Management of Rodent Viral Disease Outbreaks: One Institution’s (R)Evolution

Abigail L. Smith

Abstract
At first blush, an outbreak of mouse hepatitis virus or epizootic diarrhea of infant mice virus in a research colony of laboratory mice may not seem like a “disaster.” However, irrespective of magnitude, such an outbreak at an academic institution is disruptive for researchers at all levels. It can be a disaster for the graduate student who may have just a few experiments to finish before writing the thesis or for the postdoctoral fellow who is in the lab for only 1 or 2 years. Infectious disease outbreaks also limit the ability of principal investigators to share their animals with collaborators at their home institution as well as with those at extramural sites, thereby thwarting the expectation that research materials supported by federal funds will be made readily available to colleagues. This article traces the evolution of a change in “culture” at a large, well-funded academic institution with over 1,800 active IACUC protocols, more than 1,000 of which include mice. During a period of less than 5 years, the institution evolved from virtual paralysis in the face of such outbreaks to the implementation of policies and practices that enable effective outbreak management and the timely resumption of research functionality. This evolution required not only support from the highest levels of leadership in the university and its school of medicine but also a huge outlay of financial resources.

Key Words: biosecurity; epizootic diarrhea of infant mice (EDIM) virus; mouse hepatitis virus (MHV); outbreak management; quarantine

Introduction

Between 2004 and 2008 the University of Pennsylvania contended with four viral outbreaks in various laboratory rodent facilities, both barrier-maintained and conventional; two took place in the School of Medicine (SOM1: 2004-2005 and 2008), one in the School of Veterinary Medicine (SVM; 2005), and one in the School of Arts and Sciences (SAS1; 2007). The first of the SOM outbreaks is described in detail, followed by a discussion of impacts and policy implementation in the subsequent SVM, SAS, and SOM outbreaks. Each incident strained resources and led to important adaptations of policy.

The Two Viruses

The outbreaks were of two fairly common murine viruses. Mouse hepatitis virus (MHV1) is a coronavirus that infects wild and laboratory mice. Although the name suggests a single agent, it is actually a group of viruses that, due to their RNA genomes and high mutation rates, comprise multiple strains. Although MHV does not cause clinical signs in most mice, it can induce functional defects in the immune system and interfere with tumor biology and many other types of studies (see Barthold and Smith 2007 for a review). There are two basic, partly overlapping biotypes: (1) respiratory and (2) enterotropic/enterotropic-polytropic; the latter is the one most frequently detected in contemporary laboratory mouse colonies and is transmitted via the fecal-oral route (Barthold and Smith 2007).

Epizootic diarrhea of infant mice (EDIM1) virus is a group A rotavirus that shares with MHV the fecal-oral route of transmission and causes clinical disease only in mice less than 2 weeks of age, although mice can be infected at any age (Ward et al. 2007). Rotaviruses are generally highly transmissible among members of the natural host species and, as nonenveloped viruses, are very stable in the environment (Ward et al. 2007).

Mice that are immunodeficient (e.g., by virtue of genetic or sustained pharmacologic intervention) do not clear either virus and shed high concentrations of infectious virus in feces.

Institutional Practices and Accreditation

The following broad definitions apply to rodent vivaria at the University of Pennsylvania. A barrier-maintained facility may

Abigail L. Smith, MPH, PhD, is a professor of pathobiology and Associate Director of University Laboratory Animal Resources at the University of Pennsylvania School of Veterinary Medicine in Philadelphia.

Address correspondence and reprint requests to Dr. Abigail L. Smith, Department of Pathobiology and University Laboratory Animal Resources, University of Pennsylvania, 3800 Spruce Street, Suite 177E OVQ, Philadelphia, PA 19104 or email abigail4@upenn.edu.
contain either static, filter-topped cages or cages on ventilated racks; both types are supplied with sterilized food and bedding (corn cob unless there are special experimental needs). Animals may not return to a barrier facility once they have been removed. Conventionally housed rodents are housed in static filter-topped cages on conventional racks; food and bedding are not sterilized and rodents may be removed from these vivaria for studies in laboratories or core areas (such as imaging facilities) and then returned to their home vivaria. Water treatment varies by facility. Water temperature in cage washers is monitored continuously and autoclaves, where present, are validated monthly. All rodent housing rooms contain change stations or laminar flow biological safety cabinets for husbandry and experimental manipulations.

Each school at the University of Pennsylvania is responsible for the purchase and repair of capital equipment in its vivaria. Although the institution’s experiences with the outbreaks described resulted in some changes in equipment and supplies, the focus of this article is on the significant adaptations to policy and practice rather than on these particulars.

The SOM program has been accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International since 1993.

School of Medicine Multifacility Outbreak #1: MHV, 2004-2005

MHV was introduced via at least two shipments of infected mice from an approved vendor into five rooms of two suites (of six in the facility) in a barrier-maintained vivarium2 plus two rooms in a conventional facility. There was a delay in notification from the vendor and by then many animal relocations—room to room and facility to facility—had occurred, ultimately affecting multiple rooms in three large SOM rodent vivaria. One of the three (the only one discussed here) was “barrier-maintained” (ventilated rack housing with feed and bedding autoclaved into the facility and an automatic, reverse-osmosis water system), but change stations/biological safety cabinets, installed when the facility was commissioned, had been removed in order to accommodate more cage space in all of the housing rooms. Thus, at the time of the outbreak, cages were being manipulated on open counter tops, often in shared procedure rooms.

