Diseases of Laboratory Animals, Health Monitoring & Prevention of Diseases

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Objectives

The main objectives of the presentation are as follows:

1) To **understand** the pathogenic agents that may affect the animals within a colony

2) To early **recognize** clinical signs that may be associated with pathogenic agents

3) To outline **management** measures in response to the presence of a pathogen

4) To present **preventive** measures and strategies for protecting the health status of a colony

Disease Terminology

Disease: Any condition that impairs the normal functioning of the body, which is typically manifested by distinguishing signs and symptoms that affect normal homeostatic processes

Infectious diseases: Conditions caused by pathogenic agents that invade and multiply within animal's body, often resulting to illness
(Infected animals are called hosts)

Contagious diseases: Transmitted directly or indirectly, from an infected animal to a healthy one

Disease Terminology

Infectious diseases

- Bacteria, viruses, fungi, protozoa, parasites
- Detected only after clinical signs of disease develop
  (or following routine microbiological screening!!)

Contagious diseases

- Most infectious diseases are contagious
- Direct contact = infected animals must touch healthy ones
- Indirectly = airborne or aerosol transmission
- Horizontal transmission = within the same generation
- Vertical transmission = one generation to next (mother to offspring)

Disease Terminology

Non-infectious diseases!!

- Nutritional diseases: Dominant animals may prevent subordinate from access to food (skin condition, growth abnormalities, reduced reproductive capacity)
  (Example: Mineral imbalances, protein-calorie malnutrition)

- Hereditary abnormalities: From parent to offspring through mutations in specific genes
  Often strain-dependent
  (Example: Retinal degeneration (rd1 gene) in C3H mice)

- Congenital diseases: Developmental abnormalities present at birth, but not necessarily inherited
  (Example: Hydrocephalus in Sprague-Dawley rats)

- Degenerative diseases: Progressive deterioration of tissues or organs over time, often associated with aging, metabolic imbalance, or long-term experimental conditions
  (Example: Chronic Progressive Nephropathy (CPN) in aged male rats)

Colony

SMELL

FEEL

HEAR

SEE

Locate the problem!!!

Before thinking of a pathogenic agent!!!

Odors

NH_3, CO_2

Ventilation

Quality of the air

Light

Noise

Temperature

Cage size

Number of animals

Males, Females

Bedding

Enrichment

Food

Water

Infectious agents

Classification of diseases

Etiology: refers to their cause or origin

Anatomically: body system where symptoms occur

Acute: rapid onset and brief duration

Chronic: gradual onset and long duration

Peracute: sudden onset and violent

Local: confined to a small area

Systemic: affect a large part or several body systems

How to classify a condition?

Detect the "different" condition

Early **recognize** clinical signs

Combine all information to **classify**

Use advanced screening tools to **confirm** the classification

How to classify a condition?

Detect the “different” condition

- Smell, feel, hear, see
- Macro-environment (room, facility)
- Micro-environment (cages, bedding)
- Naked-eye check (what’s wrong? what’s different?)

How to classify a condition?

Detect the “different” condition

If no……. Keep doing the good job!

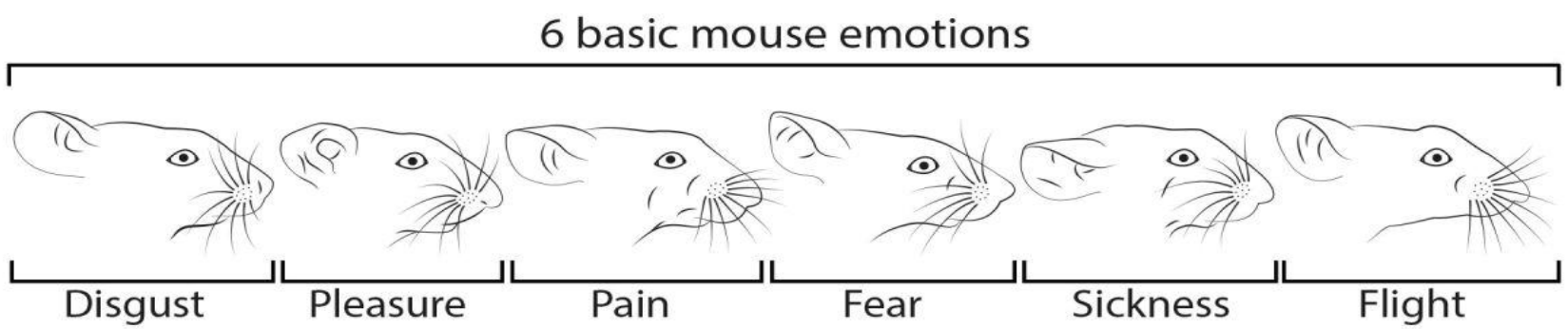
If yes???……. Be an epidemiologist!!!

- Number of patients (age, sex, strain)
- Mortality, fertility rate
- New imports (animals, material, personnel)
- Macro- & micro- environmental conditions

How to classify a condition?

Early recognize clinical signs

- Daily and frequent check of the colony
- When arrive – when leave the colony
- Trained personnel



https://www.the-scientist.com/news-opinion/facial-expressions-decoded-in-mice-67370

How to classify a condition?

Early recognize clinical signs

How many 0, 1 or 2 in my checklist???

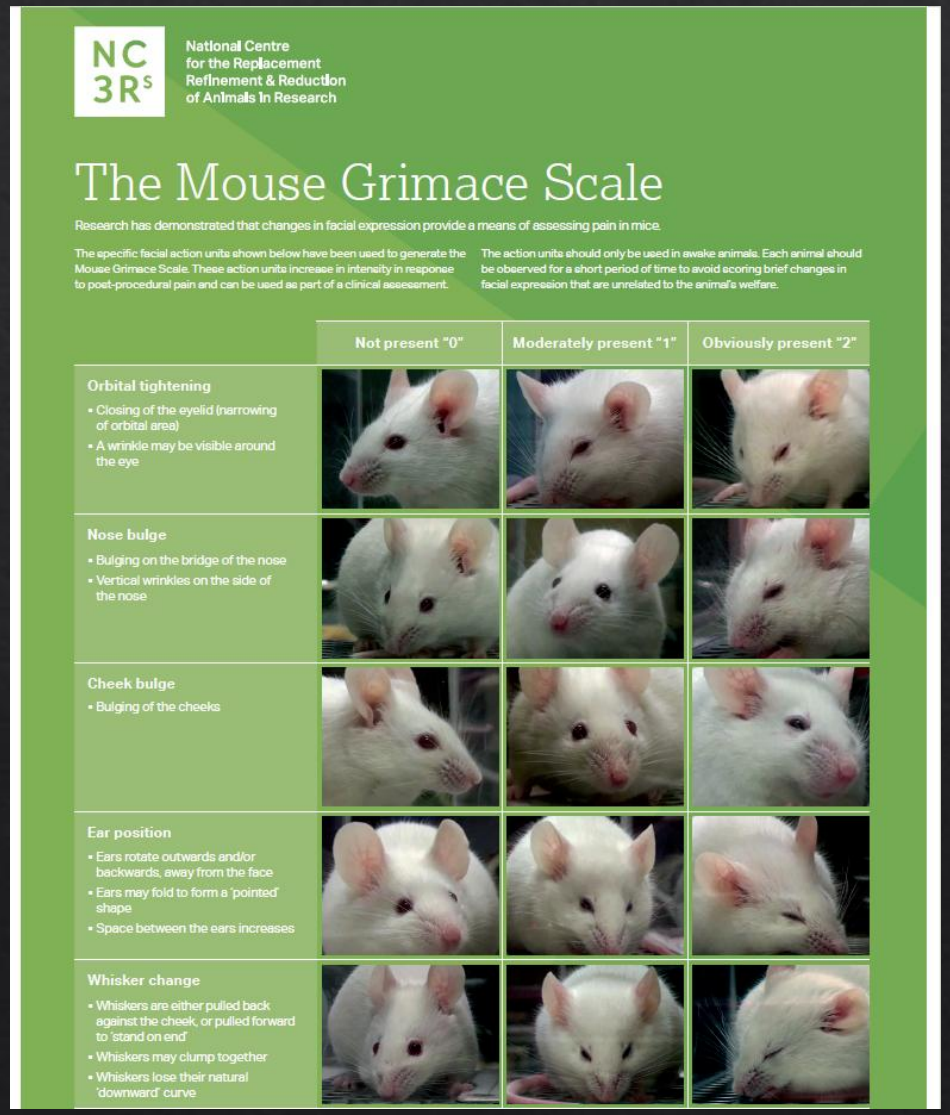
If 0, am I ok? → NO!

If 1, am I ok? → NO!

If 2, am I ok? → TOTALLY NOT!

If I have a suspicion, I will NEVER be ok!!!

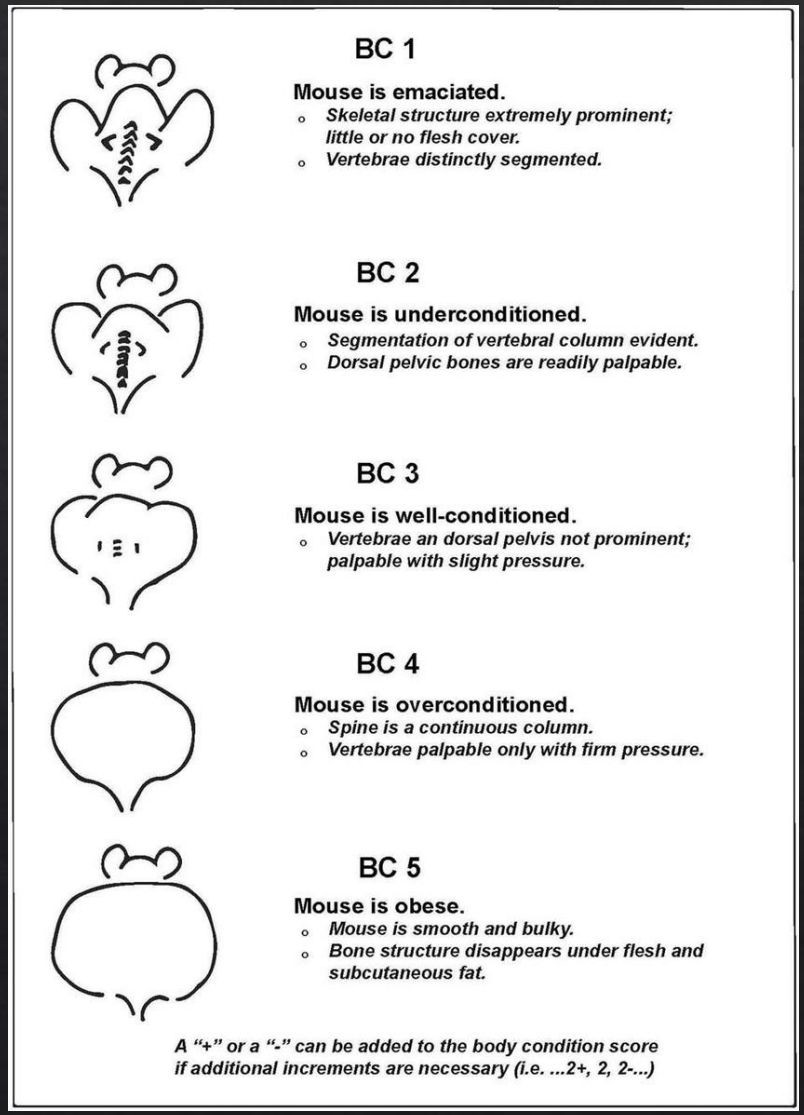
Animal's grimace and emotions
are not my diagnosis but an **indication!!!**



How to classify a condition?

