Module 07 - Basic Diseases and the Animal Facility

Objectives.

At the end of this module, the reader should:

1. Understand how animals become infected
2. Understand how diseases spread
3. Understand how diseases may be introduced to an animal facility and what steps should be taken to exclude disease
4. Understand how diseases may be contained if they do gain access to a facility
5. Understand the basics of health monitoring programs

Introduction

The spectre of disease in the animal facility sends shivers down the backs of facility managers, veterinarians and investigators who have experienced outbreaks previously. This is partly because they understand the devastating effects some diseases have on the research program, and the huge amount of work and cost involved in cleaning up after a disease outbreak.

Facility managers and veterinarians are not alone in their efforts to prevent infectious organisms from gaining entry to the animal facility. All people who regularly work in the animal facility (e.g., technicians, researchers, research staff, and graduate students) must understand how diseases may be introduced and spread. Facility guidelines and standard operating procedures (SOPs) designed to limit the risk of introducing or spreading disease must be followed by everyone. Mechanical systems must work as expected (e.g., to sterilize cages, to maintain air pressure gradients) so even the facility maintenance staff is involved.

Diseases may be broadly classified as infectious or non-infectious. This module concentrates on infectious disease. Infectious diseases are caused by a variety of organisms, such as viruses, bacteria, yeast, fungi, and parasites.

Laboratory animals, like people, are regularly exposed to potentially infectious microorganisms; however, not all such exposures result in infection. Whether a laboratory animal becomes infected depends on a number of factors related to the infectious organism, and the animal host. For example, microorganisms vary in virulence, or the animal may be exposed to only a small number of infective particles. The animal, species, or strain may be partially or

entirely resistant to infection or more susceptible because it has a deficient immune system, is stressed, or poorly nourished.

If an organism does infect an animal, there are several possible outcomes. The infection may be silent or latent, in which case the animal displays no outward evidence of infection; or the infection may cause overt disease with the animal showing a variety of clinical signs depending upon the organs or systems affected. The disease may run its course with complete recovery with or without treatment, leave some damage (residual pathology from the disease), or even lead to the death of the animal. Any animal that recovers from the disease and those animals that have had a latent infection may become carriers of the infectious organism.

Infectious diseases pose a threat to animal colonies through a wide variety of mechanisms. Clinically ill animals are poor research animals because of the disruption to their normal physiology and biochemistry. Recovery may be prolonged and recovered animals often continue to carry and shed the organisms that caused the disease, acting as potential sources of infection for healthy animals. Latent or silent infections may also adversely affect the results of an experiment due to changes in the animals' biochemistry or immune system. In thinking of means of controlling infectious diseases, it is important to know how diseases spread, the routes of infection and the routes of excretion of the organism from an infected animal.

Routes of Infection

There are just a few portals of entry into an animal for infectious agents. The two most common are by inhalation into the lungs and by ingestion through the mouth into the gastrointestinal tract. Inoculation through the skin represents a special case (e.g., insects or needles). In these cases, the disease may spread beyond the skin. Some diseases are sexually transmitted. Occasionally, infection of the skin, eyes or ears may result in disease spreading beyond these organs.

Routes of Shedding

Organisms are excreted by a variety of routes. For example, organisms may be excreted from the lungs by coughing and sneezing or from the gastrointestinal tract through faeces. Organisms may also be excreted in urine, saliva, milk, pus, from the reproductive tract, or

through vectors such as mosquitoes. Skin-based diseases (e.g., fungal infections) may cause shedding of organisms from the skin.

Disease Spread

There are only a few ways for disease to spread between animals (or between people and animals). Diseases spread: (a) by direct contact between animals, (b) via the environment, or (c) by means of fomites.

a. **Direct contact**. Animals must be in direct contact with each other for the disease to spread. This applies particularly to skin diseases (e.g., ringworm) but could also include sexually transmitted diseases.

b. **Indirect contact through the environment**. The environment is important for disease transmission. Respiratory disease is a prime example of this form of transport where the infectious organisms are in the air before they are inhaled. Water and bedding may also be considered part of the environment and so contamination of these by one animal may result in transmission to others.

c. **Fomites.** Fomites are inanimate objects that have inadvertently become carriers of infectious organisms. Contaminated cages or food, or the utensils for delivering them, are examples, but there are a variety of objects which animals come in contact with which may help transmit the infectious organisms.

Intermediate hosts or vectors are special cases for disease transmission that should not occur in animal facilities. These include biting insects (e.g., mosquitoes) in which infectious organisms may just be transported between animals (vector) or the infectious organism may undergo some development in the intermediate host.

