# CCAC training module on: infectious diseases

## Companion Notes

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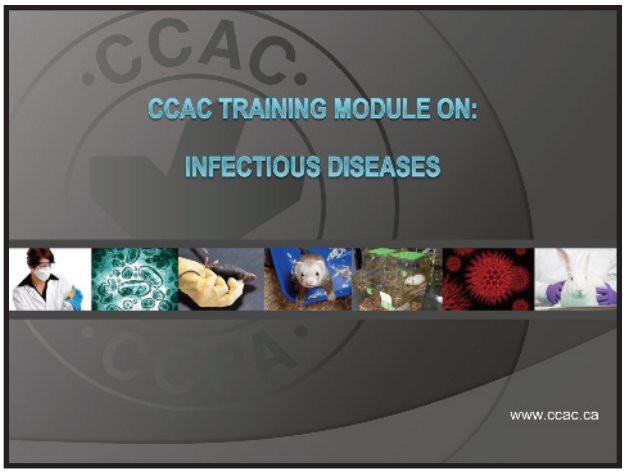
### Slide 1 CCAC training module on: infectious diseases

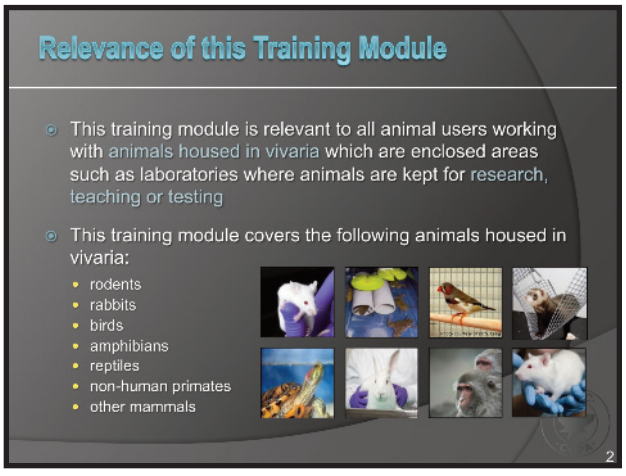



All people who regularly work in the animal facility (e.g., technicians, researchers, research staff, graduate students, etc) must understand how diseases may be introduced and spread. Equipment, facility guidelines and standard operating procedures (SOPs) are designed to limit the risk of introducing or spreading infections, and must be understood by everyone.

Infectious diseases are one of the most important variables that can interfere with research. They can have devastating effects on the research program (see *CCAC training module on: biomedical research* (2010)). Infectious disease outbreaks also limit the ability of principal investigators to share their animals with collaborators at their home institution as well as with those at extramural sites. A huge amount of work, time and cost are involved in cleaning up after a disease outbreak.

Before any research is carried out, all personnel involved must receive practical instruction, and be able to not only carry out their own work appropriately, but also work in a way that minimizes the risk of carrying infectious agents into the facility and the risk of spreading any such agents within the facility.

### Slide 2 Relevance of this Training Module




This training module is relevant to all animal users working with **animals housed in vivaria** (enclosed areas such as laboratories) where animals are kept for **research, teaching or testing**.

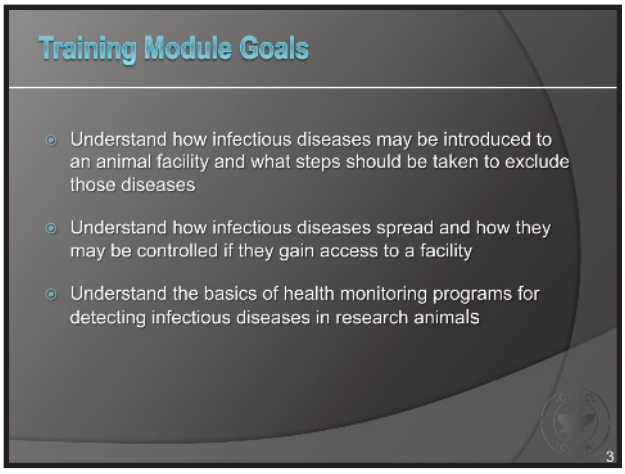
This training module covers rodents, rabbits, birds, amphibians, reptiles, non-human primates and other mammals housed in vivaria.

**Note**: This module does not cover fish; training materials relevant to fish users are available in the Fish Stream. This module does not cover farm animals; training modules relevant to farm animal users are available in the Farm Animal Stream.