Early recognize clinical signs

- Body condition scale is used to determine the housing conditions, the quality and quantity of food
- It is a useful tool to be included to our personal score sheet
- Combined with other clinical (or not) signs, could be helpful to disease's diagnosis



How to classify a condition?

Early recognize clinical signs

Physical Exam: In most cases, exam is a quick visual check to ensure that the animal is eating, drinking and behaving in a normal manner

- Cage should be observed for blood signs, abnormal feces or excessively wet or dirty bedding
- Physical examination is the first single-most important step in evaluating the health status of an animal and a colony

TPR: body Temperature, Pulse rate and Respiration rate (Larger animals)

- Physical exam includes close inspection of eyes, ears, mouth, nose, hair coat, legs and tail for evidence of clinical signs

TIP: Before attempting to restrain the animal for check, observe closely so that its movement and breathing can be assessed prior to the excitement and stress condition!!!

How to classify a condition?

Combine all information to classify

IT'S TIME TO NAME THE PATHOGENIC AGENT??? → **NO!!!**

First, we must exclude the **non-infectious** conditions

How to classify a condition?

Other **non-infectious** conditions

- Cannibalism
- Barbering, aggression
- Hydrocephalus
- Incisors overgrowth – malocclusion
- Ring tail

TIP: Keep in mind the strains of your facility. The genetic background of your animals could be the answer you are looking for…

Cannibalism

Barbering

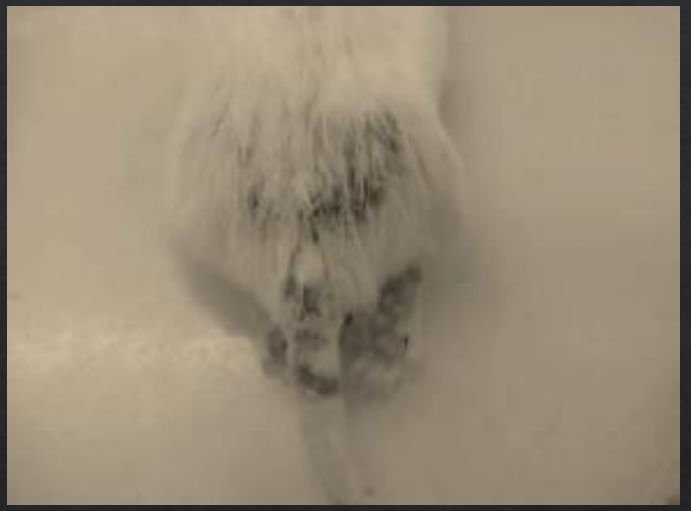
In rodents (mice), a dominant animal often chews hair off the head, neck and back of cage mates

Barbering - Aggression

- Adult male mice can be very aggressive towards each other. Fight wounds are most commonly seen on the rump and back of the animals. When fighting cage mates are found, the aggressor (if known) should be removed, or all mice separated

- If housing male mice together, they must be introduced at weaning

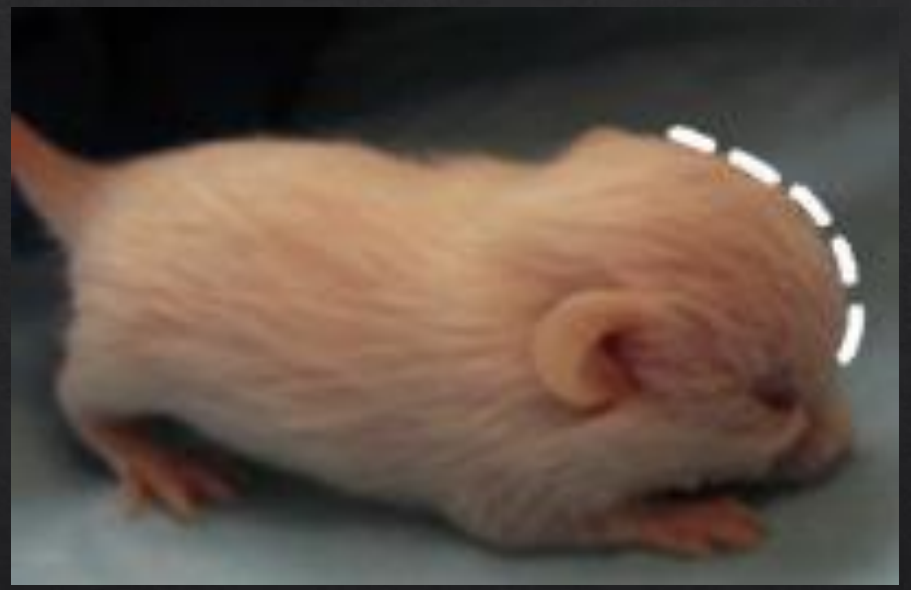
- Once separated, male mice should **NEVER** be recombined



Mouse placed with males three months Older than himself

TIP: Most of the times, the aggressor-dominant animal is the one without any barbering signs…

Hydrocephalus



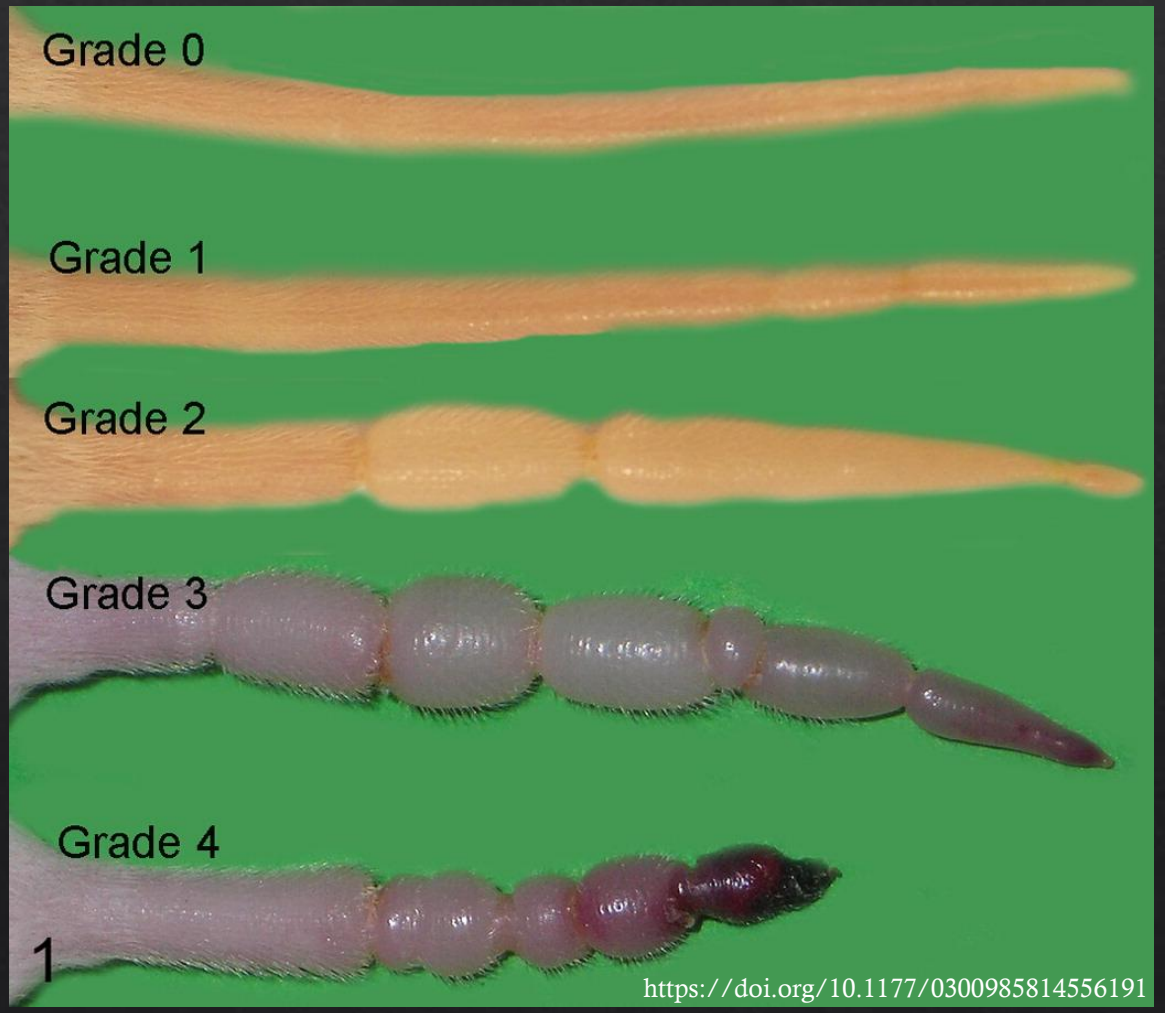
TIP: This condition is assumed to be painful. Best to use these animals quickly or euthanize when found…

Incisors overgrowth - malocclusion

- Overgrowth of the teeth and/or jaw makes it hard for the individual to eat
- The teeth can eventually grow through the tissues of the face and skull
- Teeth may be trimmed if an individual is valuable to the research
- Weekly trimming causes the animal a great deal of stress

Ring tail

- Syndrome caused by housing with low ambient relative humidity (<20%) in winter season
- Seen in suckling or pre-weaned rats
- Ring tail appears as one or more annular constrictions of the tail
- Prevented by solid-bottomed cages with adequate bedding and 50% relative humidity



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Infectious Diseases

- Viruses
- Bacteria, mycoplasma, fungi, protozoa
- Parasites

VIRUSES

Mouse Hepatitis Virus (MHV)

Classification	RNA virus, enveloped
Family	Coronaviridae
Affected species	Mice
Frequency	Common in both wild and laboratory mice.