In addition, once the outbreak was recognized, many investigators were unwilling to cull mice from their populations and laboratory staff access to the affected housing rooms was not restricted. However, investigators and their staff were asked not to remove cages from rooms in which seropositive mice had been identified.

Management of the outbreak initially entailed a test-and-cull program based on serologic testing of one mouse per cage in each confirmed positive room, irrespective of the proportion of cages that contained seropositive mice in any particular room. If one room in a suite contained seropositive mice, the entire suite was placed under “quarantine” (which dictated traffic flow) and mice in 25% of the cages in that suite’s “negative” rooms were tested. Because of the magnitude of the outbreak, University Laboratory Animal Resources (ULAR) staff could not bleed all the animals that had to be sampled and enlisted members of investigators’ laboratory staff to help with collecting blood from their mice. Although requested, information about the immunologic phenotypes of mice (which are admittedly not always known) was not communicated by many investigators.

Transmission

Traffic of Mice among Facilities

At the time of this outbreak there was no process (such as scanning bar codes) for tracking movement of cages within or between facilities. Humans and mice were permitted to move directly (without any testing) from barrier to barrier, barrier to conventional, or conventional to conventional areas; the only restriction was on movement from conventional to barrier housing. The policy was—and is—that mice destined for relocation from a conventional to a barrier-maintained facility must transition through the quarantine facility used for importation of rodents from nonapproved sources; however, tracking such moves was problematic. As indicated in the section below on SOM Outbreak #2, such interfacility movement may have contributed to the spread of MHV and EDIM virus in 2008.

Biosecurity

As mentioned, the barrier facility did not contain change stations or biological safety cabinets at the time of this outbreak. The other two affected SOM facilities, one entirely conventional and the other partly conventional and partly barrier, also did not have this equipment in place.

Based on what is known about the biology of contemporary MHV strains, the absence of change stations probably played a major role in the wide dissemination of the virus.

Resolution

After several frustrating months of continuing positive serologic reactivity, large populations of mice in affected facilities were culled and unique strains/genotypes rederived by embryo transfer at a commercial breeding facility. The university’s Office of Risk Management and Insurance paid for these services once faculty applications were approved by the vice dean for research and research training in the SOM. The cost of this endeavor exceeded $2 million. The cost to ULAR for sampling and testing during the course of the outbreak, estimated at more than $100,000, was not reimbursed.

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2This vivarium was designed based on a suite concept, with five to seven housing rooms and one procedure room in each suite.
Important Actions During and After the Outbreak

Formation of the School of Medicine Animal Research Committee

This MHV outbreak prompted the formation of the School of Medicine Animal Research Committee (SOMARC) as a policymaking body. SOMARC’s members, senior faculty who have animals in the SOM vivaria, primarily use rodents but also include some investigators who use large animals (e.g., sheep, swine, nonhuman primates). Each rodent housing facility has at least one representative, who chairs a facility-specific user group that meets more or less regularly, whether on a monthly schedule or only when there is a significant event or situation requiring a time-sensitive response (such as the detection of an outbreak).

The first SOMARC chair was the SOM vice dean for research and research training. The committee has subsequently been chaired by an individual holding a newly created position, the associate (or assistant) dean for animal research in the SOM. The ULAR director and leadership responsible for husbandry and facilities and for diagnostic services (rodent health surveillance, outbreak investigation, and rodent importation, quarantine, and export) are ex officio members and provide information and guidance to the committee during policy deliberations. In addition, both the chair and the director of animal welfare of the institutional animal care and use committee (IACUC) regularly attend SOMARC meetings. The functions of the SOMARC, ULAR, and IACUC are complementary but administratively separate.

Development of New Policies by the SOMARC

The SOMARC chair is to be notified by the associate director of ULAR Diagnostic Services within 24 hours of a suspected or confirmed MHV reaction. ULAR then presents a comprehensive outbreak management plan (OMP) for the facility to the SOMARC within 48 hours of that notification and to the user group of the affected facility within about 4 business days. The OMP is revised on a continuous basis pursuant to results of further testing.

Quarantine: Phase I

A room that has had a confirmed positive test is immediately placed on quarantine, and if it is in a facility with a suite design the entire suite is placed on quarantine. Under the initial policy, faculty could euthanize mice in positive cages or mark cages for euthanasia by ULAR personnel; the policy has changed based on restricted access of investigators’ lab staff to quarantined rooms (see section below on Resulting Refinements). Breeding must be suspended (males and females separated) and neonates euthanized to minimize the number of new susceptible animals introduced into the population. For the same reason, shipments of mice from outside sources (approved vendors) destined for quarantined rooms are cancelled if at all possible; there is a central ULAR procurement office through which all animal orders are placed, so this process is relatively easy to control. If the time frame does not permit the cancellation of an order (e.g., animals are already in transit), principal investigators (PIs) are encouraged to use the mice acutely in their laboratories rather than housing them in another vivarium or room—as illustrated below in the section on SOM Outbreak #2, the housing of an investigator’s animals in multiple rooms increases biosecurity risks.

