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Maternal Antibodies or Nonproductive Infections Confound the Need for Rederivation

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After rederivation of a mouse parvovirus (MPV)-contaminated transgenic mouse strain, serology and PCR testing of the surrogate dam showed it to be infected with mouse parvovirus strain 1 (MPV1). The rederived pups (*n* = 3) also were MPV-positive, according to serology. Despite MPV seropositivity, fecal PCR tests of the pups were negative, as were serologic results from direct-contact sentinels. Only one rederived pup survived, and this male was bred successfully. None of its mates or progeny seroconverted to MPV. At 14.5 mo of age, the rederived male mouse was euthanized; tissues were collected and submitted for MPV testing; both serologic tests and PCR analysis of mesenteric lymph nodes were MPV-negative. One explanation for the rederived pups' MPV seropostivity is passive transfer of maternal antibodies or a nonproductive MPV infection. This case illustrates that although routine serological testing of surrogate mothers and pups is appropriate, any positive results should be further investigated by using transmissibility testing (fecal PCR or contact sentinels or both) prior to repeat rederivation.

Abbreviations: IFA, indirect fluorescent antibody assays; MFIA, multiplexed fluorometric immunoassays; MLN, mesenteric lymph nodes; MPV, mouse parvovirus.

Mouse parvovirus (MPV) is a small, nonenveloped, DNA virus in the family Parvoviridae, subfamily Parvovirinae.[17] Like several other parvoviruses, the host cell must enter the S-phase of mitosis for productive MPV viral replication to occur.[13,17] MPV has a tropism for lymphocytes and can modulate the immune response both in vitro and in vivo.[3,14] Depending on the experimental system, MPV infection can either enhance or suppress immune reactions, thereby potentially confounding results.[24,25]

Despite efforts to eradicate MPV, it remains one of the most common viral pathogens in contemporary research mouse colonies.[22,28] MPV is notoriously difficult to detect and eradicate in research vivaria.[15,16,20,31] This intractability is due in large part to the resistance of MPV's nonenveloped virions to inactivation by environmental conditions (for example, desiccation) and commonly used disinfectants;[8,18] its low prevalence in enzootically infected immunocompetent mouse colonies (estimated to be less than 1%,[21] thus making surveillance technically difficult due to the large number of cages that must be sampled); its ability to infect adults as well as younger mice;[34] its persistence in vivo, which can be prolonged for months in immunodeficient strains;[4,7,11] its low infectivity;[5,9,35] and the absence of overt clinical signs and pathologic lesions that could herald an outbreak.[4,12,34] In addition, sex, age, mouse strain or stock, and housing conditions can influence virus transmissibility, persistence in vivo, and length of shedding, thus complicating the surveillance of colonies by using dirty-bedding sentinels.[7,11,32,35]

Transmission of MPV is presumably through the fecal–oral route or direct contact.[3,34] In addition, MPV was first isolated from cloned mouse T-cell lines,[23] and the inoculation of experimental mice with MPV-contaminated cell lines or other biologic materials is therefore another potential source of entry into a facility. Although vertical transmission has not been documented, PCR assays have detected MPV in ovaries, male and female gametes, and embryos from infected mice and during nonproductive infections of female mice that were implanted with infected embryos have been documented.[1,6] Currently, the detection of MPV in mouse colonies is primarily by serology for specific antibodies or PCR amplification of MPV DNA in fecal pellets, mesenteric lymph nodes (MLN), or spleen.[2,13,19,20,29]

Mice from unapproved vendor sources can enter Stanford University's AAALAC-accredited animal facilities either through a quarantine or rederivation process. The quarantine option is restricted to mice from colonies with a clean health record; mice from colonies known to be contaminated with institutionally excluded pathogens must be rederived. Embryo transfer rederivation is the 'gold standard' for the elimination of pathogens from enzootically infected colonies.[26,30,36] Testing of the surrogate mother and, in some cases, the rederived pups is necessary to confirm that the pathogens have been eliminated. If the surrogate mother or pups are positive for a pathogen, management options include euthanasia of involved mice, transfer of mice to another facility, and repeating the rederivation process.