Transmission: Through aerosols, fomites, and direct contact. The virus is highly contagious, although not persistent in the environment

VIRUSES

Mouse Hepatitis Virus (MHV)

Affects mostly immunodeficient animals

≠

Immunocompetent animals are asymptomatic

Polytropic (respiratory) strains

- Uncommon
- Replicate in nasal cavity (first)
- White foci on the liver (classic lesion)
- Same lesions in lymphoid organs

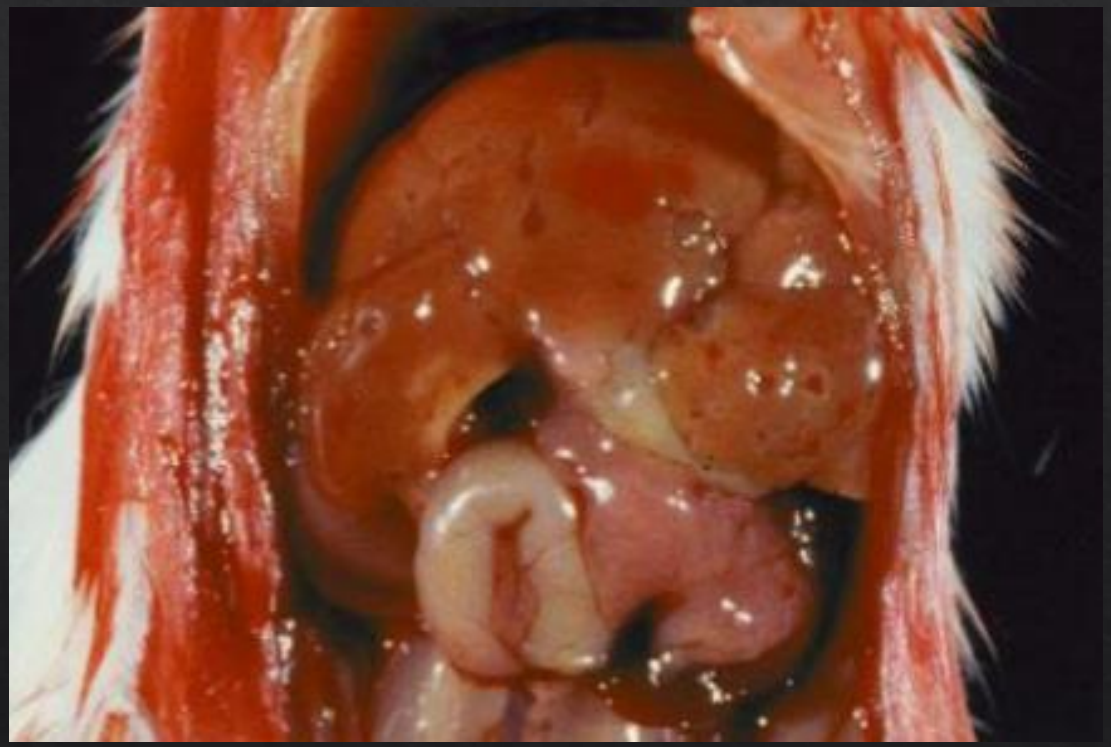
Enterotropic strains

- Common tropism of the virus
- Lesions to the intestine
- Similar lesions to ascending colon
- Excretion in feces

⚠ **Watery diarrhea and mortality in suckling mice**

VIRUSES

Mouse Hepatitis Virus (MHV)



White foci on the liver

VIRUSES

Mouse Hepatitis Virus (MHV)

- Diagnosis: ELISA, IFA

- Interference with Research: Due to lymphatic tissue infection, MHV has significant effects on the immune system, **even in immunocompetent mice**

- Depopulation, cleaning and restocking: Recommended

- Rederivation: Recommended

VIRUSES

Mouse Parvoviruses (MPV)

Classification

DNA virus, nonenveloped

Family

Parvoviridae

Affected species

Wild and laboratory mice

Frequency

Common among both laboratory and wild mice.

Transmission: Through urine, feces, and oronasal secretions. High persistence in the environment

VIRUSES

Mouse Parvoviruses (MPV)

Minute Virus of Mice (MVM)

- Pathogenicity: High (for hematopoietic cells)
- Clinical signs: None
- Clearance: Self-limiting (~ 4 weeks) in healthy mice
- Histologic lesions: Rare

MPV-1, MPV-2, MPV-3

- Pathogenicity: Very low
- Clinical signs: None
- Clearance: Can persist longer
- Histologic lesions: None

VIRUSES

Mouse Parvoviruses (MPV)

- Diagnosis: ELISA, IFA, PCR (on tissue or feces)

- Interference with Research: modification of biological responses that depend on cell multiplication
  MPV-1 infection affects immunology research in the mouse by causing derangement of immune function

- Depopulation, cleaning and restocking: Recommended
- Rederivation: Recommended

⚠️ Parvoviruses can survive in dust and debris found in ventilation systems, so aggressive decontamination is needed (detergents, oxidizing disinfectants, autoclaving)

VIRUSES

Murine Norovirus (MNV)

Classification

RNA virus, nonenveloped

Family

Caliciviridae

Affected species

Mice

Frequency

Currently, by far the most common virus in laboratory mice. The prevalence is unknown in wild mouse populations.

Transmission: By the fecal-oral route

VIRUSES

Murine Norovirus (MNV)

- Pathogenicity: **Low** in immunocompetent mice and **moderate to high** in mice with severe innate immune defects
- Clinical signs: **None** in most mice, in STAT1$^{-/-}$ or interferon-deficient mice: **weight loss, ruffled fur, diarrhea, death**
- Clearance: **Persistent infection** in both immunocompetent and immunodeficient mice
- Histologic lesions: Only in severely immunodeficient mice: **hepatitis, peritonitis, interstitial pneumonia**

VIRUSES

Murine Norovirus (MNV)

- Diagnosis: ELISA, IFA, PCR (on tissue or feces)

- Interference with Research: None (in mice with certain defects of innate immunity, the animals become ill, rendering them unsuitable for research)

- Depopulation, cleaning and restocking: Recommended

- Rederivation: Recommended

⚠️ MNV is difficult to eradicate from the environment, so aggressive decontamination is needed (detergents, oxidizing disinfectants, autoclaving)

VIRUSES

Mouse Rotavirus (Epizootic Diarrhea of Infant Mice – EDIM)

Classification

RNA virus, nonenveloped

Family

Reoviridae, Group A rotaviruses

Affected species

Laboratory and wild mice

Frequency

Common among both laboratory and wild mice.

Transmission: Feces and by the fecal-oral route

VIRUSES

Mouse Rotavirus (Epizootic Diarrhea of Infant Mice – EDIM)

- Pathogenicity: **High** in mice younger than 14 days, **low to moderate** in older
- Clinical signs: Oily, yellow diarrhea and distended abdomen (low mortality)
- Clearance: **Persistent infection** in the environment (maternal antibody provides protection)
- Histologic lesions: Rare

VIRUSES

Mouse Rotavirus (Epizootic Diarrhea of Infant Mice – EDIM)

- Diagnosis: ELISA, IFA

- Interference with Research: Experiments that use young mice. Infection modifies intestinal absorption

- Depopulation, cleaning and restocking: Recommended

- Rederivation: Recommended

⚠️ EDIM is difficult to eradicate from the environment, so aggressive decontamination is needed (detergents, oxidizing disinfectants, autoclaving)

VIRUSES

Pneumonia Virus of Mice (PVM)

Classification

RNA virus, enveloped

Family

Paramyxoviridae

Affected species

Mice, rats, cotton rats, gerbils, guinea pigs, and rabbits

Frequency

Rare in laboratory mice and rats; uncommon in wild mice (this may vary from population to population).

Transmission: Through aerosol and by direct contact with respiratory secretions

VIRUSES

Pneumonia Virus of Mice (PVM)

- Pathogenicity: **None** in immunocompetent mice, **progressive** in immunodeficient

- Clinical signs: Progressive interstitial pneumonia, weight loss and dyspnea

- Clearance: Virus not stable in the environment, losing 99% infectivity at room temperature in an hour

- Histologic lesions: Lesions in immunocompetent mice are rare. In nude mice, progressive bronchointerstitial pneumonia develops. Hyperplasia of bronchiolar epithelium occurs and is accompanied by alveolar consolidation with cellular debris and edema

VIRUSES

Pneumonia Virus of Mice (PVM)

- Diagnosis: ELISA, IFA, PCR
- Interference with Research: None (immunodeficient mice unsuitable)
- Depopulation, cleaning and restocking: Recommended
- Rederivation: Recommended

VIRUSES

Ectromelia Virus (Mousepox)

Classification

DNA virus, enveloped

Family

Poxviridae

Affected species

Laboratory mice, wild mice, and other wild rodents

Frequency

Rare in laboratory mice, uncommon in wild mice.

Transmission: Direct contact and exposure to cutaneous trauma, scabs and feces

VIRUSES

Ectromelia Virus (Mousepox)

- Pathogenicity: Depends on the **strain**
  Resistant strains: C57BL/6, C57BL/10, AKR (None)
  Susceptible strains: A, CBA, C3H, BALB/c, DBA/2 (High, 80-90% mortality!!!) ⚠️

- Clinical signs: Resistant strains (no clinical signs)
  Susceptible strains: hunched posture, facial swelling, death
  Intermediate susceptibility strains: ruffled fur, facial edema, ulceration of the muzzle, limbs, ears, tail

- Clearance: Virus very stable in the environment

- Histopathologic lesions: white spots on the liver (necropsy), acute hepatocellular necrosis, necrosis of the spleen

VIRUSES

Ectromelia Virus (Mousepox)

- Diagnosis: ELISA, IFA, PCR (of skin lesions)
- Interference with Research: High mortality in susceptible strains
- Depopulation, cleaning and restocking: Recommended
- Rederivation: Recommended

⚠️ Mousepox is difficult to eradicate from the environment, so aggressive decontamination is needed (preferably with gaseous formalin or vaporized hydrogen peroxide)

VIRUSES

Theiloviruses

Murine Encephalitis Virus (TMEV)

Mice

Classification

RNA virus, nonenveloped

Family

Picornaviridae

Affected species

Mice, rats (and experimentally, hamsters and guinea pigs)

Frequency

Common in laboratory colonies, common in wild rats and mice.

Rat Theilovirus (RTV)

Rats

Transmission: Through the fecal oral route

VIRUSES

Theiloviruses

- Pathogenicity: Low to moderate

- Clinical signs: In most cases asymptomatic
  GDVII of TMEV (virulent strain) can induce acute encephalitis
  DA of TMEV (less virulent strain) can induce a chronic demyelinating disease

- Clearance: Virus very persistent in the environment

- Histopathologic lesions: Rare (only in experimental inoculation of TMEV)

VIRUSES

Theiloviruses

- Diagnosis: ELISA, IFA, PCR (of skin lesions)
- Interference with Research: Probably with research on the nervous system, the immune system, and musculoskeletal system
- Depopulation, cleaning and restocking: Recommended
- Rederivation: Recommended

VIRUSES

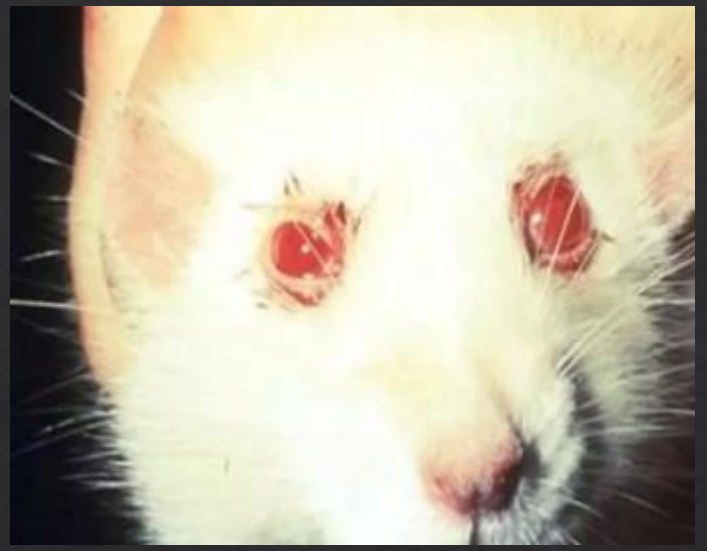
Rat Coronaviruses

Feature	Details
Classification	*Murine coronavirus* (rat-specific strains: SDAV and RCV)
Family	**Coronaviridae** → Subfamily: **Orthocoronavirinae**, Genus: *Betacoronavirus*
Affected Species	Primarily **rats** (e.g., Sprague-Dawley, Wistar); no natural infection in mice
Common Strains	- **SDAV**: Sialodacryoadenitis virus - **RCV**: Rat coronavirus
Frequency	**Moderate to high** in non-SPF colonies; **rare** in barrier-maintained facilities due to routine screening and biosecurity

Transmission: Through aerosol, direct contact

VIRUSES

Rat Coronaviruses



- Pathogenicity: Highly contagious (cause inflammation of salivary and lacrimal glands)

- Clinical signs: eye squinting, swelling of ventral cervical region and jaw, protrusion of eye
  (keratoconjunctivitis is the only sign in outbreaks)

- Clearance: Virus very persistent in the environment (low mortality)

- Histopathologic lesions: Enlarged salivary glands, edematous cervical lymph nodes, swollen lacrimal glands

VIRUSES

Rat Coronaviruses

- Diagnosis: ELISA, IFA, PCR (of skin lesions)
- Interference with Research: Rare
- Depopulation, cleaning and restocking: Recommended
- Rederivation: Recommended

VIRUSES

Rat Parvoviruses

Classification

DNA viruses, nonenveloped

Family

Parvoviridae

Affected species

Rats

Frequency

Common among laboratory and wild rats.