ULAR Diagnostic Services identifies rooms that house mice belonging to investigators who have mice in the quarantined room(s) and tests all sentinels—or, depending on the perceived risk, one mouse per resident (nonsentinel) cage—in the “collateral” location(s). Although the original policy called for the random testing of 25% of cages in all other rooms (in all facilities) that house cages of PIs from the primary infection site, ULAR modified that to cover 100% of such cages. One mouse in every cage in the quarantined room is sampled, either by bleeding or the collection of fecal pellets for reverse transcription–polymerase chain reaction (RT-PCR) in the case of known or suspected immunodeficient mice. It is important to avoid relocating cages (or mice) either within a quarantined room or to/from rooms under quarantine for MHV. All cages and supplies to be removed from a quarantined room are bagged and autoclaved before transport to the dirty cage wash area.

Quarantine: Phase II

Two consecutive negative screens of a quarantined room are required as evidence of clearance; thus if a 100% sampling of cages in the quarantined room yields no positive cages, 100% of the cages are resampled approximately 21 days after that screen (3 weeks was chosen as a reasonable interval between exposure/infection and expected seroconversion to MHV). If either screen reveals positive cages, the PI to whom the mice belong is notified and the cage(s) culled. The 100% tests continue every 3 weeks until two consecutive screens are 100% negative.

Quarantine: Phase III

The SOMARC policy states the following:

If the results of Phase II testing show that there are still positive animals, the room and/or suite will be depopulated and essential mice relocated to another facility or entity, as determined by the SOMARC. Essential mice are defined as unique strains that cannot be purchased or

3Based on experience with occasional false-positive results, the ULAR associate director has made it a practice to confirm positive reactions before notifying the SOMARC chair.
rederived from another source, or ongoing experiments that are not possible to repeat in a reasonable time frame. Exceptions to this policy will be considered by the SOMARC on a case-by-case basis if Phase II results indicate that the vast majority of infected animals have been eliminated. In this instance, one additional test/cull phase may be permitted. The SOMARC and ULAR will jointly convene a meeting of all affected faculty to communicate the depopulation plan, including timelines, within 2-3 days of receiving results of Phase II testing. Investigators who have mice in a room that is scheduled for depopulation will identify essential mice for relocation and submit a request to the SOMARC which documents:

- A description of all essential strains
- Estimated number of animals per strain
- Estimated number of required breeding and non-breeding isolators
- Brief justification of why individual strains need to be preserved

The SOMARC will evaluate individual faculty requests and forward decisions to the faculty within 48 hours. Exceptions to this timeline will be identified in the OMP developed by ULAR in concert with the SOMARC.

Because satellite space in which to house animals from quarantined rooms is usually not available on the campus and it is considered very risky to continue to house mice potentially infected with MHV (or other viral diseases with similar biology) on campus, the SOMARC has generally approved the shipment of such mice to commercial rodent breeding facilities for rederivation, housing, and/or breeding. Once the chair of the SOMARC has notified the Office of Risk Management and Insurance of the committee’s approval, that office deals with the business processes and the ULAR export coordinator arranges for shipment of the animals to an off-site facility.

After room depopulation, ULAR husbandry staff decontaminate the room surfaces according to established standard operating procedures (SOPs) and coordinate the introduction of new mice into the room.

Collaborative Efforts by the SOMARC and ULAR

Enhancements to the Biosecurity Program

Several biosecurity initiatives were undertaken after the MHV outbreak was concluded. Most importantly, the SOM committed to purchasing change stations or biological safety cabinets and mandating their use for 42 rodent housing rooms in the nonsatellite SOM vivaria. The purchase of this equipment was phased in over about 18 months and in some cases required electrical upgrades. ULAR and research staff received intensive training to ensure proper use of the equipment. Each housing and procedure room in all (not just SOM) newly constructed rodent vivaria (three have been constructed, one is due to open in 2010) has since been outfitted with change stations or biological safety cabinets.

An additional policy change to improve biosecurity required serological testing for a basic panel of viruses (including MHV and EDIM virus) of one mouse per cage any time cages of mice were to be relocated from room to room (even within a suite). If the mice to be relocated were immunodeficient, they were housed for 3 weeks with contact sentinel mice, which underwent serologic testing before the planned relocation. The PI was required to bear the cost for the serologic testing.

Another major investment that resulted from SOMARC support was the purchase of additional semirigid isolators for the quarantine facility that houses rodents arriving from “nonapproved” sources (i.e., most noncommercial facilities). In 2004, the facility contained 29 large isolators that can accommodate up to 12 shoebox cages. The waiting time for rodent imports was frequently in excess of 6 months from the date of approval of health reports (necessitating, of course, updated health reports prior to receipt of the mice). Most investigators needed to import only one or two breeding pairs of mice, so the capacity of the large isolators was frequently wasted. Since the purchase of 45 “mini-isolators” (each accommodating four shoebox cages) and electrical upgrades, there have been no waiting lists for routine importations from nonapproved sources.

During the MHV outbreak, ULAR filled a newly created position, associate director for diagnostic services and rodent quality assurance. At the time, there was (remarkably) a single rodent health monitoring technician for all ULAR-managed rodent facilities on the campus—three major SOM vivaria plus smaller satellites with a total of about 18,000 cages, one relatively small SVM vivarium, and two small SAS facilities for which veterinary care and rodent health monitoring services were provided by ULAR on a contractual basis. Health monitoring was performed quarterly and was based on a soiled bedding sentinel program using young adult female outbred rodents in cages that were somewhat haphazardly placed, contained only one sentinel animal, and sometimes monitored entire racks or even multiple racks.

