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Case Study: Mouse Parvovirus Outbreak Likely Caused by a Contaminated Commercial Lyophilized Antibody Powder

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Summary

An MPV-contaminated lyophilized antibody product obtained from a commercial vendor was the probable cause of an outbreak of mouse parvovirus (MPV) in an academic research institution. The outbreak was initially discovered by the seroconversion of the mouse sentinels receiving soiled bedding from the affected cage(s). After further investigation, a suspected antibody product was submitted to a diagnostic laboratory and the sample tested positive for MPV via polymerase chain reaction (PCR). To confirm administration of this product to mice could produce MPV infection, we inoculated the MPV-positive antibody product into experimental mice (n=5). We collected faecal pellets at Days 0, 5, 9, 12, and 14 post-inoculation. At the end of the experimental period, we collected mesenteric lymph nodes (mLN) and submitted both mLN and faecal pellets for MPV analysis via PCR. While all faecal pellets were negative for MPV, we were able to detect MPV in mLN from one of the five mice, thus replicating the likely method of transmission and the cause of the MPV outbreaks.

Introduction

Mouse parvovirus (MPV) is a small, non-enveloped single-stranded DNA virus. Murine parvoviruses may affect a variety of studies, particularly investigations of immunology, transplantation, hematopoiesis and oncology (*Janus et al., 2012)*. Non-enveloped viruses, parvoviruses are remarkably resistant to conditions like heat, desiccation, and acidic and basic pH values demonstrating a high degree of environmental stability (*Janus et al., 2012)*.

MPV has a history of being one of the more common viral contaminations reported in animal facilities (*Carty et al., 2008; Jacoby et al., 1998; Mähler & Köhl, 2009; Pritchett-Corning et al., 2009*). In 1998, a survey indicated that between 10% to >30% of institutions reported their mouse colonies were positive for coronaviruses, parvoviruses, and ecto- and endoparasites (*Jacoby et al., 1998*). More recently, in a 2008 survey, the viral diseases of mice reported most frequently (in decreasing order) were MPV, mouse hepatitis virus (MHV), minute virus of mice (MVM), and mouse rotavirus (MRV, a.k.a. EDIM) (*Carty et al., 2008*). Infectious agents can enter colonies via incoming rodent shipments, in unscreened biological materials, on people (especially husbandry or

investigative staff), or by introduction of contaminated food, bedding material or other fomites (*Compton et al., 2012; Labelle et al., 2009; Lipman et al., 2000; Watson J, 2013*). According to the 2008 survey, fifteen percent of the 35 respondents reported that biological materials were responsible for outbreaks of disease in their colonies (*Carty et al., 2008*).

Case Study

The animal holding room in this case study was used only to house mice assigned to a single investigator. The investigator's research goal was to provide new insight into the pathophysiology of both graft versus leukemia reactivity and graft versus host disease (GVHD) in order to develop better strategies for enhancing the therapeutic index of allogeneic bone marrow transplantation. The room had a history of periodic (approximately once per year) MPV outbreaks dating back more than 5 years. The room contained 3 racks of static microisolation cages. One rack was dedicated to experimental animals and the two remaining racks to animals being held for experimentation. The animals in holding served as the source of experimental animals, while the same investigator's in-house breeding colony located in an adjacent vivarium as well as animals from Jackson Laboratories (Bar Harbor, ME, USA) were the sources of mice for the holding racks. Each MPV outbreak was diagnosed through serologic conversion of soiled bedding sentinels in the room, but only sentinels from the experimental racks ever seroconverted. Neither sentinels from the holding racks nor from the investigator's breeding colony ever seroconverted to MPV. Furthermore, follow-up serology and PCR analysis of faecal pellets from randomly selected colony animals on both the holding racks and the experimental rack (at least 10% of the total cages in the room) failed to confirm the presence of MPV during any of the diagnosed outbreaks. After each episode of sentinel seroconversion to MPV, the room was placed in quarantine. The room was allowed to depopulate through normal experimental attrition. Once emptied, the room was decontaminated by a ceiling to floor spray application of a chlorine dioxide solution.

After the experimental rack's sentinel cage seroconverted again to MPV during the most recent outbreak, it prompted a more detailed investigation of the materials administered to the animals in the course of the research. This search revealed that the experimental mice had been dosed with several different types of monoclonal antibodies obtained from external sources. These antibodies were procured from other academic research institutions as well as from commercial vendors and all were of murine origin. Subsequently, aliquots of each antibody solution used were submitted to a diagnostic laboratory (IDEXX BioResearch, Columbia, MO, USA) for MPV screening via PCR. One such sample, an IgG1 antibody, produced by the mouse ascites method at a commercial laboratory (anonymous) tested positive for MPV.

