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Detection of Rodent Parvoviruses by Use of Fluorogenic Nuclease Polymerase Chain Reaction Assays

Amanda J. Redig and David G. Besselsen, DVM, PhD*

Polymerase chain reaction (PCR) assays have proven useful for detection of rodent parvoviruses in animals and contaminated biological materials. Fluorogenic nuclease PCR assays combine PCR with an internal fluorogenic hybridization probe, eliminating post-PCR processing and potentially enhancing specificity. Consequently, three fluorogenic nuclease PCR assays were developed, one that detects all rodent parvoviruses, one that specifically detects minute virus of mice (MVM), and one that specifically detects mouse parvovirus 1 (MPV) and hamster parvovirus (HaPV). When rodent parvoviruses and other rodent DNA viruses were evaluated, the rodent parvovirus assay detected only rodent parvovirus isolates, whereas the MVM and MPV/HaPV assays detected only the MVM or MPV/HaPV isolates, respectively. Each assay detected the equivalent of 10 or fewer copies of target template, and all fluorogenic nuclease PCR assays exceeded the sensitivities associated with previously reported PCR assays and mouse antibody production testing. In addition, each fluorogenic nuclease PCR assay detected the targeted parvovirus DNA in tissues obtained from mice experimentally infected with MVM or MPV. Results of these studies indicate that fluorogenic nuclease PCR assays provide a potentially high-throughput, PCR-based method to detect rodent parvoviruses in infected mice and contaminated biological materials.

Minute virus of mice (MVM) and mouse parvovirus 1 (MPV) are among the most prevalent infective agents found in contemporary laboratory mouse colonies (1). Although the immunosuppressive strain of MVM (MVMi) can induce potentially lethal renal hemorrhagic disease when experimentally inoculated into neonatal mice (2), clinical disease and histologic lesions have not been observed in mice naturally infected with MVM. Similarly, clinical disease and histologic lesions have not been observed in mice naturally or experimentally infected with MPV (3, 4). Despite the absence of clinical disease and histopathologic changes, murine parvoviruses can have appreciable deleterious effects on research due to their immunomodulatory effects in vivo and in vitro (5-10). In addition, MVM is a common contaminant of cell cultures, tissues, and other specimens of mouse origin (5, 11-13), and MPV has the potential to be a contaminant of biological materials, as indicated by its initial isolation from mouse splenocyte cultures (7). Finally, there is substantial potential for MVM and MPV to be transmitted among animal facilities and stocks of biological materials due to high degree of environmental stability (14). Therefore, identification of infected laboratory mice and contaminated biological materials is critical to minimize the impact of murine parvoviruses on research.

Several methods are currently used to detect MPV infections in mice and contaminated biological materials. Serologic evaluation for presence of anti-parvovirus antibodies has typically been used to diagnose MVM and MPV infections in mice (15-18). However, serologic assays cannot be used to detect parvovirus infections directly in immunodeficient strains of mice that do not generate a humoral immune response. In addition, false-positive and false-negative serologic test results can be problematic. For example, serologic assays that use MVM nonstructural protein antigens to detect MPV, currently the most common approach, often fail when mice are infected with MPV after 12 weeks of age (19). High-throughput MPV serodiagnosis by use of assays that contain cell culture-propagated MPV as antigen is limited as high-titered stocks of MPV are difficult to obtain and not readily available, and therefore these assays are generally used only for confirmatory testing. As a result, polymerase chain reaction (PCR) assays that specifically detect rodent parvovirus DNA have been developed and have proven to be a useful adjunctive diagnostic method to detect or confirm the presence of MPV in mice (20, 21). These PCR assays also are an attractive alternative to the mouse antibody production (MAP) test for detection of MPV contamination in biological materials. Compared with MAP testing, PCR assays confer substantial advantages of greatly reduced turnaround time and cost while also providing an alternative to whole animal testing (22). Despite these advantages, PCR assays are still labor intensive and costly, the requirement of post-PCR processing by gel electrophoresis limits its application as a high-throughput diagnostic assay, and the potential for false-positive results secondary to carry-over contamination is substantial.