We here report a case of MPV infection in a surrogate mother and MPV seropositivity of her rederived transgenic pups. Even though the rederived pups were MPV-seropositive, results from MLN and fecal PCR assays and contact sentinels suggested that the pups were not shedding MPV and that their MPV-seropositivity may have been due to the passive transfer of maternal antibodies or to a nonproductive MPV infection. The single surviving rederived mouse was bred and successfully used to propagate the transgenic strain. This case report demonstrates that rederived pups born to MPV-infected surrogate mothers, despite being MPV-seropositive, should be tested for MPV shedding. If testing results indicate that the seropositive pups are not infectious, a second rederivation attempt may not be necessary.

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Case Report

An investigator submitted a request to import from another institution a triple transgenic strain, designated HY^{cd4}, that was on a C57BL/6 (B6) genetic background. Examination of the health reports of the donor institution showed that the colony was MPV-positive, necessitating rederivation of the transgenic strain. The investigator elected to perform embryo transfer rederivation in his lab, using his colony of IcrTac:ICR (Taconic Farms, Germantown, NY) female and vasectomized male mice to generate the surrogate mothers. All mice in the investigator's colony were housed in autoclaved, static cages (Allentown, Allentown, NJ) with filter cage-tops (Ancar, Bellmore, NY) and supplied with autoclaved food and water.

The investigator's laboratory received fresh embryos at embryonic day 3.5 in M2 media by overnight courier. According to conventional procedures,[37] the embryos were washed 10 times in M2 media, with the pipette being changed after each wash. Fourteen embryos were transferred into the uterine horns of a 2.5 d postcoitus pseudopregnant female mouse under tribromoethanol (375 mg/kg IP) anesthesia, as stipulated by the investigator's IACUC-approved protocol. Lidocaine (3 mg/kg SC) administered at the surgical site immediately after surgery provided postsurgical analgesia. On recovery from anesthesia, the recipient female mouse was immediately placed in an autoclaved static cage with a filter top and the cage housed in a quarantine room to which access was limited to veterinary and husbandry staff only. Three (1 male, 2 female) pups were born, and all were successfully weaned. After weaning, the surrogate female mouse was euthanized by CO_2, blood obtained by cardiocentesis, and samples of organs stored at −20 °C for future testing. The surrogate's serum was screened by the Stanford University Veterinary Service Center's Diagnostic Laboratory by ELISA to detect antibodies against viral, bacterial, and parasite pathogens (mouse hepatitis virus, Sendai virus, minute virus of mice, pneumonia virus of mice, Theiler murine encephalomyelitis virus, lymphocytic choriomeningitis virus, reovirus 3, enzootic diarrhea of infant mice virus, ectromelia virus, K virus, mouse adenovirus, mouse cytomegalovirus, polyoma virus, mouse parvovirus [recombinant VP2 antigen], *Mycoplasma pulmonis*, and *Encephalitozoon cuniculi*). The ELISA plates and reagents were obtained from a commercial laboratory (Charles River Laboratories, Wilmington, MA). All ELISA-positive results are routinely confirmed by a commercial laboratory (Charles River Laboratories) using multiplex fluorometric immunoassays (MFIA), indirect fluorescent antibody assays (IFA), or PCR on stored tissues.

The surrogate female mouse was seropostive for MPV by ELISA, MFIA, and IFA (Table 1). To confirm the serologic results, the surrogate's MLN were submitted for MPV-specific PCR, which yielded positive results also (Table 1). The cumulative serologic and PCR results identified the MPV strain as MPV1.

The quarantine room that housed the MPV1-positive surrogate female mouse held other surrogate female and rederived mice. In light of the MPV1-positive result, all cohoused surrogate female mice and pups were tested for MPV by serology or fecal PCR; all results were negative.

A dirty-bedding sentinel from the investigator's colony had seroconverted to MPV a few weeks prior this incident. Subsequently, 51 cages on the rack from which the MPV-positive sentinel originated were screened either by MPV serology (1 mouse per cage) or MPV PCR of pooled fecal pellets from cages housing immunodeficient lines or recently weaned mice. No MPV-positive cages were identified (data not shown).

In the investigator's colony, mice used to generate pseudopregnant recipient females are replaced rapidly, typically within 3 mo of entry into the colony. Therefore, our epidemiologic investigation focused on the vasectomized male colony. The dirty-bedding sentinels monitoring the rack housing the vasectomized male colony were all MPV negative. However, when euthanized and tested, 5 of the 11 vasectomized male mice were found to be MPV1-positive by ELISA, MFIA, IFA, and MLN PCR (Table 1). We therefore concluded that the most probable origin of the MPV1 contamination was the investigator's colony.