Given these results, we concluded the administration of MPV-contaminated antibody to study mice had been the likely cause of the intermittent MPV infections in this room. To confirm this, we set out to determine if MPV replication and shedding occurred in animals that had been dosed with the suspect antibody. To this end, we collected faecal pellets at designated time points during a study and mesenteric lymph nodes at the end of a study from a group of experimental mice that received the antibody as part of the scheduled protocol. These samples were then submitted to the diagnostic laboratory for PCR testing.

Materials and Methods

Animal housing and care

Experimental, colony, and sentinel mice were housed in static microisolation cages (model JAG75, Allentown Caging Inc., Allentown, NJ, USA) bedded with hardwood chips (SaniChip®, PJ Murphy Forest Products, Montville, NJ, USA), enriched with shredded paper (Enviro-Dri®, Shepard Specialty Papers, Watertown, TN, USA), and placed on Metro® racks (InterMetro Industries Corporation, Wilkes-Barre, PA, USA). They were maintained under controlled environmental conditions (14:10-h light:dark cycle, an average daily relative humidity of 35%, and a temperature range of 20.5-21.7° C (69-71° F). The mice were fed 5LOD diet (PMI Nutrition International, Brentwood, MO, USA) *ad libitum* and given acidified, autoclaved municipal water in bottles. All cage supplies (bottoms, wire bar lids, water bottles, and microisolation tops) were sanitized prior to use. Sanitization was accomplished by washing the cage bottom and lids, wire bar lids, and water bottles in a Getinge tunnel washer model 2236 (Getinge Life Science Americas, Lake Mary, FL, USA), utilizing a citric acid cleaner (Labsan 230C, Sanitation Strategies, Holt, MI, USA) with a final rinse with water at 82.2° C (180° F). Cage bottoms were changed at least once per week, wire-bar lids at least once every 12 weeks, and microisolation tops at least once every 24

weeks. All cage manipulations and changes were performed in a HEPA filtered, small animal cage changing station (model NU-612, NuAire Inc., Plymouth, MN, USA). The hood was disinfected between each cage using a chlorine dioxide solution (Labsan™ C-Dox, Sanitation Strategies, Holt, MI, USA). Nitrile gloves (High Five Products Inc., Chicago, IL, USA) were worn and changed between each cage handling procedure (including cage changes and faecal pellet collection) of the experimentally infected cages. All activities in this study (including, but not limited to, irradiation, GVHD-induction, and antibody administration) were approved by the Medical College of Wisconsin institutional animal care and use committee. The animal care and use program at the Medical College of Wisconsin is accredited by AAALAC International.

Routine disease surveillance program

Three week-old female Crl:CD1(ICR) mice, procured from a commercial vendor, were housed in pairs in static microisolation cages identical to that used for colony animals. One sentinel cage was placed on each cage rack unit, which achieved a ratio of approximately 50 colony cages per sentinel cage in the affected animal room. At each scheduled cage change, at least 1 teaspoon of soiled bedding from each colony cage (including experimental cages) was placed in the designated sentinel cage. The sentinel cages received soiled bedding only from investigator cages on the same rack. The animals were housed in this manner for a minimum of 12 weeks prior to sample collection and replacement with new sentinels. Every 12 to 16 weeks, sentinel animals were tested for seroconversion to MHV, murine norovirus (MNV), MPV, MRV, MVM, *Mycoplasma pulmonis,* Sendai virus, and Theiler's murine encephalomyelitis virus. The sentinels were also tested for the presence of fur mites by microscopic evaluation of the pelt or by PCR, and pinworms by faecal flotation or PCR. Annually, in addition to the previously listed agents, sentinel animals were also tested for seroconversion to cilia-associated respiratory bacillus, ectromelia virus, *Encephalitozoon cuniculi*, lymphocytic choriomeningitis virus, mouse adenovirus type 1 (FL) and type 2 (K87), mouse cytomegalovirus, mouse thymic virus, pneumonia virus of mice, polyoma virus, Prospect Hill virus, and reovirus. No bacteriological monitoring is performed as part of the routine rodent health monitoring program. The sentinels have consistently tested positive for MNV, intermittently positive for MPV as described above, and negative for all other agents.