Transmission: Through urine, feces and oronasal secretions

VIRUSES

Rat Parvoviruses

- Pathogenicity: Highly contagious (high persistence in infected animals)

- Clinical signs: Only in Kilham's rat virus (RV / KRV) strain → scrotal hemorrhage, loss of body fat
- Clearance: Virus very persistent in the environment **(resistance to non-oxidizing disinfectants)**
- Histopathologic lesions: Rare (maybe in lymphoid tissue due to its tropism)

VIRUSES

Rat Parvoviruses

- Diagnosis: ELISA, IFA, PCR (on tissue or feces)
- Interference with Research: Moderate to immune system, oncology
- Depopulation, cleaning and restocking: Recommended
- Rederivation: Recommended

⚠️ Rat Parvoviruses are difficult to eradicate from the environment, so aggressive decontamination is needed (preferably with detergents and oxidizing disinfectants, autoclaving of materials)

VIRUSES

Sendai Virus

Classification

RNA virus, enveloped

Family

Paramyxoviridae
Note: Parainfluenzaviruses are subdivided into 3 groups, PI-1, PI-2, and PI-3. Sendai is a member, but not the only member of PI-1.

Affected species

Mice, rats, hamsters, and guinea pigs have been reported to be serologically positive, i.e., to have antibodies that react with the Sendai antigen commonly used in serology assays. Whether or not guinea pigs and some other rodents are truly susceptible to Sendai virus is controversial.

Frequency

Very rare in modern animal facilities; common in pet and wild rats and mice.

Transmission: Through aerosol and contact with respiratory secretions

VIRUSES

Sendai Virus

- Pathogenicity: Highly contagious (the infection does not persist in immunocompetent animals)

- Clinical signs: One of the few viruses that may cause clinical signs in immunocompetent rodents.
  Mice: dyspnea, chattering teeth, and death
  Rats: generally asymptomatic, may have problems with reproduction

- Clearance: Low persistence in the environment

- Histopathologic lesions: Dark, consolidated foci in the lungs, rhinitis, bronchitis, bronchiolitis (necropsy)

VIRUSES

Sendai Virus

- Diagnosis: ELISA, IFA, PCR
- Interference with Research: Lung changes, infertility, death of susceptible strains **(unsuitable for research purposes)**
- Depopulation, cleaning and restocking: Recommended
- Rederivation: Recommended

VIRUSES

Rat Rotavirus (Infectious Diarrhea of Infant Rats – IDIR)

Classification

RNA virus, nonenveloped

Family

Reoviridae

Affected species

Rats, humans, and swine are probably infected by distinct rotaviruses.

Frequency

Unknown, but apparently very rare in laboratory or wild rats.

Transmission: Via the fecal-oral route

VIRUSES

Rat Rotavirus (Infectious Diarrhea of Infant Rats – IDIR)

- Pathogenicity: **High** in rats younger than 14 days, **low to moderate** in older
- Clinical signs: Poor growth, diarrhea and perianal dermatitis (low mortality)
- Clearance: High persistence in the environment
- Histopathologic lesions: Rare

VIRUSES

Rat Rotavirus (Infectious Diarrhea of Infant Rats – IDIR)

- Diagnosis: PCR
- Interference with Research: Infection modifies intestinal absorption
- Depopulation, cleaning and restocking: Recommended
- Rederivation: Recommended

⚠️ IDIR is difficult to eradicate from the environment, so aggressive decontamination is needed (detergents, oxidizing disinfectants, autoclaving)

BACTERIA, MYCOPLASMA, FUNGI

Murine Respiratory Mycoplasmosis (MRM)

Classification	Small, pleomorphic bacteria that lack a cell wall
Family	Mycoplasmataceae
Affected species	Mice and rats are primary hosts; guinea pigs and hamsters are susceptible to experimental infections.
Frequency	Rare in modern laboratory animal populations; common in pet and wild populations of rats and mice; guinea pig and hamster prevalence data is not available.

Transmission: Direct contact, aerosol, transplacentally

Murine Respiratory Mycoplasmosis (MRM)

- Clinical signs: Clinical signs in **mice** include weight loss, ruffled hair coat, dyspnea ("chattering"), hunched posture, and reluctance to move.
  In **rats**, clinical signs are similar, although dyspnea in rats is described as "snuffling," and rats may also exhibit porphyrin staining of the nose and eyes

- Clearance: Are not considered to be viable for long periods of time outside of a host

- Histopathologic lesions: Gross lesions noted on necropsy vary with the duration of infection and the tissue infected. Mycoplasmosis causes suppurative infection of the respiratory and reproductive tracts
  Chronic suppurative bronchopneumonia with prominent hyperplasia of bronchus-associated lympoid tissue (microscopically)

Murine Respiratory Mycoplasmosis (MRM)

- Diagnosis: ELISA, PCR
- Interference with Research: Animals not suitable for use in research
- Depopulation, cleaning and restocking: Recommended
- Rederivation: Recommended

⚠️ Treatment: No effective treatment. Tetracycline limits losses

Helicobacter spp.

Classification

Gram-negative bacteria; spiral, fusiform, or curved; some with flagella

Family

Helicobacteriaceae

The species currently described in rats and mice are: *H. bilis*, *H. ganmani*, *H. hepaticus*, *H. muridarum*, *H. mastomyrinus*, *H. rappini*, *H. rodentium*, and *H. typhlonius (mice)* and *H. bilis*, *H. muridarium, H. rodentium*, *H. trogontum*, and *H. typhlonius (rats)*. The *Helicobacter* species associated with clinical disease in rats and mice are primarily *H. bilis* and *H. hepaticus*.

Affected species

Almost every species of mammal examined appears to have at least one associated *Helicobacter* species.

Frequency

Common in both wild rodents and laboratory animal facilities.

Transmission: The fecal-oral route, through the movement of dust and fomites

Helicobacter spp.

- Clinical signs: Disease in immunocompetent animals caused by Helicobacter is almost exclusively limited to susceptible strains of mice infected with either *H. bilis* or *H. hepaticus*
  Immunodeficient animals seem susceptible to disease due to a broader range of Helicobacter spp.
  In susceptible animals, the main clinical sign associated with Helicobacter infection is rectal prolapse secondary to typhlitis or typhlocolitis. Helicobacter-infected animals can also present with diarrhea.
  H. hepaticus may also be associated with the development of liver and colon cancer in some strains of **mice**, such as the A/J.

- Clearance: Low persistence (organism highly sensitive to desiccation)

- Histopathologic lesions: Typhlocolitis, hepatitis and gastritis

Helicobacter spp.

Rectal prolapse

Helicobacter spp.

- Diagnosis: PCR
- Interference with Research: Low (only susceptible strains are excluded)
- Depopulation, cleaning and restocking: Recommended
- Rederivation: Recommended

⚠️ Treatment: Combination of amoxicillin, clarithromycin, metronidazole, and omeprazole

Clostridium piliforme (Tyzzer's disease)

Classification

Gram-negative filamentous rod-shaped bacterium

Family

Clostridiaceae

Affected species

Laboratory rodents and rabbits are susceptible to this organism, as are many other mammals. There is evidence for host species specificity among *C. piliforme* strains, but it is not certain if this is absolute.

Frequency

Varying. In general, modern laboratory rodent populations are free of *C. piliforme*, but pockets of increased prevalence may exist. Prevalence in pet and wild animal populations is unknown. Tyzzer's disease, as distinct from asymptomatic infection, primarily occurs in conditions of poor husbandry or with immunosuppression.

Transmission: Through ingestion of spores from the environment or in the feces

Clostridium piliforme (Tyzzer's disease)

- Clinical signs: Animals may harbor C. piliforme with no clinical signs, and immunocompetent animals clear infection within a few weeks
  The typical presentation of Tyzzer's disease is seen in recently weaned animals. Animals are inappetent, thin, and have ruffled fur. **Rats** may have a greatly distended abdomen
  There may or may not be diarrhea in rodents; there is usually diarrhea in rabbits
  Acute death with no clinical signs may also be seen

- Clearance: High persistence (due to spores)

- Histopathologic lesions: White spots on and in the liver (necrotizing hepatitis). There is often a necrotizing ileitis, typhlitis, or colitis, and the mesenteric lymph nodes may be enlarged

Clostridium piliforme (Tyzzer's disease)

- Diagnosis: ELISA
- Interference with Research: High (unsuitable for use in research)
- Depopulation, cleaning and restocking: Recommended
- Rederivation: Recommended

⚠️ Treatment: Tetra- and oxytetracyclines to reduce losses

Salmonella spp.

Classification

Gram-negative, rod-shaped, aerobic bacterium

Family

Enterobacteriaceae

Salmonella nomenclature is complicated and in a state of flux. It is easiest to refer to *S. enterica* as an unit, although it is divided into six subspecies, and over 1500 serotypes. Differentiation of subspecies or serotypes should be left to diagnostic laboratories.

Affected species

All laboratory rodents are susceptible to *Salmonella* infection. *Salmonella* serotypes are also found in cold-blooded animals and free-living in the environment. *Salmonella* is potentially a zoonotic infection that may cause serious illness or even fatalities in immunocompromised caretakers.

Frequency

Vanishingly rare in modern laboratory animal colonies. Varying prevalence among pet animals, and common among wild animals.

Transmission: Via the fecal-oral route and fomites

Salmonella spp.

- Clinical signs: Commonly subclinical
  When clinical signs are present, they include diarrhea, anorexia, ruffled coat, weight loss and porphyrin staining (rats)
  Moderate to high morbidity and mortality

- Clearance: High persistence

- Histopathologic lesions: Most evident lesions in the liver, spleen and intestinal tract
  Splenomegaly, hyperemia and thickening of the intestinal tract

Salmonella spp.

- Diagnosis: Direct culture of feces, PCR
- Interference with Research: High (unsuitable for use in research)
- Depopulation, cleaning and restocking: Recommended
- Rederivation: Recommended

⚠️ Treatment: Recommended to ameliorate clinical signs

Staphylococcus aureus

Classification

Gram positive, non-motile cocci, often found in grape-like (staphylo-) clusters

Family

Staphylococcaceae

Affected Species

All known mammalian species, including common laboratory rodent and lagomorph species, are susceptible to colonization with *S. aureus*. Due to its ability to colonize a wide range of species, *S. aureus* can be readily transmitted from one species to another, including from humans to animals and *vice versa*.

