

Recovering from a Microbiological Contamination in Your Animal Facility

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Every animal facility, whether at an academic institution, a pharmaceutical company, a contract research organization, a government agency, or an animal breeder will eventually experience a contamination with a microorganism on the institution's bioexclusion list. Unless significant thought and preparation has been given to such an event, it can result in responses ranging from fear, panic, and a feeling of helplessness to a rush to plan and execute some means of control based on limited information. Eventually, a course of action is developed and implemented, the organism is controlled or eliminated, and things return to normal.

The review aims to provide insight into how to deal with viral or bacterial contaminations. Given the prevalence of agents such as coronaviruses (MHV, SDAV), parvoviruses (KRV, MVM, MPV, RPV), and mycoplasma in research facilities (Jacoby and Lindsey, 1998), coupled with the increasing numbers of rodents being used in biomedical research, it is likely that every institution will eventually experience a contamination. The probability that such a contamination will occur is compounded by the movement of animals and biological materials between institutions and the use of air transport to make transfers of live research animals in environments in which large numbers of animals of an unknown health status travel (Johnson, 1999).

Making a Diagnosis

In laboratory animal medicine, we are accustomed to seeing negative laboratory results, and hence the presence of a positive serologic or culture finding immediately stirs action often before a diagnosis is confirmed. This is particularly true of serologic results, but can also happen with bacteriologic culturing and even PCR diagnostic methods. While it may be prudent to institute certain control measures upon finding a positive result, undertaking any drastic action that is comprehensive in nature and that may involve culling of research animals should only be done in the presence of clear, confirmatory results that establish a diagnosis.

Serologic cross-reactions can and do occur with agents of unknown or no research significance. False-positive results can occur for a variety of reasons ranging from laboratory error to incorrect sample preparation and peculiarities of individual serum samples. Positive results should be repeatable by alternative tests or by screening additional samples. If infection has truly occurred, the incidence of positive results should increase over time. It is important to look for sustainable patterns in serologic or culture results and not get hung up on reconciling consistency in incidence of positive results between individual groups of samples submitted concurrently. Remember that the population is being sampled and not completely surveyed and that individual animals may be at different time-points in the process of exposure, development and recovery from active infection. Errors in selection of animals for sampling based upon their age, exposure history, immunologic status, or physical barriers to complete transmission

between groups of animals can all cause inconsistencies in individual groups of results but not in general trends.

Establishing a Perimeter and Identifying Infected Groups

Just like a military operation, a “battlefield map” that is regularly updated and supplemented with other tactical analyses is important in coordinating the response to any contamination. A floor plan of the animal facility should be used to designate areas in which infection has been detected and areas at risk as well as those that test free of it. Types and numbers of animals, as well as the investigators and the projects in which potentially infected and non-infected animals are being used, should be recorded separately. A separate floor plan should be used to trace, using colored markers, the route of various husbandry activities as well as animal, equipment, people and supply movement to and from the infected areas. This will identify other areas of concern and significant cross-over points which may require additional disinfection measures. These may include common corridors, support areas, loading docks, procedural rooms, supply areas, break areas, and frequently used animal holding areas.

For the purposes of applying control measures, a perimeter around the areas of risk should be established. In some cases, this might be limited to only a portion of the animal facility, whereas in others, it may involve the whole facility. Since not all mechanisms of transmission are equally effective, it is important to determine what materials, animals, equipment, personnel or other items cross the established perimeter and to make a judgment as to the likelihood of them being infected as well as appropriate disinfection measures that need to be applied. Screening should be continued both in and outside of the perimeter to determine if changes need to be made to the location of the perimeter or the screening or disinfection practices. The map and contamination program should be reviewed and updated daily. Investigators served by the facility should be updated regularly of progress and actions that require their help. If remote facilities are also present in addition to the one that the infection has been detected in, screening should continue in these locations until confidence can be gained that the infection has not spread. A single set of negative results is never very reassuring.