Freshly obtained fecal pellets obtained from the rederived male and 2 female pups underwent PCR analysis to determine whether the pups were shedding MPV. The assay was MPV-negative (Table 1). The male and one female pup were anesthetized with isoflurane and bled from the retroorbital sinus at 6 wk of age; the remaining female pup was euthanized at 7 wk of age because of hydrocephaly, and its blood and MLN were harvested. Serologic analysis by ELISA, MFIA, and IFA showed all 3 pups to be MPV1-positive (Table 1). PCR assay of the MLN from the hydrocephalic female pup was MPV-negative (Table 1). Two female ICR mice were cohoused as contact sentinels with the remaining MPV-seropositive male and female pups for at least 3 wk. Both contact sentinels were euthanized and their sera tested by ELISA; both were MPV-negative (Table 1). The remaining transgenic female pup subsequently was found dead; its carcass was too autolyzed to obtain samples for testing.

At approximately 7.5 mo of age, the surviving transgenic male mouse was retested serologically and by fecal PCR. Both tests were MPV-negative. To propagate the line, the transgenic male was rotated among 5 C57BL/Ka female mice obtained from the Stanford breeding colony. An additional 2 CD1 female mice that served as contact sentinels were cohoused with the male mouse for at least 3 wk. After confirmation that the transgenic strain was successfully rescued, the C57BL/Ka female mice, the CD1 contact sentinels, and 8 nontransgenic progeny were euthanized and screened for MPV by ELISA. None of these mice had seroconverted to MPV (Table 1). In addition, the MLN of the C57BL/Ka female mice were tested by PCR assay for MPV; all were negative. The transgenic male mouse was euthanized at approximately 14.5 mo of age and retested by serology and MLN PCR. Both tests were MPV negative.

Discussion

Surrogate mothers of rederived pups are routinely checked for pathogens by direct examination, serologic analysis, and, if required, PCR assay. For economic reasons, we do not routinely test the progeny when the surrogate mother is found to be pathogen-free. In the presented case, the surrogate female mouse was MPV1-positive both by serology and PCR analysis of its MLN. Because the transgenic line was valuable, the pups (1 male, 2 female) were tested for MPV by serology and pooled fecal PCR assay. The data showed that, like the surrogate, they were MPV1-positive by serology but MPV-negative by fecal PCR. One female pup's MLN were MPV-negative by PCR assay at 7 wk. The male mouse's MLN were tested at 14.5 mo and were MPV-negative. There are 2 hypotheses for the cumulative results. First, the transgenic pups were infected and seroconverted, but a productive infection either did not occur or the infection was cleared. This scenario could explain the absence of MPV in the MLN and the lack of fecal shedding. This transgenic line was on a B6 genetic background, which is resistant to MPV infection.[7,10,33] The second hypothesis is that the positive serologic results represent antibodies that were transferred to the pups from the MPV-infected dam, that is, maternal antibodies.[4] The fact that as the seropositive male mouse aged it became seronegative suggests either the passive transfer and

Table 1. Results of MPV serologic and PCR tests of samples

Sample date	Mice evaluated	ELISA	MFIA (titer)	IFA	PCR Fecal	PCR MLN	Comments
6/19/09	Surrogate dam	+	+ (26)	+	nd	+	MPV1+
7/01/09	Embryo-transfer rederived male and female (nos. 1 and 2) pups	nd	nd	nd	—	nd	Pooled fecal sample
7/07/09	Embryo-transfer rederived male pup (age, 6 wk)	+	+(20)	+	nd	nd	MPV1+
7/07/09	Embryo-transfer rederived female pup no. 1 (age, 6 wk)	+	+ (17)	+	nd	nd	MPV1+
7/10/09	Embryo-transfer rederived female pup no. 2 (age, 7 wk)	+	+ (25)	+	nd	—	Euthanized for hydrocephaly, MPV1+
7/14/09	Vasectomized male mice (n = 11)	+	+ (25, 26)	+	nd	+	5 of 11 male mice were MPV1 +
8/28/09	ICR mice (n = 2)	—	nd	nd	nd	nd	2 contact sentinels cohoused with embryo-transfer rederived male and female (no. 1) for at least 3 wk
1/14/10	Embryo-transfer rederived male mouse (age, 7.5 mo)	—	nd	nd	—	nd	Retesting
1/20/10	C57BL/Ka (n = 5)	—	nd	nd	nd	—	5 female mice that mated with embryo-transfer rederived male mouse
2/10/10	CD1 (n = 2)	—	nd	nd	nd	nd	2 contact sentinels cohoused with embryo-transfer rederived male mouse for at least 3 wk
8/10/10	Progeny (n = 8) of C57BL/Ka × embryo-transfer rederived male mouse	—	nd	nd	nd	nd	
8/10/10	Embryo-transfer rederived male mouse (age, 14.5 mo)	—	nd	nd	nd	—	Euthanized

