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This edition of the *BRL Bulletin* will discuss rodent biosecurity and methods to mitigate risks to mice and rats. In the most basic sense, biosecurity is defined as the procedures intended to protect humans and/or animals against disease or harmful biologic agents[2]. In modern vivaria, barrier housing and routine health monitoring aid in reducing risk associated with disease outbreak; however, unwanted microorganisms still can enter animal colonies from various sources. As much as animal facility staff strives to eliminate all agents on a specific pathogen exclusion list, organisms may evade even the best control mechanisms. Bearing in mind this risk, there are several ways to mitigate and minimize disruptions in biosecurity to maintain a clean research environment.

Primary Biosecurity Risks

The most important entryway for murine pathogens into research colonies is through the addition of animals to the colony. For this reason, rodents are preferentially procured from approved commercial vendors. Approved commercial vendors practice regular health status monitoring and maintain high management standards. All rodent suppliers must meet UIC's pathogen status requirements. The veterinary staff receives comprehensive health status reports from vendors, which are used to monitor the quality of animals from each supplier. In addition, the approved commercial vendors have a copy of the UIC specific pathogen exclusion list and are instructed to provide notification if an agent from the exclusion list is identified in their colonies. Animals from approved sources that are the same health status as UIC colonies are assumed to be clean, and generally are allowed into animal rooms without quarantine and testing.

The risk to biosecurity lies primarily in the shipment of rodents to UIC animal facilities. Approved vendors typically ship animals directly via a dedicated ground transportation network in specially constructed trucks engineered with environmental and temperature control systems that facilitate sanitation and air flow. Ground shipment of animals directly from approved vendors helps reduce the risk of exposure to murine pathogens during shipment. Under some circumstances, animals may need to be shipped by alternative methods. When animals are shipped by air or by common courier, there may be contact between animals of various health statuses and from different origins. This increases the risk of pathogen exposure, so these animals may be housed in the BRL quarantine room due to the potential for infection or cross-contamination.

Non-approved animal sources are those that are not on the approved list of commercial suppliers. Some of these sources may be rodents from other academic institutions, rodent repositories, transgenic cores (e.g., University of Chicago or Northwestern University), and Jax Research or NIH colonies. When rodents are requested from non-approved sources, the veterinary staff reviews health reports from these institutions. After reviewing the documents, the veterinarians determine the appropriate quarantine tier for the animals. For more information on rodent quarantine, please reference the "Rodent Quarantine Program" and FAQs found on the UIC BRL website under Policies & Guidelines.

Mice and rats from non-approved sources are typically housed in the BRL quarantine room. The purpose of the quarantine room is for rederivation of animals to eliminate unwanted pathogens. This room is not intended for long-term colony maintenance or procedures. When health reports from non-approved sources indicate the animals are carriers of pathogens excluded from UIC colonies, animals must be rederived. Commonly, incoming mice are positive for *Helicobacter sp.* and norovirus which have been excluded from UIC colonies. During rederivation, animals are housed in the BRL quarantine room, treated for ecto- and

endoparasites, and tested for pathogens. Because the quarantine room is of a different pathogen status than the rest of the colonies, this room is maintained under restricted access, is serviced last, and materials are autoclaved out of the room. Under no circumstances can animals be removed from the quarantine room. In addition, tissues from animals in quarantine cannot be collected for administration to other mice within UIC colonies.

Many animal models are produced using exogenous cells or tissue-derived products passaged through rodents which increases the risk of introducing murine pathogens into the colony. Contamination of biological materials with murine pathogens represents a significant biosecurity risk for the rodent colony. It is possible for rodents to become infected with a pathogen following inoculation with a contaminated biological material, and subsequently spread the pathogen to other rodents. Murine biologicals such as serum, tumor lines, and cell lines can become contaminated if passaged through rodents of different pathogen status than those housed in UIC facilities. Therefore, if the rodent biological is obtained from a non-commercial source, testing for a specific list of pathogens prior to their use in the facility must be performed by a commercial laboratory such as IDEXX, Charles River, or other comparable laboratory service. However, if the biological is obtained from a reputable commercial source, no testing is required. The pathogen exclusion list was developed based on a risk assessment of pathogenic agents and their impact on research models. For more information on the requirements for biological material testing, refer to the UIC ACC policy, “Biologic Material Testing”, and the *BRL Bulletin* Vol. 31 No. 4.