By the time any organism is detected, it has been resident in an animal facility for many days and usually weeks or months. Certain barriers to spread of the organism may be in place within your facility and could include separate facilities (buildings) used to house different groups of animals, the use of microisolation caging or isolators, the presence of separate support facilities and workforces for maintaining different groups of animals, etc. In most cases, such barriers are not present; or if they are, they often only encompass a portion of the entire animal facility. Most research animal facilities are designed for efficient flow of materials and personnel in order to be cost efficient and hence tend to combine or link areas through connecting corridors to common cage processing and support areas. Dirty cages, waste, and personnel move through common hallways, despite their designation of clean and dirty, provide a means for spread of contamination. The cagewash not only serves as a point for disinfecting equipment, but also a point for potentially contaminating it. Since most organisms of interest are shed in

the feces or otherwise contaminate the bedding, the movement and handling of this material is one of the most important considerations in minimizing the spread of an organism to other areas of the facility. The movement of soiled cages is also an important means for moving organisms around the facility.

Movement of animals between areas also imposes a significant risk for bringing infection to other areas of the facility. Rodents are the principal hosts for rodent viruses. In the initial stages of infection, they shed large numbers of organisms which may continue over time or decrease to low or even undetectable levels. Since there is no way to know when infection is occurring in any given animal, there is no way to know what risk is imposed by moving an animal from one area to the next. For this reason, normal functions including the continued conduct of research in the facility during an outbreak pose a risk of extending and maintaining the contamination in the facility. Thus, each activity must be analyzed for risk and controlled or halted.

People can spread organisms between areas; however, this is principally done on clothing. The magnitude of this risk is usually less than that associated with the movement of animals, soiled bedding or soiled equipment, but is often easier to address. Clothing procedures need to be considered in order to assure that clothing is clean/free of infectious organisms and changed frequently enough to limit its serving as a fomite.

Some forms of housing, such as microisolation caging or isolators, are effective barriers to the spread of microorganisms if used correctly. Unfortunately, devices such as microisolation cages subdivide populations into small, microbiologically separate groups making assessment of the entire population's health status imprecise at best. In breeding operations (e.g., transgenics) where animals are regularly moved between groups of cages, infections may persist undetected by the usual sampling methods only to be detected at a later date. In such circumstances, halting movement of animals until individual cages/groups can be determined to be free of the organism by serology, PCR or other means may be required. Limiting the movement of animals to small groups of cages and sampling surplus or culled animals, especially retired breeders, is another approach to infection control and elimination.

Barrier rooms or facilities embedded within larger animal facilities may also help slow or eliminate the spread of a contaminating microorganism, but they are not foolproof. Most such facilities are not operated with the rigorous practices required to withstand a high contamination pressure outside the barrier. The usual operating assumption is that the concentration of contaminants in the environment is low and that there can be tradeoffs in the completeness of the barrier or the complexity/completeness of the disinfection process for materials crossing the barrier. For ease of operation, cages are often washed outside the barrier being removed by opening a door or air lock to the barrier and either returning after their disinfection in the cagewash by a similar air lock or being autoclaved in a pass-through autoclave back into the barrier. These procedures leave plenty of room for recontamination, and if the autoclave is not properly load configured and calibrated/validated, contaminants may escape even this substantial means of disinfection or be recontaminated in the transfer process.

In the face of such external contamination pressures, barrier maintenance practices must be re-examined and safeguards increased. This may include additional chemical disinfection steps for caging and materials that are brought into the barrier, more frequent perimeter disinfection as well as entry lock disinfection, increased gowning practices and clothing changes and limitation of personnel entry/access.

It is important to realize that barrier rooms/facilities are usually designed to keep things from entering—not escaping. If an organism gains entrance to a barrier room, there is little in the normal husbandry practices to prevent it from escaping to contaminate the rest of the facility. The best course of action in such cases is to try to practice two-way disinfection across the barrier and to double bag and disinfect contaminated waste. Entry lock system procedures also need to be modified as does soiled garment handling and disinfection. Animal movement across the barrier should also be halted.

