

# Identification of novel murine parvovirus strains by epidemiological analysis of naturally infected mice

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Random-source DNA samples obtained from naturally infected laboratory mice ( $n=381$ ) were evaluated by PCR and RFLP analysis to determine the prevalence of murine parvovirus strains circulating in contemporary laboratory mouse colonies. Mouse parvovirus (MPV) was detected in 77 % of samples, *Minute virus of mice* (MVM) was detected in 16 % of samples and both MVM and MPV were detected in 7 % of samples. MVMm, a strain recently isolated from clinically ill NOD- $\mu$  chain knockout mice, was detected in 91 % of MVM-positive samples, with the Cutter strain of MVM (MVMc) detected in the remaining samples. The prototypic and immunosuppressive strains of MVM were not detected in any of the samples. MPV-1 was detected in 78 % of the MPV-positive samples and two newly identified murine parvoviruses, tentatively named MPV-2 and MPV-3, were detected in 21 and 1 % of the samples, respectively. The DNA sequence encompassing coding regions of the viral genome and the predicted protein sequences for MVMm, MPV-2 and MPV-3 were determined and compared with those of other rodent parvovirus strains and Lull virus. The genomic organization for the newly identified viral strains was similar to that of other rodent parvoviruses, and nucleotide sequence identities indicated that MVMm was most similar to MVMc (96.1 %), MPV-3 was most similar to hamster parvovirus (HaPV) (98.1 %) and MPV-2 was most similar to MPV-1 (95.3 %). The genetic similarity of MPV-3 and HaPV suggests that HaPV epizootics in hamsters may result from cross-species transmission, with mice as the natural rodent host for this virus.

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## INTRODUCTION

*Minute virus of mice* (MVM) and mouse parvovirus (MPV) are among the most prevalent infectious agents detected in contemporary laboratory mouse colonies, with approximately 45 % of USA research institutions harbouring these infectious agents (Jacoby *et al.*, 1996) and MPV being among the most prevalent viruses detected in research mice (Livingston & Riley, 2003). Various clinical disease syndromes in mice have been associated with MVM infection (Brownstein *et al.*, 1991; Kimsey *et al.*, 1986; Lamana *et al.*, 2001; Segovia *et al.*, 1991, 1995, 1999) and both MVM and MPV can have deleterious effects on research due to *in vitro* and *in vivo* immunomodulatory effects (Bonnard *et al.*, 1976; Engers *et al.*, 1981; Kimsey *et al.*, 1986; McKisic *et al.*, 1993, 1996; McMaster *et al.*, 1981), tumour suppression (Giese *et al.*, 2000; Guetta *et al.*, 1986; Kimsey *et al.*, 1986; McKisic *et al.*, 1993, 1996) and contamination of cell cultures and tissues originating from mice (Bonnard *et al.*,

1976; Collins & Parker, 1972; Crawford *et al.*, 1969; Garnick, 1996, 1998; McKisic *et al.*, 1993; Nicklas *et al.*, 1993). There is also significant potential for MVM and MPV to be transmitted among research facilities due to a high degree of environmental stability (Harris *et al.*, 1974), their potential to induce persistent infection in mice and cell lines (Fikrig & Tattersall, 1992; Jacoby *et al.*, 1995; Segovia *et al.*, 1999) and the difficulties associated with eradicating these viruses from infected laboratory mouse colonies. As a result, murine parvovirus infections comprise one of the most significant infectious disease problems encountered in contemporary laboratory animal research facilities.

The first strain of MVM was isolated in 1966 from a preparation of adenovirus (Crawford, 1966) and, as the prototypic virus for the genus *Parvovirus*, was designated MVMp. A second strain of MVM was isolated in 1976 from contaminated EL4 lymphocytes (Bonnard *et al.*, 1976); it was later shown to be immunosuppressive *in vitro* (Engers *et al.*, 1981; McMaster *et al.*, 1981) and was therefore designated MVMi. A third strain of MVM, designated MVM-Cutter (MVMc), was isolated during the 1980s as a contaminant of BHK-21 cells that were being used to

The GenBank/EMBL/DDBJ accession numbers for the sequences determined in this work are DQ196317 (MVMm), DQ196319 (MPV-2) and DQ196318 (MPV-3).

produce a recombinant protein at a commercial laboratory (Besselsen *et al.*, 1996). A recent report associated naturally occurring MVM infection with growth retardation, reduced fecundity and premature death in NOD mice homozygous for a targeted mutation of the immunoglobulin heavy chain 6 or  $\mu$  chain (Naugler *et al.*, 2001). Our laboratory isolated a strain of MVM from one of these infected mice that, upon initial sequence analysis, appeared to be distinct from the aforementioned MVM strains and henceforth is referred to as MVMm in reference to its isolation from mice obtained from the University of Missouri.

Routine serological testing for MVM in the 1980s indicated that another mouse parvovirus existed in laboratory mouse colonies. *Mouse parvovirus 1* (MPV-1) was subsequently isolated, with three strains designated MPV-1a, MPV-1b and MPV-1c. MPV-1a was isolated from cultures of L3 cytolytic T lymphocytes and splenocytes in 1993 (McKisic *et al.*, 1993). This isolate was then adapted to growth in CTLL-2 cytolytic T lymphocytes. Subsequent sequencing of these two strains showed that several mutations had occurred during adaptation of the virus to the CTLL-2 cells and therefore the adapted virus was designated MPV-1b (Ball-Goodrich & Johnson, 1994; Besselsen *et al.*, 1996). A field strain of MPV-1 was sequenced directly from tissues collected from a naturally infected mouse and also displayed several nucleotide differences from the two cultivated strains of MPV-1; this strain was designated MPV-1c (Besselsen *et al.*, 1996). Initial genomic sequence analysis indicated that MPV-1 was most closely related genetically to MVM and LuIII, a parvovirus species of unknown host origin, with approximately 88% nucleotide sequence similarity. Subsequently, hamster parvovirus (HaPV) was isolated and sequenced and was demonstrated to be more closely related to MPV-1 with 94.6% nucleotide sequence similarity (Besselsen *et al.*, 1996).

HaPV was isolated from a large commercial colony of Syrian hamsters that experienced high morbidity and mortality among suckling and weanling hamsters (Besselsen *et al.*, 1999). It is questionable whether the Syrian hamster is the natural rodent host for this virus, as other rodent parvoviruses are subclinical in their natural rodent hosts, yet all induce a clinical disease syndrome in experimentally infected fetal or neonatal Syrian hamsters similar to the syndrome observed in HaPV-infected hamsters (Brownstein *et al.*, 1991; Garant *et al.*, 1980; Kilham, 1960, 1961; Kilham & Margolis, 1964, 1970; Toolan, 1960). These findings suggest that the hamster is probably an aberrant host for HaPV. Given the nucleotide sequence similarity of HaPV and MPV-1 and their serological cross-reactivity by haemagglutination inhibition (Besselsen *et al.*, 1996), the mouse is a primary candidate as a natural rodent host for HaPV. However, there have been no attempts to identify HaPV in laboratory mice.

The molecular biology of MVM has been characterized and is representative of a subgroup of related autonomous parvoviruses, including MPV-1 and HaPV (Ball-Goodrich

& Johnson, 1994; Besselsen *et al.*, 1996; Cotmore & Tattersall, 1987). MVM produces three mRNA species, R1, R2 and R3, which all terminate at a single polyadenylation site at genomic map unit (m.u.) 95 (Clemens & Pintel, 1987). R1 arises from the P4 promoter (m.u. 4) and encodes the multifunctional 83 kDa phosphoprotein NS1. NS1 exhibits ATPase and helicase activities (Jindal *et al.*, 1994; Nüesch *et al.*, 1995; Wilson *et al.*, 1991), sequence-specific DNA-binding properties (Christensen *et al.*, 1995; Cotmore *et al.*, 1995) and is required for viral DNA replication and transactivation of the P38 promoter (m.u. 38) (Cotmore & Tattersall, 1986, 1995). R2 also arises from the P4 promoter, but a large intron between nt 514 and 1989 is spliced out of the primary transcript. R2 generates the smaller 23–25 kDa phosphoprotein NS2, which is required for virus replication, capsid assembly and nuclear egress of progeny virions in a cell-type-dependent manner (Cotmore & Tattersall, 1986; Cotmore *et al.*, 1997; Eichwald *et al.*, 2002; Miller & Pintel, 2002; Naeger *et al.*, 1990, 1993). R3 arises from the P38 promoter and encodes two structural viral proteins, the 83 kDa VP1 and the 64 kDa VP2 (Labieniec-Pintel & Pintel, 1986). A third structural viral protein, the 61 kDa VP3, is produced by proteolytic processing of VP2 near the trypsin-sensitive RVER motif located at VP2 aa 19–22 (Tattersall *et al.*, 1976). Alternative splicing of two donor sites and two acceptor sites between m.u. 44 and 46 determines the relative ratio of VP1:VP2 produced during infection and also results in three isoforms of NS2 that have different C termini (Clemens *et al.*, 1990; Cotmore & Tattersall, 1990; Jongeneel *et al.*, 1986; Morgan & Ward, 1986).