Frequency

Common to rare, depending on type of housing, contact with humans, and initial health status of the animal.

Transmission: Through aerosol or direct contact with fomites and infected animals

Staphylococcus aureus

- Clinical signs: In healthy, immunocompetent animals, *S. aureus* colonization of the skin, intestinal tract, or nasopharynx is generally asymptomatic
  In susceptible strains of mice or rats, or immunocompromised or immunodeficient animals, *S. aureus* may cause pyogenic (abscessing) infections of the conjunctiva and adnexa of the eye, the skin and adnexa, or the genital tract

- Clearance: Moderate (susceptible to most common disinfectants)

- Histopathologic lesions: Rare

Staphylococcus aureus

- Diagnosis: Culture on blood agar, commercial test kits
- Interference with Research: Rare
- Depopulation, cleaning and restocking: Recommended
- Rederivation: Recommended

Treatment: Recommended to ameliorate clinical signs

Pneumocystis (*P. murina*, *P. carinii*)

Classification
Fungus (*Ascomycota*)

Family
Pneumocystidaceae

Affected species
All mammals may have host species-specific *Pneumocystis*. Among laboratory rodents and rabbits, *P. murina* has been described in mice, *P. carinii* and *P. wakefieldiae* in rats, and *P. oryctolagi* in rabbits.

In immunodeficient animals of all species, *Pneumocystis* infection causes chronic progressive pneumonia. In immunocompetent rats, *P. carinii* has recently been found to cause infectious interstitial pneumonia (IIP), the condition previously informally attributed to Rat Respiratory Virus. Immunocompetent mice become infected with the fungus *P. murina*, but clear the infection without developing lesions. In rabbits, *P. oryctolagi* infection causes transient pneumonitis near weaning. There is no cross-species transmission, even among immunodeficient individuals (the human organism has been renamed to *P. jirovecii*).

Frequency
IIP caused by *P. carinii* is among the most common diseases of laboratory rats, more common than any of the parvoviruses, for example. *Pneumocystis* is typically excluded from contemporary, well-managed colonies of immunodeficient animals, so lesions are infrequently seen in these.

Transmission: Direct contact, fomites and aerosol

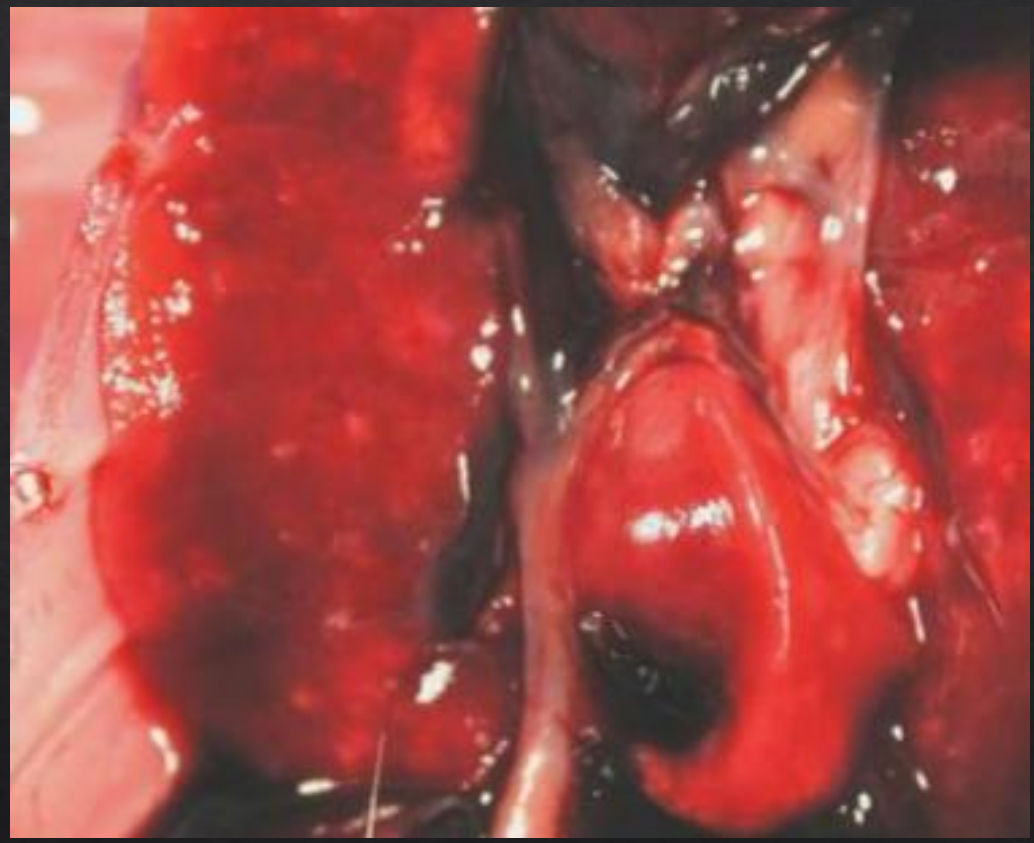
Pneumocystis (*P. murina*, *P. carinii*)

- Clinical signs: Immunodeficient **mice** and **rats** with pneumonocystosis present weight loss, ruffled fur or dry skin
  Wasting (cachexia), labored breathing, cyanosis and death

- Clearance: Not specified (environment)

- Histopathologic lesions: Lungs do not deflate, rubbery, enlarged and there may be pale, gray or red areas (necropsy)
  Alveolar septa are thickened (microscopically)

Pneumocystis (*P. murina*, *P. carinii*)



Enlarged lungs

Pneumocystis (*P. murina*, *P. carinii*)

- Diagnosis: ELISA, PCR
- Interference with Research: High morbidity and mortality (immunodeficient animals)
- Depopulation, cleaning and restocking: Recommended
- Rederivation: Recommended

⚠️ Treatment: Trimethoprim/sulfamethoxazole 50 mg and 250 mg/kg/day) in the drinking water.
Does not eliminate the organism, but reduces morbidity

PARASITES

Intestinal Protozoa

Classification	Eukaryotic, one-celled intestinal parasites or commensals. Many are flagellated
Family	Various

Affected species

All rodents are susceptible to protozoal infection. Some possible infections are listed below by species, but this is not an exhaustive list. Intestinal (and hepatic) coccidial infections in rabbits are discussed in a separate technical information sheet. Wild-caught animals may have species of protozoa not described here. Some of these protozoa may be zoonotic. Generally, care should be taken with *Giardia* and *Cryptosporidium* spp. although *Giardia* usually has a limited host-range and transmission to humans from laboratory rodents has not been reported.

Frequency

Rates of protozoal infection vary between individual colonies. Most of the generally pathogenic parasites have been eradicated from modern laboratory mouse, rat, and guinea pig colonies, but may be present in hamster and gerbil colonies. Protozoal infection rates among wild and pet animals are high.

Transmission: Via contact with infected cysts (the fecal-oral route)

Intestinal Protozoa

Mice: *Chilomastix bettencourti, Cryptosporidium muris*, Cryptosporidum parvum*, Eimeria spp.*, Entamoeba muris, Giardia muris*, Hexamastix muris, Spironucleus muris*, Trichomonas muris, Tritrichomonas muris*, and others

Rats: *Chilomastix bettencourti, Cryptosporidium muris*, Cryptosporidum parvum*, Eimeria spp.*, Entamoeba muris, Giardia simoni*, Hexamastix muris, Monocercomonoides sp., Retortamonas sp., Spironucleus muris*, Trichomonas muris, Tritrichomonas muris*, and others

Intestinal Protozoa

- Clinical signs: There are no clinical signs associated with protozoal infection
  In immunocompromised animals, or young animals with heavy infestations → non-specific signs, such as weight loss, runting, hunched posture, or rough hair coat, diarrhea

⚠️ We can see protozoa in the stomach, intestinal crypts, or free in the lumen of the gastrointestinal tract on microscopic examination

- Clearance: Moderate (must eliminate all the fecal matter in the environment)

- Histopathologic lesions: None

Intestinal Protozoa

- Diagnosis: Examination of feces or direct smears of intestinal contents
- Interference with Research: Animals showing clinical signs are unsuitable
- Depopulation, cleaning and restocking: Recommended
- Rederivation: Recommended

Treatment: Recommended to ameliorate clinical signs

Fur, skin and ear mites (Acariasis)

Classification

External parasites

Family

Arachnida

Affected species

There are many species of mites that may affect the species listed below. The list below illustrates the most commonly found mites, although other mites may be found.

- Mice: *Myocoptes musculinus*, *Myobia musculi*, *Radfordia affinis*
- Rats: *Ornithonyssus bacoti**, *Radfordia ensifera*

Frequency

Rare in laboratory guinea pigs and gerbils. Occasional in rabbits and rats. More common in mice. Almost universal in hamsters. Many of these mites are commonly found in wild and pet populations of the above species.

Transmission: Direct contact with an infected animal or the environment

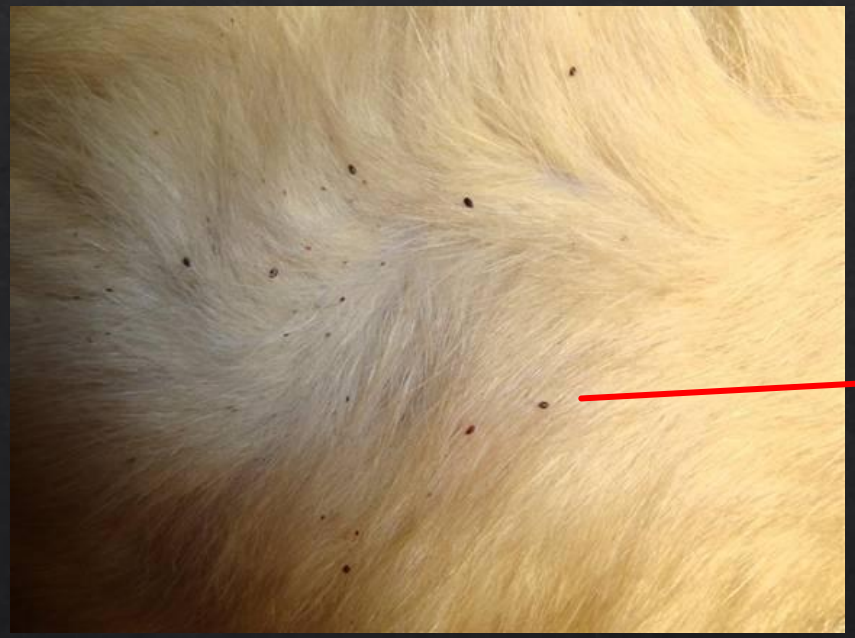
Fur, skin and ear mites (Acariasis)