## Slide 3 Training Module Goals

At the end of this training module, the reader should understand:

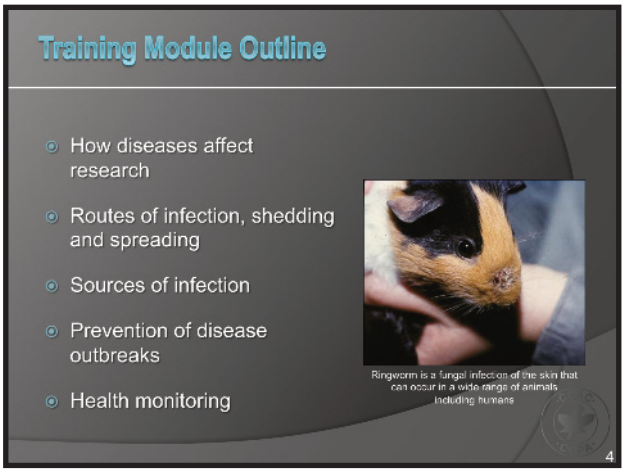
- how infectious diseases may be introduced to an animal facility and what steps should be taken to exclude those diseases;
- how infectious diseases spread and how they may be contained if they do gain access to a facility; and
- the basics of health monitoring programs for detecting infectious diseases in populations of research animals.




## Slide 4 Training Module Outline

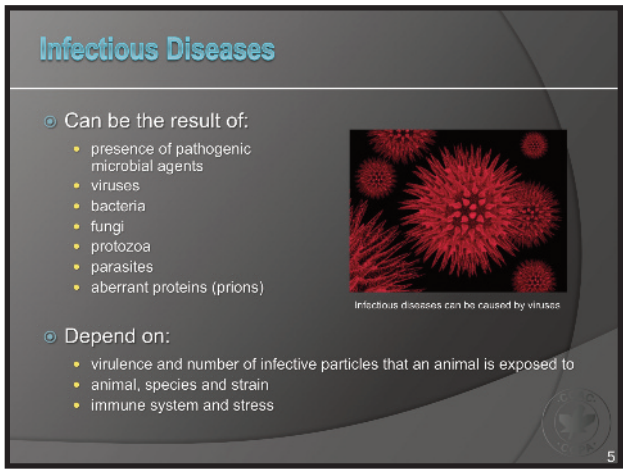
This training module will provide an overview of:

- how diseases affect research;
- the routes of infection, shedding and spreading;
- the sources of infection;
- the prevention of disease outbreaks; and
- health monitoring.

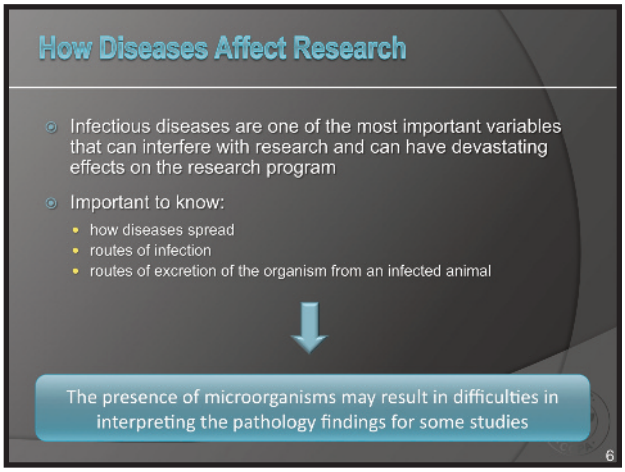



## Slide 5 Infectious Diseases

Infectious diseases result from the presence of pathogenic microbial agents, including pathogenic viruses, bacteria, fungi, protozoa, multicellular parasites and aberrant proteins known as prions. Whether a laboratory animal becomes infected depends on a number of factors related to the infectious organism and the animal host, for example, the virulence of the microorganism or the amount of infective particles that the animal was exposed to. The animal, species, or strain may be partially or entirely resistant to infection or more susceptible because of its genetic background, or because it has a deficient immune system, is stressed, or poorly nourished.