Tumor cell lines are the most common biological brought into a rodent barrier facility. There are numerous reports of contamination of biologicals with lymphocytic choriomeningitis virus (LCMV), ectromelia virus, and lactate dehydrogenase-elevating virus (LDHV)[1]. LCMV is a virus that can be propagated in a variety of cell lines with minimal cytopathic effects which contributes to persistent contamination of murine biologicals. LCMV infection within a mouse colony can stimulate or suppress immunological responses and result in immune complex disease. Ectromelia virus is the causative agent of mousepox, and although rarely present in laboratory colonies, can cause up to 100% mortality in susceptible strains of mice. However, the risk stems from viral contamination of murine biologicals from non-commercial sources, especially serum. LDHV is one of the most common contaminants of murine biological materials. LDHV can alter immune responses and tumor growth[4]. Historically, transplantable tumors have been a common source of LDHV, including an outbreak at UIC stemming from a patient-derived xenograft contaminated with this pathogen. This demonstrates the necessity of screening murine biologicals to avoid breaches in rodent biosecurity.

Routine Health Monitoring

The health monitoring program for mice and rats utilizes sentinel animals to assess the pathogen status of UIC colonies. Sentinel mice and rats are tested quarterly by serology, PCR, and parasitology. Comprehensive serologic testing of sentinel mice is performed annually. Sentinel animals are maintained in a dirty bedding cage system. At weekly cage changes, soiled bedding from each of the cages within a section of the room is added to a sentinel cage, thus exposing the sentinels to diseases that may be carried by colony animals. Dirty bedding sentinels are most effectively used to detect pathogens transmitted primarily by fecal-oral contamination[3]. This method of sentinel surveillance detects infectious agents without the need to test investigator animals. Occasionally, a direct contact sentinel mouse is used wherein a female, pathogen-free sentinel animal is placed directly in a cage with an investigator’s animals. The direct contact sentinel system is used to detect pathogens that are not readily transferred via dirty bedding. This type of sentinel allows assessment of the health of a specific cage or a single colony without needing to sample or sacrifice an investigator’s animal. Monitoring colony health with direct contact sentinels is very uncommon at UIC and used only after consultation with the investigator.

Primary Methods to Control Pathogen Transmission

Animal housing is used to control the spread of

infectious agents. Most mice and rats are housed in sterilized static microisolator cages, which provide an effective barrier to the entry and spread of microbial agents at the cage level. The microisolator cage can be likened to a petri dish where the outside of the container is considered dirty and the inner contents are clean. The microisolator cages and bedding are autoclaved, and autoclaved water bottles and irradiated rodent diet are added during cage changes, thus providing the animals with a clean environment. Microisolator cages provide enhanced protection against disease transmission and require specific handling procedures. These handling procedures are known as microisolator technique and are based on the handling of petri dishes. Cages and gloved hands are sprayed with disinfectant prior to removing the lid of the microisolator and manipulating the contents inside. Microisolators are opened only within a functioning biosafety cabinet (BSC) or animal transfer station (ATS), both known as laminar flow work surfaces. Opening cages outside of a BSC or ATS exposes the animals in the cage to potential pathogens. As another precaution to prevent cross contamination between cages or between colonies, only one cage is opened within a BSC or ATS at a time. In addition, disruption to the airflow within the cabinet by blocking the grates or vents with equipment or paper impairs its protective function. Therefore, only necessary supplies should be placed within the BSC or ATS to maintain proper air circulation. Inappropriate microisolator technique within the BSC or ATS puts animals at risk of pathogen exposure.

Any object or substance may potentially transfer a rodent pathogen from one location to another. Therefore, disinfection of all animal use areas, work surfaces, and equipment in direct contact with animals or cages must be carried out both before and after use. It is recommended to spray all equipment and instruments upon entry into the facility using the disinfectant spray provided at entryways, and then to again disinfect all equipment within the laminar flow work surface prior to manipulation of the animals. The laminar flow work surface, outer surfaces of the cage, and gloved hands should also be sprayed with disinfectant to minimize the opportunity for pathogen transfer. Fomite transmission can be mitigated by having dedicated instruments and equipment for each room. If an investigator is performing work at another institution, the BRL veterinary staff should be notified. Not all institutions in the Chicagoland area are of the same microbial status as the BRL.