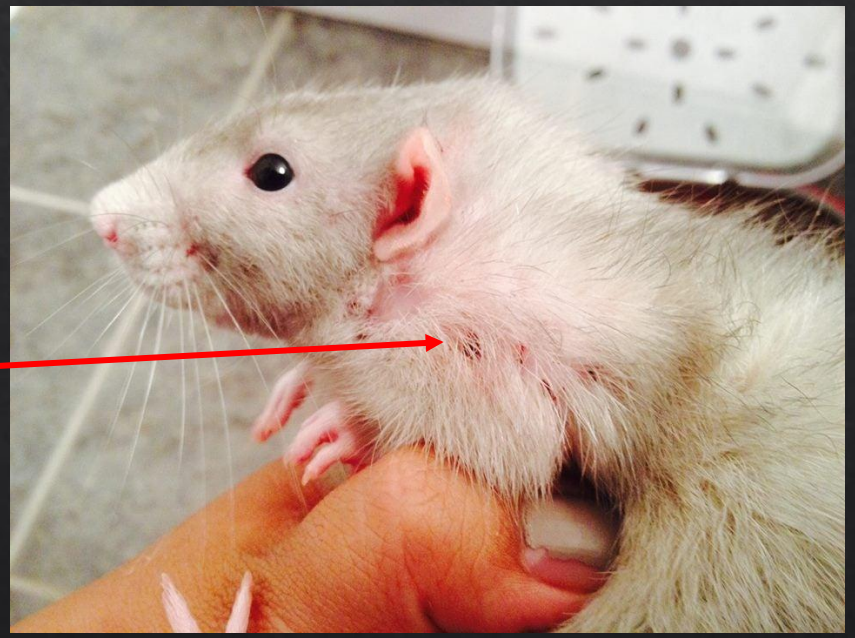
- Clinical signs: Varying clinical signs from none to mild alopecia to severe pruritus and ulcerative dermatitis (Damaged skin by scratching → worsening or leading to ulcerative dermatitis

- Clearance: Moderate to high (environment, low susceptibility to cleaning agents)

- Histopathologic lesions: None

Fur, skin and ear mites (Acariasis)





Fur, skin and ear mites (Acariasis)

- Diagnosis: Fur mites are visible on the fur using stereomicroscopy
- Interference with Research: Animals showing clinical signs are unsuitable
- Depopulation, cleaning and restocking: Recommended
- Rederivation: Recommended

Treatment: Ivermectin applied topically

Pinworms

Classification

Metazoan internal parasite

Family

Oxyuridae

Affected species

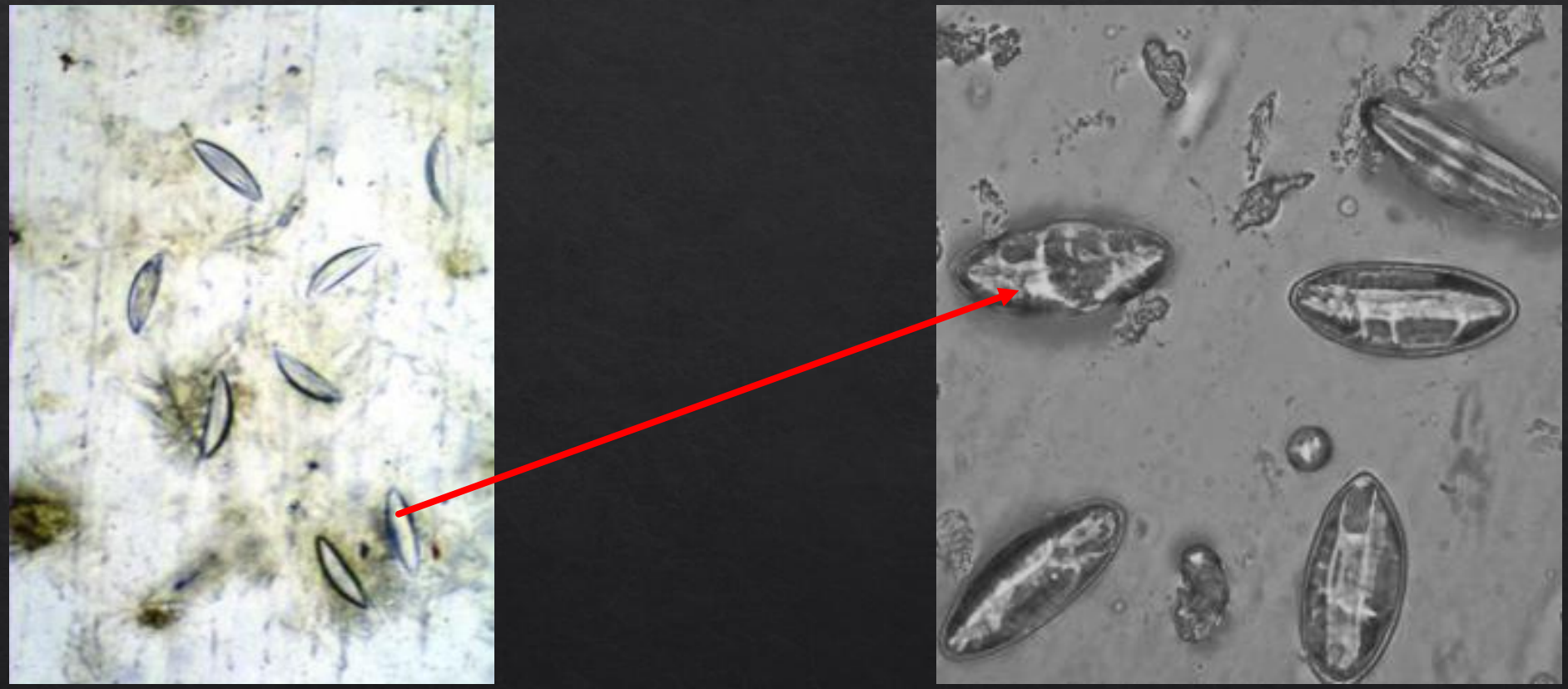
Syphacia obvelata, *S. muris* and *Aspiculuris tetraptera* are the prevalent pinworms in rats and mice. In rabbits, the prevalent pinworm is *Passalurus ambiguus*; in gerbils, *Dentostomella translucida*, although infections with *S. obvelata* and *S. muris* have been reported; and in hamsters, *S. criceti* and *S. mesocriceti*, although *S. obvelata* and *S. muris* have been reported. A true pinworm has not been described in the guinea pig, although a cecal worm in the family Heterakoidea, *Paraspidodera uncinata*, has been reported.

Frequency

Pinworms are common in wild and pet animals, with varying prevalence in laboratory animals.

Transmission: Through fecal-oral route and fomites

Pinworms



Eggs of *Syphacia muris* (microscopy)

Pinworms

- Clinical signs: In immunocompetent animals, infection is generally clinically silent
  Rectal prolapse, poor hair coat, and weight loss as signs of pinworm infection

- Clearance: High (eggs very resistant)

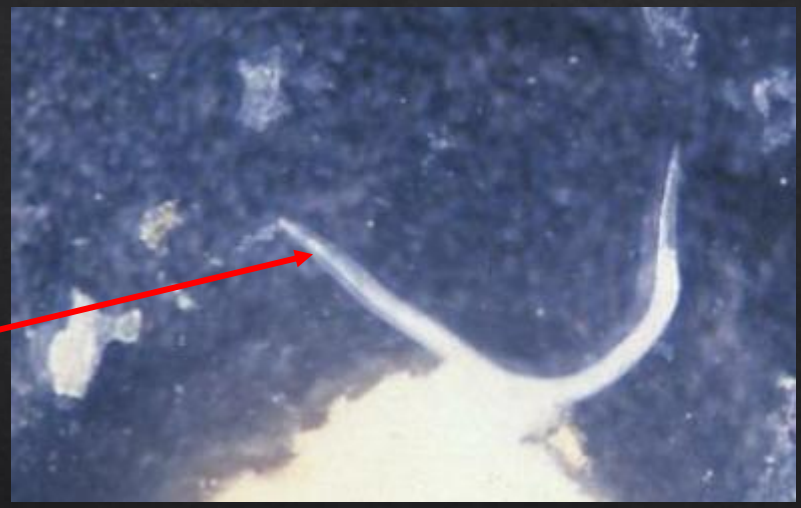
- Histopathologic lesions: None

Pinworms

- Diagnosis: ova via the perianal tape test & microscopy, anal swabbing
- Interference with Research: Animals showing clinical signs are unsuitable
- Depopulation, cleaning and restocking: Recommended
- Rederivation: Recommended

Treatment: A week-on, week-off feeding regimen with feed containing fenbendazole (as needed)

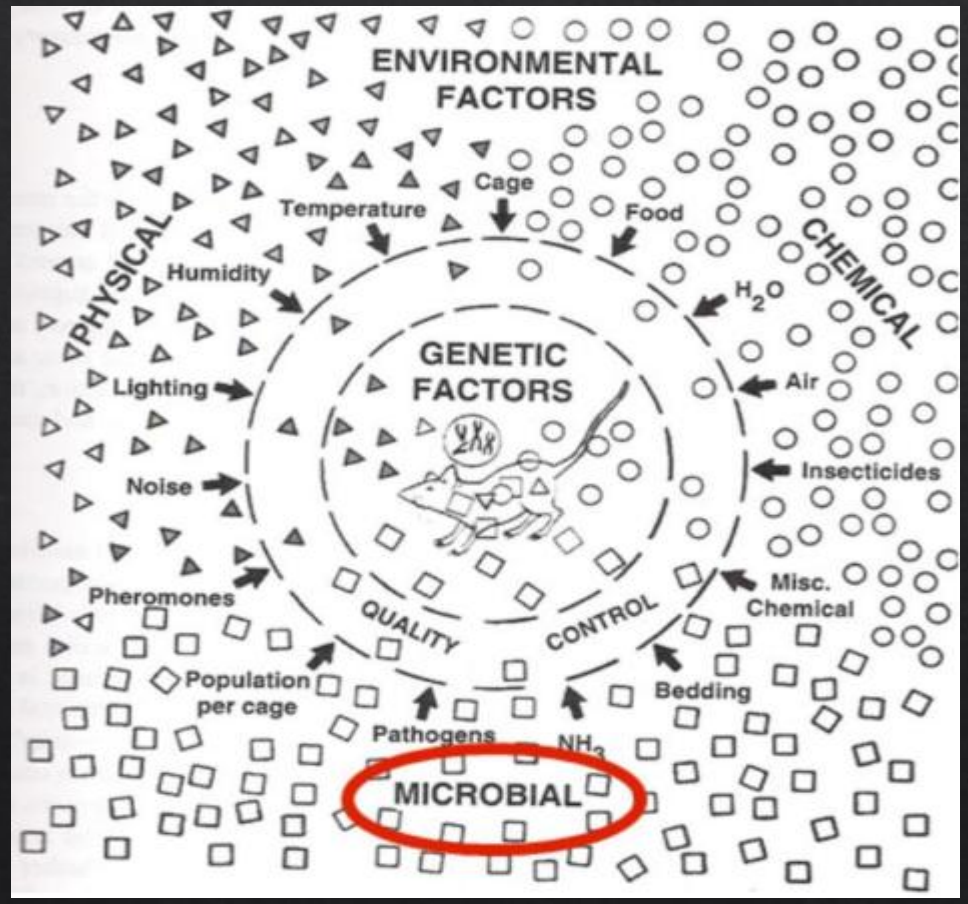
Pinworms



Intestinal content in Petri showing adults

Disease prevention in laboratory animals

What can we standardize?



Standardize = Refinement + Reduction + Replacement

Why do we need to prevent infectious diseases?