## Slide 6 How Diseases Affect Research




Infectious diseases are one of the most important variables that can interfere with research and can have devastating effects on the research program. Clinical disease is only one of several ways that infectious agents may be hazardous to research and the absence of clinical symptoms should not be interpreted as the absence of problematic infectious agents. Subclinical (or latent or silent) infection may disturb essential parameters and thus affect research results, as the following examples illustrate:

- Subclinical viral infections may affect body weight in rats.
- Behaviour will often be affected by subclinical disease, leading to disturbances in the open field test, etc.
- The presence of some microorganisms may cause changes in organs, cells or blood parameters, resulting in difficulties in the interpretation of pathology findings for some studies.
- Most animal-based experiments require animals with a normal immune system. Microorganisms may perform immunomodulation even in the absence of clinical disease, and the effect may be suppressing or activating immune functions or both at the same time, but in different parts of the immune system. Viruses are normally described as the most frequent immune modulators.
- Some microorganisms have a specific effect on enzymatic, hematological and other parameters monitored in the animal during an experiment.
- Some microorganisms affect fertility, which is particularly problematic for breeding colonies.
- Infectious agents may induce cancer, enhance the oncogenic effects of certain oncogens, or reduce the incidence of cancer in certain laboratory animals.

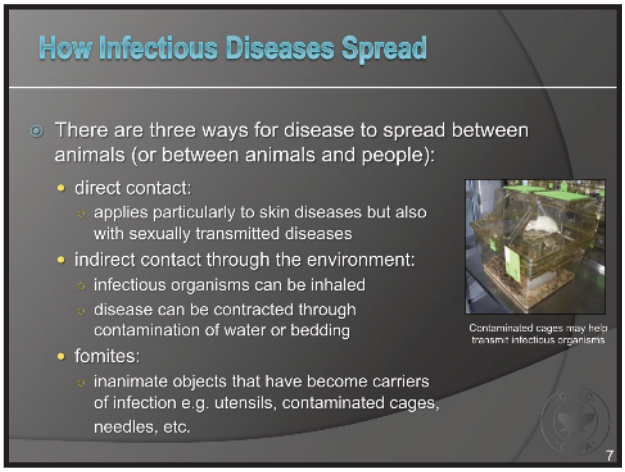
Any animal that recovers from the disease and those animals that have had a latent infection may become carriers of the infectious organism.

In thinking about 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.

## Slide 7 How Infectious Diseases Spread

There are only a few ways for disease to spread between animals (or between people and animals). Diseases spread by:

- direct contact between animals (or between animals and people);
- indirect contact (through the environment); or
- by means of fomites.




### 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.

### Indirect contact:

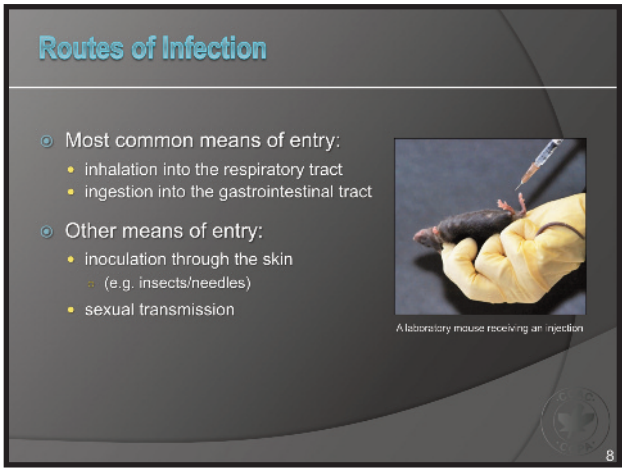
The environment is important for disease transmission. Respiratory disease is a prime example of this form of transport where the infectious organisms are in the air before they are inhaled. Water and bedding may also be considered part of the environment and so contamination of these may result in transmission to animals.

### 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 with which animals come in contact that may help transmit infectious organisms.

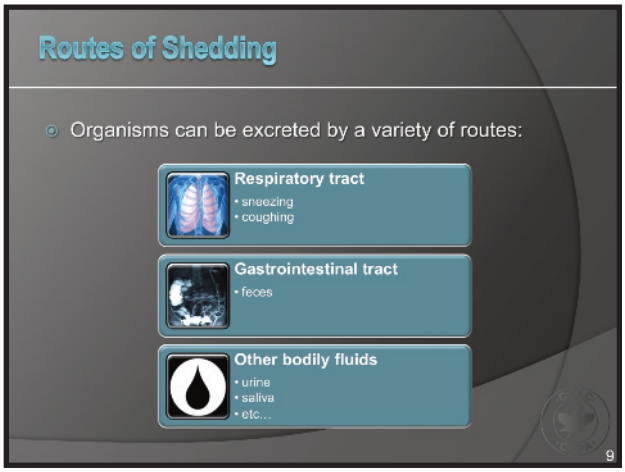
## Slide 8 Routes of Infection

There are just a few means of entry into an animal for infectious agents. The two most common are by inhalation into the respiratory tract and by ingestion 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.