The three-dimensional structure of the capsid has been determined for MVM and several other parvoviruses (Kaufmann *et al.*, 2004; Llamas-Saiz *et al.*, 1997; McKenna *et al.*, 1999; Padron *et al.*, 2005; Simpson *et al.*, 2002; Tsao *et al.*, 1991; Walters *et al.*, 2004) and phenotypic characteristics displayed by parvoviruses have been mapped to specific surface structures of the virus capsid (Agbandje-McKenna *et al.*, 1998; Chang *et al.*, 1992; Parrish, 1991). For example, MVMp is fibrotropic, whilst MVMi is lymphotropic. Mutational analyses initially mapped these specific tropisms to two amino acid loci in the viral capsid proteins (Ball-Goodrich & Tattersall, 1992). However, forward mutants induced on to either of these mutations mapped to other amino acid loci that are not proximally located in the amino acid sequence. Structure determination of the MVM capsid subsequently revealed that each of these amino acid loci clustered at the surface of the three-fold spike of the capsid (Agbandje-McKenna *et al.*, 1998; Llamas-Saiz *et al.*, 1997). Therefore, minor structural alterations induced by changes at different amino acid loci confer the distinct cell tropisms of MVMp and MVMi. Differences in the pathogenesis of MVMp and MVMi infections in mice, with MVMp infection restricted to the enteric tract with no associated pathology and MVMi producing a systemic infection associated with renal papillary necrosis and haematopoietic disruption (Brownstein *et al.*, 1991; Kimsey *et al.*, 1986; Lamana *et al.*, 2001; Segovia *et al.*, 1991, 1995, 1999), are

thought to be a result of the differing cell tropisms conferred by capsid surface alterations. Other phenotypic alterations such as haemagglutination have also been associated with VP2 amino acid changes that result in altered capsid surface structure (Ball-Goodrich & Tattersall, 1992; Chang *et al.*, 1992; Parrish, 1991).

The discovery of several novel rodent parvovirus strains over the past decade (Ball-Goodrich *et al.*, 1998; Besselsen *et al.*, 1999; McKisic *et al.*, 1993; Wan *et al.*, 2002), the potential for significant differences in phenotype to be conferred by a minimal number of VP2 amino acid substitutions that alter capsid surface topography, questions about the natural rodent host for HaPV and the significance of murine parvovirus infections in research animal facilities indicate a need for a survey of mice naturally infected with parvoviruses to assess murine parvovirus strains circulating in contemporary laboratory mouse colonies. The objectives of this study were to determine the prevalence of known murine parvovirus strains circulating among contemporary laboratory mouse colonies and to identify novel murine parvovirus strains, including HaPV-like strains, that may be circulating in these colonies. The coding regions of the genomes of newly identified murine parvovirus strains were sequenced and the DNA and predicted protein sequences for each isolate were then compared with those of other rodent parvoviruses to examine their genetic relatedness and genomic organization.

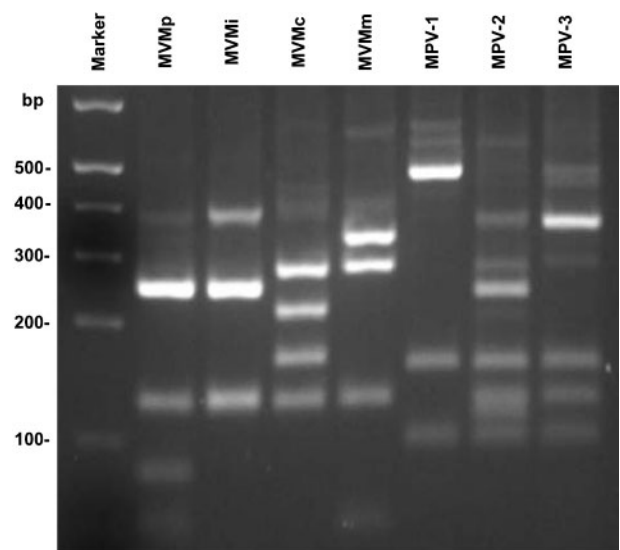
## METHODS

**Viral isolates.** MVMp, MVMi, MVMc, MPV-1b, HaPV and LuIII were propagated as described previously (Besselsen *et al.*, 1996). MVMm was isolated and propagated as follows. Spleen was harvested from an NOD- $\mu$  chain knockout mouse, homogenized in 50 mM Tris/HCl (pH 8.7), 10 mM EDTA, freeze-thawed once and centrifuged. The supernatant was filtered through a 0.2  $\mu$ m filter. The sterilized homogenate was co-cultivated with murine A9<sub>2L</sub> fibroblasts (Tattersall & Bratton, 1983) at 37 °C and passaged until a cytopathic effect was observed. Crude lysates were prepared from infected A9<sub>2L</sub> cells by subjecting the media and cells to four freeze-thaw cycles. The TCID<sub>50</sub> was then determined.

**Molecular epidemiology analysis.** DNA or tissue samples from a total of 381 parvovirus-infected animals were obtained from two large rodent diagnostic laboratories and nine other animal facilities within the USA. The vast majority of samples ( $n=334$ ) were obtained from the two large rodent diagnostic laboratories; both laboratories routinely evaluate DNA and tissue samples for murine parvoviruses by PCR for a wide variety of research animal facilities throughout the USA, with samples obtained from both sexes and many different strains and ages of mice. Murine parvovirus DNA had previously been detected in each of the samples submitted by the two large rodent diagnostic laboratories by the PCR assays utilized at each of these laboratories. Samples submitted directly to our laboratory from other animal facilities were from mice that were previously determined to be positive for murine parvovirus infection by serology and/or PCR, with each mouse obtained from colonies with historical evidence of enzootic murine parvovirus infection. As required, DNA was extracted from tissue samples (generally mesenteric lymph node or spleen) with a Qiagen DNA Tissue kit (Qiagen). DNA was screened using a fluorogenic nuclease PCR

(fnPCR) assay that detects all rodent parvovirus species (target template is a conserved region of the NS1 exon), with positive samples then evaluated by MVM- and MPV-specific fnPCR assays (target templates within the VP2 exon), as described elsewhere (Redig & Besselsen, 2001). Early in the course of these studies, discrepancies among the quantitative viral DNA levels detected in some DNA samples by the panel of fnPCR assays (i.e. high rodent parvovirus DNA levels coupled with absent MVM and low MPV DNA levels) led to the identification of a novel strain of MPV (henceforth named MPV-2). The published MPV/HaPV-specific fnPCR assay was shown to display poor sensitivity for detection of MPV-2, so an alternative primer/probe set (forward primer 5'-CTGCGCCAATTTATCCAA-CAA-3'; reverse primer 5'-AGAAGTTGACCTGGAGCATTGTT-3'; probe 5'-TGGGACAAAGAACTTGATCTTGAACACAAACC-3') was designed to provide a sensitive (less than 10 template copy level detection in a single reaction) and specific fnPCR assay for the detection of MPV-1, MPV-2, HaPV and LuIII (data not shown).

Samples in which MVM DNA was detected by fnPCR were analysed using a PCR/RFLP approach that discriminates the MVMp, MVMi, MVMc and MVMm strains. An 800 bp amplicon that encompasses a heterologous region of the VP2 gene corresponding to MVMi nt 3760–4559 was generated by MVM-specific PCR (forward primer 5'-TAACCTGGGTGAGTGAAGCAAT-3'; reverse primer 5'-GTTAGTAA-GTATTCTAGCAACAGGTCT-3'). Amplicon DNA was digested overnight with the restriction enzyme *Hpy8I*, DNA digests were electrophoresed on a 3% NuSieve agarose gel and banding patterns were used to determine the MVM strain present in each sample (Fig. 1). Similarly, samples that contained MPV/HaPV DNA by fnPCR were subjected to MPV/HaPV-specific PCR (forward primer 5'-GATGAATTTGCTACTGGAACCTAC-3'; reverse primer 5'-GT-AAGGTTTGGTGAAGTCTAAC-3') to produce a 731 bp amplicon corresponding to MPV-1a nt 3581–4311, which was subsequently digested with the restriction enzymes *NlaIV* and *BfuCI* to discriminate among MPV-1, MPV-2 and mouse-origin HaPV (henceforth referred to as MPV-3) (Fig. 1). High-fidelity PCR was performed for all PCR/RFLP analyses to reduce polymerase-induced errors. Reactions were performed in 25  $\mu$ l in a Perkin Elmer model 2400 thermocycler. Each reaction mixture contained 2  $\mu$ l template DNA, 0.2  $\mu$ M each oligonucleotide primer and 22.5  $\mu$ l Platinum PCR Supermix High



**Fig. 1.** RFLP banding patterns for MVM and MPV strains. Marker, 100 bp DNA ladder.

Fidelity (Invitrogen). Thermocycling parameters were 30 s of denaturation at 94 °C, followed by 45 cycles of 30 s of denaturation at 94 °C, 30 s of annealing at 57 °C (MVM) or 53 °C (MPV/HaPV) and 60 s of elongation at 72 °C.

