

I. Introduction

Parvoviruses are among the smallest viruses, measuring just 15-28 nm in diameter; they possess a single-stranded DNA genome of 5 kb that encodes two highly conserved non-structural proteins, NS1 and NS2, and two major virion (or coat) proteins, VP1 and VP2. Due to their biochemical simplicity, parvoviruses depend on host cell factors produced during cell division and differentiation to replicate productively (i.e., produce infectious virus). This requirement for productive infection and the related tendency of parvoviruses to infect mitotically active cells substantially account for the pathogenicity and research complications caused by adventitious parvovirus infections^{28,30,33,59,61}.

NS proteins are required for viral gene expression and replication^{18,39,45}; they can be found in parvovirus-infected cells, but as their name denotes, do not become part of the virus particle, or virion. Coat proteins (i.e., VP) determine viral host range and tissue tropism; not surprisingly, they differ substantially from one parvovirus serotype to the next. These differences can be demonstrated by serum neutralization and, as shown in the following table, by hemagglutination inhibition, i.e., HAI^{3-6,9,25,56}.

Specificity of Rodent Parvovirus HAI

Antiserum	HAI Titer ^a		
	KRV	H-1	MVM
KRV	160	-	-
H-1	-	20,480	-
MVM	-	-	10,240

^a - = titer less than 20.

Adapted from Siegl, 1976.

Until the early 1980's, the HAI was the principal serologic method by which mouse and rat colonies were screened for antibodies to the three known parvovirus serotypes represented by minute virus of mice (MVM), Kilham rat virus (RV) and Toolan's H-1 of rats. The switch by our laboratory and others to more generic and sensitive solid-phase immunoassays, including the enzyme-linked immunosorbent assay (ELISA) and the indirect fluorescent antibody test (IFA), coincided with serologic evidence suggesting the existence of novel parvovirus serotypes in both mice and rats. Specifically, rodent colonies that had consistently been parvovirus seronegative by HAI were found to be seropositive by IFA. The cross-reacting antibodies detected by IFA were by and large missed by corresponding ELISA for reasons to be discussed later. The additional serotypes were initially referred to as "orphan" parvoviruses, but after further characterization were named mouse parvovirus 1 (MPV)^{3,35,58}, rat parvovirus 1 (RPV)⁴ and rat minute virus 1 (RMV)⁶⁵. Although MPV was discovered not long ago, retrospective serology indicates that it was prevalent in mouse colonies over 30 years ago³⁰. RPV-1a has been shown to be genetically and antigenically quite distinct from the other rodent parvoviruses^{4,65}.

While improvements in laboratory rodent husbandry and biosecurity practices over the past two decades have dramatically reduced the occurrence of once common adventitious viral infections (such as those with Sendai virus), excluding parvoviruses from specific pathogen-free (SPF) rodent colonies continues to be problematic for various reasons including:

- Parvovirus infections can be difficult to diagnose. They are typically asymptomatic. Some rodent parvoviruses, e.g., MPV and RPV, are non-pathogenic even in immunodeficient and infant rodents, which are, in general, highly susceptible to viral disease^{4,58}. The parvovirus seroconversion rate is often low for rodents kept in microisolation cages and ventilated racks. Some inbred mice, including DBA/2 and C57BL/6, appear to be resistant to infection with MPV and therefore, rarely seroconvert to this virus¹⁰.
- Parvoviruses are comparatively common contaminants of animal tissues and transplanted tumor cell lines^{12,17,44} because they persist in the tissues of infected animals^{21-23,35-37,46} and have a predilection for rapidly dividing cells^{59,61}.
- Once shed into the environment, parvoviruses are exceptionally stable^{32,69}, that is, resistant to disinfection. They can withstand prolonged exposure to heat and, because they are non-enveloped, resist denaturant disinfectants such as alcohols, phenolics and quaternary ammonium compounds that readily inactivate enveloped viruses including Sendai and mouse hepatitis virus (MHV). Like other viruses, parvoviruses pass through microfilters that remove larger microorganisms such as bacteria. These physiochemical characteristics increase the probability of introducing parvoviruses to a facility via contaminated equipment and supplies (i.e., fomites) or people acting as mechanical vectors; they make disinfection a daunting and often futile undertaking.