Often assumptions are made about daily animal care and use practices. In larger facilities where veterinary rounds or supervision is less frequent, small changes in animal care and use practices from prescribed methods set forth in standard operating procedures may not be recognized, yet, could substantially increase the risk of spread of contamination. Similarly, changes in animal care practices instituted to address an ongoing contamination may not be conducted in the manner in which those coordinating the effort had envisioned. Thus, frequent observation and supervision of the facilities during this critical time is necessary.

If a contamination team has been established, the team or at least the veterinarian and facility manager should make regular rounds at various times during the day so that all types of activity are observed. Risky practices should be documented and modifications instituted. All changes in the procedures should be put in writing and reviewed with those conducting them to assure that there is adequate understanding. It is important to assure that technical staff understand the rationale behind changes instituted and that consideration be given to the practicality of the changes to be implemented especially when they cause significant disruption to a normal routine or require complex clothing changes that may not be well thought out.

To Cull or Not to Cull

The first reaction to a contamination is to kill infected animals, as the most direct and effective method of decreasing the risk to the rest of the facility. This presupposes that one can identify which animals are infected. It also puts a heavy burden on the testing methodology and its accuracy. Moreover, culling infected animals can have a devastating impact on research, and in some cases may not be warranted by the nature of the organism and the type of research. In such instances, alternatives may be necessary at least for portions of the effected research animal population. If certain infected animals must be kept for short periods, this is best accomplished by establishing one area for biocontainment. Ideally, this should be done at a remote location off site or with a contract quarantine service provider. If biocontainment must be conducted on site, it

should be done using isolators or similar secure housing devices which, unfortunately, will probably not be readily available at the time of the contamination. Simply designating a room as infected with no other means of containment will only leave a nidus of infection in the facility assuming one is capable of cleaning up the rest of the facility. The use of microisolation caging for biocontainment can be considered, but decontamination should be done within the room prior to moving caging to any central cage washing facility. Remember, few, if any, microisolation caging systems are designed for reliable biocontainment purposes. Moving soiled cages to other areas, no matter whether they have been disinfected or how well they are covered, only increases the risk to the rest of the facility. Additional considerations in culling decisions revolve around the biology of the organism, including whether or not the contaminating organism causes a persistent infection, whether or not it has a phase that persists in the environment, how easily infection is transmitted, and whether or not the organism is zoonotic.

The handling of waste generated within the room/area and the handling of clothing worn by personnel are also important concerns. Nothing should leave a containment area that has not been chemically disinfected or otherwise completely enclosed/sealed with plastic. The exterior surfaces of such materials, even if placed in clean bags for autoclaving or other treatment, should not be considered risk free unless disinfectants have been applied.

If the decision is made is to cull animals, it should be done within the room or area, and the carcasses should be adequately packaged in strong, liquid-impervious wrapping whose exterior surfaces can be adequately disinfected. Equipment used for culling should be considered contaminated. Carcasses and any associated dirty bedding should be removed from the facility immediately for incineration or other safe disposal as contaminated waste. Further processing or storage within the facility poses an unacceptable risk. Manual cleaning of materials within the room and subsequent disinfection of the room before movement of any materials out of the room is critical.

Burn Out Procedures for Viruses

Certain enveloped viruses that do not cause persistent infections may be amenable to the control process termed “burnout”. Only a few agents (e.g. Coronaviruses) can be managed in this way, and only in immunocompetent animals. Coronaviruses such as MHV and SDAV are highly infectious and may approach 100 percent prevalence in a naïve population. That being said, there is no guarantee that all animals will be infected by any given point in time and hence clear the infection by a certain time. Introduction of naïve animals during the course of infection will prolong the burnout period by reestablishing active infection. Breeding of animals will continue to produce naïve animals and will also prolong/sustain infection. The presence of maternal antibodies may further confound the time course of infection making the length of the burnout period even more unpredictable. At a minimum, the burnout period should be at least six to eight weeks during which time no naïve animals should be introduced and no newborn animals produced. Any animals born during that period of time should be immediately

culled, and no breeding should occur. If the burnout period can be extended, the chances of success are increased if appropriate steps are taken to ensure uniform transmission (e.g. open cages, transfer of dirty bedding) within the population has occurred and if significant and repeated disinfection of caging, equipment, as well as all surfaces within the room, is conducted.