**Sequence analysis.** MVMm DNA was amplified by PCR using overlapping primer sets that together encompassed the entire coding region of the MVM genome. Due to the inability to propagate MPV-2 and MPV-3 in cell culture and the limited amounts of DNA available for these two strains, high-fidelity PCR was performed to produce overlapping 2500 bp amplicons that were ligated into the pT7Blue-2 vector (Novagen), amplified in Tuner (DE3) pLacI Competent Cells (Novagen) and purified using a Qiagen Plasmid Purification kit. Purified plasmid DNA was then used as template to generate sequence data. Oligonucleotide primers were synthesized by Sigma-Genosys and primer sequences were selected on the basis of sequence alignments performed in the GCG software package (Genetics Computer Group) to produce overlapping 700–800 bp segments of the viral genome. Primers were initially designed from sequences that were highly conserved among characterized murine parvovirus strains. As sequence data for MVMm, MPV-2 and MPV-3 became available, these data were also utilized for primer design. PCRs were performed as described previously for the PCR/RFLP analyses except that 50 µl reactions were performed. The 700–800 bp amplicons were electrophoresed on a 2% NuSieve agarose gel, bands of the expected size were excised and DNA was extracted with a QIAquick Gel Extraction kit (Qiagen) and gel-purified amplicon DNA was sequenced at the University of Arizona's Molecular Core Facility. The overlapping sequence fragments for each viral strain were then assembled into a consensus sequence that spanned >90% of the genome (4687 nt for MVMm, 4740 nt for MPV-2, 4750 nt for MPV-3) and encompassed the entire coding region of each virus. Nucleotide sequences for MVMm, MPV-2 and MPV-3 were aligned with those of other rodent parvoviruses and the percentage nucleotide similarity with other rodent parvoviruses was determined. GenBank accession numbers for the parvovirus sequences that were included in the analyses are: J02275 (MVMp), M12032 (MVMi), U34256 (MVMc), U12469 (MPV-1a), U34255 (HaPV), M81888 (LuIII), X01457 (H-1), AF321230 (*Kilham rat virus*, KRV), AF332882 (rat minute virus, RMV-1a), AF036710 (rat parvovirus, RPV-1a) and M38367 (*Porcine parvovirus*, PPV). Amino acid sequences for the predicted NS1, NS2, VP1 and VP2 proteins were translated and aligned with predicted protein sequences for other rodent parvoviruses and percentage amino acid identity and similarity were determined. The GCG software program used is indicated in each figure legend and the parameters used were default settings unless otherwise indicated.

## RESULTS

### Molecular epidemiological analysis

Screening of DNA samples by the rodent parvovirus fnPCR assay yielded 381 parvovirus-positive samples. Early in the course of these studies, we observed a subset of samples in which high quantities of viral DNA were detected by the rodent parvovirus fnPCR assay coupled with very low to absent quantities of viral DNA detected by the MPV/HaPV-specific fnPCR assay and no viral DNA detected by the MVM-specific fnPCR assay. Several of the samples were analysed by PCR using the MPV/HaPV-specific and MVM-specific primers developed for the RFLP strain typing analysis; all of the samples were positive by the MPV/HaPV PCR, but negative by the MVM PCR. Amplicons from these MPV/

HaPV-specific PCRs were sequenced and, when aligned with MPV-1 and HaPV, revealed a distinct strain of MPV (tentatively named MPV-2) present in these samples. Alignment of the published primers and probe for the MPV/HaPV-specific fnPCR assay used initially (Redig & Besselsen, 2001) with the MPV-2 sequence revealed numerous nucleotide mismatches within the primers and probe. Therefore, a new primer/probe set was designed that would sensitively and specifically detect MPV-1, MPV-2 and HaPV (data not shown). After implementation of the new MPV/HaPV-specific fnPCR assay, all 381 samples that were positive by the rodent parvovirus fnPCR assay were also positive by the MVM-specific and/or the MPV/HaPV-specific fnPCR assays. Of the parvovirus-positive samples, 77% were positive by the MPV/HaPV assay, 16% were positive by the MVM assay and 7% were positive by both the MVM and the MPV/HaPV assays indicating dual infection (Table 1). Of the 87 MVM-positive samples, we were able to determine the MVM strain in 69 samples by RFLP analysis; the remaining samples had too little viral DNA present to strain type. The most recent isolate MVMm was detected in 91% of these samples, with MVMc detected in the remaining 9% of samples. MVMp and MVMi were not detected. RFLP analysis was successful for 276 of the 322 MPV/HaPV fnPCR-positive samples, with the remaining samples having too little viral DNA present to strain type. MPV-1 was detected in 78% of these samples and the newly recognized MPV-2 was detected in 21% of the samples. HaPV was detected in four (1·4%) of the samples, with all four of these mice originating from the same colony of mice. The 731 bp RFLP amplicon for each of the four HaPV-positive samples was sequenced to confirm the presence of HaPV. All four samples displayed an identical sequence, which differed from the published sequence of hamster-origin HaPV by only 8 nt in this region. As this viral strain was detected in naturally infected mice and was distinct

**Table 1.** Molecular epidemiology survey results

Assay performed	No. positive /no. tested	Positive (%)
<b>fnPCR assays</b>		
Rodent parvovirus (NS1)	381/381	100
MVM	59/381	15·5
MPV	294/381	77·2
MVM/MPV	28/381	7·3
<b>MVM PCR/RFLP strain type</b>		
MVMp	0/69	0
MVMi	0/69	0
MVMc	6/69	8·7
MVMm	63/69	91·3
<b>MPV PCR/RFLP strain type</b>		
MPV-1	215/276	77·9
MPV-2	57/276	20·7
MPV-3	4/276	1·4

from MPV-1 and MPV-2, this strain was tentatively named MPV-3.

### DNA sequence analysis

Genomic sequence was obtained for MVMm, MPV-2 and MPV-3 that corresponded to MVMi nt 141–4827. Sequence alignment of these viruses with other rodent autonomous parvoviruses revealed that portions of the genome determined previously to be important in transcription, mRNA processing and translation of prototypic rodent parvoviruses were conserved among the three newly identified murine parvovirus strains (Fig. 2). Conserved regions included the P4 and P38 TATA boxes and SP1-binding sites, the TAR element associated with the P38 promoter, the small and large intron donor and acceptor sites, the polyadenylation site and the start and stop codons for the NS1, VP1 and VP2 proteins. Variations of the 65 nt repeats located in the 3' untranslated region of MVMp were observed in the newly identified murine parvoviruses. MVMm completely lacked one repeat (like MVMi), whilst MPV-2 and MPV-3 each had a 12 nt deletion in the first repeat compared with MVMp.

Nucleotide sequence similarities were determined for the newly identified murine parvoviruses and other autonomous parvoviruses. Percentage sequence identities for the three new viral strains and the other rodent parvoviruses and LuIII are shown in Table 2. The nucleotide sequence for MVMm was most similar to that of MVMc, with marginally lower similarity to MVMi and MVMp. MPV-2 was most similar to MPV-1, with slightly less identity to MPV-3 and HaPV. MPV-3 was highly similar to HaPV. When the nucleotide sequences of MPV-2 and MPV-3 were compared with those of other rodent parvoviruses, a relatively high degree of similarity was also observed with MVM strains and LuIII, whilst relatedness to rat-origin parvoviruses was significantly lower. A dendrogram generated from the genomic nucleotide sequences of the rodent parvovirus strains indicated that the murine parvoviruses appeared to fall into an MVM clade (MVMp, MVMi, MVMc, MVMm) and an MPV-like clade (MPV-1, MPV-2, MPV-3, HaPV), with LuIII and the rat-origin parvoviruses being more distantly related (Fig. 3).

### Protein sequence analysis

The amino acid sequences for the predicted proteins for each newly identified murine parvovirus were translated from the genomic DNA sequences, aligned with those of other rodent parvoviruses and LuIII and the percentage amino acid similarity and identity for each protein comparison was calculated. The NS1 proteins of MVMm and MPV-3 were most similar to HaPV, whilst the NS1 protein of MPV-2 was most similar to MPV-1. However, all of the rodent parvoviruses exhibited a high percentage NS1 protein similarity (Table 3). Translation of the NS2 proteins for the newly isolated parvoviruses revealed no amino acid changes in the second exon or in the C terminus of the two

predominant isoforms compared with MVMi. However, several variations were observed at aa 185, 187 and 195 of the rare isoform of NS2 that utilizes the small intron donor one and acceptor two sites, with MVMm identical to MVMp, MPV-3 identical to HaPV and MPV-2 having a unique C-terminal sequence that was most similar to MPV-1c (Fig. 4). In addition, the nuclear export signal (NES) amino acid sequence for MVMm NS2 was LTKKFSTLTI compared with MTKKFGTLTI for MVMp and the other murine parvoviruses (including MPV-2 and MPV-3), with the initial leucine indicating a shift towards the consensus NES sequence (Eichwald *et al.*, 2002). Comparisons of the predicted VP1 unique N-terminal amino acid sequences showed that MVMm was most similar to MVMp and MVMi, MPV-2 was most similar to MPV-1 and MPV-3 was identical to HaPV. The latter is notable, as HaPV includes a three-codon insertion in the VP1 unique N terminus compared with the MVM strains, MPV-1 and MPV-2. The predicted VP2 amino acid sequences showed that MVMm was most similar to MVMc, MPV-2 was most similar to MPV-3 and MPV-3 was most similar to HaPV (Table 3). Alignment of the VP1 amino acid sequences showed conservation of the VP2/VP3 cleavage site, the polyglycine sequence located at the N terminus of VP2, the basic amino acids within the nuclear localization sequences and motif (Lombardo *et al.*, 2000, 2002), the C-terminal leucine of the  $\beta$ -strand sequence of VP2 that forms the cylinder at the fivefold symmetry axis (Farr & Tattersall, 2004) and the phospholipase A<sub>2</sub> calcium-binding and catalytic residues (Zadori *et al.*, 2001) (Fig. 5). This alignment also revealed that the two amino acids that are the primary determinants of the fibroblast-specific tissue tropism of MVMp (Ball-Goodrich & Tattersall, 1992) were identical for MVMm and MVMp, whilst amino acids involved in haemagglutination were identical for MVMm and MVMc (Agbandje-McKenna *et al.*, 1998).