II. Pathogenesis

Natural parvovirus infections of rodents are predominantly asymptomatic, supporting the notion that the relationships between rodent parvoviruses and their natural hosts are longstanding and highly evolved. Rodents infected *in utero* or as neonates, however, are susceptible to disease because they provide large numbers of dividing cells for virus replication; in addition, neonates are unable to recover from infection because their immune system is immature. The newly recognized rodent parvoviruses MPV and RPV, however, are non-pathogenic, even for neonatal and immunodeficient hosts.

A characteristic result of perinatal parvovirus infection of rodents and other animal species is cerebellar hypoplasia and ataxia due to a selective viral attack on the rapidly proliferating external germinal layer of the cerebellum. Rodent parvoviruses are particularly pathogenic for neonatal hamsters injected with virus by the intracerebral route. In addition to cerebellar hypoplasia, neonatally infected hamsters develop mongoloid-like deformities due to viral destruction of proliferating skeletal and dental tissues^{1,27,59,62}.

The vascular endothelium and hemopoietic and lymphoreticular systems (which comprise diverse cell types) proliferate and differentiate in adults as well as newborns and hence, are common targets of parvovirus infection; lesions and disease signs are due to infection of these tissues. Mice of certain inbred strains develop lethal renal or intestinal hemorrhage when inoculated as newborns with the immunosuppressive (i) variant of MVM; DBA/2 mice infected with MVM (i) as infants show accelerated involution of hepatic hematopoiesis^{14,51,52}. RV is the only rodent parvovirus that is naturally pathogenic. Epizootic RV infection of naïve dams initially decreases the number and size of litters. Neonatally infected rats develop necrosis of the lymphoreticular and hemopoietic tissues, with the latter leading to anemia and thrombocytopenia. As dams become RV seropositive, fetuses and pups are protected from infection and consequently, production returns to normal. Rarely, RV produces a disseminated hemorrhagic disease in adult rats^{20,22,29,31}.

With the exception of MVM, rodent parvoviruses often persist in individual hosts, even after seroconversion. This has been demonstrated by virus isolation⁴⁶ and by detection of viral genomic sequences using *in situ* hybridization^{21,35,36,58} and PCR^{7,10,60}. Virus persists in various cell types such as lymphocytes, vascular endothelium and vascular smooth muscle^{4,35-37}. RV persists longer in immunodeficient and neonatally infected rats than in post weaning immunocompetent rats^{21-23,34}, whereas MPV persists for long periods in the tissues of immunocompetent mice infected as adults. On the other hand, MPV shedding by immunocompetent mice is ephemeral^{53,59}.

III. Epizootiology

Transmission of rodent parvoviruses is generally horizontal, although congenital infection does occur when naïve dams are infected during pregnancy. Vertical transmission of RV has been experimentally produced by virus inoculation of dams just prior to mating or during gestation^{29,38}.

Horizontal transmission of parvoviruses occurs more efficiently by animal-to-animal contact or fomites than by aerosol^{34,69}. The route of entry for natural parvovirus infections is oral; parenteral inoculation of parvovirus-contaminated biological materials is another important entry route. Subsequent to oral exposure, the primary replication site for RV is the respiratory tract²⁰, while the other rodent parvoviruses, including RPV, MVM and MPV, are enterotropic. Parvoviruses are shed in the feces and urine. RV is also shed from the respiratory tract. Like persistence, shedding of RV has been shown to last longer in rats that are immunodeficient or infected as infants^{21,34}. Mice infected with MPV as infants also shed virus for prolonged periods, whereas virus shedding by mice infected after weaning is short-lived^{55,58}.

IV. Research Effects

Much of the research disruption attributed to rodent parvoviruses is due to infection of proliferating cells - particularly tumor cells and lymphocytes - and the distortion of biological responses that depend on these cells^{33,55,58}. A number of the prototype rodent parvovirus strains, including RV, H-1 and MVM (i), were originally isolated as tumor cell contaminants that interfered with research. Parvoviruses can suppress the growth of tumor cells *in vivo*, which is referred to as oncosuppression, and prevent the growth in culture of cells from infected hosts^{30,59}.

Parvovirus infection of lymphocytes has been associated with suppression and dysregulation of the immune response. MVM (i) was found to be the inhibitory "factor" released by a murine lymphoma that suppressed the generation of cytotoxic T lymphocytes in mixed mouse lymphocyte cultures¹² and was then shown to inhibit a variety of T cell functions and the clonogenic capacity of different hemopoietic precursors^{19,51,52}. The first MPV isolate, MPV-1a, was discovered as a contaminant that caused mouse T-lymphocyte clones to lose function and viability⁴¹. After adult immunocompetent rats were inoculated with RV, their lymphocytes had diminished *in vitro* proliferative and cytolytic responses, which correlated with infection of both T and B lymphocytes⁴². As examples of dysregulation, diabetes resistant (DR) BB rats developed autoimmune insulinitis following RV infection¹³; MPV-infected mice rejected tumor and skin allografts faster than non-infected mice, and paradoxically rejected isografts⁴³. The potentiation of graft rejection by MPV was not due to infection of the grafts and appeared to be T-cell mediated, even though *in vitro* measures of specific alloantigen reactivity by T lymphocytes were diminished.

