were included for studies of SCID and BSW mice. Clinical observation of both HaPV- and mock-infected mice was performed daily throughout the course of the study. Mice were euthanized at 1, 2, 4, or 8 wk after inoculation by carbon dioxide inhalation. Blood was collected by cardiocentesis from each mouse. Serum was diluted 1:5 (v/v) in PBS and stored at –80 °C until evaluated by multiplex fluorescent immunoassay (MFI). Samples of spleen, mesenteric lymph node, and thymus were taken immediately after euthanasia and placed in RNAlater (Qiagen, Valencia, CA). Various tissues and bodily excretions were collected from each mouse as follows: mesenteric lymph node, spleen, jejunum (all mouse strains); peripheral lymph node, thymus, heart, lung, salivary gland, colon, liver, pancreas, kidney, gonad, bone marrow (BALB/c, BSW, and SCID strains only); brain, feces, urine, and nasopharyngeal lavage fluid (BALB/c, SCID strains only; feces collected only at 4 and 8 wk after inoculation from neonates). Representative samples of each tissue were fixed in 4% paraformaledehyde for histopathology or stored frozen at –80 °C for DNA extraction. Routine histotechnology was performed on fixed tissues with paraffin-embedding, 5-µM sections, and hematoxylin and eosin staining (SCID, BALB/c mice only). DNA was extracted from fresh frozen specimens (approximately 20 mg tissue or 50 to 200 µL fluid) by using a MagneSil KF Genomic DNA extraction kit (Promega, Madison, WI) and a KingFisher robotic extraction station (Thermo Scientific, Franklin, MA) according to the manufacturer's recommendations. Extracted DNA was stored at –20 °C until evaluated by quantitative PCR (qPCR).

qPCR. Extracted DNA was screened by an MPV- and HaPV-specific qPCR assay as previously described.[7,37] Reactions were performed on an Mx3000P qPCR System (Stratagene, Cedar Creek, TX) and products were analyzed by using the accompanying software. Each 20 µL reaction consisted of 1× *Taq*Man buffer (50 mM KCl, 10 µM EDTA, 10 mM Tris-HCl [pH 8.3] and 60 nM Passive Reference); 5.5 mM $MgCl_2$; 200 µM (each) dATP, dCTP, dGTP; 400 µM dUTP; 300 nM primers; 125 nM probe; 0.2 U AmpErase uracil-N-glycosylase; 0.5 U Ampli*Taq* Gold Polymerase (Applied Biosystems, Foster City, CA); and 2 µL template DNA. Thermal cycling conditions consisted of 50 °C for 2 min for uracil-

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N-glycosylase incubation, 95 °C for 10 min for polymerase activation, and then 45 cycles of 95 °C for 15 s followed by 60 °C for 1 min. Samples were considered positive if they exhibited a cycle threshold of less than 35.

Serology. The multiplex fluorescent immunoassay (MFI) format was used to evaluate sera for the presence of virus-specific antibodies. Baculovirus-expressed recombinant nonstructural protein 1 (rNS1) from MVM prototype strain, recombinant MPV1b viral capsid protein 2 (MPV rVP2), and recombinant HaPV viral capsid protein 2 (HaPV rVP2) were prepared essentially as previously described.[26,38] Purified rNS1 and rVP2 antigens were covalently coupled to carboxylated polystyrene microspheres (Luminex, Austin, TX) at a coupling concentration of 25 µg protein per 5 × 10^6 microspheres according to the manufacturer's recommended protocols. Ovalbumin, $A9_{2L}$ mouse fibroblast cell lysate, and Hi-Five (Invitrogen, Carlsbad, CA) insect cell lysate were similarly coated to microspheres to serve as control antigens, with cell lysates prepared by 3 freeze-thaw cycles. Microspheres were stored at 4 °C in the dark until use. Evaluation of mouse sera for rNS1- and rVP2-specific antibodies was performed automatically (LiquiChip Workstation, Qiagen) as described previously.[3] 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 (heavy + light chain) secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA), washed twice, and resuspended in stop solution containing formalin. The microplate then was shaken for 5 min and analyzed. Baseline values of 275 (MPV rVP2, HaPV rVP2) and 125 (rNS1) were used to discriminate negative and positive samples. Baselines were determined previously as the mean plus 5 SD of results obtained for 50 serum samples from mice known to be negative for murine parvovirus infection. Results are reported as the median fluorescent intensity (mfi) of 50 antigen-coated microspheres.