When the technician left to pursue an educational opportunity, two individuals were hired to replace her. One of the first things they noticed was how clean the bedding in the sentinel cages was! Discussions with animal caretakers revealed that they had aesthetic concerns about placing soiled bedding in the cages. Educational programs, quarterly staff meetings attended by all ULAR staff, and facility-specific husbandry staff meetings were necessary to impress upon caretakers the importance of transferring soiled bedding from colony cages to sentinel cages. It was a long process and reminders are still occasionally necessary but the situation is much improved.

The cost of the rodent health monitoring program has increased substantially as a result of several changes. For mice, two newly purchased sentinels are placed in each cage at the beginning of each calendar year and one cage is placed on each side of every rack in every facility for which ULAR is responsible. When each quarter 1 (Q1) sentinel is removed,
a newly purchased animal is placed in the cage and the cage mate of the Q1 sentinel is sampled in Q2, and so on. This approach ensures that there is always a second animal that can be sampled in the event of an unexpected result that requires confirmation. It also reduces the risk of missing a monitoring period if a sentinel is lost for any reason. The veterinary technicians, staff veterinarians, research staff, IACUC staff, and animal husbandry staff are all asked to inform ULAR Diagnostic Services if they notice that a sentinel animal or cage is missing.

Communication of Policies: Electronic, Intranet, Room Postings, and Public Presentations

Policies concerning procedures that are and are not permitted during outbreaks are communicated in a variety of ways. SOMARC-vetted policies appear on both the SOM and ULAR internal websites. Broadly applicable ULAR policies that are not school-specific are available on the internal ULAR site, either as SOPs or free-standing policies, and are generally also communicated via email if they pertain to new outbreaks. Certain ULAR policies are also posted on the IACUC website.

When quarantine is imposed due to an outbreak of infectious disease, laminated signs are posted on the doors of affected animal rooms, specifying the infectious agent and outlining approved and nonapproved practices for the rooms under quarantine.

In addition, at the request of the SOMARC chair the author developed a presentation that (1) summarizes the specific policies that pertain to outbreaks of viral infections, pinworms, and mites in vivaria that house laboratory rodents and (2) provides a brief overview of the university’s rodent biosecurity (health monitoring and incoming rodent quarantine) programs. All individuals who use rodents (based on IACUC protocol-listed personnel) in the SOM had to attend one of seven sessions over a 5-month period. Investigators and their staff are reminded to refer to the presentation on the ULAR website when user meetings are convened to discuss new outbreaks.

Subvention to Encourage Cryopreservation

The SOM instituted a subvention program to underwrite 50% of the cost of cryopreserving unique mouse strains by the school’s Transgenic and Chimeric Mouse Facility core. This measure (1) ensures that unique strains will not be lost during an outbreak or other catastrophic event, (2) has the potential to save money associated with per diem costs for strains kept “on the shelf” but not frequently used by investigators, and (3) conserves much-needed cage space. Although not explicitly stated, the letter offering this perquisite to SOM PIs implied that the school might not, in the future, assist financially with redervation of lines that become infected with viral agents such as MHV or EDIM virus.

New Policy Implementation by Other Schools on Campus

School of Veterinary Medicine MHV Outbreak, 2005

The SVM, which had a rodent user group led by one of the department chairs, sustained an MHV outbreak in June 2005, several months after the SOM outbreak concluded.

Q2 sentinel testing revealed three MHV-seropositive sentinel animals in two rooms of a single suite, and further testing revealed that 13 of 23 tested cages in one of these rooms (a single-investigator room) contained MHV-seropositive mice. Two weeks after detection of the first positive sentinels, testing of all remaining sentinels (cage mates of the previously tested Q2 sentinel mice) in the facility revealed three additional MHV-seropositive sentinels in the room with positive colony (nonsentinel) mice, resulting in the depopulation of the room. The second sentinel-positive room in the suite was depopulated at the end of July after the detection of seropositive colony mice earlier in the month. Remarkably, there was no indication of spread beyond these first two rooms. But because some investigators had mice in both rooms in the positive suite as well as in a room in another suite, colony mice in the second suite room were tested despite the fact that sentinels had been MHV seronegative. The sequence of testing and the serology results for this second suite are shown in Table 1.

ULAR leadership met with investigators housing mice in the positive room of the second suite to discuss the SOM policy and encourage the affected SVM investigators to implement it. The policy was to depopulate a room if a third round (which at that point had been exceeded) of testing detected positive cages after two sequential rounds of a test-and-cull approach failed. The investigators opted to depopulate by a combination of euthanasia and shipment to an off-campus site for redervation. New sentinel mice were ordered for the entire facility and underwent monthly serological monitoring for MHV until the next scheduled quarterly testing. No further positive mice were detected and the facility remained MHV negative until it was depopulated for the commissioning of a newly constructed facility during the first quarter of 2007.

Table 1 Sequence of sampling and mouse hepatitis virus serology results obtained in the second University of Pennsylvania School of Veterinary Medicine vivarium suite, 2005

| Date    | No. cages tested (%) of cages in room | No. cages positive (%) |
|---------|---------------------------------------|------------------------|
| Jul 7   | 43 (30%)                              | 3 (7%)                 |
| Jul 19  | 136 (100%)                            | 5 (4%)                 |
| Aug 10  | 132 (100%)                            | 4 (3%)                 |
| Aug 30  | 115 (100%)                            | 2 (2%)                 |

*All positive cages were culled.*
School of Arts and Sciences Dual MHV/EDIM Virus Outbreak, 2007

In March 2007, seroconversion to both MHV and EDIM virus was detected in Q1 sentinel mice in a conventional facility in the SAS. The facility was extremely old, had not been primarily designed as a vivarium, and comprised four rooms with doors that were usually propped open, meaning the facility was effectively one very large room. In addition, investigators regularly moved mice to their laboratories and back.