## DISCUSSION

A molecular epidemiological survey of random-source DNA samples obtained from naturally infected laboratory mice was performed to determine the prevalence of murine parvovirus strains circulating in contemporary laboratory mouse colonies. MPV strains were the most commonly detected, with approximately one quarter of the samples evaluated being positive for MVM strains alone or both MPV and MVM (dual infection). This indicates a higher prevalence of MPV circulating in contemporary laboratory mouse colonies, a finding consistent with recent serological surveys (Livingston & Riley, 2003; Livingston *et al.*, 2002). Alternatively, MPV-1 DNA can persist for weeks to months in lymphoid tissues in experimentally infected immunocompetent mice (Besselsen *et al.*, 2000; Jacoby *et al.*, 1995), whereas MVM is thought to induce only acute infections in immunocompetent mice (Jacoby *et al.*, 1996; Smith, 1983); thus, a higher prevalence of MPV DNA in tissue samples may be reflective of the longer period of time during which viral DNA can be detected in MPV-infected mice compared

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1          Spl       P4 TATA          → R1 (NS1) and R2 (NS2) mRNA START
MVMi : CCAAGGCGCGAAAAGGAAGTGGGCGTGGTTTAAAGTATATAAGCAAATGCTGAAGTCAGTTACTTATCCTTTCTTCATTCTGTGAGTCGAGACGCGCAG
MVMm : ---T-----C-A-CA---G-----T-----A-A-A---
MPV-2 : ---A-A-----C-TAT-----T-----A---
MPV-3 : ---A-----TG-----A-T-----TA---A---

101         NS1 and NS2 START
MVMi : AAAGAGAGTAACCACTAACCATGGCTGGAATGCTTACTCTGATGAAGTTTGGGAACAACCAACTGGTTAAAGGAAAAAGTAACCAGGAAGTGTCT
MVMm : -----A-G-----
MPV-2 : -G-----C-----G-----
MPV-3 : -G-----C-----A-----

201
MVMi : CATTTGTTTTTAAACTGAGGATGTTCACTAAATGGAAAAGATATCGGATGGAATAATTACAAAAGGAGCTGCAGGAGGACGAGCTGAAATCTTTACA
MVMm : -----A-A-----G-C-----G-----A-A-----
MPV-2 : -----G-----G-----
MPV-3 : -----A-A-----G-----

301         R2 LARGE SPLICE DONOR
MVMi : ACGAGGAGCGGAAACTACCTGGGACCAAGCGAGGACATGGAATGGGAATCTACAGTGGATGAAATGACCAAAAAGCAAGTATTCATTTATGACTCTTTA
MVMm : -----G-----T-----C-A-----T-----
MPV-2 : -----
MPV-3 : -----G-----C-A-----

401
MVMi : GTTAAAAATGTTTGGTTGAAGTGCTTAGCACAAAAATATAGCTCCTGCTGATGTTACTTGGTTTGTGCAGCATGAATGGGGAAAGACCAAGGCTGGC
MVMm : -----G-T-----G-C-----AG-----A-----A-----
MPV-2 : -----G-C-----G-----A-----A-----A-----
MPV-3 : -----G-T-----G-C-----AG-----A-G-----A-----

501
MVMi : ACTGCCATGTACTAATTGGAGCAAGGACTTTAGTCAAGCTCAAGGAAAATGGTGGAGAAGGCAGCTAAATGTTTACTGGAGCAGATGGTTGGTAACAGC
MVMm : -----T-----C-----T-----A-----A-----T-----A-----
MPV-2 : -T-T-----A-----G-----A-----A-----T-----A-----
MPV-3 : ---T---G-C-----C-----T-----A-----A-----

601
MVMi : CTGTAATGTGCAGCTAACACCAGCTGAAAGAATTAAACTAAGAGAAATAGCAGAAGACAGTGAGTGGGTACTTTACTCACTTATAACATAAGCAAACC
MVMm : ---G-----T-----G-G-----G-----CAA-A-----C-----G-T-----G-----
MPV-2 : -----T-----C-G-----G-----CAA-A-----C-G-----G-----
MPV-3 : -----T-T-----G-G-----G-----CAA-A-----C-G-T-----G-----

701
MVMi : AAAAAGGACTATACTAAATGTGTTCTTTTGGAAATATGATGCTTACTACTTTTAAACAAAAGAAAATAAGCACCAGTCCGCCAAGGACGAGGCT
MVMm : ---A-----C-G-----TGC-----AG-----T-----G-----T-----A-----
MPV-2 : -----C-----C-----A-----T-----A-----A-----
MPV-3 : -----C-G-----AG-----G-----A-----A-----A-----

801
MVMi : ATTTTCTAAGCAGTGACTCTGGCTGGAAAACTAACTTTTTAAAGAGGGCGAACGCCATCTAGTGAGCAAATTATACACTGATGACATGCGGCCAGAAAC
MVMm : -----T-----A-----C-----
MPV-2 : -----G-----C-----
MPV-3 : -----G-----C-----A-----

901
MVMi : GGTGAAACCACAGTAACCACTGCGCAGGAACTAAGCGCGGCAGAATTCAACTAAAAAGAGGTTTCTATTAAACCACACTTAAAGAGCTAGTGCAT
MVMm : -----A-----C-----G-A-----
MPV-2 : -----G-----A-G-----
MPV-3 : -----G-----C-----G-----

1001
MVMi : AAAAGAGTAACCTCACCAGAAGACTGGATGATGATGCAGCCAGACAGTTACATTGAAATGATGGCTCAACCAGGTGGAGAAAACCTGCTGAAAAATACGC
MVMm : ---G-----T-----
MPV-2 : -----
MPV-3 : -----

1101
MVMi : TAGAGATTGTACGCTAACTCTAGCCAGAACAAAACAGCATTGACTTGATTTAGAAAAAGCTGAAACCAGCAAACCTAACCAACTTTTCACTGCCTGA
MVMm : ---A-C-----T-----C-----G-----
MPV-2 : -----T-----C-----T-----
MPV-3 : -----A-----C-----G-----

1201
MVMi : CACAAGAACCTGCAAGATTTTGTCTTTTCATGGCTGGAACATGTTAAAGTTTGCCATGCTATTGCTGTGTTCTAAACAGACAAGGAGGCAAAAGAAAT
MVMm : -----C-----
MPV-2 : -----CA-----
MPV-3 : -----C-----A-----

1301
MVMi : ACTGTTTTATTTTACGGACCAGCCAGTACAGGCAAATCTATTATTGCACAAGCCATAGCACAGGCAGTTGGTAATGTTGGTTGCTATAATGCAGCTAATG
MVMm : -----T-----C-----A-----
MPV-2 : -----T-----C-----A-----
MPV-3 : -----T-----C-----A-----

1401
MVMi : TGAACCTTCCATTTAATGACTGTACCAACAGAAGCTTATTGGGTAGAAGAAGCTGGTAACTTTGGACAGCAAGTAAACCAAGTTTAAAGCCATTTGCTC
MVMm : -----C-----A-C-----G-----
MPV-2 : -----C-----G-----
MPV-3 : -----C-----A-C-----A-----

1501
MVMi : TGGTCAAACCTATTGCGATTGATCAAAAAGGAAAAGGCAGCAAAACAAATTGAACCAACACCAGTCATCATGACCACAAATGAGAACATTACAGTGGTCAGA
MVMm : -----C-----G-G-----A-----
MPV-2 : -----A-----G-----A-----
MPV-3 : -----A-----G-----A-----

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1601
MVMi : ATAGGCTGCGAAGAGAGACCAGAACACACTCAACCAATTAGAGACAGAATGCTCAACATTCATCTAACACATACATTGCCTGGTGACTTTGGTTTGGTTG
MVMm : -----G-----G-----C-----G-----
MPV-2 : -----T--G-----C-----T-----
MPV-3 : -----T--A-----C-----T-----T-----

1701
TAR ELEMENT
MVMi : ACAAGAATGAATGGCCCATGATTTGTGCTTGGTTGGTAAGAATGGTTACCAATCTACCATGGCAAGCTACTGCGCTAAATGGGGCAAAGTTCCTGATTG
MVMm : -----T-----A-----
MPV-2 : -----A-G-----C-----T-----
MPV-3 : -----AC-----T-----

R2 LARGE SPLICE ACCEPTOR
1801
Sp1 P38 TATA → R3 (VP1 and VP2) mRNA START
MVMi : GTCAGAAAACCTGGGCGGAGCCAAAGGTGCCGACTCCTATAAATTCCTAGGTTTCGGCAGCTCACCATTACGACACCGAAAAGTACGCCTCTCAGCCAG
MVMm : -----A-----G-----A-----A-----
MPV-2 : -----A-----G-----T-----
MPV-3 : -----A-G-----G-----

1901
MVMi : AACTATGCAATAACTCCAATTGCATCGGATCTCGAGGACCTGGCTTTAGAGCCTTGGAGCACACCAAATACTCCTGTTGCGGGCACTGCAGAAACCCAGA
MVMm : -----C-----
MPV-2 : -----C-----
MPV-3 : -----C-----

2001
MVMi : ACACCTGGGAAGCTGGTTCCAAAGCCTGCCAAGATGGTCAACTGAGCCCACTTGGTCAGAGATCGAGGAGGATTGAGAGCGTGCTCGGTGCGGAACC
MVMm : -----
MPV-2 : -----
MPV-3 : -----