RT-PCR. RNA was extracted from tissues stored in RNAlater by using an RNeasy Mini Kit (Qiagen). Primer sequences were kindly provided by Dr Ken Henderson (Charles River Laboratories) for detection of viral RNA encoding VP1 and VP2, as previously described.[3] All RT-PCR reactions were performed with GeneAmp 2400 PCR System (PE Applied Biosystems). The reverse transcription step consisted of 1× *Taq*Man buffer (50 mM KCl, 10 mM Tris-HCl [pH 8.3]); 5.5 mM $MgCl_2$; 500 µM dATP, dCTP, dGTP, and dTTP; 8 U RNase inhibitor; 25 U of murine leukemia virus reverse transcriptase (Applied Biosystems); 2.5 µM random hexamers; and 2 µL of template RNA in a 20 µL reaction. Thermal cycling conditions consisted of 25 °C for 10 min for hexameric primer extension, reverse transcription at 48 °C for 30 min, and 95 °C for 5 min for reverse transcriptase inactivation. The PCR reaction consisted of 1× *Taq*Man buffer (50 mM KCl, 10 µM EDTA, 10 mM Tris-HCl [pH 8.3] and 60 nM passive reference); 5.5 mM $MgCl_2$; 200 µM dATP, dCTP, dGTP; 400 µM dUTP; 0.5 U of AmpliTaq Gold; 0.2 U of uracil N-glycosylase; 150 nM forward and 300 nM reverse primers; and 2 µL cDNA in a 20-µL reaction. Thermal cycling conditions consisted of 50 °C for 2 min for uracil N-glycosylase incubation, denaturation, and *Taq* polymerase activation at 95 °C for 10 min, and 40 cycles of 95 °C for 15 s, 57 °C for 15 s, and 72 °C for 25 s. DNA amplicons were electrophoresed on a 3% NuSieve agarose gel, and banding patterns were used to determine the presence of viral RNA in a given tissue.

Results

HaPV infection in immunocompetent neonatal mice. Neonatal mice of 3 immunocompetent strains (C3H, BALB/c, BSW) were inoculated with HaPV. Serum samples collected at 1, 2, 4, and 8 wk after inoculation were monitored for antibody response to rNS1, MPV rVP2, and HaPV rVP2 by MFI (Table 1). Seroconversion to NS1 was observed in mice representing all 3 mouse strains at 2 wk after inoculation and later, although seroconversion to this antigen was inconsistent. Seroconversion to MPV and HaPV rVP2 antigens were detected in almost all BSW and C3H mice at the 4- and 8-wk time points, whereas seroconversion to these antigens was less consistent in BALB/c mice. Although the MPV and HaPV rVP2 antigens were prepared identically and coated onto microspheres at the same concentration for use in the MFI, mean antibody levels detected by HaPV rVP2 antigen were higher than those detected by MPV rVP2 antigen. Mock-infected BSW mice did not seroconvert to rNS1, MPV, or HaPV VP2 antigens (data not shown).

In addition, tissue DNA extracts from infected neonatal mice were evaluated by qPCR (Table 1). Results from individual tissues obtained from each mouse were compiled into 3 tissue groups (lymphoid, intestinal, and other) for interpretation. Viral DNA was detected most consistently in lymphoid tissues in all 3 mouse strains. Viral DNA was detected in intestinal tissues in several mice from 1 to 4 wk after inoculation but in none of the mice at 8 wk after inoculation. Viral DNA was detected in tissues other than lymphoid and intestinal tissues, with sporadic detection in salivary gland, pancreas, and lung. Viral DNA was not detected in feces of mice inoculated as neonates at 4 and 8 wk after inoculation. Viral DNA was not detected in samples from any mock-infected neonatal BSW mice (data not shown).

Histology was performed on all BALB/c mice inoculated as neonates. No lesions were observed in lymph node (mesenteric, submandibular), spleen, thymus, heart, lung, salivary gland, jejunum, colon, liver, pancreas, kidney, gonad, or bone marrow.