## Slide 9 Routes of Shedding

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




## Slide 10 Excluding Diseases and Sources of Infection

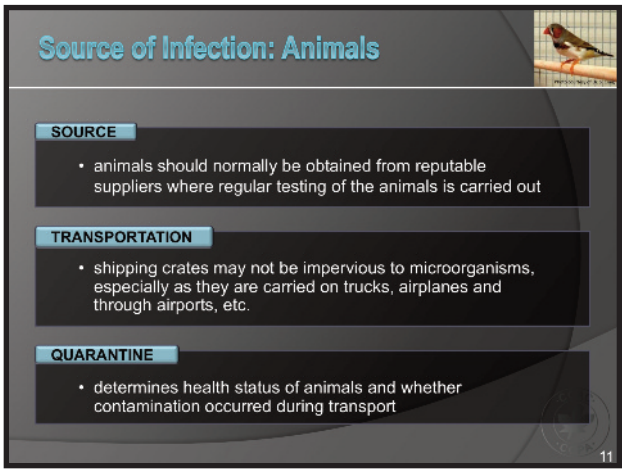
Many facility design features, specialized equipment, and facility management SOPs have as their main objective the exclusion of undesirable organisms and the containment of disease if it should occur.


- Facility design features primarily include isolating the animal facility from public access and from other activities, and may also include physical barriers, filters that exclude microorganisms and positive relative air pressure as further barriers to infectious agents entering the facility;
- Specialized equipment may include filtered (and individually ventilated) cages, as well as cage changing stations and procedural hoods that isolate animals from potentially contaminated air.
- Facility management SOPs that are designed to exclude undesirable organisms focus mainly on entry procedures for people (change into facility specific clothing), supplies (disinfection) and animals (reception and quarantine), work traffic patterns within the facility and entry procedures for rooms that may contain infectious agents.

There are four potential sources of infection: animals, environment, people and experimental procedures.

## Slide 11 Source of Infection: 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 obtained from institutions which cannot supply clean bills of health should be regarded as potential carriers of infectious disease, and measures (quarantine, rederivation) should be taken to protect disease-free colonies.

### Transportation:

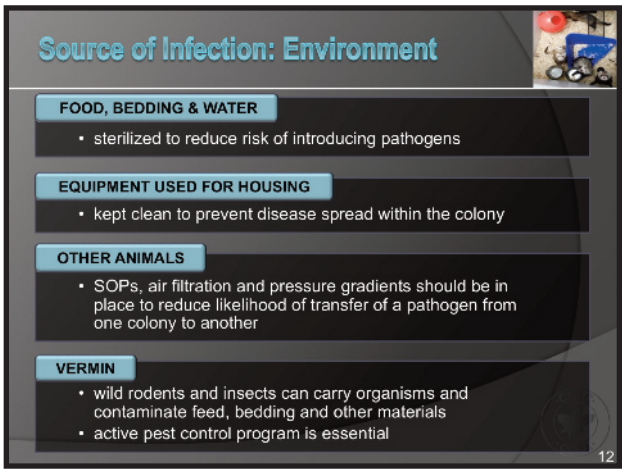
Research animals arriving at institutions have passed through a number of areas where their health status may have been compromised (trucks, airport freight areas, airplane compartments, etc.). Shipping crates are usually not entirely impervious to microorganisms and airports may be infested with wild 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 quarantine of incoming animals 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 determine the health status of the animals and to demonstrate that contamination did not occur during transport.

## Slide 12 Source of Infection: Environment

### Sources of food, bedding, water:




There is the possibility of food, water or bedding becoming contaminated before it reaches the animal. 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 immunocompromised animals. Food, bedding and water may be sterilized to reduce the risk of introducing a pathogen.

### Equipment used for housing:

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 in addition to thorough washing procedures (automatic cage washers).