Antibody product information

According to the company's product information, the antibody was a mouse IgG1, κ(MOPC-21), clarified ascites. It was provided lyophilized from 0.01 M phosphate buffered saline, pH 7.2, with no preservatives added. The MOPC-21 tumor line that produced the mouse IgG1,κ was a mineral oil induced plasmacytoma originated and carried intraperitoneally in BALB/c mice. The hapten binding specificity of the MOPC-21 line is unknown. The ascites produced from the MOPC-21 tumor line contained IgG1,κ in addition to normal levels of other mouse immunoglobulin and serum proteins. The ascites fluid was clarified by centrifugation and filtration. Each vial contained at least 5 mg of mouse IgG1, κ myeloma protein, determined by densitometry of electrophoresed ascites fluid (*Mouse IgG1 Product Information, anonymous, 2003*).

Animal Model Preparation

The investigator's mouse model was prepared by an initial total body irradiation (TBI), followed by bone marrow transplant (BMT) that would elicit GVHD, and the subsequent administration of the antibody product.

TBI: Five, group-housed, male BALB/c J (H-2^d) mice were conditioned with a TBI of 900cGy administered as a single exposure using a Shepherd Mark I Cesium Irradiator (J.L. Shepherd and Associates, San Fernando, CA, USA). Mice were placed in a plastic pie cage with partitions so that each mouse received the same dose. After irradiation, the mice were returned to their home cages.

Bone Marrow Transplantation and GVHD Induction: Bone marrow (BM) was obtained by flushing femurs and tibias of male C57BL/6J (H-2^b) mice, with Dulbecco's modified media and passing through sterile mesh filters to obtain single cell suspensions. The C57BL/6J spleen cells were passed through sterile mesh screens to obtain single cell suspensions and analyzed by flow cytometry to determine the percentage of T cells. GVHD was elicited by a single intravenous injection used to deliver 10 x 10^6 C57BL/6J BM cells plus 0.7 x10^6 C57BL/6J T cells to each irradiated BALB/c J mouse.

Antibody Administration: Mice were administered a purified monoclonal antibody as part of the research project (to determine if this antibody could prevent GVHD). As a control, an IgG1isotype antibody

was obtained from a commercial supplier (anonymous). The powdered antibody preparation was initially reconstituted using sterile (0.2 micron filter) reverse osmosis water, to a concentration of 5 mg/mL and then further diluted to a working solution of 0.75 mg/mL using sterile PBS. Each mouse received 150 µg/dose, three times per week for 2 weeks via intraperitoneal injection beginning on day 7 post-transplant.

Faecal pellet collection

Faecal pellets were collected for PCR analysis for MPV. At least 1 faecal pellet was collected per sampling period directly from each mouse's rectum and pooled into a single container for that time point. Collections were performed at time 0 (baseline, prior to TBI and antibody solution administration) and then twice weekly for 2 weeks (Days 5, 9, 12, and 14) after initiation of antibody solution administration. The baseline and each time point sample were stored frozen in a -20° C freezer. At the end of the study period, the samples were submitted to IDEXX BioResearch for MPV analysis via PCR.

Collection of mesenteric lymph nodes

Twenty-one days post transplantation (14 days post-antibody solution initiation) mice were euthanized by cervical dislocation and mesenteric lymph nodes (mLN) were collected. mLNs were placed into cryovials and immediately frozen (-20° C) and submitted to IDEXX BioResearch for analysis.

Real-time PCR MPV testing

Total nucleic acids were extracted from 50 µL of reconstituted antibody solutions, mouse faecal pellets or mesenteric lymph nodes with standard protocols using a commercially available platform (One-For-All Vet Kit, Qiagen, Valencia, CA, USA). The MPV PCR tests were based on the IDEXX BioResearch proprietary service platform (IDEXX Laboratories, Inc., Westbrook, ME, USA). Briefly, the MPV real-time PCR assay targets a region of the VP2 gene conserved among all MPV genomic sequences deposited in GenBank and utilizes a FAM/TAMRA labeled hydrolysis probe. Hydrolysis probe-based real-time PCR assays targeting a mammalian gene (18S rRNA) or bacterial gene (16S rRNA) were used to ensure DNA recovery and the absence of PCR inhibitors in nucleic acid extracted from mesenteric lymph node and antibody test samples or faecal samples, respectively. Real-time PCR was performed with standard primer and probe concentrations using a commercially available mastermix (LC480 ProbesMaster, Roche Applied Science, Indianapolis, IN, USA) on a commercially available real-time PCR platform (Roche LightCycler 480). The copy number estimate of MPV DNA given to each mouse was calculated by plotting the real-time crossing point (Cp) values from the MPV PCR assay of the working stock antibody solution on a standard curve of log-fold dilutions of a known copy number positive control and accounting for volume of material tested and the amount of antibody given to each mouse.