The use of a burnout regimen is most successful when applied to small groups of animals with substantial research importance. It is also most successful and poses the least risk to other animals in the facility when applied under cage level (microisolation) or group level (isolator) containment. A burnout regimen on a multiple room level or a facility-wide level is less successful, especially if cage level or group level containment is not applied. Success is measured by assaying naïve animals exposed to the population for a sufficient period of time to allow them to acquire the infection if present. Unfortunately, this may be complicated if microisolation caging is used since cage to cage transmission is impeded. This sentinel monitoring process requires at least four to six weeks of exposure prior to sampling in order to allow enough time for organism transmission from other animals or the environment and for the development of serologic titers. Additional groups of sentinels should be exposed and sampled over time to increase confidence. If PCR is used, less time may be required, but inherent problems with this type of assay and its interpretation, as well as cost, may decrease its usefulness. Thus, the minimum time to complete the process from the initiation of the burnout process until one can begin to have some confidence that the procedure has worked is 10 to 14 weeks with 16 weeks being a more likely time period given the need to receive and interpret results as well as test additional sentinel animals.

It must also be remembered that any progeny born from virally infected mothers will, in all likelihood, have maternal antibodies that will confound interpretation of any serologic determination. For that reason, serology done on progeny or their parents will be difficult to interpret unless negative. Only F₂ generation animals are likely to not have this interference. Hence, the use of seronegative sentinels of known health status is the most expedient way to reliably assess colony status.

Room Disinfection

Once a decision has been made on the disposition of the animals, an orderly process of disinfection of rooms and equipment should begin. This process of disinfection and reestablishing of the room to house, use or support animals is referred to as “recycling”. Once the animals and dirty bedding have been removed from the room under controlled conditions, the room should be closed to other traffic until such time as it is ready to be populated with animals of known health status. The door to the room should be kept closed and preferably kept locked at all times when personnel are not in the room. Signs on the room door should indicate that disinfection is in process, and the room should only be entered by authorized personnel.

The first step in this process is to determine the integrity of the room. An examination should be made of all surfaces for the presence of cracks or other

penetrations that will need to be sealed. Next, dirt and debris should be removed from existing racking and fixtures and the floor swept. This is best done manually with utensils which can later be disposed of as contaminated materials. Unless HEPA filtered, vacuum cleaners can aerosolize materials to a degree that may coat surfaces and make them more difficult to disinfect. Moreover, the cost of vacuum cleaners would make it difficult to rationalize their disposal between rooms, and the ability to disinfect them would also be questionable. If a non-HEPA filtered vacuum cleaner must be used, one with a disposable bag is preferred. All collected materials should be disposed of as infectious waste.

Animal rooms will generally have some source of water that can be used in the washing process even if a sink of suitable size is not present. Similarly, disposal of contaminated water will usually be possible in animal rooms although occasionally sinks used for hand washing will need to be used for this purpose. In those instances where such fixtures are not available, large containers of wash and rinse water may need to be brought into the room and be removed as contaminated waste materials during the process after a suitable disinfectant has been added to them.

All surfaces of the room should be scrubbed with a detergent with good degreasing properties. The ceiling and associated fixtures should be washed first, while being careful to avoid electrical hazards. This initial cleaning should focus on external surfaces such as light covers, ceiling surfaces, walls, etc. Scrubbing pads, sponges on mop handles or other similar devices that assure that surfaces can actually be well-washed and rinsed should be used. Personnel involved in this process should preferably be attired in disposable clothing and, where appropriate, water-resistant garments such as boots and substantial gloves. Appropriate personal protective equipment should be provided including eye protection, and in later stages of the disinfection process, respiratory protection should be used. All water generated during the washing process should be removed by sponge mops or similar absorbent devices from which water can be wrung out. The usual string mops and ringers are not particularly appropriate for these tasks nor for application of detergent solutions and rinse. All clothing, cleaning equipment and supplies should be considered infected.