### Other animals:

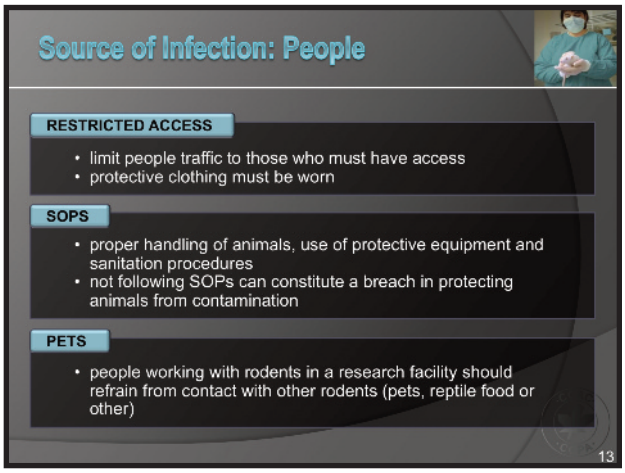
It is common for an institution to house both disease-free animals and animals of unknown status in the same general areas. There must be SOPs in place to reduce the likelihood of transfer of a pathogen from one colony to the other. The use of air filtration and pressure gradients assist in separating 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 can occur.

### Vermin:

The entry of wild rodents and insects into an animal facility is cause for concern. Some of these animals may carry organisms that facilities want to exclude. Vermin 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 program is essential to reduce this risk.

## Slide 13 Source of Infection: People

**People constitute an important risk factor for introducing and/or spreading infection.**




### Restricted access:

One of the risk factors for infections in disease-free colonies is the people who have access to the animals. It is important to limit persons entering animal areas to those who must have access. Access may be restricted for people who have very recently visited another animal facility. Bioexclusion SOPs may require that a specific time period occur from the previous animal facility contact.

In addition to the restrictions mentioned above, entry requirements should be enforced. Equipment may need to be brought in that cannot be sterilized and appropriate disinfection should be undertaken prior to introducing it into the animal facility.

Microorganisms that are carried by people without causing disease in healthy persons may be a source of animal disease in some cases. Immunocompromised animals are often susceptible to organisms that do not cause disease in immunocompetent animals. For example, *Staphylococcus aureus* and *Klebsiella pneumoniae* are two organisms that some people carry which may cause disease in immunocompromised mice.

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 specific pathogen free (SPF) or immunocompromised 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.

### Standard operating procedures:

People must be appropriately trained and adhere to SOPs for the proper handling of animals, use of protective equipment (e.g. micro-isolation caging, change station or biosafety cabinets, etc.) and sanitation procedures (e.g. surface decontamination, cage washing, autoclaving, etc.). Not complying with the established procedures can constitute a breach in the barrier protecting the animals from infectious contamination.

### Pets:

Pet rodents are known to be highly contaminated with viruses, bacteria and parasites. People can become a mechanical vector for those pathogens. People working with rodents in a research facility should refrain from contact with rodents outside the facility (whether the rodents are considered as pets or reptile food).

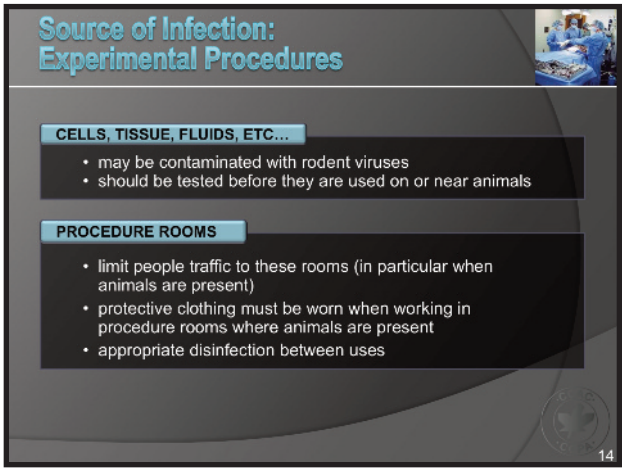
## Slide 14 Source of Infection: Experimental Procedures

### Cells, tissues, fluids, etc:

Cell lines may be contaminated with rodent viruses or mycoplasma. These materials should be tested for contamination before they are used on animals, or even before they are used in proximity to animals.