Fluorogenic nuclease PCR assays (23), a recently developed technique also known as real-time PCR or the *Taq*Man PCR assay, confers several advantages over the gel detection PCR assay. Similar to PCR assays, the fluorogenic nuclease PCR assay amplifies DNA between two specific oligonucleotide primers by thermocycling in the presence of *Taq* polymerase. However, included in the fluorogenic nuclease PCR reaction mix is an internal fluorogenic hybridization probe with covalently-linked fluorogenic and quencher dyes in close proximity. The *Taq* polymerase nucleolytically cleaves the probe during each round of

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University of Arizona, University Animal Care Building 101, Central Animal Facility, Room 116, 1127 East Lowell Street, Tucson, Arizona 85721-0101.
**Corresponding author.*

amplification, thereby releasing the fluorogenic dye from the quencher. The increase in fluorescence is measured optically at the end of each thermocycle, this data is transmitted to an attached computer in "real" time, and subsequent computer analysis results in a quantitative, closed-tube detection system for specific PCR products. The fluorogenic nuclease PCR assay, therefore, eliminates post-PCR processing and carry-over contamination and potentially imparts improved specificity via the internal probe. As a result, the goal of the study reported here was to develop three fluorogenic nuclease PCR assays that detect all known rodent parvoviruses or, specifically, detect MVM or MPV/HaPV.

Materials and Methods

Viruses. Minute virus of mice prototype strain (MVMp), minute virus of mice immunosuppressive strain (MVMi), minute virus of mice Cutter strain (MVMc), mouse parvovirus 1b (MPV-1b), hamster parvovirus (HaPV), Toolan's parvovirus (H-1), Kilham rat virus (KRV), mouse cytomegalovirus (MCMV), mouse adenovirus 1 (MAD-1), mouse adenovirus 2 (MAD-2), polyoma virus, and vaccinia virus were obtained from intramural stocks. Each parvovirus was propagated in a permissive cell line as described (24), MCMV was propagated in 3T3 cells (ATCC CCL-92), MAD-1 and MAD-2 were propagated in CMT-93 cells (ATCC CCL-223), polyoma was propagated in 3T6 cells (ATCC CCL-96), and vaccinia was propagated in Vero cells (ATCC CCL-81). Cell lysates (or concentrates for MPV-1b) were prepared, and the 50% tissue culture infective dose ($TCID_{50}$) for each was determined as described (24). All viral stocks were stored at –70°C until use.

Fluorogenic nuclease PCR assays. Genomic nucleotide sequences for MVMp, MVMi, MVMc, MPV-1a, MPV-1b, MPV-1c, HaPV, H-1, KRV, RPV-1a, RV-Umass, and LuIII were obtained from GenBank. Rat minute virus (RMV) sequences were kindly provided by Dr. Cho-hua Wan (University of Missouri, Columbia, Mo.). Sequences were aligned by use of the ClustalW and Pretty software programs (Genetics Computer Group, Madison, Wis.), and the primers and probe for each fluorogenic nuclease assay were identified, using the Primer Express software (PE Applied Biosystems, Foster City, Calif.). All fluorogenic nuclease PCR reactions were performed by use of a PE Applied Biosystems GeneAmp 5700 Sequence Detection System, and products were analyzed by use of the accompanying software. Each 25-µl reaction consisted of 1X *Taq*Man buffer (50 m*M* KCl, 10 µ*M* EDTA, 10 µ*M* Tris-HCl [pH 8.3], and 60 n*M* Passive Reference), 5.5 m*M* $MgCl_2$, 300 n*M* dATP, dCTP, dUTP, and dGTP, 0.05% gelatin, 0.01% Tween-20, 0.625 U of Ampli*Taq* Gold, 0.25 U of uracil-N-glycosylase (UNG), 300 n*M* both forward and reverse primer, an optimized concentration of probe, and 2.5 µl of template DNA.

Thermal cycling conditions consisted of 50°C for 2 min for UNG incubation, polymerase activation at 95°C for 10 min, then 40 cycles of 95°C for 15 sec., followed by 60°C for 1 min. Samples were considered to have positive results if they had mean fluorescence (Rn) > 0.1 and a cycle threshold (Ct) < 40.