HaPV infection in immunocompetent weanling mice. Weanling immunocompetent mice (C3H, BALB/c, BSW) were inoculated with HaPV (Table 2). Sera collected from mice were evaluated by MFI serology at 1, 2, 4, and 8 wk after inoculation. Seroconversion to NS1 was observed in mice representing all 3 mouse strains at 2 wk after inoculation or later, although seroconversion to this antigen was inconsistent. Seroconversion to MPV and HaPV rVP2 antigens were detected in most mice at 2 wk inoculation or later, with generally higher antibody responses observed to HaPV rVP2 antigen as compared with MPV rVP2 antigen. Mock-infected BSW mice did not seroconvert to rNS1, MPV, or HaPV VP2 antigens (data not shown).

We used qPCR evaluation to detect HaPV DNA in individual tissue DNA extracts from weanling C3H, BALB/c, and BSW strains (Table 2). Again, results were grouped into lymphoid, intestinal, and other tissue types. Viral DNA was detected only in lymphoid tissues of C3H and BSW mice at 1, 2, or 4 wk after inoculation. HaPV DNA was detected in lymphoid, intestinal, and several other tissues (salivary gland, pancreas, lung, liver, heart, and kidney) of BALB/c mice at multiple time points after inoculation, with the highest levels of viral DNA detected in lymphoid tissues. Fecal virus was detected by qPCR in BALB/c mice at 2 and 4 wk after inoculation but not at 8 wk after inoculation. Viral DNA was not detected in samples from mock-infected weanling BSW mice (data not shown).

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Table 1. Serologic and qPCR results for neonatal immunocompetent mice inoculated oronasally with HaPV

			MFI (mean mfi for all seropositive mice)			MPV PCR (mean log_{10} viral copies/20 mg tissue)		
Strain	Viral dose[a]	Time after inoculation (wk)	rNS1	MPV	HaPV	Lymph node, spleen, thymus	Intestine	Other[b]
C3H	4.0	1	0/2[c]	0/2	not done	2/2 (5.4)	2/2 (6.6)	not done
C3H	4.0	2	0/2	2/2 (1424)	not done	2/2 (4.8)	2/2 (4.0)	not done
C3H	4.7	4	3/4 (300)	4/4 (5246)	4/4 (11956)	4/4 (4.5)	2/4 (4.1)	not done
C3H	4.7	8	1/5 (584)	5/5 (15046)	5/5 (13597)	3/5 (5.8)	0/5	not done
BALB/c	3.7	1	0/4	0/4	0/4	2/4 (5.4)	4/4 (4.7)	1/4 (4.3)
BALB/c	3.7	2	1/3 (135)	0/3	1/3 (1141)	2/4 (5.4)	1/4 (4.7)	1/4 (4.4)
BALB/c	3.7	4	3/4 (729)	2/4 (1732)	3/4 (4286)	1/4 (6.6)	0/4	1/4 (6.7)
BALB/c	3.7	8	2/4 (754)	2/4 (886)	2/4 (6436)	2/4 (4.4)	0/4	0/4
BSW	4.0 ID_{50}	1	0/8	0/8	0/8	4/4 (5.3)	2/4 (3.9)	1/4 (5.9)
BSW	4.0 ID_{50}	4	2/4 (141)	4/4 (1744)	4/4 (9838)	1/4 (4.3)	0/4	0/4
BSW	4.0 ID_{50}	8	2/4 (396)	3/4 (4919)	4/4 (8329)	2/4 (4.6)	0/4	0/4

[a]For C3H and BALB/c mice, viral dose is expressed as 50% tissue culture infective dose (log_{10}); for BSW, viral dose is expressed as the 50% infective dose of HaPV inoculum propagated in vivo.
[b]Other = bone marrow, brain, pancreas, liver, kidney, gonad, salivary gland, heart, lung
[c]no. positive animals/total no. animals tested

Table 2. Serologic and qPCR results for weanling immunocompetent mice inoculated oronasally with HaPV

