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Rodent biosecurity was previously reviewed in *BRL Bulletin* Vol. 32 No. 2 (2017) and covered methods to mitigate risks to mice and rats housed at UIC. It can be found on the BRL website (https://brl.uic.edu/training). This issue is a follow-up that reinforces key concepts about rodent biosecurity as well as discusses lessons learned from four recent cases involving breaches in rodent biosecurity.

Biosecurity is defined as the procedures intended to protect humans and/or animals against disease or harmful biologic agents. From an infectious disease standpoint, laboratory rodents pose very little risk to their human caretakers or research personnel. However, people and other sources from outside the rodent colony are potential contamination risks for these animals, and therefore biosecurity strategies designed for rodent colonies focus most heavily on preventing the accidental introduction of infectious agents. This approach to rodent colony biosecurity relates to the concept of "specific-pathogen-free," or "SPF," animals. Rodents bred for use in biomedical research are SPF for known murine pathogens and are maintained that way through strict biosecurity practices and frequent testing. Pathogens are generally excluded from a facility for three main reasons – to ensure quality animal welfare, to minimize zoonotic disease risk, and to prevent confounding effects on research. Some of the most infectious and difficult to eradicate agents excluded from SPF rodents do not have any noticeable health effects, but are known to cause significant biophysiological abnormalities that can have major impacts on experimental outcome measurements including cell counts, blood serum parameters, histologic lesions, and tumor growth kinetics. It is the mission of the BRL to facilitate the critically important animal research conducted at UIC which starts with ensuring healthy and reproducible animal models.

Figure 1 illustrates potential sources of contamination to an SPF rodent colony which have been identified as high risk critical control points, and toward the center of the figure are the primary biosecurity strategies utilized to mitigate risk from those sources. For example, the acquisition of research animals from other facilities is among the highest risks for the introduction of contaminants due to individual facility differences in health status and health monitoring techniques. Therefore, at UIC, all incoming animals which do not come directly from an approved commercial vendor (i.e., Charles River Laboratories, The Jackson Laboratory, Envigo, Taconic) must first be housed in a designated quarantine room for testing or undergo re-derivation before they can be housed within the general rodent colony population. Although the risk of a contaminated research animal entering UIC's SPF rodent colony is largely diminished by the quarantine program implemented and closely overseen by the BRL staff, other sources of contamination require more active cooperation by the animal users to ensure biosecurity. This includes diligent use of personal protective equipment (PPE), appropriate microisolator technique, sterilization and/or testing of any compounds or materials introduced to an SPF animal or their environment, and other operating procedures to prevent cross-contamination. The remainder of this *Bulletin* will expend upon these biosecurity strategies through the description of four unique cases of breaches in biosecurity, each from sources that may not have been initially apparent to the persons involved, but