Excluding Disease

Many facility design features, facility equipment, and standard operating procedures have as their main objective the exclusion of undesirable organisms and the containment of disease if it should occur. There are three potential sources of infection: animals, people and experimental procedures. For each of these, the following points will be discussed in terms of excluding disease or preventing it from entering an animal facility.

Animals

- Sources of animals
- Arrival procedures
- Quarantine
- Interlopers
- Other animals

People

- Restricted access
- Entry requirements
- Clothing requirements
- Pets

Animal and Facility Related Procedures

- Cells, tissues, fluids, etc.
- Sources of food, bedding, water
- Equipment used for housing, changing, feeding, watering
- Surgical facilities
- Facility functions (e.g., ventilation, cage washing, sterilizing facilities, caging systems, barriers, airlocks etc.)

Animals

Sources of animals. Animals should normally be obtained from reputable suppliers where regular testing of the animals is carried out and where the results of these tests are available. Animals, especially genetically modified animals, obtained from institutions which cannot supply clean bills of health should be regarded with a high index of suspicion if they are to be introduced into a disease-free colony. Ensuring the disease-free status of immunocompromised animals from such sources is a particular problem, as they may not develop antibodies to pathogens which is the most common method used for health monitoring.

Arrival procedures. Research animals arriving at institutions have passed through a number of areas where their health status may have been compromised (trucks, airport freight areas,

airplane compartments). Shipping crates are usually not entirely impervious to micro-organisms and airports may be infested with wild rodents. Thus, there is a real possibility that the disease-free animals that left the supplier are no longer disease-free when they arrive. In some cases, when the delivery can be controlled either by the vendor or the purchaser, the risk may be less (e.g., when the vendor is close to the purchaser and purpose specific vehicles are used for transport).

Quarantine. The conditioning/quarantine of all incoming animals serves two purposes: it allows the animals to be acclimated after a long trip, and it provides the opportunity to determine if the health status of the animals meets the requirements for entry into the disease-free colony. The quarantine period should be long enough to demonstrate that contamination did not occur during transport. Remember immune-compromised animals may not develop antibodies, and sentinel animals will be required. When rodents come from sources where their health status cannot be assured, a longer period of quarantine may be required to determine their health status.

Interlopers. The entry of wild rodents into an animal facility is cause for concern. Some of these animals may carry organisms that facilities want to exclude. Wild rodents may come into close contact with colony animals, but may also contaminate feed, bedding and other materials destined for colony use. An active pest control programme is important.

Other animals. It is common for an institution to house both disease-free animals and animals of unknown status in the same general areas. There may be SOPs in place to reduce the likelihood of transfer of a pathogen from one colony to the other. The use of air pressure gradients or airflow patterns assist in separating the colonies. It should be remembered that procedures and facilities are only as good as the people who work with them and that human or mechanical failures do occur. The main focus of this module is the laboratory animal facility in which animals known to be free of certain specified micro-organisms are housed - primarily rodents and rabbits. However, many other research animals do not have highly defined health profiles (e.g., dogs, cats, farm animals, nonhuman primates, fish, etc.). While the precautions regarding entry of these animals into the facility may be less stringent, the requirement to prevent disease spread remains.

People

Restricted access. One of the risk factors for infections in disease-free colonies is the number of people who have access to the animals. It is important to limit the people traffic to those that must have access. Access may be restricted for people who have visited another animal facility during the same day. Bio-exclusion SOPs may require that a specific time period occur from the previous animal facility contact.

Entry requirements. In addition to the restrictions mentioned above, entry requirements for all other people (e.g., physical plant workers, visitors, accreditation teams, etc.) should be enforced. Equipment may need to be brought in that cannot be sterilized. It may be desirable to have all visitors put on fresh clothing or shower before entering the unit.

Clothing requirements. Protective clothing should be worn when working with animals. A complete change of clothing into facility clothing may be desirable for entry into some areas such as SPF colonies. Showering in may be necessary in some areas. Protective wear could include bonnets, masks, gloves and foot wear depending on the level of protection required both for the animals and the people.

Pets. It has been documented that people who have rodents at home, for whatever reason, may spread rodent viruses. This is really a special case of transferring unwanted organisms from an infected colony to a disease-free colony. It is recommended that people working with disease-free rodents should not keep rodents as pets. Normal microorganisms carried by people may be a source of animal disease in some cases. Immuno-compromised animals are often susceptible to organisms that do not cause disease in immune-competent animals. *Staphylococcus aureus* and *Klebsiella pneumoniae* are two organisms that some people carry which may cause disease in immune-compromised mice.