SMALL SPLICE DONOR 1 NS1 STOP ↓ VP1 START SMALL SPLICE DONOR 2
2101
MVMi : GTTGAAGAGAGACTTCAGCGAGCCGCTGAACCTTGACTAAGGTACGATGGCGCCTCCAGCTAAAAGAGCTAAAAGAGGTAAGGGTTTAAAGGGATGGTTGG
MVMm : -----A-----A-----
MPV-2 : -----A-----
MPV-3 : -----

SMALL SPLICE ACCEPTOR 1 SMALL SPLICE ACCEPTOR 2
2201
MVMi : TTGGTGGGGTATTAATGTTTAATTACCTGTTTACAGGCCTGAAATCACTTGGTTTGTAGTTGGGTGCCTCCTGGCTATAAGTACCTGGGACCAGGGAAC
MVMm : -----C--C-----C-----C-----C-----
MPV-2 : -----C-----C-----A-----A-----G-----
MPV-3 : -----C-----C-----A-----C-----

2301
MVMi : AGCCTTGACCAAGGAGAACCAACCAATCCATCTGACGCCGCTGCCAAAGAGCACGACGAGGCCCTATGATCAATACATCAAATCTGGAATAATCCTTACC
MVMm : -----A-----
MPV-2 : -----C--C-----
MPV-3 : -----A-----C--C-----

2401
MVMi : TGTACTTCTCTGCTGCTGATCAACGCTTTATTGACCAAAACCAAGGACGCCAAAGACTGGGGAGGCAAGGTTGGTCACTACTTTTTTAGAACCAAGCGCGC
MVMm : -----A-----T-----C-----
MPV-2 : -----A-----T-----C-----
MPV-3 : -----G-----G-----C-----C-----GT--A-----

2501
MVMi : TTTTGCACCTAAGCTTGCTACTG...ACTCTGAACCTGGAATTC...TGGTGTAAAGCAGAGCTGGTAAACGCCTAGACCACCTGCTTACATTTTT
MVMm : -----T-----G-----A-----
MPV-2 : -----G--A...G-----G-----G-----G-----T--C--A-----C-----
MPV-3 : -----A--TT-----C--GACACTG-----C-----G-----AACTGG--A--C--G-----A--GT--A-----A--AC--A--

2601
VP2 START
MVMi : ATAAACCAAGCCAGAGCTAAAAAAACTTACTTCTTCTGCTGCACAGCAAAGCAGTCAAACCATGAGTGATGGCACCAGCCAACCTGACGGCGGAAACG
MVMm : --T-----G-----CT-----A-----A--C--G--A-----G--GAG--T-----A-----A-----
MPV-2 : --T-----G-----CT-----A-----A--C--G--A-----G--GAG--T-----A-----A-----
MPV-3 : G-----G-----GCG-----CT-----T--A--G--A--C--G--A-----G--GAG--T-----A-----A-----

R V E R
2701
MVMi : CTGTCCACTCAGCTGCAAGAGTTGAACGAGCAGCTGACGGCCCTGGAGGCTCTGGGGGTGGGGGCTCTGGCGGGGTGGGGTTGGTGTCTTCTACTGGGTC
MVMm : -----G-----T-----G-----G-----T-----T-----C-----AG
MPV-2 : -----G-----T-----G-----G-----T-----T-----C-----AG
MPV-3 : -----G-----T-----G-----G-----T-----T-----C-----AG

2801
MVMi : TTATGATAATCAGACGCATTATAGATTCTTGGGTGACGGCTGGGTAGAAATTAAGTCACTAGCACTAGACTAGTACATTTAAACATGCCTAAATCAGAA
MVMm : -----A-----
MPV-2 : -----AG--T--A-----T-----TTACAGC--AC--CA-----T-----C-----
MPV-3 : -----G--T--AAC--T--T-----TTACAGC--AC--CA-----T-----C-----

2901
MVMi : AACTATTGCAGAATAAGAGTTCAACAACACAACAGACACTTCAGTCAAAGGCAACATGGCAAAGATGATGCTCATGAGCAAATTTGGACGCCATGGAGCT
MVMm : -----C--A-----AACA-----C-----
MPV-2 : -----G--C--C--G-----A--AT-----AGAC--AGC--GC--G-----TCT-----A-----A-----C-----
MPV-3 : -----G--GC--C--G-----AT-----ACA--ACAGC--A-----A-----A-----A-----

3001
MVMi : TAGTGGATGCTAATGCTTGGGGAGTTTGGCTCCAGCCAAGTGACTGGCAATACATTTGCAACACCATGAGCCAGCTTAAGTGGTCTCACTTGATCAAGA
MVMm : -----A-----
MPV-2 : -----A--T-----T-----T--T--A-----TTC-----AT-----T--CA--G--T--CAT-----C-----
MPV-3 : -----A--T-----T-----T--T--A-----G--TT-----T-----A-----T--TG--A--T--CA-----

3101
MVMi : AATATTTAATGTAGTGCTGAAAACTGTTACAGAGCAAGACTCAGGAGGTCAAGCTATAAAAATATACAACAATGACCTCACAGCTTGCATGATGGTTGCA
MVMm : -----A-----CC-----

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MPV-2 : GC-G-----CA-T----A-C--T--A--A-C---GCC-G-C--C--C--GG-----T--T---CA-----T
MPV-3 : GC-G-----G--CA-T----A----T--A--A-A-----C-G---C--C--GG-----T--T---CA-----T

3201
MVM1 : GTAGACTCAAAACAACATTCTGCCATACACACCTGCAGCAAACTCAATGAAACACTTGGTTTCTACCTTGGAAACCACTATAGCATCGCCATACAGGT
MVMm : -----C-----A-----
MPV-2 : C-G---T-----A---T-----A--A-G--AACCAA-----T--T-----CC-GC--AGT--T-----
MPV-3 : C-T--T--T-----A---T-----A--G--AACCAA-----T--T-----C--GC--AGT--T-----

3301
MVM1 : ACTATTTCGCGTTGACAGAGATCTTTTCAGTAACCTATGAAAATCAAGAAGGCACAATTGAGCATAATGTAATGGGAACACCAAAAGGAATGAATTCTCA
MVMm : -----G-----
MPV-2 : ---C--TAA-TG-----T-G---G--T---C-----AC--A-----...AC--CAG---CTG-TG-C-GT--G---GC----
MPV-3 : ---AA-TG-----TC-T-G---G--T--C-C-G-A--AC--A-AC---...GT-G-CAC---CT-ATG-C-GC-----GC----

3401
MVM1 : ATTTTTCACCATTGAGAACACACAACAAATCACATTGCTCAGAACTGGTGATGAGTTTGCTACTGGAACCTACTACTTTGACACAAACCCAGTTAACTT
MVMm : -----G-----A-----
MPV-2 : ---A-----T--G-GC--TCAGC---T-----A-----T-----A--G-A--A-C-----
MPV-3 : -----T---GC--TCAGC---T-----A-----T-----T-----G-A--A-C-----

3501
MVM1 : ACACACACATGGCAAACTAACCGTCAACTTGGACAGCCTCCACTGCTGTCAACCTTTCCTGAAGCTGACACTGATGCAGGTACACTTACTGCTCAAGGGA
MVMm : -----GA-----
MPV-2 : T-T-----T-A--A-G--G--G--T-----AAA-CA-TGAGC-A--AACTT-----AC--CA--C--TT-AGTA--AG--TG
MPV-3 : T-T-----T-A--A-G--G--G--T-----AAA-TA-TGA--A--AAGT-----AC--AAAT-C--TT-AGTA--AG--TG

3601
MVM1 : GCAGACATGGAGCAACACAGAT...GGAGGTTAACTGGGTGAGTGAAGCAATTAGAACACGACCTGCTCAAGTAGGATTTTGTGAGCCACACAATGACTT
MVMm : -----A-----A---...GA-----C-----A-----
MPV-2 : ---TCA--TTTG--C--A--TTAT-GCAGC--TGCT--C-----T-C---GTG--G---A-----T-----A--T--TG-CA-T--
MPV-3 : ---TCA--T-TG--C---TTTT-GCAGC--TGAT--A-C-----T-C---GTA--G--G--A-----T-----A--T--TG-CA-T--

3701
MVM1 : TGAAGCCAGCAGAGCTGGACCATTTGCTGCTCCAAAAGTTCCAGCAGATGTTACTCAAGGAGTGGACAGAGAAGCCAATGGCAGTGTAGATACAGTTAT
MVMm : -----G-----T-----A--A--G-----C-T-A-----T-----CA-----
MPV-2 : ---A--G--C-C--A--C--T--AAG-T---GT--G---A-CA--C---CCAA--CAT-C---C---CC-A-----CA---
MPV-3 : ---A-TG-----A--T--T--AAG-T---GT--G---A-CA-C-A---CCAA--CAT-C---C---CC-A-----CA---

3801
MVM1 : GGCAACAGCATGGTGAAAATTTGGGCTGCACACGACCAGCACCAGAGCGCTACACATGGGATGAAACAACTTTGGTTTCAGGAAGAGACACAGAGATG
MVMm : -----A-----T-----T-----G-----T-----G-----G-----G-T--T-----T-----
MPV-2 : -A-----A-----C---C-AT--AAGCA-T...ACA-CAG--CA-A-TT-T-----GT-TT-G--A--A-----C---TGG--TGACAGAT
MPV-3 : -A-----C---C---C-AT--AAGCA-T...ACACAG--CA-A-----T-----GT-TT-G--A--A-----C---TGG--TGACAGAT