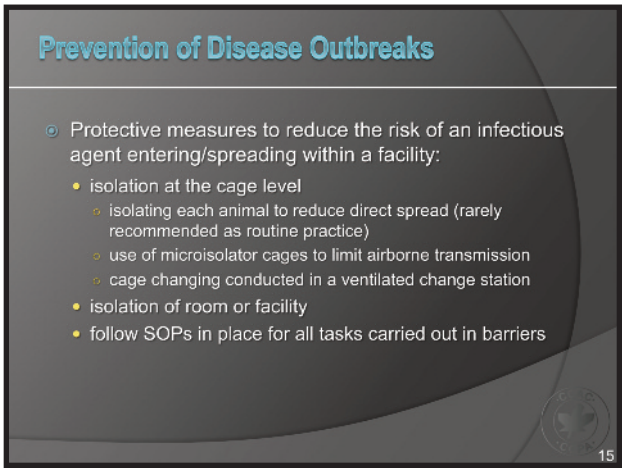
### Procedure rooms (e.g. surgical or imaging facilities):

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 same space at the same time, it is important to ensure that there is proper disinfection between uses.




## Slide 15 Prevention of Disease Outbreaks

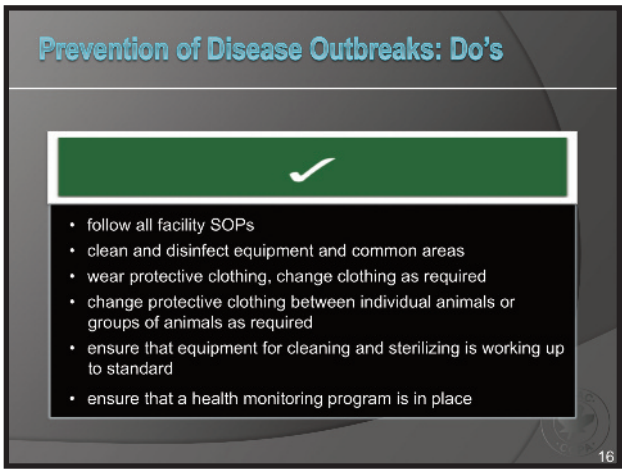
To reduce the risk of infectious agents entering or spreading within animal facilities, certain protective measures can be taken. For example, animals may be isolated from each other, thereby eliminating direct spread of an agent. This may not be practical and is generally detrimental to the well-being of social animals.




The most efficient containment occurs at the cage level. Microisolator 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. 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.

## Slide 16 Prevention of Disease Outbreaks: Do's

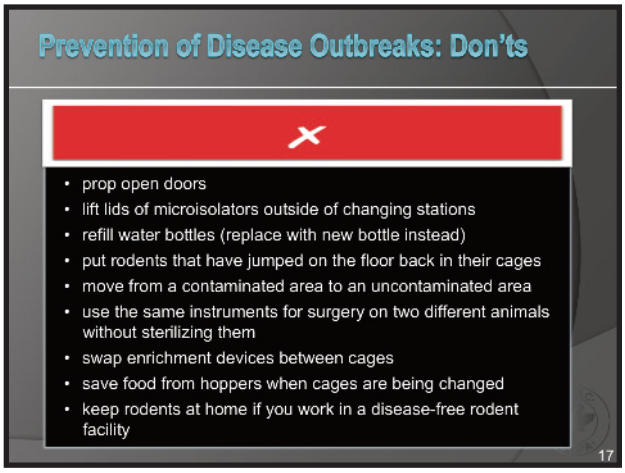
### To prevent disease outbreaks, do:




- follow all facility SOPs;
- clean and disinfect common equipment and areas after each use (e.g. procedure tables, anesthetic machines);
- wear protective clothing (gloves, masks, caps, gowns, shoe covers, etc.) as required by facility guidelines;
- change protective clothing between individual animals or groups of animals as required;
- ensure that equipment for cleaning and sterilizing is working up to standard; and
- ensure that a health monitoring program is in place (the design of a health monitoring program is specific to a facility).