Amplicon cloning. Amplicons generated by each fluorogenic nuclease PCR assay primer set were resolved by use of agarose gel electrophoresis, and the target band was eluted by use of a QIAquick kit (Qiagen Inc., Valencia, Calif.) according to the manufacturer's instructions. The 154-base pair (bp) parvovirus product, 71-bp MVM product, and 78-bp MPV product were then ligated into the pT7Blue-2 vector (Novagen, Madison, Wis.), sequenced at the University of Arizona's Molecular Core Facility, amplified in Tuner (DE3) pLacI competent cells (Novagen), and purified, using the Qiagen plasmid purification kit. The concentration of the purified plasmid DNA was determined by use of an MBA 2000 UV-vis spectrophotometer (PE Applied Biosystems). The plasmid DNA concentration and the calculated molecular weight of the plasmid vector with amplicon insert were then used to calculate the concentration of each sample in units of template copy number.

Specificity: The specificity of each fluorogenic nuclease PCR assay was determined by evaluation of DNA extracted from viral cell preparations of MVMp, MVMi, MVMc, MPV-1b, HaPV, H-1, KRV, MCMV, MAD-1, MAD-2, polyoma virus, and vaccinia virus. Viral DNA from each lysate or concentrate was extracted, using a QIAamp DNA kit following the manufacturer's protocol (Qiagen). The RPV-1, RMV-1a, RMV-1b, and RMV-1c DNA also were used to evaluate specificity and were kindly provided by Dr. Cho-hua Wan (University of Missouri, Columbia, Mo.).

Sensitivity. The absolute sensitivity of each fluorogenic nuclease PCR assay was determined through evaluation of 10-fold serial dilutions of cloned amplicon DNA (range, 10^8 to 10^0 template copies). The relative sensitivities of each fluorogenic nuclease PCR assay, compared with that of previously reported gel detection PCR assays (21), was determined through evaluation of 10-fold serial dilutions of DNA extracted from MVMp and MPV-1b viral stocks. Gel detection PCR assays were performed, using a PE Applied Biosystems GeneAmp 2400 as described (21).

Mice. Four- and six-week-old male Hsd:ICR(CD-1) mice, 12-week-old male DBA/2NHsd mice, and C3H/HeNHsd breeding pairs were obtained from Harlan Sprague-Dawley (Indianapolis, Ind.). Neonatal C3H/HeNHsd mice were obtained from breeding the C3H/HeNHsd adults on site. All mice were specified by the supplier to be free of murine viruses, pathogenic bacteria, and endo- and ectoparasites. Each control or experimental group was housed separately in microisolator cages, and all animal manipulations were performed in a class-IIA biological safety cabinet, using standard microisolation technique. Animals were housed in a biocontainment facility at a temperature of 22 to 24°C, humidity of 40 to 60%, 12 to 15 air exchanges/h, and a 12:12-h light:dark cycle. The University of Arizona Institutional Animal Care and Use Committee approved all animal procedures.

Mouse antibody production test. Six-week-old ICR mice were separated into groups of four animals each and administered 10-fold serial dilutions of either MVMp or MPV-1b.Viral dilutions ranged from 10^6 to 10^2 $TCID_{50}$/mouse for MVMp and 10^{-2} to 10^{-6} $TCID_{50}$/mouse for MPV-1b. Ten percent of each dose was administered oronasally, with the remainder injected intraperitoneally. Dose ranges were established from the endpoint dilution of the MVM- and MPV/HaPV-specific fluorogenic nuclease PCR assay for MVM and MPV, along with the addition of 10-fold and 100-fold more and less concentrated dilutions. Mice were euthanized by carbon dioxide inhalation four weeks after inoculation, and blood was collected by cardiocentesis. Serum was diluted 1:5 in phosphate-buffered saline and stored at –20°C until evaluated by use of indirect fluorescent antibody (IFA) serologic assays specific for MVM or MPV as reported (19).