			MFI (mean mfi for all seropositive mice)			MPV PCR (mean log_{10} viral copies/20 mg tissue)		
Strain	Viral dose[a]	Time after inoculation (wk)	rNS1	MPV	HaPV	Lymph node, spleen, thymus	Intestine	Other[b]
C3H	4.5	1	0/2[e]	1/2 (322)	2/2 (820)	1/2 (5.5)	0/2	not done
C3H	4.5	2	1/2 (194)	1/2 (8121)	2/2 (2988)	1/2 (3.9)	0/2	not done
C3H	5.0	4	1/4 (162)	4/4 (3678)	not done	0/4	0/4	not done
C3H	5.0	8	2/4 (172)	4/4 (10524)	not done	0/4	0/4	not done
BALB/c	4.0	1	0/4	0/4	0/4	0/4	0/4	1/4 (3.8)
BALB/c	4.0	2	3/4 (484)	3/4 (639)	3/4 (3324)	3/4 (5.5)	2/4 (4.0)	3/4 (4.3)
BALB/c	4.0	4	4/4 (1092)	4/4 (5615)	4/4 (9831)	4/4 (6.4)	3/4 (4.9)	4/4 (4.3)
BALB/c	4.0	8	3/4 (449)	3/4 (3834)	3/4 (10948)	3/4 (5.3)	0/4	3/4 (5.0)
BSW[d]	4.0 ID_{50}	1	1/4 (153)	0/4	0/4	0/4	0/4	0/4
BSW[d]	4.0 ID_{50}	4	1/4 (255)	2/4 (6080)	2/4 (11964)	1/4 (4.3)	0/4	0/4

[a]For C3H and BALB/c mice, viral dose is expressed as 50% tissue culture infective dose (log_{10}); for BSW, viral dose is expressed as the 50% infective dose of HaPV inoculum propagated in vivo.
[b]Other = bone marrow, brain, pancreas, liver, kidney, gonad, salivary gland, heart, lung
[c]no. positive animals/total no. animals tested
[d]Inoculated by gastric gavage

HaPV infection in SCID mice. Neonatal and weanling SCID mice were inoculated oronasally with varied doses of HaPV. Individual tissues were surveyed for viral DNA by qPCR and grouped (lymphoid, intestinal, or other tissues) for analysis (Table 3). HaPV DNA was detected in multiple tissues in most neonatal SCID mice at multiple time points after inoculation. Consistently high levels of HaPV that greatly exceeded those present in the inoculum were detected in neonatal SCID mice at all time points, with the highest levels detected in the lymphoid tissues. In addition to lymphoid and intestinal tissues, HaPV DNA was sporadically detected in brain, salivary gland, lung, pancreas, gonad, heart, liver, bone marrow, and kidney, and fecal virus was detected by qPCR consistently at 4 and 8 wk after inoculation. In weanling SCID inoculates, low levels of viral DNA were detected in tissues, predominantly during the first 2 wk after inoculation. Viral DNA was not detected in samples from mock-infected SCID mice (data not shown).

Tissues from several SCID mice inoculated as neonates were collected at the 8-wk time point and preserved in RNA later. RNA from these samples was evaluated by RT-PCR for the presence of viral transcripts. Bands corresponding in size to that expected for unspliced and spliced HaPV transcripts were detected in HaPV-inoculated mice but not in mock-inoculated mice (Figure 1). Differential splicing at the small splice donor and acceptor sites was visualized by the presence of the 2 smaller bands (290 and 276 bp) on the gel in addition to a band corresponding in size to unspliced RNA (372 bp).

Histology was performed on all neonatal and weanling SCID mice. No lesions were observed in lymph nodes (mesenteric, sub-

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Table 3. qPCR results for neonate and weanling SCID mice inoculated oronasally with HaPV