V. Diagnosis

A. Serology

The humoral immune response of rodents to parvovirus infection comprises antibodies to the viral non-structural (NS) proteins and to virion proteins (VP). Because NS proteins are highly conserved, NS antibodies formed in response to one parvovirus serotype cross-react strongly with others. On the other hand, VP antibodies tend to be selective (i.e., serotype-specific) in that they usually do not cross-react with heterologous viruses.

Assays for selective VP antibodies, such as the HAI, are valuable for distinguishing between MVM and MPV infections of mice and among the various serotypes that infect rats. For screening assays, however, low selectivity is often preferable to high selectivity because it allows for detection of cross-reacting antibodies to heterologous and perhaps novel viral serotypes. It was the low selectivity of the IFA that provided the initial serologic evidence for the existence of additional rodent parvovirus serotypes. Although the parvovirus ELISA and IFA are both heterogeneous, solid-phase immunoassays, they typically use dissimilar antigen preparations that account for important differences in their selectivity. Conventional ELISA antigen is prepared by purifying virus particles from infected cell culture and adsorbing the purified virions to wells in 96-well microtiter plates. The IFA antigen, by contrast, consists of infected cells fixed with acetone to wells on glass microscope slides. As the infected cells that constitute IFA antigen contain NS proteins, whereas the virus particles that compose ELISA antigen do not, parvovirus ELISA are more selective than corresponding IFA. This difference in selectivity explains why cross-reactive MPV and RPV serum antibodies were initially detected by IFA, but not by ELISA.

The development of serologic assays for VP antibodies to MPV and RPV has been slow because isolates of these newfound parvoviruses are difficult to propagate in culture. Only the first isolate of MPV, MPV-1a, is being routinely propagated *in vitro*, albeit with difficulty. The titer of MPV-1a produced in culture is sufficient to prepare IFA and HAI antigens, but is far below that required to prepare ELISA antigen. RPV has been propagated in a T lymphoma line⁶³. The difficulty in cultivating MPV and RPV is being circumvented by the production of recombinant viral proteins, made possible by recent advances in genetic engineering. The first rodent parvovirus antibody assay to utilize recombinant (r) protein antigen was an rNS1 ELISA⁴⁹. This assay was initially touted as a generic screen for parvovirus seroconversion, but subsequent experience and experimental evidence have shown that the NS1 antibody response can be absent or delayed vis-à-vis the antibody response to VP. In a recent study¹⁰, ICR mice were shown to seroconvert to MPV, irrespective of the age at which they were inoculated with the virus. However, while mice inoculated with MPV at 4 or 8 weeks of age produced detectable NS antibodies, those inoculated at 12 weeks of age did not. Consequently, to minimize the occurrence of false negative results, rodent parvovirus serology screening panels should include VP antibody assays for each parvovirus serotype.

VP2 is the main constituent of parvovirus capsids and accordingly, is the coat protein from which recombinant antigens have been prepared for MPV and other parvoviruses. MPV rVP2 ELISA have been described that utilize antigen from *E. coli*² and insect cells infected with recombinant baculovirus⁴⁰. Recombinant VP2 produced in baculovirus-infected insect cells self assemble into virus-like particles (VLP), which display serologic selectivity equivalent to natural virus particles. *E. coli* rVP2 antigen appears to be less selective, probably because it is denatured as part of the extraction and purification process.

The table below lists the types of assays commonly used for rodent parvovirus serology and whether they detect cross-reactive NS or selective VP antibodies. The IFA is the only method that detects both.

Assay		Antibodies	
Method	Antigen	NS	VP
HAI	Viral hemagglutinin	-	+
IFA	Infected Cells	+	+
ELISA	Purified virions	-	+
	rVP2	-	+
	rNS1	+	-

As mentioned, it is not unusual for NS antibodies to be undetectable in rodent parvovirus antisera. The presence of NS antibodies and whether antigen is homologous or heterologous determine the result obtained for a serum sample by a particular method.