Cage racking and other fixed equipment should have all surfaces washed and rinsed. In the case of automatic watering tubing attached to walls and other wall-mounted non-electrical fixtures, fasteners should be loosened so that washing and disinfection can occur behind them. Cover plates for light switches and electrical outlets should be removed as part of the cleaning process or at least have their outside surfaces disinfected. Where appropriate, these could be discarded and new plates put in place. It is important that all such electrical fixtures have adequate seals as it is often difficult to completely clean electrical boxes or other pieces of equipment. Sealing conduits, electrical boxes and cover plates with room temperature vulcanization compound or dimeric caulking following the disinfection process is usually an adequate alternative to more complex disruption of surface-mounted systems, including lights and light timers. There is often a trade-off between the level of disinfection and cleaning possible for such devices and the cost for replacement. Most risk involves dust particles that may cling to

areas that are shielded by the fixtures themselves, hence reestablishing a seal around them (e.g., lights) may be an adequate alternative. Follow the philosophy: if there is a hole or a crack, eliminate it!

Supply and exhaust grills are points for collection of dust. Exhaust grills pull dust and debris from the room into the exhaust ducts and hence serve as an important source of contaminants. The exhaust diffuser should be removed, and all surfaces washed and disinfected. Room level exhaust pre-filters should be removed and replaced with new ones. Where possible, ducts should be brushed out, washed and disinfected to a depth of 3 feet. This is not always possible, but some attempt should be made in order to prevent possible retrograde movement of infected dust particles back into the room. In most applications, supply ducts do not have to be similarly disinfected; however, supply diffusers should be disinfected since they may participate in eddying patterns that lift dust particles from the floor up to the supply diffuser where they mix with incoming air and move back down to the floor. High velocity changes in direction cause impaction of particulates riding in the air stream resulting in deposit of the particles on the ceiling and supply grill. For the same reason, attention should be paid to cleaning and disinfecting all surfaces of lights that are exposed to the room.

Following cleaning with detergents and rinsing, the surfaces should be allowed to dry. The first of two disinfectants should then be applied. The choice of disinfectants should be matched to the contaminating organism, and aqueous disinfectants with different modes of action should be chosen (Block, 1991). At least one of the disinfectants should be active in the vapor phase, thereby not requiring direct contact with the disinfectant solution. For this purpose, CRL prefers chlorine dioxide based agents or bleach. Where possible, a 1:5:1 dilution of chlorine dioxide based agents is recommended and is most effective for initial disinfection. Some surfaces, however, are very susceptible to their oxidizing action of chlorine dioxide, and hence dilutions of 1:9:1 or 1:18:1 may be tried. If household bleach is used, it should be diluted to a 1 percent solution (39 ml of a 5.25% stock bleach/gallon of water). Dilution should be made with water of pH less than or equal to 7.0. All use of these agents should be done with respiratory and eye protection. Appropriate protective clothing including gloves and water resistant covering for the skin is mandatory. These materials can be applied using sponge mops, or depending upon the nature of the surfaces, with hand-held pump or garden sprayers. All surfaces in the room should be disinfected. The disinfectant should be allowed to dry completely before applying the second disinfectant.

A second disinfectant with a different mode of action should be chosen. Quaternary compounds, peroxides, and phenolics are commonly available. Use dilutions should be chosen that minimize contact time. Appropriate safety precautions should be taken as described above. The second disinfectant should be allowed to go to dryness before the room is entered for removal of any equipment or the conduct of any repairs that are necessary prior to restarting the room. Traffic into the room should be controlled, and personnel with work assignments in potentially infected areas within the perimeter should not enter the room without proper decontamination such as clothing change. Equipment and materials brought into the room should be disinfected. Only

materials of known disinfection status should be returned to the room during any set up procedure. When there is any doubt regarding the contamination status of materials, it is better to assume that they are contaminated and re-disinfect.

If large areas are being disinfected such as multiple rooms and support areas with subsequent set up and re-population occurring over a period of time or if the area remains unused for a period of time while other areas are being disinfected, it may be appropriate to minimize the risk that recontamination has occurred by repeating the cleaning and disinfection process immediately prior to restarting the room.