Diagnostic Results

It was estimated that each experimental mouse received 2.7 x 10^4 copies of MPV DNA in each 150 µg dose of antibody solution. The faecal pellets for the experimental cage (5 mice) collected at each individual time point (days 0, 5, 9, 12, and 14) tested negative for MPV by PCR. For the mesenteric lymph nodes (collected at day 14), 1 of 5 mice tested positive for MPV by PCR.

Discussion

The history of repeated MPV outbreaks on a single investigator's rack that housed mice dosed with an MPV positive commercially available antibody strongly implicated the antibody as the source of these outbreaks. The amount of MPV present in the lot of the commercial antibody used in this study is one factor that likely led to detecting MPV DNA in the mesenteric lymph nodes from only 1 of 5 experimental mice dosed. We estimated that each mouse received 2.7 x 10^4 copies of MPV DNA, but as PCR cannot determine the difference between infectious and noninfectious virus, the dose of MPV each mouse received was at or below 2.7 x 10^4 virions which is near the minimum infectious dose of MPV. In a previous study (*Besselsen, et al., 2000*), MPV DNA was detected in the mesenteric lymph nodes from one of five 12 week-old BALB/c mice given a 5 x 10^5 $TCID_{50}$ dose of MPV which is consistent with our experimental outcome. While the current vials of the antibody product had been from the same box and lot used during the past several MPV outbreaks, we cannot confirm which lots may have been purchased prior to this one. Therefore, we were not able to test and estimate the amount of MPV in the previously used vials of antibody that were associated with the earlier MPV outbreaks. It is possible that the dose of MPV was higher in these lots than in the one we tested. Another factor that may have influenced the

low detection of MPV in the antibody dosed mice of our study was the number of doses of antibody received compared to the prior studies conducted by this investigator linked to the MPV outbreaks.

The specific experiment involving the animals from which we collected faeces and lymph nodes required the animals to receive thrice weekly doses of the antibody for 2 weeks rather than the thrice weekly for a 4-week schedule used in prior experiments conducted by this investigator. As such, the mice used in our study received half the number of doses of the MPV contaminated antibody as the mice used when the MPV outbreaks were observed, which may have led to the low number of MPV-positive mice observed in this case study. We did not have the opportunity to sample animals receiving the usual number of antibody doses because the investigator discontinued the use of the suspect antibody in any subsequent studies. Furthermore, we note that previous detections of MPV-positive sentinels were found after the investigator had performed the 4-week protocol.

While there are other potential sources of MPV contamination to consider such as food, bedding, water, caging supplies, vendor-contaminated sentinels, etc., in this case, the MPV-contaminated antibody is the most likely source given the pattern of seroconversion of sentinel animals. The only sentinel animals to seroconvert to MPV in the entire animal facility were located on the single experimental rack in this one animal holding room. The animals on this rack were the only ones that had been administered the MPV-contaminated antibody product. Since the animal care practices, including food, water, bedding and caging sanitation, were the same for all mice in this particular facility, if one of these was the source of MPV contamination, one would expect seroconversion of other sentinels on racks in the same room as well as in other mouse rooms. In addition, since the investigator switched antibody product vendors to an antibody that is free of MPV contamination, there has not been a single outbreak of MPV for over 3 years.

This outbreak scenario highlights the importance of having a robust assessment of all biological materials that are to be introduced into the animals. The recommendations for screening of biological materials for pathogens are reiterated in both the Guide for the Care and Use of Laboratory Animals (*National Research Council, 2011*) and in the FELASA recommendations on rodent and rabbit colony health monitoring (*Mähler, et al., 2014)*. This assessment may range from reviewing the pathogen screening results from the vendor to submitting aliquots of the biological materials in question to a diagnostic laboratory. When these outbreaks occur, the costs, in both time and labor, of diagnosis, quarantine and eradication are considerable. The vivarium or veterinary staff are often tasked with investigating the source of infection by testing investigator animals and submitting those samples to a diagnostic laboratory in the hope of finding positive results. The suspect rooms are typically placed in quarantine and consequently may have increased personal protective equipment requirements as compared to standard procedures. In addition, there are often restrictions on the movement of animals within the institution and on shipping them to other institutions. Hence, scientific collaborations can be hindered.

With the history of the MPV outbreaks, in conjunction with the MPV-positive results from the antibody aliquot and the subsequent MPV-positive mLN post-inoculation of an experimental animal, we are confident in a cause-effect relationship of these outbreaks. As a result of this case, commercial antibody preparations administered to animals should be considered potential sources of MPV infections in mouse colonies.

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