The pattern of sentinel reactivity is shown in Table 2A. During the next 2 weeks one mouse from each cage in the facility was bled for serological testing for MHV and EDIM virus (there were no mice known to be immunodeficient housed in the facility); the results are shown in Table 2B. Overall, 17.5% of the tested cages in the vivarium were EDIM virus seropositive.

Members of ULAR leadership held two meetings with the three investigators using mice in the vivarium; one individual used pups less than 12 hours after birth (housed in Room 1), another performed behavioral experiments in two rooms (mice housed in Room 2), and the third studied gene expression in mouse embryos (housed in Rooms 5 and 5A). The investigators opined that neither viral agent would affect their experiments and expressed reluctance to participate in an eradication or management program. ULAR staff discussed the biology of the two viruses as well as the physical limitations of the facility: no change stations (so cages were husbanded in open rooms), doors propped open, clean and soiled cages commingled in the cage washing area, cage washer not reliably functional. In addition, there were concerns about known collaborations between two of the three SAS investigators and SOM faculty.

Given the anticipated opening of a new barrier-type SAS vivarium in May, ULAR leadership made the following recommendations:

1. Cull all mice that could be replaced by purchase from approved commercial sources.
2. Cull all mice that were not essential to ongoing experiments.
3. Send unique mouse strains/genotypes to a commercial facility for rederivation and/or cryopreservation.
4. For mice too old to breed, send to a commercial breeding facility to reestablish colonies by in vitro fertilization, ovarian transplant, or intracytoplasmic sperm injection, then rederive and/or cryopreserve.

Despite written and verbal explanations and requests, however, cage numbers in the facility actually increased and breeding, which was to have ceased as soon as the infections were detected and quarantine imposed, continued. ULAR leadership also learned that SOM personnel had been observed entering the facility, despite SOMARC policy prohibiting such access. Discussions between ULAR leadership and the university’s vice provost for research led to implementation of the recommendations listed above. The facility was depopulated and closed in July 2007.

SOM Multifacility Outbreak #2: Dual MHV/EDIM Virus, 2008

One of the most challenging aspects of managing outbreaks at a large, decentralized academic institution is the phenomenon of “investigator creep”: a single PI may initially house mice in one or two rooms in a single vivarium but may, over time and for a variety of reasons, end up housing mice in multiple rooms of several facilities. ULAR has, with the support of the SOMARC, been attempting over the last 3 years to consolidate mice belonging to SOM PIs as opportunities have arisen (e.g., space becomes available with the departure of a PI).

Table 2A First-quarter mouse sentinel monitoring serology results for University of Pennsylvania School of Arts and Sciences vivarium (March 20, 2007)\textsuperscript{a}

| Room | Number tested | Number seropositive | MHV | EDIM | Total |
|------|---------------|---------------------|-----|------|-------|
| 1    | 6             | 0                   | 0   | 0    | 0     |
| 2    | 12            | 1                   | 0   | 1    | 1     |
| 5    | 6             | 2                   | 0   | 2    | 2     |
| 5A\textsuperscript{b} | 4 | 1 | 2 | 3 |

\textsuperscript{a}EDIM, epizootic diarrhea of infant mice; MHV, mouse hepatitis virus
\textsuperscript{b}Room 5A was a small cubicle contiguous with Room 5 and the two were essentially a single space.

Table 2B Serologic reactivity of sera from colony mice housed in University of Pennsylvania School of Arts and Sciences vivarium (April 11, 2007)\textsuperscript{a}

| Room | Number tested | MHV Positive | MHV % | EDIM Positive | EDIM % |
|------|---------------|--------------|-------|---------------|-------|
| 1\textsuperscript{b} | 233           | 2            | 0.9   | 97            | 1.0   |
| 2    | 303           | 1            | 0.3   | 462           | 13    | 2.8   |
| 5    | ND\textsuperscript{c} | 155       | 104   | 67.1          |       |
| 5A   | ND            | 82           | 21    | 25.6          |       |

\textsuperscript{a}EDIM, epizootic diarrhea of infant mice; MHV, mouse hepatitis virus; ND, not done
\textsuperscript{b}Sentinel mice in cages in Rooms 1 and 5 that had been MHV seronegative on March 20 were MHV seropositive on April 11.
\textsuperscript{c}Not all colony mice sampled were tested for both viruses once the extent of the EDIM virus infection became evident.

Building A (Conventional Facility): First Detection of MHV

In early 2008 such an opportunity arose for “Dr. Brown” who had mice housed in five rooms in three facilities: two
rooms of a conventional facility (Building A), one room in each of two barrier facilities, and in an IACUC-approved satellite facility. The plan was to move all of his mice (except those in the satellite facility) into two rooms in a suite of one of the barrier facilities. SOM and ULAR policies dictate that mice cannot be relocated from a conventional to a barrier facility without transitioning through the quarantine facility for rodents from nonapproved sources. However, the quarantine facility could not accommodate the number of Dr. Brown’s cages (approximately 700) in the two Building A (conventional) rooms (536 and 537). Therefore, ULAR leadership, in consultation with the SOMARC, devised a plan to facilitate the consolidation without the need for quarantine.