Animal infections. One-day-old C3H/HeN mouse pups were inoculated oronasally with 1.5×10^7 $TCID_{50}$ MVMp or mock inoculum (50 m*M* Tris base, 10 m*M* EDTA [pH 8.7]) and euthanized 8 days after inoculation. Four-week-old ICR mice under isofluorane

anesthesia were inoculated via gastric gavage with 5×10^5 $TCID_{50}$ MPV-lb or mock inoculum, then were euthanized one or four weeks after inoculation. Twelve-week-old DBA/2 mice were inoculated via gastric gavage with 5×10^4 $TCID_{50}$ MPV-1b or mock inoculum, then were euthanized four weeks after inoculation. All mice were euthanized by carbon dioxide inhalation, and blood samples were collected from the adult animals by cardiocentesis. The resulting sera were diluted 1:5 in phosphate-buffered saline and stored at –20°C until use. Approximately 30 milligrams each of spleen, mesenteric lymph node, and small intestine specimens were harvested from each animal. Each tissue specimen was snap-frozen in liquid nitrogen immediately after harvest and stored at –70°C until tissue DNA was extracted. Tissue DNA was subsequently stored at –20°C until evaluated by use of fluorogenic nuclease PCR assays.

Results

Selection and evaluation of primer and probe sequences. Sequence alignment of rodent parvovirus genomic DNA revealed several regions in the nonstructural 1 (NS1) gene that were highly conserved among rodent parvovirus species and several regions in the viral protein (VP) genes that were heterologous among the different species but were conserved among strains within a species. Conserved regions within the NS1 gene were used to design the rodent parvovirus fluorogenic nuclease PCR primers and probe, and heterologous sequences within the VP2 gene were used to design fluorogenic nuclease PCR primers and probes specific for MVM and MPV/HaPV (Table 1). All primers were optimized at a concentration of 300 n*M* through the use of checkerboard titrations of 50 n*M*, 300 n*M* and 900 n*M* concentrations of each primer with a 125 n*M* probe. Similarly, the parvovirus, MVM, and MPV probes were optimized at 75, 100, and 125 n*M* concentrations, respectively, through evaluation of 25, 50, 75, 100, 125, 150, 175, 200 and 225 n*M* concentrations of probe with the optimal primer concentrations.

Specificity. The specificity of each fluorogenic nuclease PCR assay was determined by evaluation of MVMp, MVMi, MVMc, MPV-1b, HaPV, H-1, KRV, RPV-1, RMV-1a, RMV-1b, RMV-1c, MCMV, MAD-1, MAD-2, polyoma virus, and vaccinia viral DNA. The rodent parvovirus assay detected all rodent parvoviruses, the MVM assay detected only MVMp, MVMi, and MVMc, and the MPV/HaPV assay detected only MPV-1b and HaPV. The MCMV, MAD-1, MAD-2, polyoma virus, and vaccinia virus were not detected by use of any of the fluorogenic nuclease assays.

Sensitivity. The absolute sensitivity of each fluorogenic nuclease PCR assay was determined through evaluation of serial dilutions of cloned amplicon DNA. The parvovirus and MVM assays detected the equivalent of 10 copies of the targeted template, whereas the MPV/HaPV assay detected the equivalent of one copy of template (Fig. 1). The relative sensitivity was also determined for each fluorogenic nuclease PCR assay, compared with that of previously published gel detection PCR assays specific for rodent parvoviruses, MVM, or MPV/HaPV. Identical 10-fold serial dilutions of MVMp and MPV-1b DNA were used as templates for both types of assays. The parvovirus fluorogenic nuclease PCR assay detected as little as 100 fg of viral DNA, an increase in sensitivity of five logarithm dilutions, compared with that of the parvovirus gel detection PCR assay, which could detect 10 ng of MVM and MPV DNA. The MVM and MPV/HaPV assays also detected 100 fg of MVM and MPV DNA, respectively, a 100-fold increase in sensitivity, compared with that of their corollary gel detection PCR assays, which could detect 10 pg of viral DNA.