1) Clinical disease and mortality → animal welfare

2) Interference with experimental results

3) Quality of biological products

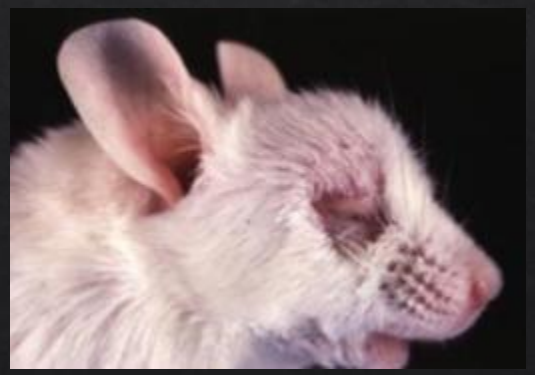
4) Zoonoses

Preventing infectious diseases

Clinical disease and mortality → animal welfare

1) Ethical aspects

2) Most infections do not lead to clinical symptoms (subclinical infections, carrier animals)



Preventing infectious diseases

Interference with experimental results

1) Importance of subclinical infections/carriers

2) Subclinical infections: not always detected, leading to false results

3) Interference: a) With reproduction
b) Modulation of oncogenesis
c) Immunomodulation
d) Physiological modulation

Preventing infectious diseases

Quality of biological products

1) Spread of agents

2) Contamination of tissue samples, transplantable tumors, cells, sera

3) Effects on *in vitro* trials

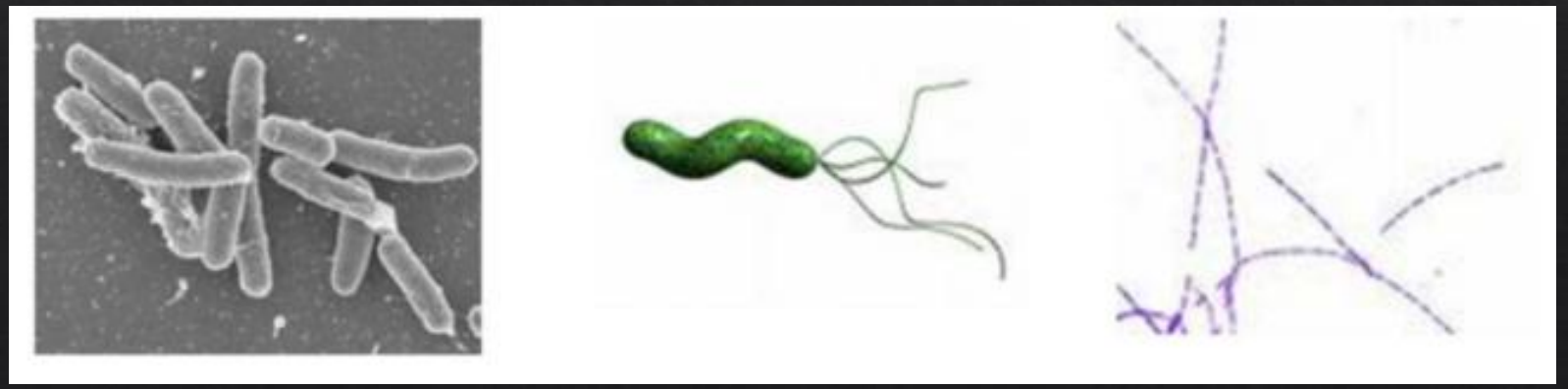
4) Mostly viruses

Preventing infectious diseases

Zoonoses

1) Risk of transmission of certain agents to personnel

2) Examples: *Salmonella* spp., *Helicobacter* spp.



Importance of prevention infectious diseases

1) Clinical disease and mortality → animal welfare

2) Interference with experimental results

3) Quality of biological products

4) Zoonoses

PREVENT – MONITOR - CONTROL

Prevention and control of infectious diseases

1) Introduction of non-contaminated animals

2) Eradication methods

3) Containment facilities

4) Hygienic measures

5) Treatment

6) Other control measures

Introduction of non-contaminated animals

⚠️ **Suppliers should supply health report** ⚠️

1) Commercial manufacturers

2) Conventional livestock animals

3) Transgenic animals? Universities?

	Test frequency	Latest test date	Latest results	Testing laboratory	Test method	Historical results (< 18 months)
Viruses						
Mouse hepatitis virus	2 weeks	28/01/03	0/41	M&B	ELISA	NEG
Mouse rotavirus (EDIM)	2 weeks	28/01/03	0/20	M&B	ELISA	NEG
Parvoviruses						
Minute virus of mice	2 weeks	28/01/03	0/47	M&B	ELISA	NEG
Mouse parvovirus	2 weeks	28/01/03	0/47	M&B	ELISA	NEG
Pneumonia virus of mice	3 months	28/01/03	0/10	M&B	ELISA	NEG
Sendai virus	3 months	28/01/03	0/10	M&B	ELISA	NEG
Theiler's murine encephalomyelitis virus	3 months	28/01/03	0/10	M&B	ELISA	NEG
Ectromelia virus	Annually	05/11/02	0/10	M&B	ELISA	NEG
Lymphocytic choriomeningitis virus	Annually	05/11/02	0/10	M&B	ELISA	NEG
Mouse adenovirus type 1 (FL)	Annually	05/11/02	0/10	M&B	ELISA	NEG
Mouse adenovirus type 2 (K87)	Annually	05/11/02	0/10	M&B	ELISA	NEG
Mouse cytomegalovirus	Annually	05/11/02	0/10	M&B	ELISA	NEG
Reovirus type 3	Annually	05/11/02	0/10	M&B	ELISA	NEG
Additional organisms tested:						
Bacteria, mycoplasma and fungi						
Citrobacter rodentium	3 months	28/01/03	0/10	M&B	CULT	NEG
Clostridium piliforme (Tyzzer's disease)	3 months	28/01/03	0/10	M&B	ELISA	NEG
Corynebacterium kutscheri	3 months	28/01/03	0/10	M&B	CULT	NEG
Mycoplasma spp.	3 months	28/01/03	0/10	M&B	ELISA	NEG
Pasteurella spp.	3 months	28/01/03	0/10	M&B	CULT	NEG
Salmonella spp.	3 months	28/01/03	0/10	M&B	CULT	NEG
Streptococci β-haemolytic (not group D)	3 months	28/01/03	0/10	M&B	CULT	NEG
Streptococcus pneumoniae	3 months	28/01/03	0/10	M&B	CULT	NEG
Helicobacter bilis	Annually	05/11/02	POS	M&B	PCR	POS
Helicobacter hepaticus	Annually	05/11/02	POS	M&B	PCR	POS
Other *Helicobacter* spp.	Annually	05/11/02	POS	M&B	PCR	POS
Streptobacillus moniliformis	Annually	05/11/02	0/10	M&B	CULT	NEG
Additional organisms tested:						

Health report

Organism	Sample Tested	Test Method	Jun 9 '14	Apr 28 '14	Mar 17 '14	Feb 3 '14	Previous 12 months
PARASITES & PROTOZOA							
Encephalitozoon cuniculi	Serum	MFI	0/16	0/16	0/15	0/16	0/128
Fleas	Fur	Visual	0/06	0/06	0/06	0/06	0/47
Fur mites, lice	Fur	Stereoscope	0/06	0/06	0/06	0/06	0/47
Follicle mites	Subcutis	Visual	0/22	0/16	0/21	0/22	0/176
Pinworms	Cecum	Visual	0/06	0/06	0/06	0/06	0/48
Opportunistic protozoa (e.g., Giardia, Spironucleus)	Intestine	Micro	0/06	0/06	0/06	0/06	0/48
Roundworms and other helminths	Intestine	Visual	0/06	0/06	0/06	0/06	0/48
Tapeworms	Intestine	Visual	0/06	0/06	0/06	0/06	0/48

OTHER ORGANISMS MONITORED (SHIPPING NOT STOPPED) -

Most of these organisms are excluded from most Repository barriers. When an excluded organism is found an investigation is undertaken to identify and eliminate all infected mice from the barrier. Positive results - including results from investigations - are noted in this report, but shipping from the area is not suspended.

Organism	Sample Tested	Test Method	Jun 9 '14	Apr 28 '14	Mar 17 '14	Feb 3 '14	Previous 12 months
Klebsiella pneumoniae	Oropharynx, intestine, or feces	Culture	0/22	0/16	3/21	0/22	0/176
Klebsiella spp. other than *K. pneumoniae*	Oropharynx, intestine, or feces	Culture	6/22	8/16	3/21	4/22	46/176
Nonpathogenic protoza (e.g., Trichomonads)	Intestine	Micro	0/06	0/06	0/06	0/06	0/48
Pneumocystis murina	Lung	PCR	0/06	-	0/06	0/06	0/48
Proteus mirabilis	Oropharynx, intestine, or feces	Culture	0/22	0/16	0/21	0/22	0/176
Pseudomonas spp.	Intestine or feces	Culture	0/22	0/16	0/21	0/22	0/176
Staphylococcus aureus	Oropharynx	Culture	0/22	0/16	0/21	0/22	0/176
Streptococcus spp.	Oropharynx	Culture	0/22	0/16	0/21	0/22	0/176

GROSS PATHOLOGY	Sample Tested	Test Method	Jun 9 '14	Apr 28 '14	Mar 17 '14	Feb 3 '14	Previous 12 months
Necropsy findings		Exam, histopath	0/22	0/16	0/21	0/22	0/176

Health Report – Why? When?

1) The needs of the research groups (transgenic animals, susceptible strains)

2) The health status of the facility (SPF or conventional)

3) Management tools for “positive animals” (caging system, quarantine)

4) Education and experience of the personnel

Introduction of non-contaminated animals

In case of “unsafe” suppliers:  Prefer to avoid if possible…

1) Quarantine, barrier systems
2) Sentinel animals
3) Small units with separate entry/exit
4) Rederivation

Quarantine

All animals entering an area must be quarantined

- If a “clean” health report exists → quarantine for acclimatization

- If the health report does not exist → quarantine for acclimatization

→ quarantine for health control/check

Barrier systems

1) Full barrier shower in / autoclave materials

2) Individually Ventilated Cages (IVC)

3) Isolators

4) Clean areas

Barrier systems

What does "Full Barrier" mean?

A **full barrier facility** enforces strict separation between:

- **"Dirty" areas** (**outside the barrier** – general environment, humans, supplies) and

- **"Clean" areas** (**inside the barrier** – animal rooms, equipment, cages)

This ensures **biosecurity**, especially in **SPF** **or immunocompromised rodent colonies**, where even minor infections can affect health or research validity.

Barrier systems

What does "Full Barrier" mean?

🚿 "Shower-in" Requirement

Personnel entering the barrier facility must:

1) **Shower upon entry** using provided showers

2) **Change into facility-specific clothing** and sterile gear (scrubs, gloves, masks, caps, etc.)

3) In some setups, showering out is also required to prevent reverse contamination

☑ Purpose: Removes **contaminants (dust, microbes, pathogens)** from skin and hair to reduce pathogen introduction via humans

Barrier systems

What does "Full Barrier" mean?

♨ **"Autoclave Materials In“**

All equipment, bedding, feed, cages, instruments, and other supplies:

1) Must be **autoclaved (steam sterilized) before** entering the clean barrier area

2) Typically go through **pass-through autoclaves** that open only one side at a time, preserving separation between clean and dirty sides.