3901
MVM1 : GTTTTATTCAATCAGCACCTCTAGTTGTTCCACCACCACTAAATGGGATTCTTACAAATGCAAAACCTATTGGAACATAAAATGACATTATTTTCAAA
MVMm : -----A-----A-----G-----
MPV-2 : -C-----G--A---ATATACA-AAA---G-T--T--CAAC--A--A---CT-TG-T-C---GAC---C---T--A---A-CAG--
MPV-3 : -C-----GG--A---AT-TACATCA-----G-T--T--CAAC--A--A---CT-G--C-----GC---C--G---A---A-CA-C-

4001
MVM1 : TGTTTTAAACAGCTATGGTCCACTAAGTGCATTTTTCACACCAAGTCTGTATACCCCTCAAGGACAAATATGGGACAAAGAAGTATAGTCTTGAACACAAA
MVMm : ---A-----A-----T-----G-----A-----T-----G-----A-----
MPV-2 : --A-----A-----A-----G--T--C---T--TGCG--GA-T-T-A---G---C-----T-----
MPV-3 : AACAA-----A-----G--T--C---T--TGCG--GA-T-T-A---G---T-----T-----

4101
MVM1 : CCTAGACTTCACATAACTGCTCCATTTGCTGTGTAATAACAATGCACCTGGACAAATGTTGGTTAGATTAGGACCAATCTAACTGACCAGTATGATCCAA
MVMm : ---T-----T-----A-----C-----A-----
MPV-2 : --A-----A---G--CAA---T---T--C--G-----T--AA-T--C-TC-T---GC-T-C---C--T-----C-
MPV-3 : --A-----G--CAA---T---T--C--G-----T--A--T--C-TC-A-----C-T-C---C--T-----C-

4201
MVM1 : ACGGAGCCACACTTTCTAGAAATTGTGACTTATGGTACATTTTCTGGAAGGAAAACTAACCATGAGAGCAAACTTAGAGCTAACACCACCTTGAACCC
MVMm : -T-----T-----A-----T-----A-----
MPV-2 : --A-TAG---T-----C-----T-----G---C-A-A-----C--TG-T-----
MPV-3 : --A-TAG---T-----A-----C-----C-A-A-----C--TG-T-----

4301
MVM1 : AGTGATACCAAGTAAGTGTGAAGACAA...TGGCAACTCATACATGAGTGTACTAAATGGCTACCAACTGCTACTGGAACATGCAATCTGTACCGCTT
MVMm : -----C-----A-----G-----G-----
MPV-2 : --T-----C-C--T--C-ATC-AA---TGAC-----CA--CAC-----G---G--C---C-----GAA--G--TT-G
MPV-3 : --T-----C-C--T--C-ATC-AA---TGAC-----CA-CCAC-----G-----C-----GAA-----TT-G

4401
VP1 and VP2 STOP
MVM1 : ATAACAAGACCTGTTCTAGAAATACCTTACTAACTAACCATGTTTTCTCTGTACTTCATATATTATTAAGACTAATAAAGATACACATAAAAAATA
MVMm : -----C---T-----G-----
MPV-2 : C-TT-T-----C-----C--T-----G-----
MPV-3 : C-TT-T-----C-----C--T-----A-----G-----

4501
MVM1 : TAATATTACATATAGATTTAAGAAATAGAATAATATGGTACTTAGTAACCTGTAGAAATAATAGAACCTTTGAAATAACAAGATAATAGTTGGT...<----
MVMm : -----G-----G-----
MPV-2 : -----C-----T---G-----G---AA-TAATG
MPV-3 : -----CGC-----T---G-----G---AC-----

4601-----DIRECT REPEAT 1----->-----DIRECT REPEAT 2-----
MVM1 : .....TAATGTAGATAGAATAAAGAGATTATGTATAATGGGTAAAGGGTGAAGG
MVMm : .....A-----A-----
MPV-2 : TTAGATAGAATAAAGAGATGATGTACATGGATAAATAGTTAGTTGGT-----AG-----A-----A-----
MPV-3 : -----AA-C-----AG--C-----

4701----->-----POLY A
MVM1 : GTGGTTGG.TTGGTATTCCTTAGACATGATGTTAAGGACCAAAAAATAATAAAA
MVMm : ---A-----T-----C-----
MPV-2 : -----T-A-----C-----
MPV-3 : -----G-----T-----C-----

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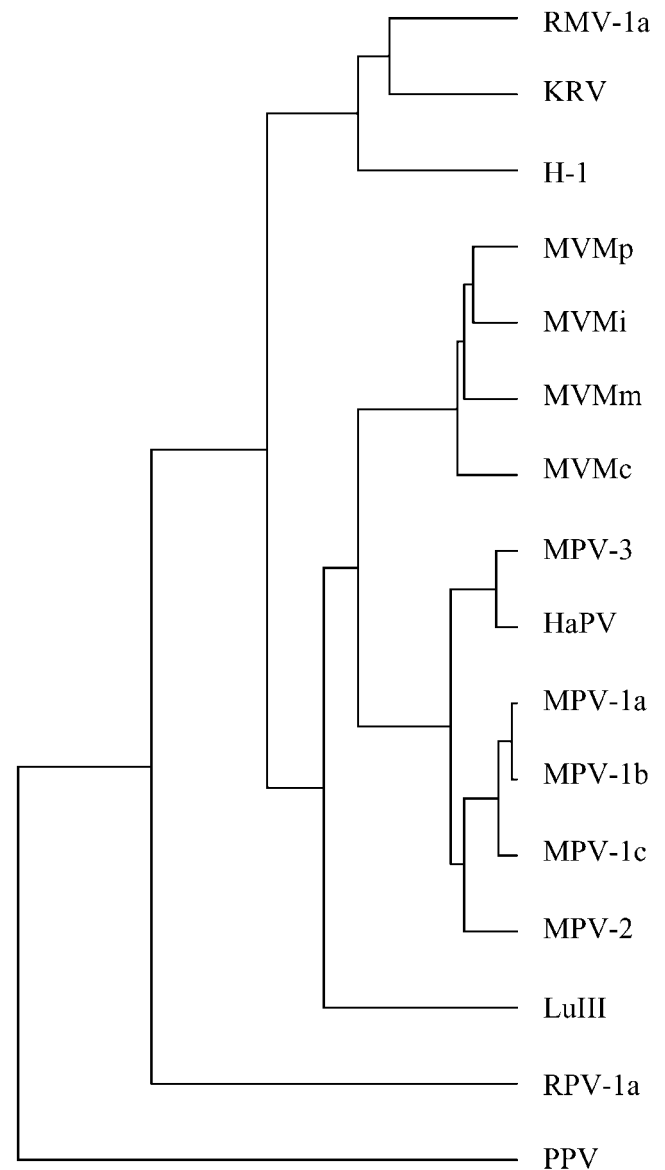


**Table 2.** Percentage nucleotide identity among rodent parvoviruses

Determined by the GCG GAP program. Percentage identities were based on sequence alignments that excluded the terminal hairpin region.

Strain	MVMm	MPV-2	MPV-3
MVMp	95.5	87.5	86.8
MVMi	96.0	88.0	87.5
MVMc	96.1	87.8	87.4
MVMm	100	87.2	87.7
MPV-1a	87.5	95.3	94.8
MPV-2	87.2	100	94.7
MPV-3	87.7	94.7	100
HaPV	88.4	94.5	98.1
LuIII	84.1	88.3	88.2
H-1	80.5	79.8	79.9
KRV	81.0	80.3	80.3
RMV-1a	81.6	80.9	81.2
RPV-1a	71.0	71.6	71.6

with MVM-infected mice. Of significant interest was the strain prevalence observed within the samples evaluated. MVMm was the most prevalent strain of MVM detected, at 91 % of samples evaluated. This is not surprising, as MVMm was isolated in 2002 and is the only characterized MVM strain isolated directly from naturally infected mice. It is significant, as MVMm is the only MVM strain documented to induce clinical disease in naturally infected mice (Naugler *et al.*, 2001), as previous reports of MVMi-associated clinical disease and pathology have resulted from experimentally induced infections. MVMc, the second most recently isolated strain, was the only other MVM strain detected in the survey. The significance of MVMc infections in mice is unknown, as studies to assess its pathogenesis have not yet been performed. The failure to detect MVMp and MVMi, the two best-characterized murine parvovirus strains, in the survey was somewhat surprising. These strains could be circulating at extremely low levels that were not detectable among the samples evaluated in our survey. Alternatively, as MVMp and MVMi were isolated more than three decades ago as cell-culture contaminants, genetic mutations that occurred during adaptation to cell culture, or possibly genetic drift of the origin strains that have been circulating in laboratory mouse colonies since that time, could explain the failure to detect them in naturally infected mice in the present survey. MPV-1 was detected in the majority of the MPV/HaPV-positive samples. Given the high sequence similarity of MPV-1a, MPV-1b and MPV-1c, the RFLP approach used was unable to discriminate among these

**Fig. 3.** Phylogeny using genomic nucleotide sequences for rodent parvoviruses and LuIII. Porcine parvovirus (PPV) was used as the outgroup. Generated with the GCG PILEUP program.

strains, so they were grouped as MPV-1. A novel murine parvovirus was detected in most of the remaining MPV-positive samples and has tentatively been named mouse parvovirus 2 (MPV-2). Little else is known about this virus, as it has not yet been propagated successfully in mice or in cell culture, although there is evidence that sera obtained from MPV-2-infected mice may react more consistently

**Fig. 2.** DNA sequence alignment of the newly identified murine parvovirus strains with MVMi nt 141–4827. Overlined nucleotide sequences indicate genomic regions previously determined to be important in transcription, mRNA processing and translation for the prototypic rodent parvoviruses. Sequence alignments were generated with the GCG CLUSTAL\_W and PRETTY programs. Dashes represent identical sequence.