Comparison of fluorogenic nuclease PCR assays and mouse antibody production (MAP) tests. The relative sensitivities of fluorogenic nuclease PCR assays and MAP testing were determined. Ten-fold dilutions of MVMp and MPV-1b viral preparations were evaluated directly by use of the fluorogenic nuclease PCR assays, and the endpoint detection limit for each assay was determined. A viral dose equivalent to the endpoint detection limit of the fluorogenic nuclease PCR assay was then administered to each of four 6-week-old ICR mice. Similarly, 10- or 100-fold more and 10- or 100-fold less virus was administered to each of four 6-week-old ICR mice in each of four other groups to provide a range of five logarithm dilutions of virus. Four weeks after inoculation serum was harvested from the mice and evaluated by use of IFA serologic testing. None of the mice inoculated with MVM or MPV was seropositive on the basis of IFA testing for each virus. Each fluorogenic nuclease PCR assay was, therefore, at least 100-fold more sensitive than was MAP testing.

Detection of parvoviral DNA in murine tissues by use of fluorogenic nuclease PCR assays. Neonatal C3H/HeN mice were inoculated with MVMp or a mock inoculum and were euthanized eight days after inoculation. The MVM fluorogenic nuclease PCR assay detected viral DNA in small intestine, mesenteric lymph node, and spleen DNA extracted from all MVM-infected mice, but not in age- and strain-matched mock-infected mice (Table 2). Similarly, four-week-old ICR mice were inoculated with MPV-1b or a mock inoculum and were euthanized one and four weeks after inoculation. The DNA extracts from

Table 1. Primer and probe sequences for rodent parvovirus, minute virus of mice (MVM)-specific, and mouse parvovirus/hamster parvovirus (MPV/HaPV)-specific fluorogenic nuclease polymerase chain reaction (PCR) assays

Assay	Sequence (5'-3')	Position (5'-3')
Rodent parvovirus		
Forward primer	GATGATGATGCAGCCAGACAGT	1181-1203[1]
Reverse primer	TTTGCTGGTTTCAGCTTTTTC	1335-1311
Probe	TCCACCTGGTTGAGCCATCATTTCAA	1207-1232
MVM-specific		
Forward primer	GCCATACACACCTGCAGCAAA	3410-3430[1]
Reverse primer	TGGCGATGCTATGGTTGGT	3481-3462
Probe	TCAATGGAAACACTTGGTTTCTACCCTTGGA	3463-3493
MPV/HaPV-specific		
Forward primer	CCAGCAGAGCAGGACCTTTT	3914-3934[2]
Reverse primer	CTGCCATTGGCGTCATGTA	3992-3973
Probe	TTCCAGTAGTGCCAGCAAATGTTACACAAGG	3938-3969

[1]Nucleotide positions from MVMp sequence (GenBank accession No. J02275).
[2]Nucleotide positions from MPV-1a sequence (GenBank accession No. U12469).

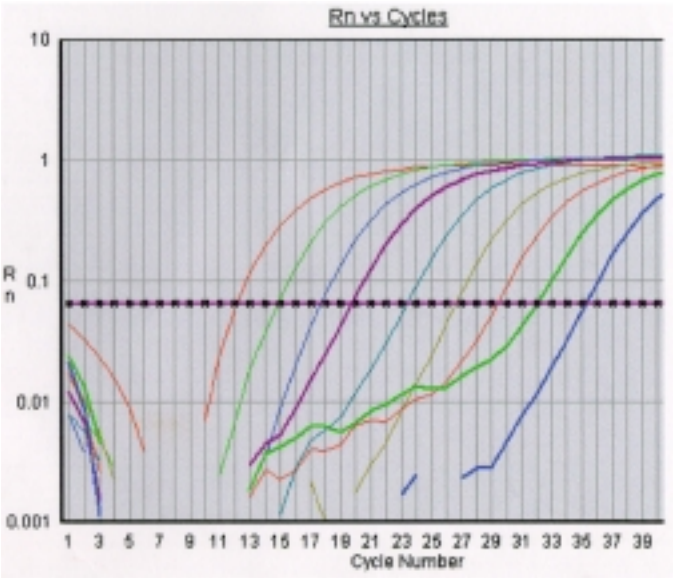

Figure 1. Absolute sensitivity of the mouse parvovirus (MPV)-specific fluorogenic nuclease polymerase chain reaction (PCR) assay as demonstrated by an amplification plot of 10-fold serially diluted cloned amplicon DNA. Dilutions from left to right on the amplification plot range from 10^8 to 10^0 copies of target amplicon. All values with mean fluorescence (Rn) > 0.1 and a cycle threshold (Ct) < 40 are considered positive results.

small intestine, mesenteric lymph nodes, and spleen of each mouse were evaluated by use of the MPV/HaPV fluorogenic nuclease PCR assay. The MPV DNA was detected by use of the fluorogenic nuclease PCR assay at both time points in all tissues obtained from MPV-infected mice, but not in the same tissues harvested from mock-infected mice (Table 2). All MPV-infected mice were also seropositive on the basis of results of MPV IFA testing at four weeks after inoculation, but were seronegative at one week after inoculation.