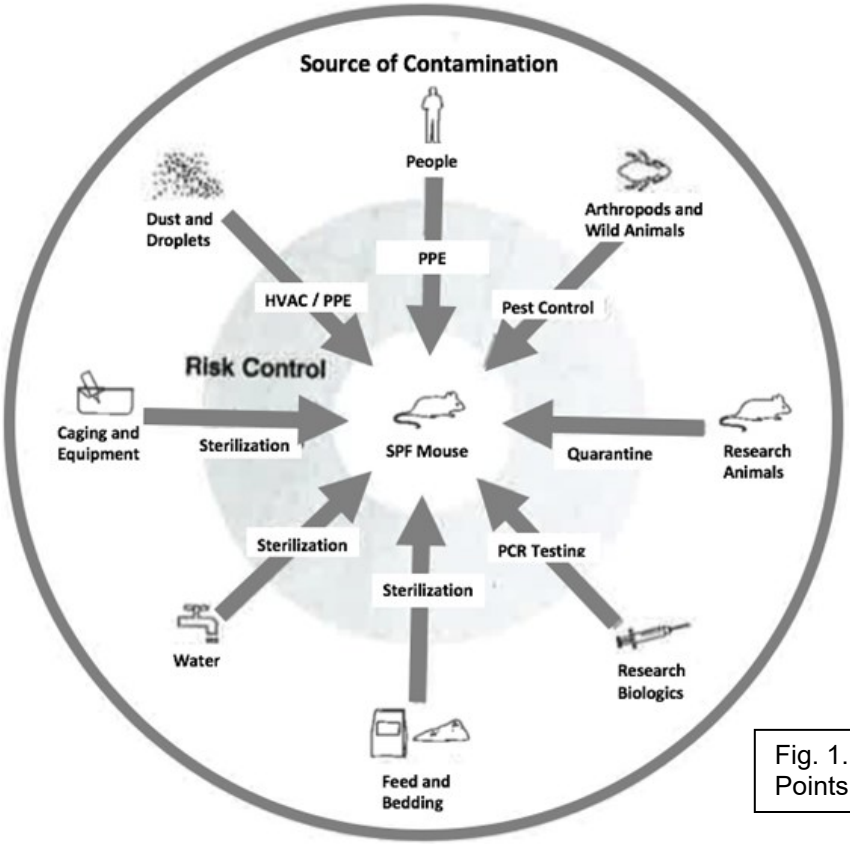

Fig. 1. SPF Rodent Biosecurity Critical Control Points (Charles River Laboratories, 2014)

provide important lessons from which we can all learn. All cases are based on real events with changes made for clarity and to protect the identity of the institutions involved. In each case, the biosecurity breach was ultimately managed, and the contaminating agent was effectively eliminated from the facility.

Case 1

Several mice of a genetically modified mouse strain deficient in the STAT1 gene, an important strain used for studying mechanisms of host defense, began to show signs of illness including weight loss, hunched posture, and ruffled fur. At around the same time period, sentinel mice in the room tested positive for mouse norovirus (MNV) infection. MNV causes clinical signs and mortality in STAT1 deficient mice specifically, but due to the virus's affinity for macrophages and dendritic cells, it also has the potential to impact mouse experiments focused on studying macrophage-driven inflammatory diseases such as obesity and atherosclerosis. MNV was discovered relatively recently, and since its discovery, some institutions have made a concerted effort to eradicate it from their animal facility; however, it still remains prevalent at many institutions. It was determined that the likely source of this biosecurity breach was contaminated equipment used by a researcher who conducted animal work between two separate facilities, one SPF for MNV and one known to be MNV-positive. MNV is very stable in the environment, which enables it to survive and be transmitted on equipment. Therefore, when conducting animal work at two different facilities, it is always recommended to designate independent sets of equipment and tools for each location.

Case 2

During the testing of sentinel mice, routinely performed several times per year at most facilities to screen for excluded pathogens, mouse cages in an animal housing room tested positive for mouse parvovirus (MPV). MPV infections are not associated with clinical signs, even in immunocompromised mouse strains,

but the virus disrupts normal biological functions and can confound studies investigating humoral and cellular immune responses. This is another virus which is very stable in the environment, and therefore has the potential to be brought into the colony on contaminated items. It was determined that the likely source of this biosecurity breach was a contaminated special diet. At most facilities, rodents are fed a diet that has been subject to a sterilization process, either by autoclave or gamma irradiation, to reduce the potential for microbial contamination. The problem in this case originated because the researchers were using a custom-made diet to deliver doxycycline as part of their experiment. Custom diets are a common way to manipulate nutritional intake or administer medications; however, these diets are not automatically subject to the same sterilization techniques as standard rodent feed. There are three general categories of diet formulations, natural-ingredient diets which are made from standard agricultural ingredients, purified diets which use pure ingredients as the sources of each nutrient, and chemically defined diets where the protein or fat sources are replaced by individual amino acids or fatty acids respectively. Natural-ingredient diets are of particular risk for their potential to contain infectious agents. Therefore, when selecting a custom diet to feed to rodents at UIC, it is a requirement that any natural-ingredient diet formulation be sterilized by autoclave or irradiation before use.