+, positive; –, negative; nd, test not done
ELISA was performed inhouse by using commercial ELISA plates and reagents. MFIA, IFA, and PCR were performed at a commercial diagnostic laboratory.

loss of maternal antibodies or the difficulty in detecting MPV antibodies in older mice. In addition, the presence of maternal antibodies could have afforded some protection of the pups from infection from the dam.[27] We did not test whether the mother was actually shedding MPV.

The investigator's goal was to rescue the transgenic line that was reduced to a single male mouse. However, this goal had to be accomplished without compromising the health status of the investigator's colony or Stanford's general mouse population. Despite evidence suggesting that MPV was not being shed, given the insidious nature of MPV, we took extra precautions before releasing the transgenic line to the investigator. These precautions included the use of additional contact sentinels and the testing of the female mice mated with the transgenic male mouse and a subset of their progeny. Importantly, the investigator was able to rescue the transgenic line and continue his research during this time instead of repeating the importation and embryo transfer process immediately after the initial serologic results. No sentinel in the investigator's room has seroconverted to MPV in the 2 y since the release of the transgenic line.

An epidemiologic investigation into the source of the MPV contamination was conducted. Hypotheses included the embryos themselves, environmental contamination (that is, quarantine room), or a source within the investigator's colony. The embryos were obtained from an MPV-positive colony at the donor institution. Infection of a recipient female mouse can occur when embryos are harvested from female mice that are viremic.[1,6] The risk of transmission is minimized (but is not eliminated) when the embryos are washed extensively prior to transfer into the recipient female.[1,6] Due to the small number of embryos received, they were not tested for MPV. Therefore we cannot eliminate the embryos as the source of the MPV contamination.

The most likely source of the MPV contamination was the investigator's colony, given the recent history of identifying an MPV-seropositive sentinel and the subsequent identification of 45% (5 of 11) of the vasectomized male mice in the investigator's colony as positive for MPV1. Although we surmise the surrogate female mouse was infected during mating with an MPV-infected vasectomized male mouse, the reverse is equally likely, that is, the pool of female mice used to generate the pseudopregnant female mice were infected first, and they subsequently infected the vasectomized male mice. It is unclear why the dirty-bedding sentinels on the rack housing the vasectomized male mice failed to detect the contamination, but this finding accentuates the shortcomings of dirty-bedding transfer sentinels, as indicated by other investigators.[5,20,35]

This case report highlights 3 main points. First, it reemphasizes the importance of testing surrogate female mice after weaning of embryo-transferred rederived pups and, if necessary, the rederived pups themselves, to confirm the absence of pathogens. Not surprisingly, many investigators are resistant to incur the additional cost of pathogen testing, which at our institution can approach as much as 50% of the rederivation service itself. Second, a clean source of pseudopregnant female mice is crucial for any rederivation effort. We now recommend that all pseudopregnant female mice be obtained from barrier-held mice or commercially from approved vendors. In addition, when an investigator is providing transgenic mice to other investigators, the colony of vasectomized males are under enhanced surveillance that includes contact

sentinels. Last, investigators should be aware that MPV maternal antibodies or nonproductive MPV infections can confound the interpretation of positive serologic results. We recommend that tests for MPV shedding by fecal PCR or of MPV transmission by the use of contact sentinels (or both assays) be performed before concluding that rederivation must be repeated. Because of the insidious nature of MPV, we also recommend that even when these test results are negative, a cautious approach is warranted with regard to the introduction of the presumably MPV-negative strain into the general mouse population, preferably by focusing on introducing serologically negative descendents.

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