Given the improved sensitivity of fluorogenic nuclease PCR assays at detecting MPV, compared with that of previously published gel detection PCR assays, DNA extracts obtained from the small intestine and mesenteric lymph node tissues of 12-week-old DBA/2 mice experimentally inoculated with MPV-1b were evaluated. These mice had been evaluated by use of the published MPV-specific gel detection PCR assay that failed to detect MPV DNA in these organs despite seroconversion of mice to MPV (19). The MPV DNA was detected in almost all tissues obtained from infected mice by use of the MPV-specific fluorogenic nuclease PCR assay (Table 2).

Discussion

Fluorogenic nuclease PCR assays that detect all rodent parvoviruses or specifically detect either MVM or MPV/HaPV were developed. Each assay specifically detected the targeted viruses without detecting non-targeted viruses. The detection of MPV and HaPV by use of the MPV/HaPV assay was expected since MPV and HaPV are closely related genetically (24), and the primer and probe oligonucleotide sequences for the MPV-specific assay differed by only one nucleotide per oligonucleotide from the aligned HaPV sequence. Each assay was also documented to be extremely sensitive at detecting its targeted template. Absolute sensitivity determinations, using cloned amplicon DNA, indicated that each assay had an endpoint detection limit of at least 10 copies of target template. Due to this extremely low sensitivity, caution during PCR set up is of paramount importance to avoid DNA contamination. Carry-over contamination in fluorogenic nuclease PCR assays is minimized by inclusion of uracil-N-glycosylase in the reaction mixture (25), and by use of the closed-tube detection system, which eliminates exposure of the laboratory environment to PCR products. These precautions, along with good bench top laboratory technique, were adequate to prevent false-positive results in our laboratory.

The sensitivity of fluorogenic nuclease PCR assays, compared with that of previously published rodent parvovirus gel detection PCR assays also was improved, especially for the rodent parvovirus assays for which the fluorogenic nuclease PCR assay was five logarithm dilutions more sensitive. This improved sensitivity may reflect the much shorter amplicons generated by the fluorogenic nuclease assays, the use of a hot-start *Taq* polymerase, which reduces non-specific primer binding, and the improved sensitivity of fluorogenic detection versus gel detection. We have found, in other experiments in our laboratory, that with use of identical PCR products, detection by the fluorogenic probe can improve sensitivity by 10- to 100-fold over detection with ethidium-stained agarose gels (data not shown). Although in most instances, this improved sensitivity may not make a difference in the ability to qualitatively detect parvovirus DNA, in some instances, it may. For example, the MPV/HaPV-specific fluorogenic nuclease PCR assay detected MPV DNA in tissues obtained from adult DBA/2 mice, whereas the previously published MPV-specific gel detection PCR did not.

Fluorogenic nuclease PCR assays also have several advantages over the MAP tests used to detect viral contamination in biological materials. The MVM- and MPV/HaPV-specific fluorogenic nuclease PCR assays were found to be at least 100-fold more sensitive then their respective MAP tests. Although this increased sensitivity does not allow distinction between infective and non-infective virions, as indicated by the detection of far fewer than one tissue culture infective dose of MPV-1b by use of the MPV/HaPV-specific fluorogenic nuclease assay, the detection of virus-specific DNA would reliably indicate biological material exposure to a parvovirus. Fluorogenic nuclease PCR assays also have appreciably reduced turnaround time, provide an alternative to a whole animal bioassay, and are less costly than MAP testing. Given the high potential of rodent parvovirus contamination of cell cultures and other biological materials (12, 13), fluorogenic nuclease assays should provide an accurate, cost-effective, and timely means of screening these materials for parvovirus. The rodent parvovirus fluorogenic nuclease PCR assay would be especially useful for this application since it can detect all known rodent parvoviruses and has much improved sensitivity over the previously published rodent parvovirus gel detection PCR assay (21).