**Table 3.** Percentage amino acid similarity and identity among rodent parvovirus isolates for NS1, the unique N terminus of VP1 and VP2

Determined using the GCG Best Fit program. Results are shown as percentage similarity, with percentage identity shown in parentheses.

Strain	NS1			N terminus of VP1			VP2		
	MVMm	MPV-2	MPV-3	MVMm	MPV-2	MPV-3	MVMm	MPV-2	MPV-3
MVMp	97.5 (96.7)	98.4 (97.6)	97.5 (96.3)	99.3 (97.9)	95.1 (93.0)	92.3 (89.4)	97.4 (95.7)	79.4 (74.4)	79.0 (74.4)
MVMi	98.2 (97.2)	98.5 (98.1)	98.5 (97.6)	99.3 (97.9)	95.1 (93.0)	92.3 (89.4)	97.1 (95.9)	78.7 (73.6)	78.3 (73.7)
MVMc	99.0 (98.1)	97.9 (97.0)	97.9 (97.2)	97.9 (95.8)	94.4 (93.0)	92.3 (88.7)	97.6 (96.1)	79.1 (74.5)	78.3 (73.9)
MVMm	100 (100)	97.5 (96.3)	98.4 (97.3)	100 (100)	94.4 (91.5)	92.3 (88.0)	100 (100)	78.7 (73.3)	78.3 (73.5)
MPV-1a	98.1 (97.0)	98.7 (98.5)	98.7 (98.1)	93.0 (90.1)	98.6 (98.6)	93.0 (90.8)	78.2 (73.7)	92.1 (89.2)	95.1 (92.9)
MPV-2	97.5 (96.3)	100 (100)	97.9 (97.5)	94.4 (91.5)	100 (100)	93.0 (90.8)	78.7 (73.3)	100 (100)	94.6 (93.0)
MPV-3	98.4 (97.3)	97.9 (97.5)	100 (100)	92.3 (88.0)	93.0 (90.8)	100 (100)	78.3 (73.5)	94.6 (93.0)	100 (100)
HaPV	99.4 (98.8)	97.6 (97.0)	98.7 (98.2)	92.3 (88.0)	93.0 (90.8)	100 (100)	78.0 (73.0)	94.1 (92.3)	98.8 (98.5)
LuIII	95.2 (94.0)	94.6 (93.4)	94.5 (93.6)	95.1 (93.0)	95.8 (95.1)	93.0 (91.5)	78.5 (73.0)	87.9 (83.9)	88.4 (84.9)
H-1	93.3 (90.9)	93.0 (90.9)	93.2 (91.1)	94.4 (90.8)	94.4 (93.0)	93.7 (93.0)	72.7 (67.2)	72.2 (67.6)	71.1 (66.6)
KRV	93.8 (91.2)	93.5 (91.2)	93.6 (91.4)	94.3 (90.0)	94.3 (92.1)	93.6 (92.1)	75.7 (72.2)	74.2 (69.5)	73.6 (69.8)
RMV-1a	93.6 (91.1)	93.2 (90.9)	93.3 (90.9)	94.4 (90.8)	94.4 (91.5)	93.7 (91.5)	76.6 (71.8)	76.2 (71.3)	76.7 (72.1)
RPV-1a	85.0 (81.5)	85.1 (81.4)	84.8 (81.3)	84.4 (78.0)	84.5 (80.3)	84.6 (81.1)	68.5 (63.2)	69.3 (64.5)	67.9 (63.7)

with baculovirus-expressed MPV-2 VP2 antigen compared with baculovirus-expressed MPV-1 VP2 antigen (Dhawan *et al.*, 2004). This is of practical importance for the serological diagnosis of MPV-2 infections in mice, although significant cross-reactivity with heterologous capsid antigens (e.g. from MPV-1, MPV-2, MPV-3) appears to occur among antisera generated against MPV-1, MPV-2 and MPV-3 (D. G. Besselsen & R. S. Livingston, unpublished data).

Interestingly, a murine parvovirus strain that is almost genetically identical to HaPV was detected in DNA samples obtained from four different mice from a single mouse colony. There were several reasons to include HaPV with the murine parvoviruses in this survey. Of all of the rodent parvoviruses, HaPV is genetically most similar to MPV-1

and there is some cross-reactivity of the MPV-1 and HaPV haemagglutinins serologically by haemagglutination inhibition (Besselsen *et al.*, 1996), one of the traditional methods used to classify viral species. In addition, the rodent parvoviruses MVM, H-1 and KRV generally induce subclinical infections in their natural rodent hosts, but induce incisor loss, domed craniums, haemorrhagic disease and cerebellar and testicular hypoplasia in experimentally infected hamsters (Brownstein *et al.*, 1991; Garant *et al.*, 1980; Kilham, 1960, 1961; Kilham & Margolis, 1964, 1970; Toolan, 1960). Likewise, MPV induces subclinical infections in mice (Jacoby *et al.*, 1995, 1996; Shek *et al.*, 1998; Smith *et al.*, 1993), whilst the closely related HaPV causes lesions similar to those induced by the prototypic rodent parvoviruses in hamsters (Besselsen *et al.*, 1999). Together, these findings suggest that the mouse may be the natural rodent host for HaPV, with the hamster an aberrant host for this virus. The detection of a parvovirus strain almost identical to HaPV in naturally infected laboratory mice provides support for this hypothesis and further suggests that a more appropriate name for this virus would be mouse parvovirus 3 (MPV-3), as it is genetically distinct from MPV-1 and MPV-2. Unfortunately, the colony of mice infected with MPV-3 had been depopulated by the time we identified this viral strain and only DNA samples from these mice were available, so attempts to propagate the field strain of this virus could not be pursued. However, the original HaPV isolate can be cultivated *in vitro* and can be used for further investigation of this virus strain in the mouse host. In addition, since the completion of this survey, we have identified MPV-3-positive samples in mouse tissues obtained from another animal facility (data not shown).

Nucleic acid and protein sequence similarities provided insights into the relatedness of the various rodent parvoviruses. MVMm and MVMc, the two most recent MVM

```

MVMp   : LGASWLQVPGTREQP@
MVMi   : -----@
MVMc   : --T-----@
MVMm   : -----@
MPV-1a : --T-R-----K--@
MPV-1b : --T-R-----K--@
MPV-1c : --T-R-----@
MPV-2   : --T-R-----A----@
MPV-3   : --T-----@
HaPV    : --T-----@
LuIII   : ----R-----@