	Viral dose[a] (log_{10} $TCID_{50}$)	Time after inoculation (wk)	MPV PCR (mean log_{10} viral copies/20 mg tissue)		
			Lymph node, spleen, thymus	Intestine	Other[b]
Neonates	3.7	1	1/4[c] (4.9)	3/4 (4.1)	3/4 (5.4)
	3.7	2	2/4 (6.6)	1/4 (5.5)	2/4 (4.6)
	3.7	4	3/4 (6.6)	2/4 (6.0)	2/4 (5.1)
	3.7	8	2/4 (7.0)	2/4 (6.1)	4/4 (5.8)
	4.0 ID_{50}	4	4/4 (5.8)	3/4 (4.9)	4/4 (4.9)
	4.0 ID_{50}	8	2/2 (6.4)	2/2 (4.8)	2/2 (4.8)
Weanlings	4.0	1	2/4 (4.0)	0/4	1/4 (4.0)
	4.0	2	2/4 (5.7)	0/4	3/4 (4.0)
	4.0	4	0/4	0/4	0/4
	4.0	8	1/4 (4.6)	1/4 (4.4)	3/4 (4.7)

[a]For SCID mice, viral dose is expressed as 50% tissue culture infective dose (log_{10}); ID_{50} indicates viral dose is expressed as the 50% infective dose of HaPV inoculum propagated in vivo.
[b]Other = bone marrow, brain, pancreas, liver, kidney, gonad, salivary gland, heart, lung
[c]no. positive animals/total no. animals tested

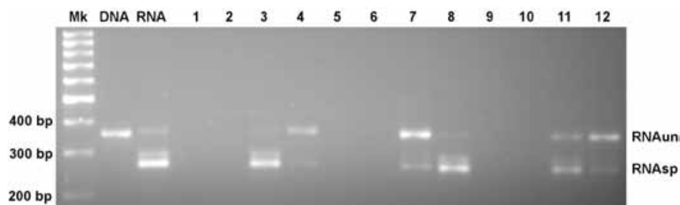

Figure 1. RT-PCR detection of HaPV transcripts in mouse tissues collected from 2 HaPV-infected SCID mice (lanes 3, 4, 7, 8, 11, 12). Results also shown for comparison tissues collected from 2 mock-infected mice (lanes 1, 2, 5, 6, 9, 10). Tissues include mesenteric lymph node (lanes 1 through 4), thymus (lanes 5 through 8), and spleen (lanes 9 through 12). Amplicons represent spliced (RNAsp) and unspliced (RNAun) viral transcripts. Mk, marker; DNA, DNA isolated from HaPV-infected cell culture; RNA, RNA isolated from HaPV-infected cell culture.

mandibular), spleen, thymus, heart, lung, salivary gland, jejunum, colon, liver, pancreas, kidney, gonad, or bone marrow.

Discussion

The rodent parvoviruses minute virus of mice, H1, and Kilham rat virus are generally subclinical in their natural mouse and rat hosts, yet they all induce clinical disease when inoculated into fetal or neonatal Syrian hamsters.[14,20-23,44] LuIII, a parvovirus of unknown host origin most closely related genetically to MPV and HaPV, similarly induces clinical disease when inoculated into neonatal hamsters.[43] The clinical signs, gross and histologic lesions associated with experimental infection of hamsters by these parvoviruses are similar to those observed in HaPV-infected hamsters. This result suggests that the Syrian hamster may be an aberrant host for HaPV and that another rodent species may serve as the reservoir. Genomic sequence analysis of HaPV and MPV3, a variant of MPV recently detected in naturally infected mice, revealed these 2 viruses exhibit extremely high genetic homology and share a unique 3 codon insertion in the VP1 capsid protein that is not present in MPV1, MPV2, or other rodent parvoviruses.[7] This finding suggests HaPV and MPV3 are variants of a single viral genotype and that transmission of MPV3 from mice to hamsters may have induced the clinical manifestations in hamsters that ultimately led to the isolation of HaPV.

The present studies evaluated the infectivity of HaPV in potentially susceptible strains and ages of mice to determine whether HaPV could serve as a surrogate isolate for MPV3, which has not yet been isolated from infected mice. Initially HaPV was propagated in vitro in cell culture, with some inconsistency observed in the results obtained for C3H, BALB/c, and SCID mice inoculated with this stock (Tables 1 through 3). In addition, HaPV was cultivated in vivo in neonatal hamsters to obtain high-titer viral stock, to determine whether more-concentrated viral inocula would induce infection more consistently. Again we obtained somewhat inconsistent results in BSW and SCID mice; therefore, data from all experimental infections of mice were used in the final analysis.