Fluorogenic nuclease PCR assays also offer several advantages as adjunct diagnostic methods for routine rodent health monitoring. Since fluorogenic nuclease PCR assays do not rely on an induced immune response to detect infection, they can be used to directly detect murine parvoviruses in tissues obtained from immunocompromised rodents. Fluorogenic nuclease PCR assays may also be suited for ante-mortem diagnostic evaluation. Data generated in our laboratories indicate that the MVM- and MPV/HaPV-specific assays can detect MVM and MPV, re-

Table 2. Fluorogenic nuclease PCR assay, gel detection PCR assay, and serologic test results for mice experimentally inoculated with MVMp or MPV-1b

Virus	Dose ($TCID_{50}$)	Strain	Age	Tissue harvest	Fluorogenic nuclease PCR[1]			MPV PCR[1]		MPV IFA
					SI	SPL	MLN	SI	MLN	
MVMp	0	C3H	2 days	8 days a.i.	0/4	0/4	0/4	ND	ND	ND
MVMp	1.5×10^7	C3H	2 days	8 days a.i.	8/8	8/8	7/8	ND	ND	ND
MPV-1b	0	ICR	4 weeks	1 week a.i.	0/3	0/3	0/3	ND	ND	0/3
MPV-1b	5×10^4	ICR	4 weeks	1 week a.i.	5/5	5/5	5/5	ND	ND	0/5
MPV-1b	0	ICR	4 weeks	4 weeks a.i.	0/4	0/4	0/4	ND	ND	0/4
MPV-1b	5×10^4	ICR	4 weeks	4 weeks a.i.	5/5	5/5	5/5	ND	ND	5/5
MPV-1b	0	DBA/2	12 weeks	4 weeks a.i.	0/4	ND	0/4	0/4	0/4	0/4
MPV-1b	5×10^5	DBA/2	12 weeks	4 weeks a.i.	3/4	ND	4/4	0/4	0/4	4/4

[1]MVM-specific assays used for MVMp-inoculated mice; MPV-specific assays used for the MPV-1b inoculated mice.
[2]Number of animals positive/number of animals tested.
IFA = indirect fluorescent antibody (test); SI = small intestine; SPL = spleen; MLN = mesenteric lymph node; a.i. = after inoculation; $TCID_{50}$ = median tissue culture infective dose; ND = not done.

spectively, in fecal pellets and cage swab specimens collected from the cages of experimentally inoculated mice (data not shown). Although consistent ante-mortem detection of murine parvovirus DNA in individual cages over long periods is unlikely due to acute and/or intermittent viral shedding (4, 15), detection within a mouse colony could potentially be accomplished by random sampling or evaluation of pooled samples. A reliable, non-invasive approach toward ante-mortem testing would be advantageous for rodent colony health monitoring, particularly among animals involved in long-term studies.

Rodent parvovirus fluorogenic nuclease PCR assays could also be applied to parvovirus research. The quantitative results generated by fluorogenic nuclease PCR assays could be useful in parvovirus pathogenesis studies when quantitation of viral load, viral shedding, or viral gene expression is desired. They could also be applied to parvovirus epizootiologic studies to help determine transmission modes and frequency. Finally, the rodent parvovirus assay could be useful in detecting novel murine parvoviruses since it indiscriminately detects all known rodent parvoviruses, whereas the MVM- and MPV/HaPV-specific assays can distinguish MVM and MPV from other murine parvoviruses.

In summary, each of the fluorogenic nuclease PCR assays developed in these studies to detect murine parvoviruses are specific and sensitive at detecting their respective targeted viral DNA in vitro and in vivo. Each assay is also more sensitive than MAP testing and previously published gel detection PCR assays that target rodent parvoviruses. These results indicate that fluorogenic nuclease PCR assays should provide a sensitive, potentially high-throughput method to detect parvovirus infections in rodents and rodent parvovirus contamination of biological materials, with the potential to also be useful in rodent parvovirus research.

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