If heavy rolling stock is brought into the disinfected room (e.g., racking or carts on wheels or large pieces of equipment), it is important to assure that those surfaces that are closest to floor be re-disinfected if such materials are moved through corridors where residual contamination may reside and can contaminate these areas.

Large pieces of mechanical equipment such as laminar flow hoods or bedding dump stations located within rooms are often difficult to disinfect. Dump stations and laminar flow hoods pull in large amounts of air through coarse filters, blower assemblies, and HEPA filters before being discharged into or out of the device. There are often complex return channels within the device for re-circulation of air as well as other mechanical fixtures and controls. If such devices are present in a room that has been contaminated, consideration should be given to in-the-room disassembly of the equipment for the purpose of replacement of filters and cleaning of internal surfaces. Pre-filters and HEPA filters must be replaced. Due to the possibility of bleed through of contaminants since no HEPA filter is 100 percent efficient, surfaces downstream of the HEPA filter may also be contaminated. All surfaces of the equipment both interior and exterior should be cleaned and disinfected. To thoroughly eliminate the risk of re-infection, some mechanical components such as pulley assemblies and blower motors may need to be replaced, since lubricants and windings are traditionally difficult to penetrate with typical disinfectants and may be damaged by them. At the very least, vacuuming out such components and replacement of filters should be done and as much of the pre-HEPA filter assembly as possible treated with disinfectants. Alternatively, commercial services exist that will wrap such devices and release paraformaldehyde in place into them to disinfect difficult to reach areas. The necessary use conditions for accomplishing disinfection by this method, however, may not be achievable and hence it is prudent to replace filters and to carry out as much surface disinfection as possible regardless of whether the extra step of application of paraformaldehyde is selected. Alternatively, such equipment may be encased in plastic wrappings and sent out for commercial ethylene oxide sterilization or irradiation. These choices are expensive and not risk free.

If the room being treated has floor drains, the protective coverings and strainers should be removed and treated with the same regimen as the rest of the room. Flushing of the drains with a degreasing agent followed by disinfectant rinses is a prudent measure. Similar treatment should be given to sinks.

Other challenges for disinfection and cleaning of equipment may arise that have not been covered in this discussion. It is important to keep in mind when trying to develop plans to address such situations that thorough cleaning of the surfaces involved using some agent capable of removing organic matter including grease and animal hair should be conducted. More than one disinfectant should be used as a “belt and suspenders” approach to ensure that the agent in question is adequately killed. Do not look for shortcuts or make assumptions about adequacy of disinfection. It is better to be thorough even if it takes more time and effort, then to do it over after re-contamination.

It is also important to realize that during times of facility disruption, there is always the possibility that other agents whose presence was not previously detected could be introduced. For this reason choosing disinfectants with overlapping spectrums of action is critical, as well as an intensified health monitoring program. In addition, thorough rinsing and repeating of cleaning and disinfection processes provides the added benefit of dilution which will further decrease the risk that an infectious dose of particulates will remain even if they are unaffected by the cleaning and disinfection process.

Taking the time to understand how things are constructed and operate is key to adequately disinfecting any space. Some items such as laminar flow hoods which draw in large amounts of air and particulates will pose a greater risk to re-infection than flat surfaces that can be easily accessed and which have a lower impact and entrapment rate for infectious particulates. Fixtures that cannot be easily removed from walls or otherwise disassembled for cleaning may be appropriately resealed, ensuring that spaces that cannot be accessed will not release particles back into the room.

Doors and doorframes can pose a significant reservoir of infectious particles especially since air from the room often accelerates over their surfaces due to air pressure differentials being maintained across them. This may be further complicated if the ventilation system in the room utilizes vents within the doors to establish air supply or return. Metal doors have ventilation holes in the top and bottom to allow moisture to be released. These areas also serve as points for accumulation of contaminated dust and debris. Hardware openings in the doors, as well as strike plates in the door jambs, often allow access to hollow internal surfaces that can accumulate infectious particles. Environmentally sensitive agents will usually soon die out in such areas; however, non-enveloped viruses, spore-forming bacteria and parasite eggs may remain infective in such locations for long periods of time. Resealing/caulking such areas is probably the only effective means of minimizing the risk from any particulates that reside in them. Care should be taken to remove and replace or completely disinfect door seals including door sweeps and sills. Vents in the doors should be removed, completely cleaned, disinfected and replaced.