In addition to extensive testing for fur mites and pinworms, one mouse from each of the 700 cages underwent serological testing (no known immunodeficient mice were involved) for the viral agents that were considered high risk at the institution and/or were excluded from the destination barrier facility: MHV, EDIM virus, and mouse parvovirus. Testing in the conventional rooms revealed one MHV-positive cage in room 536 and eight in room 537. After an independent laboratory’s confirmatory testing of these samples along with those of all cage mates, the positive cages were culled. Mice from all of the cages in both rooms were tested serologically for MHV two additional times at 3- to 4-week intervals and no further positive cages were detected. (Screening of all Dr. Brown’s cages in the satellite and two barrier facilities showed that they were negative.) Table 3 provides a summary spreadsheet (derived from a much more detailed one) that helped immensely in tracking the progress of infection in each room involved in this extensive outbreak.

During these containment efforts, the manager of the Building A vivarium alerted ULAR Diagnostic Services staff that there might be a connection between mice in rooms 536 and 537 and those in room 562, and collateral testing revealed more MHV-seropositive mice. But the mice in room 562 belonged to “Dr. Jones,” who had no connection to Dr. Brown! A sampling in early April of all the Building A vivarium sentinel mice showed that one of 106 sentinel was EDIM virus seropositive. This sentinel was monitoring mice belonging to Dr. Jones in room 549, where testing of one mouse in each of the approximately 500 resident cages yielded four positive cages, which were culled. Three weeks later another screen of 100% of the cages in room 549 yielded two positive cages belonging to Dr. Jones and these cages were culled. By the third screen, the number of resident cages in the room had been reduced by about 20% and all tested mice were seronegative. Room 549 was released from quarantine after a second negative screen of the room (which now housed only about 300 cages) 3 weeks later.

**Building B (Conventional Facility): Collaborations and Inappropriate Feed Storage**

The incidence of MHV in the Building A vivarium—a few positive cages in the rooms that were tested—did not seem compatible with the expected biology of this virus. A second vivarium, Building B, seemed the most likely location to investigate because of known PI collaborations in that facility.

In early March all of the sentinel mice in Building B were bled for Q1 surveillance and tested for a basic panel of viruses. Of 18 sentinel mice tested in room 4 (a conventional room housing approximately 700 cages on nine racks), five were MHV seropositive and three EDIM virus seropositive. Subsequent tests of mice from 100% of the cages in room 4 showed that 8% were MHV seropositive and 2% EDIM virus seropositive, thus overall 10% positive for one or the other virus (improvements to the sentinel monitoring program were evident in the very low incidence of EDIM virus–seropositive mice in the room). With support from the SOMARC, ULAR made the decision to depopulate the room without further testing.

Because most of the positive mice in room 4 belonged to two PIs, “Dr. Green” and “Dr. Black,” their mice housed in other locations underwent collateral testing for both viruses, resulting in culling and quarantine for 1,300 cages in two additional rooms in the Building B vivarium; room 8 had over 3,000 samples taken during the course of five 100% screens and room 19 had 2,000 samples collected over four 100% screens. Table 4 provides a summary of MHV and EDIM virus sentinel and resident mouse test results.

Well into the test-and-cull process, Dr. Green, who had mice in room 4, volunteered that special feed was stored in his laboratory in its original plastic bag, not in secondary containment. His staff had reported that the bag appeared to have holes chewed in it and that there were rodent fecal pellets on the floor in the vicinity of the bag. Those fecal pellets were submitted for RT-PCR for MHV and EDIM virus and were MHV positive. The lab staff were advised about proper storage of all animal feed in the vivarium (not the lab), where there is an active pest control program.

**Building C: Traffic of Mice among Facilities**

One week after identification of the positive sentinel in room 549 of Building A, ULAR learned that a single EDIM virus–seropositive sentinel mouse had been identified in Building C, which contains a large rodent vivarium that is not managed by ULAR. Testing of mice in the 168 resident cages revealed only one positive cage; however, testing of 94 cages belonging to the investigator, Dr. Jones, whose cage was positive, yielded 51 positive cages in another room in Building C. Although an interview with Dr. Jones did not reveal the origin of the EDIM virus infection, it appeared not only that it first occurred in the Building C vivarium, based on the high (54%) prevalence, but also that some mice from room 549 in Building A had been manipulated in the same procedure room as the mice housed in Building C and then returned to the Building A vivarium (this is permitted—see section above on Institutional Practices and Accreditation—although it heightens the likelihood of microbial contamination).
Table 3 Summary of mouse hepatitis virus (MHV) and epizootic diarrhea of infant mice (EDIM) virus investigation in University of Pennsylvania School of Medicine vivaria, 2008