Assay		Expected Reaction ^D	
Method	Antigen ^A	α NS +	α NS -
HAI	HO	+	+
	HE	-	-
IFA	HO	+	+
	HE	+	-
ELISA	HO VP^B	+	+
	HE VP^B	-	-
	rNS1^C	+	-

^AAntigen serotype is homologous (HO) or heterologous (HE)

^BConventional or recombinant virion (coat) protein

^CRecombinant NS1

^DNS antibodies are present (α NS +) or absent (α NS -).

We screen for parvovirus antibodies by the ELISA. Our screening panels include ELISA for VP antibodies to all serotypes except RMV and RPV. It has been our experience that RMV antibodies cross-react with KRV and H-1 by ELISA, whereas RPV antibodies do not. We therefore continue to depend on the rNS1 ELISA to detect seroconversion to RPV; we infer a diagnosis of RPV when assays for NS antibodies (including the rNS1 ELISA and KRV IFA) are positive, but those for heterologous VP antibodies (i.e., the KRV and H-1 ELISA and HAI) are negative.

CRL Parvovirus Serology Panels		
Species	Method	Antigen
Mouse	ELISA	MVM
		MPV rVP2
		rNS1
	HAI	MVM
	IFA	MPV
		MVM
KRV		
Rat	ELISA	KRV
		H-1
		rNS1
	HAI	KRV
		H-1
	IFA	KRV
		MVM

B. Polymerase chain reaction (PCR)

While antibody serology continues to be the mainstay of surveillance for rodent viruses, including parvoviruses, the diagnostic application of viral PCR is expanding and becoming routine. Some of the uses to which rodent viral PCR are being applied are:

- Detection of viral contaminants in biological specimens, such as cell lines, in place of the mouse antibody production (MAP) test. Screening PCR for this and diagnostic purposes amplify NS1 genomic sequences^{15,48,68}.
- Diagnosis of viral infection in immunodeficient rodents, incapable of mounting an antibody response.
- Environmental monitoring: This type of monitoring was not practicable prior to the advent of PCR. It has been our experience that samples from room exhaust and laminar flow air filters work best because these filters concentrate virus-laden particles²⁶.
- Release from quarantine: Post mortem or survival specimens, such as mesenteric lymph nodes or fecal pellets, can be tested to demonstrate that quarantined animals are not infected with or shedding virus.
- Serotype and strain identification and characterization by PCR that target VP2 gene sequences^{7-9,65}.
- Confirmation of serological findings: Viral infections of an immunocompetent host are often short-lived in that infectious virus cannot be recovered from host tissues following seroconversion. For such transient infections, the PCR may not be appropriate for confirming positive serological findings – although it was recently reported for non persistent MHV infections that viral genomic sequences could be detected by PCR in host tissues weeks longer than could infectious virus¹¹. On the other hand, the PCR has been especially helpful for confirming rodent seroconversion to parvoviruses that persist in the tissues of seropositive hosts, such as RV, RPV and MPV.

C. Results Interpretation

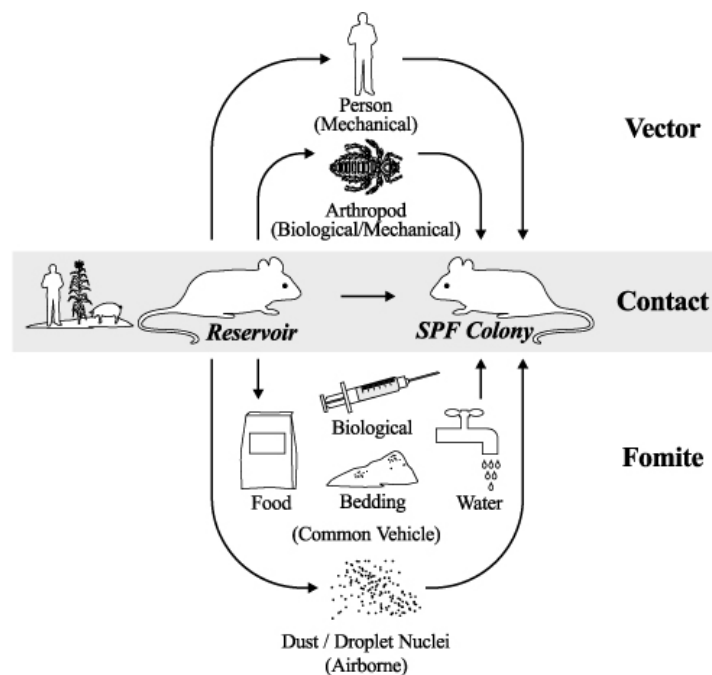
The principal goal when interpreting parvovirus test results (and when interpreting the results of tests for other microorganisms to be excluded from an SPF colony) is to determine whether an adventitious infection has occurred. Because no diagnostic test is always accurate, first-time positive findings should always be confirmed by repeat testing of the positive samples, by testing additional samples and by using alternative assays and diagnostic methodologies to corroborate primary test results. False positive results should be suspected when reactions are borderline-positive, the prevalence of positive specimens is low or primary results cannot be confirmed⁵⁴.