Animal and Facility Related Procedures

Cells, tissues, fluids, etc. Animal cell lines may be contaminated with rodent viruses or Mycoplasma. An outbreak of mouse pox in 1998 was traced to contaminated mouse serum. These materials should be tested for contamination before they are used on animals. Human cell lines that have been passaged through or maintained in animals may also be contaminated.

Sources of food, bedding, water. There is the possibility of food and bedding becoming contaminated before it reaches the animal facility. Animal facility SOPs should ensure that any damaged bags are rejected and the outsides of the other bags are disinfected. Unwanted organisms can be introduced in the drinking water, which is a particular concern for immune-compromised animals. Automatic watering systems must be thoroughly cleaned and disinfected on a regular basis.

Equipment used for housing, changing, feeding, watering. The equipment used in an animal facility must be kept clean to prevent disease spread within the colony. In some cases, routine autoclaving of the equipment is used to back up the cage washing facilities. Food, bedding and water may be sterilized at the same time. Irradiated food is often used rather than autoclaved food.

Surgical Facilities. These are usually shared facilities and may represent a crossover point for disease-free and possibly infected animals. Although it is unlikely that the two groups of animals will be in the surgery at the same time, it is important to ensure that there is proper sanitation between uses. This applies to all other shared facilities (e.g., radiology units, procedure rooms.).

Facility functions (e.g., ventilation, cage washing, sterilizing facilities, caging systems, barriers, airlocks, etc.). There are a number of facility functions that serve to minimize the risk of bringing unwanted organisms into the facility. It is important that the ventilation system does what it is supposed to in terms of the movement of air through the various areas. As an electro-mechanical system, it is subject to failure (e.g., a damper may fail to open or close when requested, or a fan may not operate as required). Add to these human foibles such as propping open a door and ventilation patterns can change. Airlocks are employed in modern facilities to limit the movement of air between discreet areas within the facilities and between the facility and

the outside. Facility SOPs should be in place to ensure that cage washing facilities and autoclaves are checked routinely to ensure that they are performing as required. The use of Standard Operating Procedures will also help ensure that the correct procedures are used each time.

Containment of Disease

Although it is most desirable to keep diseases out of a colony, sometimes it is necessary to take steps to contain a disease and limit its spread. For example, animals may be isolated from each other, thereby eliminating direct spread of an agent. This may not be practical in some cases and may be detrimental to the well-being of the animals. Micro-isolator cages may be used to limit airborne transmission. Cage changing should be conducted in a ventilated change station so that airborne particles from one open cage do not land in the next open cage. The same procedure should be used for cages on ventilated racks.

Micro-isolators represent containment at the cage level. Flexible film isolators provide containment at a higher level. These units may contain a number of cages and the air entering and exiting is HEPA filtered. Clean cages are introduced and dirty cages are removed through airlocks with appropriate controls to prevent pathogens from escaping. A number of these units may be placed in the same room, each isolating the animals from the others. Containment barriers may also occur at the room or facility level.

Specific procedures should be in place to complement physical barriers. These should be in the form of SOPs for all tasks carried out in the barriers, all aimed at maintaining the containment. Perhaps equally important, people should be aware of what they should not do (e.g., open a micro-isolator cage lid to look at the mice without using a change station). Animals that jump onto the floor should be considered contaminated.

Summary

Do not:

- Prop open doors; it interferes with the ventilation system
- Lift the lids of micro-isolators for any reason unless they are in a properly ventilated changing station
- Refill water bottles (replace with a new bottle instead)
- Put rodents that have jumped onto the floor back in their cages
- Move from contaminated areas to uncontaminated areas
- Use the same needle to inject two different animals
- Use the same instruments for surgery on two different animals without sterilizing them
- Swap enrichment devices between cages
- Save food from the hoppers when the whole cage is being changed
- Keep rodents at home if you work in a disease-free rodent facility

Do:

- Follow all facility SOPs
- Clean and disinfect common equipment and areas after each use (e.g., procedure tables, Anaesthetic machines)
- Wear protective clothing, gloves, masks, caps, gowns, shoe covers, etc. as required by facility guidelines
- Change protective clothing between individual or groups of animals as required
- Ensure that equipment for cleaning and sterilizing is working up to standard
- Ensure that a health monitoring program is in place (the design of a health monitoring program is specific to a facility, and is beyond the scope of this module; however readers should be aware that such a program is required).