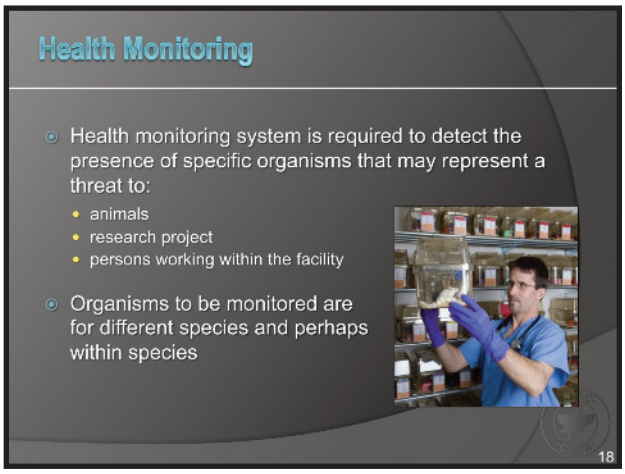
## Slide 17 Prevention of Disease Outbreaks: Don'ts

### To prevent disease outbreaks, do not:




- prop open doors; it interferes with the ventilation system (and in particular with keeping specific areas clean with positive relative air pressure);
- lift the lids of microisolators 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 within the facility from contaminated areas to uncontaminated areas;
- 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; and
- keep rodents at home if you work in a disease-free rodent facility.

## Slide 18 Health Monitoring

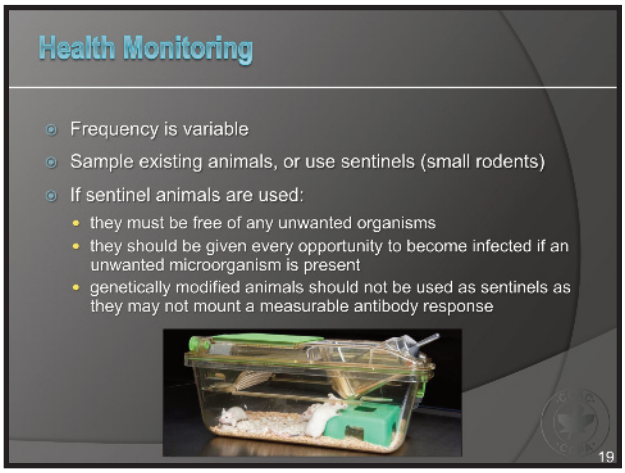



There are a number of diseases that should be excluded from animal facilities. Some organisms cause severe disease in animals and so render them unsuitable for research purposes.

Other 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. Some are transmitted to humans and may pose a risk to people.

A health monitoring program is required to detect the presence of specific organisms that may represent a threat to the animals, the research or the persons in the facility. Health monitoring has become even more of a necessity with the increase in transfers of animals, particularly mice, between institutions. The list of specific pathogens to be excluded will be different for different species and perhaps even within species.

## Slide 19 Health Monitoring




The frequency of testing for pathogens depends on a number of factors. Some factors increase the need for frequent testing (e.g. frequent deliveries of animals from many sources), and some decrease it (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.

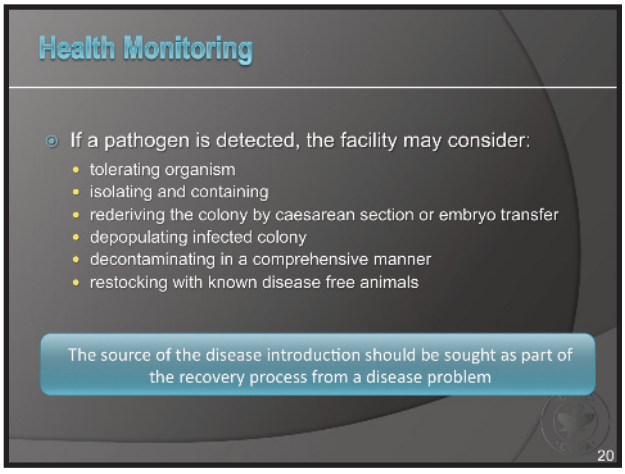
If sentinel animals are used in the health monitoring program, they must be free of any specifically unwanted organisms. The major consideration for sentinel animals is that they should be given every opportunity to become infected if such an organism is present. Their cages should be placed near the outflow of air in the room to receive the greatest possible exposure to any airborne pathogens. Dirty bedding from colony animals may be placed in the sentinel cages since a number of diseases are spread by the fecal-oral route.

Genetically modified animals should not be used as sentinels because they may not mount a measurable antibody response.