We recommend that routine surveillance for parvoviruses be accomplished by antibody serology. Our primary screening assays are ELISA because they are more amenable than other methods to automation. Parvovirus seroconversion in the ELISA is verified by HAI and IFA. Serologic findings may then be further corroborated by PCR. As adventitious parvovirus infections are relatively common, we advocate storing the mesenteric lymph nodes from euthanized serology sentinels for later confirmatory PCR testing, if necessary.

Test results can be evaluated to establish the parvovirus serotype(s) to which animals have been exposed. This may be helpful in determining the source of infection and a course of action, although many of the research complications attributed to adventitious parvovirus infections are common to all serotypes. Therefore, the ultimate goal, irrespective of serotype, will be to eliminate and prevent a recurrence of the infection.

VI. Biosecurity

Preventing adventitious parvovirus infection is accomplished by controlling the risk factors associated with probable sources of infection including wild, escaped or imported rodents, supplies, people and biological materials⁵⁴.



Animal facilities should be constructed and maintained so that potential nest areas and routes of ingress or egress for rodents are not present. A professionally managed pest control program should be put in place. Because parvoviruses are exceptionally stable, disinfection of supplies is particularly important for preventing adventitious infection. Food and bedding should be disinfected by autoclaving or gamma irradiation, although gamma irradiation is not particularly effective at inactivating parvoviruses. The outer surfaces of supply containers should be disinfected using chemicals known to be effective against parvoviruses, such as oxidants including sodium hypochlorite (i.e., bleach), chlorine dioxide and peroxygens. When using oxidant disinfectants, you should keep in mind that they not only react with living microorganisms, but also with inorganic reducing substances such as ferrous iron and organic impurities, e.g., dissolved proteins. These reactions exert a chemical demand that reduces the concentration of free disinfectant. Association with dirt and organic matter, or biofilms, has been shown to protect microorganisms from disinfectants^{24,50,67}. It is therefore crucial that soiled surfaces be sanitized before being disinfected in order to reduce chemical demand and ensure that microorganisms are adequately exposed to disinfectant.

Waterborne viruses are characteristically of small to medium size, non-enveloped (and hence stable) and shed in the feces. As these are properties of rodent parvoviruses, the possibility that untreated water could be a source of parvovirus contamination should be taken seriously. Virucidal treatments that have been utilized for animal drinking water are UV irradiation, ozonation and chlorination^{16,32,53}. While viruses cannot be reliably eliminated from animal drinking water by microfiltration, they can be by ultrafiltration or reverse osmosis.

For reasons already discussed, rodent parvoviruses are comparatively common contaminants of animal-derived cells, particularly lymphocytes and transplantable tumors. Therefore, a substantial risk of adventitious parvovirus infection is posed by inoculation of rodents with biological materials that have not been screened for extraneous viruses. Screening has traditionally been carried out by the mouse and rat antibody production (MAP and RAP) tests and by other *in vivo* and cultural isolation techniques^{66,57,64}. Investigators, though, may unwisely avoid rodent antibody production testing because of the time and expense involved. PCR assays for viruses provide an accurate and rapid alternative to MAP testing⁴⁷.

Should an adventitious infection occur, control and eradication are most reliably achieved by depopulation and disinfection, followed by repopulation with SPF replacements or rederived descendants of the infected colony. The chance of vertical transmission during Cesarean rederivation can be reduced by using older (e.g., multiparous) seropositive dams. Attempting to interrupt the cycle of infection by instituting a moratorium on the introduction of naïve hosts may not be reliable for parvoviruses because persistently infected hosts may shed virus for prolonged periods and excreted virus will remain infectious for a long time. It is worth emphasizing that it is counterproductive to depopulate and start a new SPF colony without first investigating the sources of the infection, and making the necessary procedural and facility modifications to prevent a recurrence.

VII. Reference List

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