```

**Fig. 4.** Amino acid sequence alignment of the C terminus of the rare isoform of the NS-2 protein for MVMp, MVMi, MVMc, MVMm, MPV-1a, MPV-1b, MPV-1c, MPV-2, MPV-3, HaPV and LuIII. The alignment was generated with the GCG PILEUP and PRETTY programs. @, Termination codon.

```

1      NLS1      PLA2-Ca      PLA2      PLA2      NLS2
MPV-1a : MAPPAKRAKRGWVPPGYKYLPGNSLDQGEPTNPDSAAAKEHDEAYDKYIKSGKNPYLYFSAADQRFIDQTKDAKDGGKGVGHYFFRTKRAFAPRLASSS
MPV-2 : -----Q-----
MPV-3 : -----Q-----TSK-PDTD
HaPV : -----Q-----TSK-PDTD
MVMp : -----Q-----K-TD-
MVMi : -----Q-----K-TD-
MVMc : -----Q-----K-TD-
MVMm : -----EQ-----K-STD-

101      NLS3      NLS4      → VP2 START      VP2/VP3 CLEAVAGE
MPV-1a : EP...GTSGVSIAGKRTKPPAHIFINQARAKKKRASLAAQRTQTMSDGAEQPDGSAVQSAARVERAADPGSGGGGSGGGVSTGSDYNQTHYRF
MPV-2 : ---...-----T-----S---N-----K-
MPV-3 : SQPGTS-G--TR---S-----V-----S---N-----
HaPV : SQPGTS-G--TR---S-----V-----S-C-N-----
MVMp : ---...-R---R---Y-----LT-S---SS---TS---N-H-----
MVMi : ---...-R---R---Y-----LT-S---SS---TS---G-N-H-----
MVMc : ---...-R---IR---Y-----LT-S---NS---TS---G-N-HA-----K-
MVMm : ---...-K---R---Y-----LT-S---SS---TS---GEN-H-----

201 <-----
MPV-1a : LSDGWVEITAYSTRMVLNMPKSENYCRVRVHNTNDTRTAGNMAKDDAHEQIWTWPSLIDSNAGVWVFQPSDWQFICNNMSHVNLHSLDQELFNVIKTV
MPV-2 : -G-----I-----K---DV-Q-L-----L-----M-----
MPV-3 : -T-----I-----Q-----
HaPV : -A---Q-----I-----Q-----
MVMp : -G-----LA-L-----I---T---SVK-----V-A-----L-----T-QL-V---I---L---
MVMi : -G-----LA-L-----I---T---SVK-----V-A-----L-----T-QL-V---I---L---
MVMc : -G-----LA-L-----I---T---AVQ-----V-A-----L-----T-QL-V---I---L---
MVMm : -G-----LA-L-----IK---T---NIK-----V-A-----L-----Y---T-QL-V---I---L---

301--5-FOLD CYL--->
MPV-1a : TEQNTGAEAVKIYNNDLTASMMVALDSNNILPYTPATDNQETLGFYFPWKPTMPSPYRYFYFNCDSRLSVTYTDQIGSIDV.TMANASGLSSQFFTIENTQR
MPV-2 : ---P-PD-I-V-----L-----D-----AN---T-TN.R-A---M-----
MPV-3 : ---I-V-----AE---N---M-----
HaPV : ---I-V-----A---N---A---M-----
MVMp : ---DL-GQ-I-----C---V-----ANSM-----IA-----CV-D-----EN-E-T-EHNV-GTPK-MN-----Q
MVMi : ---DS-GQ-I-----C---V-----ANSM-----IA-----CV-D-----EN-E-T-EHNV-GTPK-MN-----Q
MVMc : ---DS-GQ-I-----C---V-----ANSM-----IA-----CV-D-----EN-E-T-EHNV-GTPK-MN-----Q
MVMm : ---DS-GQ-L-----C---V-----ANSM-----IA-----CV-D-----EN-E-A-EHNV-GTPK-MN---I-----Q

401 MVM ALLOTROPIC DETERMINANTS
MPV-1a : QLLRTGDEFATGTYTFETEPIKLSHTWQSNRQLGQPPQITDLPADNENATLVTRGDRSGITQISGSNDVTEATRVRPAQVGFQCPHDNFETSRAQPFK
MPV-2 : -----D-----S-DT---A-G---L---Y---A-----G-----
MPV-3 : -----D-----S-----A-G---V---F-----G-----
HaPV : -----D-----S-----A-G---V---F-----G-----
MVMp : -T-----D-NSV--T---T-----LLSTF-E--TDAG--TAQ-S-H-T--MG.V-W-S--I-T-----ND-A-----A
MVMi : -T-----D-N-V--T---T-----LLSTF-E--TDAG--TAQ-S-H-A--ME.V-W-S--I-T-----ND-A-----A
MVMc : -T-----D-N-V--T---T-----LLSTF-DS-TDPG--IAQ-S-H-T--MG.V-W-S--I-T-----ND-A-----A
MVMm : -T-----D-N--T---T-----LLSTF-ET-TDAG--TAQ-S-H-T--MG.V-W-S--I-T-----ND-A-----A

501 * * * * *
MPV-1a : FVVVPANVTQGNHEHDANGSLRYTYDKQHGDWGSN.NSKERFTWDIAISYDSGRWADRCFINATPFTSPPALNNILTNSDPIGNKTAIHYQNVFNSYGLPT
MPV-2 : -----I---QD-----NYA--DTRD-----G-----T-----QS--Y-ET-----D-D-----S
MPV-3 : -----I---QD-----NYA--DNRD-----G-----T-----Q-----S-G---QT-----S
HaPV : -----I---QD-----NYA--DNRD-----G-----T-----Q-----K-----S-G---QT-----S
MVMp : A-K---DI---VDKE---V--S-G---EN-A-HGPAP--Y---ET-FG---DTKDG--QSA-LVV--P-G---AN---T-ND--FS-----
MVMi : A-K---D---VDRE---V--S-G---EN-AAHGAP--Y---ETNFG--DTRDG--QSA-LVV--P-G---AN---T-ND--FS-----
MVMc : -K---TE---PLDRE---V--S-G---EN-AAHGAP--Y---ET-FGA--DTRDG--QSA-LVV--P-G---AN---T-ND--FA-----
MVMm : -K---TE---ALDRE---I--S-G---EN-A-HGPAP--Y---ET-FGA--DTSDG--QSA-LVV--P-G---AN---T-ND--FS-----

601 NLM *
MPV-1a : AFPHPAPIYPQGIQWDELDLEHKPLRLHAQAPFVCKNNAPGQLLVRLAPNLTDQYDPSNSTLSRIVITYGTTFFWKGLTLKAKLRPNATWNPVYQVSAQYQ
MPV-2 : -----V-----S-----
MPV-3 : -----V-----
HaPV : T-----V-----
MVMp : --S--S-V-----IT-----M---G-----GA-----MR---A-T-----ED.
MVMi : --S--S-V-----IT-----M---G-----GA-----MR---A-T-----VED.
MVMc : --S--S-----VT-----M---G-----GA-----MR---A-T-----ED.
MVMm : --S--S-----IT-----M---G-----GA-----MR---T-T-----ED.

701
MPV-1a : NENEYMSIHKWLPATGTMQSIPLLSRPVARNTY@
MPV-2 : SN-D-----S-----NV-----
MPV-3 : SN-D-----NV-----
HaPV : SDQD-----NV-----
MVMp : -G-S---VT-----V--IT-----
MVMi : -G-S---VT-----V--IT-----
MVMc : -G-S---VT-----V--IT-----
MVMm : -G-S---VT-----V--IT-----

```

**Fig. 5.** Amino acid sequence alignment of the VP1 proteins for MVMp, MVMi, MVMc, MVMm, MPV-1a, MPV-2, MPV-3 and HaPV. The alignment was generated with the GCG PILEUP and PRETTY programs. Underlined amino acids indicate MVMi VP2 loci 364 and 366–368 of the haemagglutination loop; bold amino acids indicate MVM allotropic determinants at VP2 loci 317 and 321. NLS, Nuclear localization sequence; NLM, nuclear localization motif; PLA2, phospholipase  $A_2$  catalytic amino acid residues; PLA2-Ca, phospholipase  $A_2$  calcium-binding amino acid residues; fivefold CYL, VP2 anti-parallel  $\beta$ -strands comprising a cylinder at the fivefold symmetry axis with a conserved leucine at position 172; \*, second-site revertants for MVM allotropism; @, termination codon.

isolates, were most closely related genetically and formed a tightly associated clade with MVMp and MVMi. The MPV-1 strains, MPV-2, MPV-3 and HaPV also formed a tightly associated clade of viruses. These MVM and MPV-like clades clustered together with LuIII, whilst the more distantly related rat-origin parvovirus clades comprising KRV, H-1, RMV-1 and RPV-1 strains also clustered together with LuIII. This lends support to the hypothesis that the mouse is the natural rodent host for HaPV and possibly LuIII, as rodent parvoviruses would be expected to co-evolve with their natural rodent host over time, as has been shown previously for rodent hantaviruses (Plyusnin & Morzunov, 2001) and for Canine parvovirus (Hueffer & Parrish, 2003; Shackelton *et al.*, 2005). However, we did not detect LuIII DNA in our epidemiological survey, despite the fact that the MPV/HaPV fnPCR and the MPV PCR/RFLP assays used in the survey can detect LuIII (data not shown), leaving the host origin of LuIII in doubt.

Examination of genomic DNA sequence alignments of the newly identified murine parvovirus strains and other rodent parvoviruses indicated that all of these viruses share a common genetic organization with conservation of promoter regions, splice junctions and translation start and stop codons. Examination of the NS1 and NS2 amino acid alignments showed a high level of conservation, as would be expected for proteins that are functionally critical for virus replication and transcriptional regulation. Several regions of diversity among the newly isolated rodent parvoviruses, the prototypic rodent parvoviruses and LuIII were readily apparent upon examination of the VP1 amino acid sequence alignment. Many of these regions of diversity corresponded to regions that are located at or near the surface of the capsid, as determined by extrapolation from the three-dimensional structure of MVMi (Agbandje-McKenna *et al.*, 1998; Llamas-Saiz *et al.*, 1997). These differences in the capsid region could alter tissue tropism and haemagglutination, and ultimately *in vivo* pathogenesis, displayed by the newly identified murine parvovirus strains, as has been shown for MVMi and MVMp (Agbandje-McKenna *et al.*, 1998; Ball-Goodrich & Tattersall, 1992; Brownstein *et al.*, 1991, 1992; Kimsey *et al.*, 1986; Maxwell *et al.*, 1995) and for the *Feline parvovirus* subgroup (Chang *et al.*, 1992; Govindasamy *et al.*, 2003; Hueffer & Parrish, 2003; Parker & Parrish, 1997; Parrish, 1991; Tresnan *et al.*, 1995; Tsao *et al.*, 1991).

In conclusion, several newly identified murine parvovirus strains were detected in naturally infected mice. Nucleotide and amino acid sequence comparisons indicated that these viral strains retain the genomic organization characteristic of other rodent parvoviruses and demonstrated that the murine parvoviruses appear to form two distinct groups, the MVM group and the MPV-like group. Amino acid differences observed in the predicted capsid proteins of the newly identified murine parvovirus strains (compared with the well-characterized strains) appear to cluster at or near the capsid surface, which suggests that they may yield

phenotypes distinct from those displayed by the well-characterized murine parvovirus strains. Additional studies are therefore needed to evaluate the specific phenotypes displayed by these viral strains. In particular, studies to evaluate the *in vivo* pathogenesis of these viral strains are needed to develop methods appropriate for the detection and control of these infections in laboratory mouse colonies and to improve our understanding of the impact that infections by these viral strains may have on mouse models used in biomedical research.

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