## Slide 20 Health Monitoring

When a pathogen is detected, the following options may be considered:

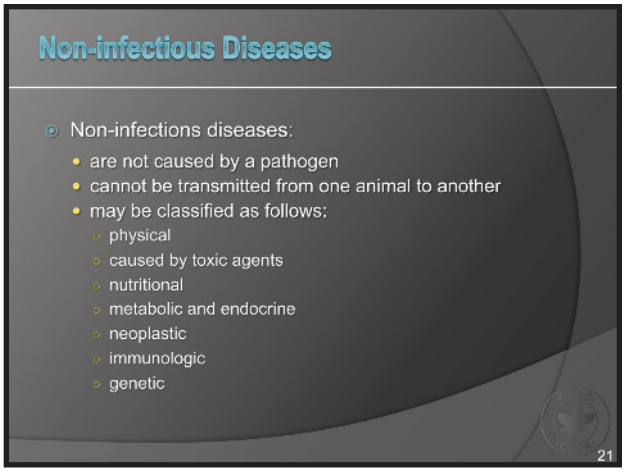
- tolerating the organism if it does not pose an immediate threat to the research, animals or people
- isolating and containing the infected colony
- rederiving the colony by caesarean section or embryo transfer
- depopulating the infected colony, doing a comprehensive decontamination and restocking 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.

## Slide 21 Non-infectious Diseases

Non-infectious diseases are those diseases that are not caused by a pathogen and cannot be transmitted from one animal to another. Non-infectious diseases may be divided as follows:




### Physical diseases:

They are caused by trauma (e.g. fighting injuries, bone fractures), chemicals (e.g. chemical burns with acids or bases), temperature (e.g. frost bite, burn), and irradiation (e.g. x-rays, ultraviolet light).

### Diseases caused by toxic agents:

These diseases are caused by poisons such as organic and inorganic toxins.

### Nutritional diseases:

There are a variety of possible nutritional deficiencies which may occur. A good example is scurvy in guinea pigs caused by vitamin C deficiency.

### Metabolic and endocrine diseases:

They are caused by disruption of a normal metabolic pathway or due to a disorder of the endocrine system (hormonal balance) e.g. pregnancy toxemia, diabetes, hypothyroidism or hyperthyroidism.

### Neoplastic diseases:

They are characterized by the abnormal and uncontrolled growth of tissue in an organ which results in the formation of a tumor (e.g. cancer, benign tumors).

### Immunologic diseases:

They are caused by alterations to the immune system such as autoimmune disease (e.g. lupus) or animal models specifically created with a deficient immune system (e.g. athymic (laboratory mice lacking thymus gland) or severe combined immunodeficiency (SCID) mice).

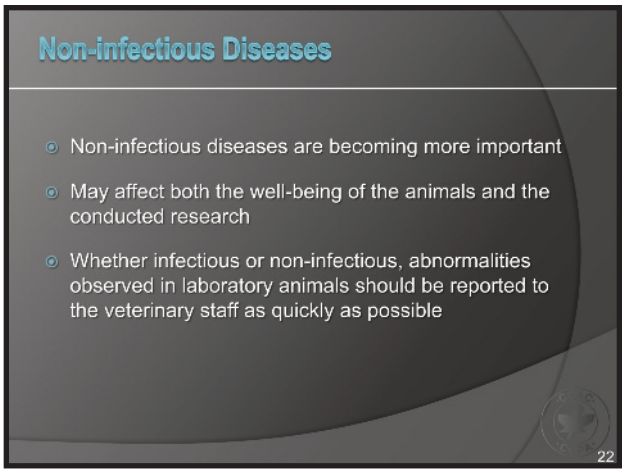
### Genetic diseases:

There are a multitude of genetic abnormalities in laboratory animals (e.g. ulcerative dermatitis in C57BL/6 mice). Some of these are well characterized and have proven to be valuable animal models for the research laboratory (e.g. the stroke-prone Severely hypertensive rat).

## Slide 22 Non-infectious Diseases

With the large numbers of genetically modified animals being produced, non-infectious diseases are becoming more important. The "secondary" diseases identified in these animals may affect both the well-being of the animals and the research being conducted on them.

When noticing abnormalities in a laboratory animal, regardless whether they are of infectious or non-infectious origin they are to be reported to veterinary staff.




## Slide 23 Summary

In this training module, the role of infectious diseases as one of the most important variables that can interfere with research was explained.

Animal users should strive to achieve their scientific goals while adhering to the best possible facility management and animal health and welfare standards. In this manner, both scientific and ethical goals are realized by using the fewest animals to generate valid and reproducible scientific data.