Case 3

The biosecurity breach in this case occurred in a rat housing room and was identified when a sentinel rat tested positive for rat parvovirus (RPV) during routine sentinel testing. As with MPV in mice, RPV is among the most prevalent viruses that contaminate SPF rat colonies. RPV is also like MPV in that the virus can survive very well on objects in the environment and does not cause overt clinical disease, but can have detrimental effects on research study data. After discussions with the research staff, it was determined that the likely source of contamination was a pet rat in the home of one of the lab members. Infectious disease surveys of rodents from pet stores have found that these animals carry numerous pathogens that are excluded from laboratory rodent colonies. Additionally, the behavioral testing experiments for this study were being done in a laboratory located outside of the centralized animal facility where there may have been limited use of PPE. The combination of exposure to a contaminated pet animal at home with the environmental stability of RPV, and potential lack of PPE, created a high risk scenario for contamination. Once infection is established in one colony animal, it then becomes a risk for all other SPF rodents in the facility. This case is an example of why personnel with rodent facility access should not keep rodents at home as pets. Another lesson from this case is the importance of PPE and why it is necessary to wear at all times when working with research animals.

Case 4

NOD scid gamma (NSG) mice are an immunodeficient strain of mouse used commonly for studying the growth of transplanted tissues. This strain will not reject grafts due to an inability to mount an immune response to foreign tissue. Patient-derived xenografts (PDX) are cancers that have been removed from human patients and subsequently transplanted into mice in order to study them for the purpose of ultimately developing cures. In this case, an NSG mouse that had received a subcutaneously implanted PDX was later found to have paralysis of both hind legs sometime after a seemingly full recovery from the PDX implantation surgery. Before initiating PDX tumor studies, it is common to first “expand” the original sample of human tumor tissue by first allowing it to grow in other laboratory mice in order to have an adequate amount of PDX to implant. This expansion process is sometimes done at a facility different from where the main research study takes place. After testing the paralyzed

mouse described in this case, it was determined that the implanted PDX tumor had been contaminated with lactate dehydrogenase-elevating virus (LDV), a mouse pathogen that remains one of the most frequent contaminants of murine transplantable tumors. LDV is detrimental to research studies because it significantly alters immune function and also interferes with the growth of tumors in cancer studies. Another ill-effect of LDV is that when infecting certain susceptible mouse strains, such as NSG, the virus can cause neurologic abnormalities including paralysis. In this case, the experimental PDX tumor had likely been infected with LDV from an infected mouse during expansion at a different facility. When the LDV-infected tumor was eventually implanted into the NSG study animal, it resulted in the clinical signs described and therefore a failure of the experiment. As this case illustrates, biologic materials represent a significant biosecurity risk to the animal colony. UIC defines biologic materials as tumors, cell lines, tissues, hybridomas, serum, antibodies, and basement membrane matrix, which includes the commonly used Matrigel. Any of these which are not derived from rodents within the general UIC colony or from a reputable commercial supplier must be tested for pathogens listed in the UIC Biologic Materials Panel. For more information, including the full list of pathogens included on this panel, see the UIC policy on Biologic Materials Testing (brl.uic.edu/policies-and-guidelines).

In conclusion, these four cases bring attention to some of the many sources for potential contamination of an SPF rodent colony. It is important to be cognizant of these sources, which can be facilitated by thinking about them in the context of the critical control points described. Because these critical control points have been identified, policies and guidelines are in place to mitigate risk at each point. It requires a combined effort from each and every person that works within the animal facility to maintain the SPF status of the animals in order to promote the highest quality standards for the biomedical research conducted at UIC. Investigators are encouraged to contact the BRL veterinary staff for any questions regarding rodent biosecurity.

References

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Announcements

This will be the last (hard copy) mailing of the *BRL Bulletin*. In the future research staff will be made aware of new issues electronically and via an announcement on the BRL website. Note the next *BRL Bulletin* will address per diems and will be sent out toward the end of February.

Congratulations are in order for Mary Urbina, a 2019 UIC Merit Award recipient. In addition, the following animal care staff were recognized by the BRL: Raul Chagoya (BRL Technician of the Year) and Greta Nekrasova and Byron Quezada (BRL Technician of the Year Honorable Mention).