Additional Considerations in Reducing Risk of Pathogen Transmission

Another means to decrease pathogen exposure and spread is through disinfection of all shared-use equipment and space prior to use. Common use areas act as a crossover point in which animals from different investigators can intersect. These areas represent a vulnerable link in the maintenance of biosecurity. The IVIS suite, shared use of ultrasonography equipment, MRI, and procedure rooms are all situations where multiple investigators and rodent colonies can overlap. Animals should be placed into clean cages before being taken to common use areas. In common use areas, microisolator cages are often opened outside of a laminar flow hood which can expose both the animals and the surrounding area to pathogens. For this reason, all work surfaces, cages, equipment, and other items in contact with the animals must be disinfected both prior to and after use. To further minimize potential exposure and breaches in biosecurity, cages should be opened for the shortest time possible and only one cage should be opened at a time. If animals are returned to the colony after being in these workspaces or an investigator's laboratory space, they are returned to BRL room 122 or other designated return room. Once housed in BRL room 122, animals cannot to be moved to any other room within the facility. This precautionary measure minimizes the risk of pathogen exposure and spread. It should be noted that the sentinel program has not detected pathogens from the pathogen exclusion list in dedicated return rooms.

There is a room entry order in the BRL which also helps minimize the spread of pathogens. Although most mice and rats within the BRL are of the same microbial status, the entry order prioritizes rooms from those that are cleanest (e.g., BRL barrier suite) to those which are presumably the dirtiest (e.g., quarantine room). Note that room 122 and any other designated

return rooms are entered after all other rodent housing rooms. It is important to keep in mind the rooms that must be entered throughout the day including animal housing rooms, laboratories, and cage storage areas to avoid entering a clean area after visiting a dirtier one.

Personnel should refrain from keeping pet rodents to further decrease the risk of pathogen transfer to UIC colonies. Exposure to pet rodents or rodents used as food for other pets is a known risk factor for the introduction of diseases into research colonies. A variety of pathogens including LCMV, *Salmonella*, *Streptobacillus*, lice, mites, pinworms, and tapeworms have been detected in rodents from pet stores[1]. In fact, many diagnostic laboratories use mice from pet stores as a source of positive controls. Research staff can act as fomites for these pathogens and inadvertently introduce them into rodent research colonies. Unintentional infection of laboratory rodents can compromise scientific research as well as the health of the animals and animal handlers.

Another means to control spread of rodent diseases is with personal protective equipment (PPE). PPE is required at all times in animal and procedure rooms. PPE must not be worn out of the animal or procedure room which increases the risk of potential pathogen spread throughout the facility. Another control method to reduce inadvertent pathogen exposure is to hang personal items such as coats, backpacks, and hats on the hooks provided outside of the animal rooms. These items should not be brought into the animal rooms.

In conclusion, there are many methods used to maintain biosecurity within the UIC animal facilities. Most of the risk can be controlled by investigative staff, animal care technicians, veterinarians, and other personnel following appropriate procedures. It is our collective responsibility to maintain constant awareness to assure the highest level of biosecurity within UIC animal facilities. In doing so, it helps ensure animal health which in turn minimizes confounding variables to research models and maintains the specific pathogen status of animal colonies.

REFERENCES

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ANNOUNCEMENTS

Streamlining mouse shipments - Effective August 1, the BRL will begin autoclaving and re-using plastic shipping crates received from Jax Labs and Taconic to fill requests to ship mice from UIC to other institutions. We currently request the receiving institution to purchase new crates and send them to UIC which delays shipments by 1-2 weeks. We will charge investigators' BRL account $15.00 per crate to recoup BRL costs for autoclaving the crates, crate dividers, and gel packs. Please note that for large shipments, there may be insufficient numbers of in-house recycled crates available, so investigators may need to purchase new crates from Taconic. Questions regarding this process can be directed to the BRL veterinary staff or BRL business office.

New postdoctoral fellow – Please help the BRL veterinary staff welcome Dr. Kat Coda, who started the postdoctoral training program in laboratory medicine in July. She will be assigned to the primate area for the first 6 months.