| Facility | Room (and PI)          | # of cages | MHV | EDIM | Action and status                                                                 |
|----------|------------------------|------------|-----|------|----------------------------------------------------------------------------------|
| A        | 536, 537 (Dr. Brown)   | ~ 400,     | +   | n.a. | First detection late January, followed by 2 negative screens; Mar 20 released from quarantine |
|          |                        | ~ 300      |     |      |                                                                                  |
|          | 562 (multiple PIs)     | ~ 500      | +   | n.a. | 2 (100%) screens yielded single positive cages (culled)                          |
|          |                        | ~ 400      | +   | n.a. | Apr 3-4 bled 100%: 2 positive cages (culled)                                      |
|          |                        | ~ 400      | +   | n.a. | May 2 bled 100%: 1 positive cage (culled; Dr. Jones)                              |
|          |                        | ~ 400      | 0   | n.a. | May 17-18 bled 100%: 1st negative screen                                         |
|          |                        | ~ 280      | 0   | n.a. | Jun 7-8 bled 100%: all negative, 4 PCR also negative; Jun 19 released from quarantine |
|          | 558 [cubicles] (multiple PIs) | ~ 700  | +   | n.a. | 1 negative screen after 1 positive (5 of 712 cages; 5 cages culled)              |
|          |                        | ~ 520      | +   | n.a. | Apr 5-7 bled 100%: 3 positive cages in cubicle H (culled)                        |
|          |                        | ~ 340      | 0   | n.a. | Apr 23 bled 100%: 1 negative screen                                             |
|          |                        | ~ 340      | 0   | n.a. | May 14-16 bled: all negative; May 22 released from quarantine                    |
|          | All sentinels (2nd quarter) | 106     | 0   | 1    | Apr 9 bled; confirmed by independent laboratory; room 549 (Dr. Jones)           |
|          | 549 (Dr. Jones and others) | ~ 500    | n.a. | +    | Apr 20-22 bled and feces collected: 4 EDIM seropositive (culled; Dr. Jones); 0/15 PCR + |
|          |                        | ~ 500      | n.a. | +    | May 10-12 bled and feces collected: 2 EDIM seropositive (culled; Dr. Jones); 0/15 PCR + |
|          |                        | 393        | 0   | 0    | Jun 16-17 bled: 1 negative screen                                               |
|          |                        | 278        | n.a. | 0    | Jul 4-5 bled and feces collected: 2nd negative screen; 0/12 PCR +; Jul 18 released from quarantine |
|          | All sentinels (1st quarter) | 214     | 5   | 3    | Positive sentinels all located in room 4 (18 tested)                            |
|          | 4 (Dr. Green and others) | ~ 700      | +   | +    | 10% of cages positive; depopulated                                              |
|          |                        | ~ 800      | 0   | +    | Mar 18 bled 100%: 1 positive cage (culled)                                       |
|          |                        | ~ 640      | 0   | 0c   | Mar 29-31 bled 100%                                                            |
|          |                        | ~ 515      | 0   | +    | Apr 18-20 bled 100%: 3 EDIM positive (culled)                                   |
|          |                        | 612        | 0   | 0    | May 3-4 bled: 1st negative screen                                              |
|          |                        | 595        | 0   | 0    | Bled Jun 2: 2nd negative screen; released from quarantine Jun 5                 |
|          | 19 (Dr. Green and others) | ~ 500    | +   | 0    | Mar 12 bled 100%: 1 MHV positive (culled)                                        |
|          |                        | ~ 500      | +   | 0    | Mar 27-28 bled 100%: 2 MHV positive (culled)                                    |
|          |                        | ~ 500      | 0   | 0    | Apr 23-28 bled: 1st negative screen                                            |
Resulting Refinements

With the support of the SOMARC, ULAR implemented three major refinements during the 2008 MHV/EDIM virus outbreak:

1. Locks on the doors of rooms containing positive mice were changed before announcing quarantine. This prevented a predictable exodus of mice after quarantine was imposed. Night and weekend access was severely restricted and required justification to and approval by the chair of the SOMARC and associate director of ULAR Diagnostic Services.

2. Access to a quarantined room was restricted to husbandry personnel and a single representative (usually the laboratory manager) from each laboratory housing mice in the room. This was significant as some laboratories have huge numbers of personnel (one affected lab had 29 pre- and postdoctoral researchers plus technical staff).

3. In many cases, laboratory staff did not need to manipulate their mice but needed information on cage cards to make decisions about culling animals or needed to see the condition of mice in their cages. In those cases, ULAR permitted the use of digital cameras to photograph cage cards or mice so that multiple staff members didn’t have to enter the quarantined rooms.

Table 4 Summary of mouse hepatitis virus (MHV) and epizootic diarrhea of infant mice (EDIM) virus sentinel results and resident mouse population testing during University of Pennsylvania School of Medicine outbreak (ULAR-managed facilities) #2, 2008

| Facility | Room | Agent | % seropositive resident mice | Overallb | Minimum | Maximum |
|----------|------|-------|-----------------------------|----------|---------|---------|
| A        | 536  | MHV   | n.a.                        | 0.25     | 0.00    | 0.25    |
|          | 537  | MHV   | n.a.                        | 2.70     | 0.00    | 2.70    |
|          | 562  | MHV   | n.a.                        | 0.29     | 0.00    | 0.50    |
|          | 558  | MHV   | n.a.                        | 0.42     | 0.00    | 0.70    |
|          | 549  | EDIM  | 6                            | 0.35     | 0.00    | 0.80    |
| B        | 4    | MHV   | 18                           | 7.60     | n.a.    | n.a.    |
|          | EDIM | 18    | 3                            | 2.10     | n.a.    | n.a.    |
|          | 8    | EDIM  | n.a.                        | 0.13     | 0.00    | 0.60    |
|          | 19   | MHV   | n.a.                        | 0.15     | 0.00    | 0.40    |

aAdditional testing of approximately 1,500 cages (one mouse per cage) was performed to determine the extent of spread of MHV and/or EDIM virus.

bOverall % seropositive resident mice = % of mice that were seropositive over the course of all 100% screens of each room

cn.a., not applicable
An additional change, though not a direct result of outbreaks and their management, has been ULAR’s implementation of a cage card system that permits the use of unique identifiers (using bar codes) for each cage. With this system it is possible to track individual cages and flag those that are not in their assigned location.