☑ Purpose: Ensures **sterility of all incoming materials** to maintain a pathogen-free environment

Individually Ventilated Cages (IVC)

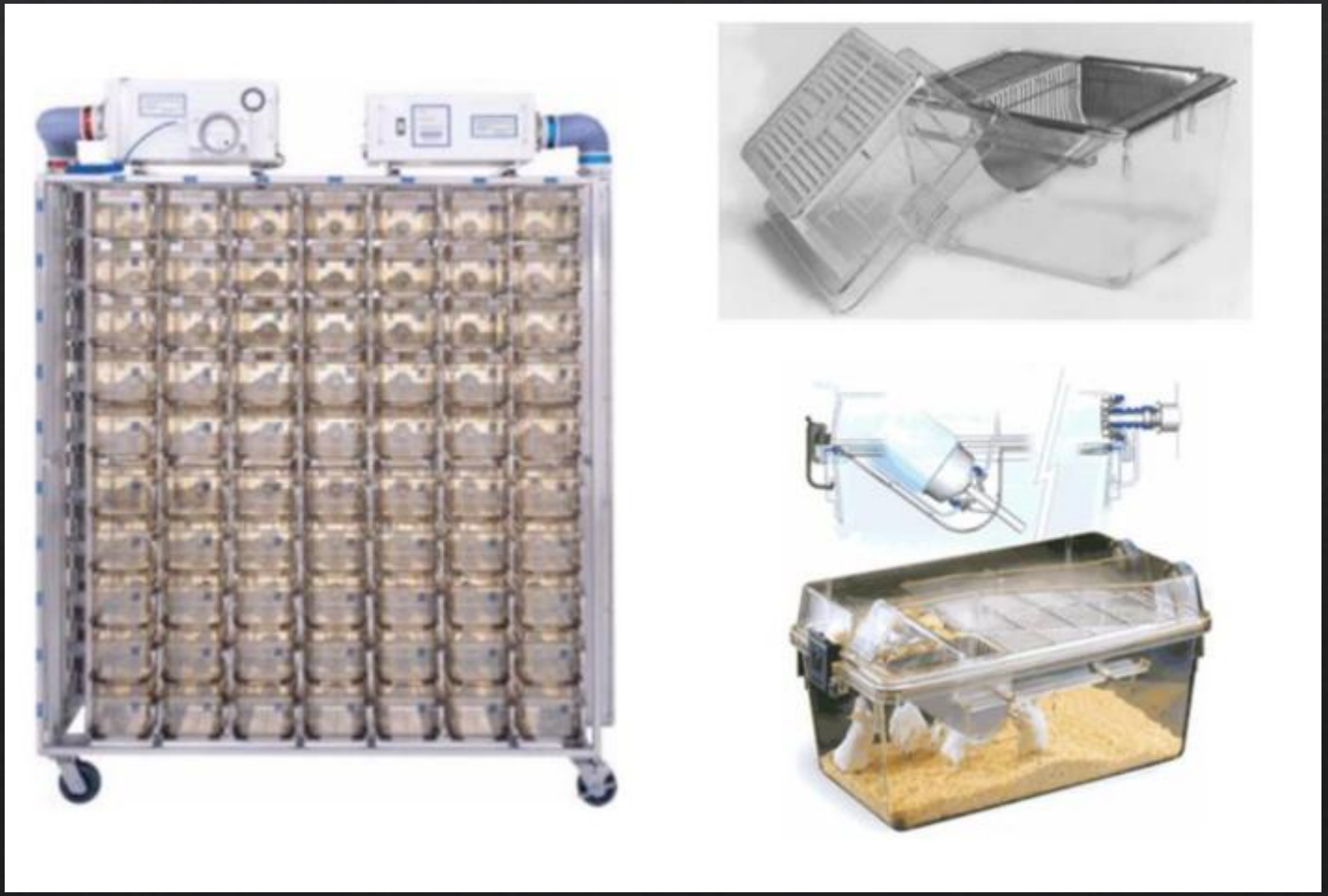
1) Used in a clean room

2) All materials sterilized

3) Personnel change going into room

4) All manipulations take place in laminar flow

Individually Ventilated Cages (IVC)



Isolators

1) Isolators: fully closed systems in which animals are only handled through gloves tightly sealed to the isolator wall

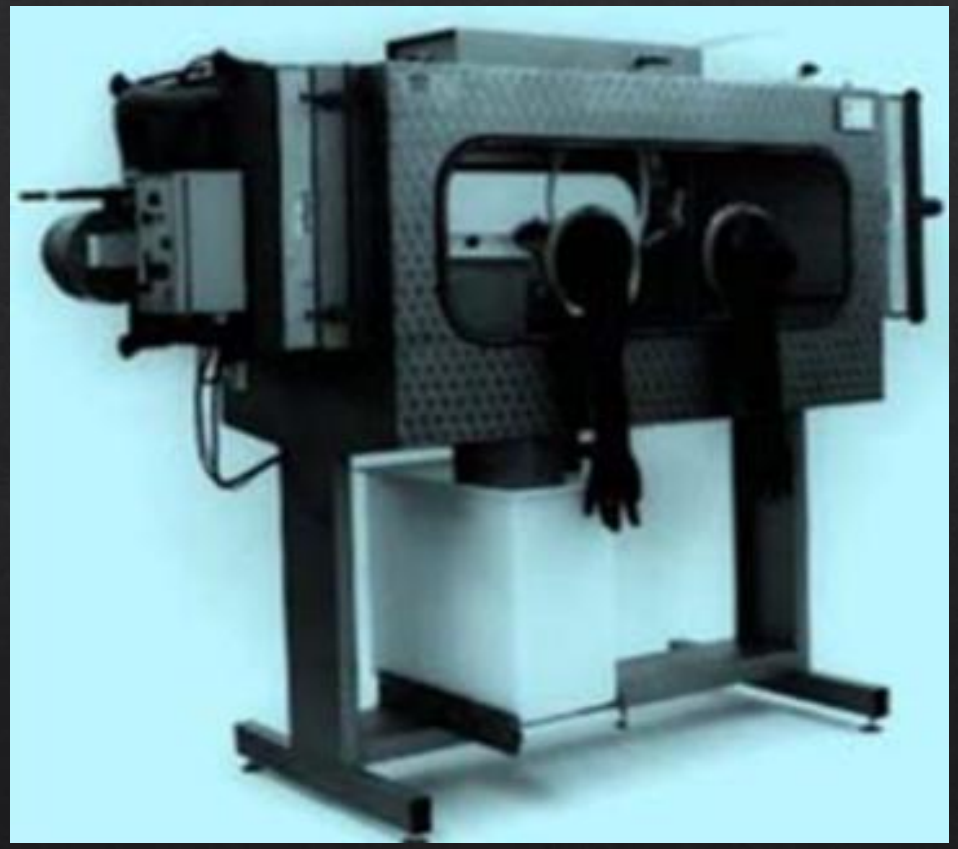
2) Needed when full knowledge of the microbiota of the animals is required

3) HEPA (High-Efficiency Particulate Air) filtered air, incoming materials and feed decontaminated

4) Water-lock for air outlet

5) Positive pressure (protection of animals)

Isolators



Clean Area

1) It is often desirable to hold animals in conventional areas for short periods while under experiment

2) This can be done under clean room

3) Not SPF but maintain barriers

Health Monitoring

Health Monitoring

1) Part of quality assurance: process and product quality monitoring

2) Used for microbiological standardization of animals

3) Aims to produce animals that meet preset requirements of microbiological quality

4) Aid in maintenance of microbiological quality during experiments

5) Keep animals suitable for experiments

Health Monitoring

- Actual practice of health monitoring can be adapted due to:

1) Research objectives

2) Local prevalence of specific agents

3) Regulations of national monitoring recommendations

Monitoring frequency / sample size

1) Monitoring of colonies at least quarterly

2) In addition: necropsy of death / diseased animals

3) Units containing different rooms / species???

4) More frequent monitoring may be needed

When more frequent monitoring is needed?

1) Frequent introduction of animals

2) Frequent entry of personnel in addition to animal care personnel

3) Frequent change of personnel working in unit

4) Introduction of animals from different breeders

5) Multipurpose units with various kinds of experiments

6) Infected animals on-site

Monitoring frequency / sample size

1) Sample size of at least 10 animals per unit is recommended (according to total population / rooms)

2) Cost of sampling size VS detection rate (false negative result if taking not enough samples)

3) Detection rate depends on test method (Serology VS direct plating)

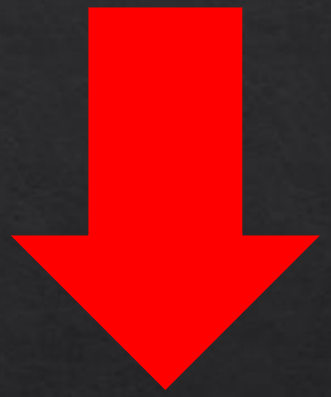
Serology VS Direct plating

Aspect	Serology	Direct Plating
Definition	Detection of **antibodies** in the blood (host response)	Detection of **live microorganisms** in samples (e.g., feces, tissue)
What it tells you	Evidence of **past or recent exposure** to a pathogen	Presence of **viable organisms** at the time of sampling
Sample type	**Blood serum**	Feces, swabs, tissue, secretions
Sensitivity	High (especially for systemic infections)	Lower if the pathogen is present in low numbers or not viable
Specificity	May show **false positives** (cross-reactivity)	More **specific** to viable organisms cultured

Serology VS Direct plating

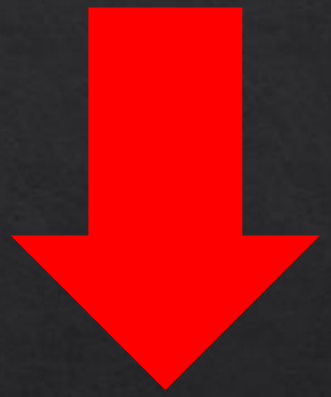
Timing relevance	Reflects **immune memory**; may persist after infection	Reflects **current colonization or infection**
Usefulness	Good for **screening populations**, especially for SPF facilities	Best for **identifying active carriers** or confirming infection
Speed	Typically 1–2 days (depending on the test)	Several days to weeks depending on organism
Limitations	May miss **very early infections** (before seroconversion); cannot detect local-only infections	Cannot detect past infections; requires viable pathogens
Examples of application	Screening for **mouse norovirus, MHV, Mycoplasma pulmonis**	Detecting **Salmonella, Clostridium piliforme, Helicobacter spp.**

What about expensive animals / small groups?



1) Increase sampling frequency
2) Non-invasive sampling
3) Sampling without killing (swabs, blood collection)

What about expensive animals / small groups?



Sentinel animals ! ! !

Sentinel Animals

Rodents for Health Monitoring purposes are sometime introduced into a rodent population, housed in open cages placed systematically throughout the colony, and designated as sentinels for use in periodic testing.

- Sentinels can also be transferred into dirty cages

Use of sentinels

- Different cage racks
- Housed in open cages

How long

- From 6 to 14 weeks depending on the cage system

Health data indicate the health status of the unit ! ! !

Sentinel Animals

References

http://www.criver.com/customer-service/resources/infectious-agent-information

National Research Council. Companion guide to infectious diseases of mice and rats. http://www.nap.edu/catalog.php?record_id=1540

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AAALAC - AAALAC

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Thank you ! !