In support areas, suspended ceilings that have washable drop-in tiles are occasionally used. If these areas are believed to be contaminated, it may be necessary to individually disinfect these tiles or to consider replacement. The space above such areas

is often not secure from particulates; however, depending upon the nature of materials above such ceilings, it may not be possible to disinfect effectively.

Communication

When a contamination occurs, those responsible for controlling it often go through a brief period of panic and denial, followed by a reluctance to discuss it before they have it well under control. While there is little to be gained in panicking investigators before a diagnosis has been confirmed and some basic control options developed, waiting too long to involve the research community at the institution in the problem can also be detrimental to the success of the control program. It is important to meet with representatives of principal investigative groups early on and present the facts and options. Input should be sought from users on concerns, as well as on their willingness to pursue certain control options and adhere to more restrictive care and use practices. The entire research community of the institution should be informed in writing of the contamination eradication program and progress towards controlling the infection. A description of the organism, as well as its research effects and appropriate references, should be made available.

Other Considerations

Some facilities conduct certain portions of their animal care operations in hallways. In addition, materials are often stored in hallways to allow convenient access and may include supplies or commonly used equipment. Items such as waste dump stations, shelves containing clothing or other supplies, supply cabinets, and sanitation areas such as sinks should be considered possible points of contamination and should be adequately disinfected. Conducting animal changing or other animal care operations in such common corridors are risky at best at any time, but especially during an outbreak. Confining such procedures to animal rooms or anterooms, at least as a temporary measure, minimizes the risk of transmission as well as the potential release of particulates in hallways from animals and caging that are incorrectly assumed to be free of the agent. In evaluating any procedure or area, ask yourself: Am I assuming that the animals, equipment or area are contained or non-contaminated? All too often such practices assume that no contamination exists.

Areas used to store animal waste or animal carcasses, as well as necropsy areas and procedural rooms in which contaminated animals could potentially be used, should be considered contaminated and should simultaneously undergo disinfection in a manner similar to animal rooms in which contaminated animals were housed. This cleaning and disinfection should be continued at regular intervals at least until the contamination has been eliminated.

Following detection of the contamination, considerable attention should be paid to the pest control program. This is especially important in older facilities where the incursion of wild rodents or harborage of feral animals that have escaped the research program can be a significant reservoir of the infectious agent. An integrated pest

management system with regular monitoring including trapping and, where appropriate, the use of bait stations in new animal housing areas will help to minimize the risk of recontamination by this route.

People seldom serve as reservoirs for rodent adventitious organisms, but may serve as transient fomites for such organisms. During the control and disinfection process, careful consideration should be given to scheduling of work assignments inside and outside of the perimeter in question. Clothing change can be effective in minimizing carryover of infectious particulates from one location to another; however, the extent of such clothing change needs to be matched with the perceived decrease in risk of transmission.

Items of clothing to be used should be chosen based upon those areas on an individual most likely to become contaminated while carrying out assignments in a suspected area of contamination. The hands, arms and chest are the most common sites of animal contact and contamination. The least risk to spread is complete covering of personnel. Shoes can become contaminated by virtue of particulates falling on the floor, and so washable footwear or water-resistant shoe covers may provide adequate protection in many instances. Footbaths sound like a good idea but often give a false sense of security unless used properly. Similarly, sticky mats only address the soles of shoes and can be easily avoided. At worst, they serve as a reservoir of infected particles. The use of gloves and adequate hand sanitation are also clearly important. More comprehensive gowning of individuals should be matched to the risk.