One of the many reasons to have an effective sentinel rodent program is to detect outbreaks early so that a test-and-cull program, with its attendant reduction/refinement benefits (compared to depopulation), can have some chance for success. Based on the experience of the past 5 years, a test-and-cull approach to MHV and EDIM virus outbreaks is successful with minimal impact on investigators if 4% or less of the cages in a room are positive. The 4% figure is based strictly on experience: raising the level to 5% substantially increased the number of rounds of testing (1 month for each round: a minimum of 3 weeks between sample collections and about a week for test results to become available) to get two consecutive negative screens of 100% of the cages in a room. At that point depopulation may be more efficient.

Costs to the Institution

Financial (Self-Insurance)

The university’s Office of Risk Management and Insurance is responsible for coordinating risk management activities in areas such as property insurance, liability insurance, workers’ compensation, and medical professional liability. Whereas initially this office covered only the cost of shipping rodents to off-site facilities and their subsequent rederivation, housing, and breeding, it has more recently reimbursed ULAR for at least a portion of the sampling (supplies, overtime) and testing performed during outbreak investigations. Future reimbursements will be contingent upon demonstration that ULAR staff were not responsible for an outbreak.

Loss of Faculty (Potential) and Research Productivity

Repeated outbreaks requiring quarantine and possibly depopulation are a source of faculty frustration and increase the risk to the institution of losing talented investigators whose career success depends on the use of rodents. The research of one SOM faculty member whose studies involve the use of unique genotypes of aged mice was halted during both the 2004-2005 and 2008 outbreaks; and another faculty member whose research was halted during the 2004-2005 MHV outbreak was being considered for tenure soon after that.

Loss of Unique Strains (Potential)

As described in other articles in this issue (Bailey et al. 2010; Goodwin and Donaho 2010; Swarengen et al. 2010), there are many threats to the viability of lines of research animals. The cryopreservation subvention was offered as one method of preserving unique strains. But something can always happen to the liquid nitrogen tanks or the buildings in which they are stored. Thus, if finances permit, germplasm should be stored at more than one location. One Penn PI distributes her unique mice to multiple collaborators at other institutions; if she loses her lines here, she can obtain her mice relatively easily from others who have them “on the shelf.”

Wish List for the Future: How Things Could Be Even Better

Facility and Housing Room-Level Security

Security currently consists largely of a programmable “black key” system for perimeter entry and hard keys for individual animal housing rooms. Theoretically, perimeter entry can be tracked electronically but, in practice, records are cumbersome and time-consuming to access. Despite advocacy for improved vivarium security, specifically the installation of biometrics, retrofitting new systems to older facilities has proven too challenging. As a result it is difficult to learn who has entered facilities and at what times. In addition, it is impossible to track entry into individual rooms as these are largely either unlocked or on hard key systems. There has been a strong push to install modern security and tracking systems in newly constructed facilities, but new construction always seems to run over budget and enhancements like fingerprint readers or iris-recognition scans are “value engineered” out of the projects, despite the fact that they have greatly reduced the potential for abuse inherent to black keys, magnetic cards, and hard keys, all of which are easily transferable from person to person.

In the absence of biometric systems or complete prohibition of investigator entry into housing areas (as is frequently done in the pharmaceutical industry), disease outbreaks will continue to occur. There has been considerable discussion about, and some enthusiasm in one facility user group for, routinely (not just during outbreaks) “locking down” the facility during particular hours (e.g., from 7:00 PM to 7:00 AM) with exceptions granted on request and with good justification. However, the impetus for such action lags as the memory of the consequences of an outbreak fades. Perhaps the next MHV or EDIM virus outbreak will revive the discussion and spur some action....

New Construction (More Space!)

As others have pointed out (Jacoby and Lindsey 1998), high-density housing conditions contribute to outbreaks of infectious disease in rodent facilities. The explosive growth in populations of genetically modified mice and the worldwide sharing of them have increased census counts beyond anyone’s expectations. And the phenotypes of these mice are often un-
anticipated as are their responses to infectious agents (Smith 1998). Although currently available housing systems and change stations have features to reduce exposure to and transmission of infectious agents, they are only as effective as the individuals using them.

One partial solution to the infectious disease curse is the availability and efficient use of more space. One of the (now older) barrier facilities on this campus that was designed according to the suite concept has rooms that are so small that the doors have to be propped open during cage changing activities in order for the supplies to be readily available. This practice has been observed even during pinworm outbreaks. But in the caretakers’ defense, short of removing racks from the rooms and reducing housing capacity, there isn’t any other way for them to perform their job duties.

Conclusion

Building on lessons learned in the experiences described above, this institution has made great strides in its viral disease outbreak management strategy. The 2004-2005 MHV outbreak in the SOM took 13 months to resolve, whereas the dual MHV/EDIM virus outbreak in 2008 was resolved in less than 6 months from first diagnosis.

There is no one-size-fits-all solution to rodent infectious disease outbreak management, especially at academic institutions, which have different cultures, sizes, arrangements (centralized or not), levels of resources, and commitment to the quality of animal-based biomedical research. University administrators who control budgets, particularly special capital outlays, may or may not have experience and/or knowledge in the area of animal-based research. At the University of Pennsylvania we are fortunate to have had an administra-

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