Health Monitoring

There are a number of diseases that should be excluded from animal facilities. Some organisms cause severe disease in the animals and so render them unsuitable for research purposes. Some organisms may be present in the animal without overt signs of disease appearing until the animal is stressed, for example after a surgical procedure. Some organisms may be present in animals without causing any disease but still represent a threat to research because of the changes they cause to the immune system, for example. These latent or silent diseases usually do not provide any clinical signs of their presence. Some are transmitted to humans and

may pose a risk to people, while in other cases the diseases do not cause overt disease in the animals but may interfere with their use in research. A health monitoring program is required for any of the reasons given above and it has become more of a necessity with the increase in transfer of animals, particularly mice, between institutions.

The health monitoring program should seek evidence for the presence of specific organisms that have may represent a threat to the animals, the research or people. The list will be different for different species and perhaps even within species. (Note: Link to this in a separate window: See list of unacceptable organisms for rats and mice) The organisms include viruses, mycoplasma, bacteria, internal and external parasites, fungi, etc. The program will generally use a number of techniques (e.g., serology, microbial culture, PCR technology, microscopy, histopathology) to evaluate the health status of the animals.

The frequency of testing of a colony depends on a number of factors, some of which increase the need for increased testing (e.g., frequent delivery of animals from many sources), and some of which decrease the need (e.g., good quarantine and management procedures).

The testing process may simply involve taking samples from existing animals for serology, culture, etc. Sheep or cattle are usually tested in this manner. For colonies of small rodents, sentinel animals are frequently employed so that the main colony is not disrupted. If colony animals are used, then there are several caveats to be recognized.

Since the whole colony will not be tested, enough animals must be tested to be reasonably sure that an infection does not exist in the colony. As an example, if the incidence of infection is 30% in a colony, 10 animals per 100 must be tested to be 95% confident that at least one infected animal will be identified.

Often older animals are picked when colony animals are used for surveillance. This may result in some interesting reports, especially from the histopathology examinations as some of the animals may be showing age-related changes.

If sentinel animals are used in the health monitoring program, they must be free of any of the unwanted organisms. **The major consideration for sentinel animals is that they should be given every opportunity to become infected if an organism is present.** Genetically modified animals should not be used as sentinels, because they may not mount a measurable antibody response.

Dirty bedding from colony animals may be placed in the sentinel cages since a number of diseases are spread by the fecal-oral route. The sentinel animals should be exposed to the air from colony animals (i.e., they should not have micro-isolator tops on their cages) and they should be close to the outflow for air in the room. (One design of ventilated rack routes all the air from the rack through the cage containing the sentinels.)

When the animals are tested, the hope is that all results will come back negative. However, it should be remembered that the tests are not infallible and so while the results of a single testing may be encouraging, repeated negative tests are more conclusive.
Sometimes there are positive results, particularly from serology. False positives do occur and if there is just one positive from an appropriate sample, then retesting may show that the colony is disease free. If a test comes back with a positive result, repeat samples are often taken. Repeat positive results are handled in a number of ways depending on the organism, its ability to spread, its effect on research, etc.

The course of action may also depend on the type of animals in the facility. Immune-compromised animals are at a greater risk than immune-competent animals. If the infected animals are unique, this will influence the course of action. Among the options a facility may consider would be: live with the organism if it does not pose a threat to the research, other animals or people; isolate and contain the infected colony; depopulate the infected colony, do a comprehensive decontamination and restock with known disease free animals.

The source of disease introduction should be sought as part of the recovery process from a disease problem in an animal facility. Animal sources, transportation, people, cell lines, etc., should all be checked to avoid a repeat of the problem.

Monitoring for Non-infectious Disease

The previous discussion dealt primarily with the detection of infectious diseases since they may spread and threaten a whole colony. However, non-infectious diseases are becoming more important with the large numbers of genetically modified animals being produced. The "secondary" diseases being identified in these animals may affect both the well-being of the animals and the research being conducted on them. A broad health monitoring program for genetically modified animals should also be in place.

Observing and Reporting Disease

Research scientists, research technicians, graduate students, animal care staff all have a role to play in minimizing the impact of disease in an animal facility. Important elements in health monitoring are the identification of sick animals and the implementation of procedures for dealing with the animals. Animals may be ill because of something unrelated to the experimental protocol. It is important that sick animals are promptly reported to the veterinary staff for diagnosis. It may be appropriate to isolate the animal during the diagnostic period or it may be necessary to euthanize it to secure a definitive diagnosis. The health monitoring program outlined above should be seen as a snapshot of colony health at the time the samples were taken. Very low levels of infection or diseases still in the incubation phase may be missed so it is important to obtain a diagnosis on sick animals between the routine tests. Standard Operating Procedures should be established for the reporting of sick animals and for subsequent procedures (e.g., isolation, euthanasia, necropsy, diagnosis).