Hanging of gowns or other items of clothing within the room for reuse is a questionable practice at best. Control of soiled clothing by provision of covered containers or bags for disposal is critical. Care should be taken to select appropriate locations for putting on any protective clothing before entering contaminated areas. While placing colored tape on floors or other marking systems may be useful in reminding personnel where practices are to be conducted, they have little bearing on precisely defining or limiting contaminated areas. Clothing change within the room is probably inappropriate unless cage or group level containment is used. Laundering of suspected contaminated clothing in the face of an outbreak is best done outside of the routine laundering practices that serve the rest of the facility. Care should be taken to use hot water, disinfectants and detergents if reusable clothing is considered. The use of disposable clothing is often much more cost effective and less problematic in the face of an outbreak than trying to deal with soiled clothing. Autoclaving reusable clothing is the only way to guarantee its complete disinfection; however, care should be taken not to use autoclaves that service critical areas of the animal facility if steps have not been taken to disinfect the clothing or completely containerize it prior to autoclaving. All materials presumed to be disinfected should be covered or otherwise wrapped in a fashion to prevent recontamination preferably prior to final disinfection.

For any disinfection or control measures to be effective, it is important not to overwhelm them by allowing large numbers of people access to areas of suspected of being contaminated. Minimizing access until the contamination is under control will

decrease the number of individuals that might serve as potential fomites for bringing the contamination to other areas of the animal facility.

Adjusting air pressure differentials between rooms and corridors in the presence of a contamination may do more harm than good especially if the system has not been designed for such changes to occur without affecting the pressures in other areas. To be done correctly, the whole facility will need to be rebalanced which is not practical in the midst of a contamination.

Identifying the Source of Contamination

Identifying the source of contamination, while an important issue in order to prevent reoccurrence, should not be the primary focus of recovering from a contamination. Other steps that have been previously outlined need to be put in place as quickly as possible. In most cases, identifying the source of contamination with certainty is never really accomplished. One can develop a list of possibilities, but it is all too easy to race to the conclusion that there is only one possible source of contamination.

In order to adequately address the possible source of contamination, the facility will need to keep track of key pieces of information on an ongoing basis prior to the contamination in order to support one or another possible methods of introduction. This will require records of animal receipt and disposition, records of health monitoring and biologics testing, records of implementation of key control point disinfection and sanitation procedures, and regular evaluation of the facility from a biosecurity perspective. Regular communication with facilities, including animal suppliers, regarding contaminations and health status is also important in trying to sort out organism introduction. Key contacts and alternatives for supplier information should be identified and immediate notification by suppliers of contamination should be assured.

If this type of information is available, a list of methods of introduction can be developed. Appropriate animal and environmental testing can then be undertaken to confirm assumptions. In practice, possible modes of introduction are relatively limited and should be part of any discussions of biosecurity and risk analysis prior to the development of any contamination. Records, while never a guarantee that critical processes were in place and functioning correctly, can be used to determine if certain modes of introduction can be eliminated, thereby leaving only a few avenues by which the present contamination could have been introduced. Unfortunately, there is usually insufficient information to absolutely identify the mode of entry or to link a contamination to a specific event, unless confirmatory data is available. Confirmatory data may include the development of a similar contamination at other institutions or the announcement of a contamination at an animal supplier. All too often, unsubstantiated assumptions are perceived to be fact rather than a probable but unsubstantiated possibility.

Conclusion

There are many organisms that can potentially infect laboratory animals. The decision as to which of these are important to the institution's research program should be made well in advance of the implementation of any screening and control program that is to be applied across the institution. Different standards may be applied to different groups of animals within an institution provided there are appropriate methods put in place to exclude the organism in question from the appropriate groups of animals.

The techniques for assessing the relative importance of the various microorganisms to an institution thereby forming the definition of contamination for the institution, as well as the existing control mechanisms, have been described (White, et al., 1998). The concept of disaster planning or, more appropriately, preplanning for contamination will make the inevitable finding of a contamination easier to address than if planning is postponed until the contamination occurs. Preplanning coupled with minimizing the risk of introduction of contaminants through an aggressive self-assessment program—a biosecurity program—will lessen the impact of such contaminations. The steps previously outlined for addressing contaminations are compatible with principles that should be familiar to animal care professionals and that have been used successfully by many institutions. Their application in an orderly fashion should enable an institution to successfully recover from the introduction of an unwanted microorganism.

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