Multiplex serology and qPCR assays specific for MPV and HaPV were used to evaluate antibody induction and tissue viral DNA loads, and histology was performed in select mice to identify microscopic lesions. Seroconversion to MPV1 and HaPV viral capsid protein was consistently observed at 4 and 8 wk after inoculation in immunocompetent mice inoculated as neonates and weanlings, with many mice seropositive at 1 and 2 wk after inoculation. Even though purified MPV1 and HaPV rVP2 antigen were coated onto microspheres at identical concentrations, and antibody response was detected in a multiplex format, MFI signal strength often was greater for HaPV rVP2 antigen as compared with MPV1 rVP2 antigen. This differential seroreactivity suggests that further investigation is needed to determine whether serologic detection of uncommon MPV variants like MPV2 and MPV3 could be improved through the use of additional rVP2 antigens. Antibody response to rNS1 was less consistent than that to rVP2 but present in many mice from 2 to 8 wk after inoculation, particularly in BALB/c mice, and signal strength in seropositive mice approached the antibody levels observed in mice infected with MPV1.[3,6] Importantly, NS1 protein is not present in infectious parvovirus virions, so antibody response to this viral protein implies viral entry and transcription, thereby indicating productive infection of mice by HaPV.

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As for the antibody response, detection of HaPV DNA by qPCR in immunocompetent mice was also inconsistent, although some trends were observed. Viral DNA was detected in intestinal tissue primarily during the early stages of infection, with detection in lymphoid tissues at all time points. In addition, viral DNA was more likely to be detected in mice inoculated as neonates as compared with weanlings and was more likely to be detected in C3H and BALB/c mice as compared with BSW. These findings are similar to previous reports on MPV1 pathogenesis which indicate acute infection of intestinal tissues with persistence of viral DNA in lymphoid tissues[19,42] and mouse age- and strain-related susceptibilities.[8,36] Notably, the viral DNA levels detected in many tissues were higher than were present in inocula, indicating viral genome replication consistent with productive infection. High levels of viral DNA in tissues and feces of SCID mice inoculated as neonates were detected throughout the course of infection, suggesting persistent productive infection similar to the pathogenesis of MPV1 in neonatal SCID mice.[3] Spliced and unspliced viral transcripts were detected in neonatal SCID mouse inoculates by RT-PCR at 8 wk after inoculation, providing additional evidence of persistent productive infection. Histology was performed on tissues from SCID and BALB/c mice inoculated as neonates, because these age and strain groups displayed the most consistently positive results and are considered to be the most susceptible to MPV1 infection. Similar to findings for MPV1 infection in mice, no gross or histologic lesions were detected in any of the tissues examined.

The above findings indicate HaPV can induce productive infection in mice, with a pathogenesis similar to that observed in mice infected with MPV1. Multiple attempts to consistently induce productive infection were made by administration of higher viral doses, administration by gavage (weanling BSW only), and use of several mouse strains and ages. Technical limitations related to the inability to generate high-titer viral stock or oronasal inoculation of neonatal mice with a consistent amount of virus may have played a role in these inconsistencies. Alternatively, virus adaptation first to the hamster host and then to cell culture may have altered the ability of HaPV to consistently induce productive infection in mice. It is well known that relatively few amino acid changes in the parvovirus capsid protein can significantly alter the in vitro cell or in vivo host range of a parvovirus.[1,2,33] The evolution of canine parvovirus from feline parvovirus[34] provides a paradigm for the interspecies transmission from mouse to hamster hypothesized for MPV3–HaPV.

In summary, we inoculated several strains and ages of mice with HaPV and observed a pathogenesis similar to that of MPV1 infection in mice. The detection of seroconversion to MPV–HaPV nonstructural and capsid antigens, viral DNA levels that greatly exceeded that present in inocula, virus in feces 8 wk after inoculation, and both spliced and unspliced viral RNA indicate a productive HaPV infection in mice. These and previous findings support the hypothesis that HaPV originally arose after interspecies transmission of MPV3 from mice to hamsters. Although interspecies transmission of parvovirus rarely occurs, these data support the common practice of separate housing for different rodent species in laboratory animal facilities.

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