



INFRAFRONTIER
mouse disease models

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EMMA (EUROPEAN MOUSE MUTANT ARCHIVE)

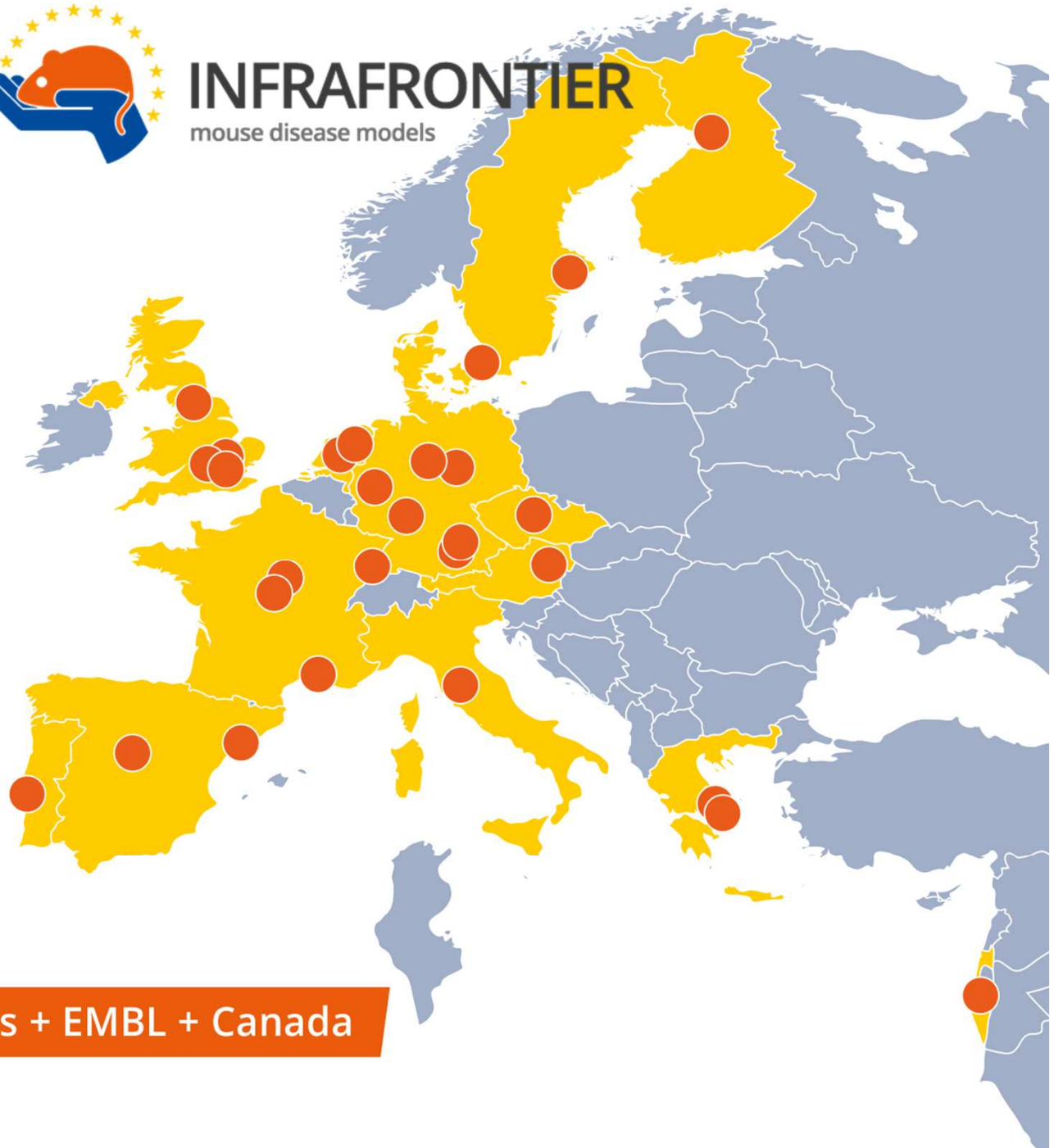


HEALTH MONITORING
Controllo, Cura, Trattamento, Profilassi sanitaria



INFRAFRONTIER
mouse disease models

European **Research Infrastructure** for the **generation, systemic phenotyping, archiving** and **distribution** of mammalian models



14 European countries + EMBL + Canada



The Model of EMMA - Infrafrontier

Mouse Mutants are
received and
genetically monitored



Cryopreserved as
embryos and gametes
In liquid nitrogen



Distributed under SPF
condition
(Specific Pathogen Free)





EMMA ACTIVITY



- **IMPORT AND CRYOPRESERVE IMPORTANT MOUSE MUTANT STRAINS**
 - **DISTRIBUTE CLEAN SPF ANIMALS AFTER EMBRYO DERIVATION TO THE SCIENTIFIC COMMUNITY.**
 - **MICE MUST BE OF THE SPECIFIC MUTATION AND GENETIC BACKGROUND MONITORED AND REPORTED**
-



Why is health monitoring so important ?

The study of mouse mutants can be altered by the infection of mouse pathogens changing their behaviour and physiology.

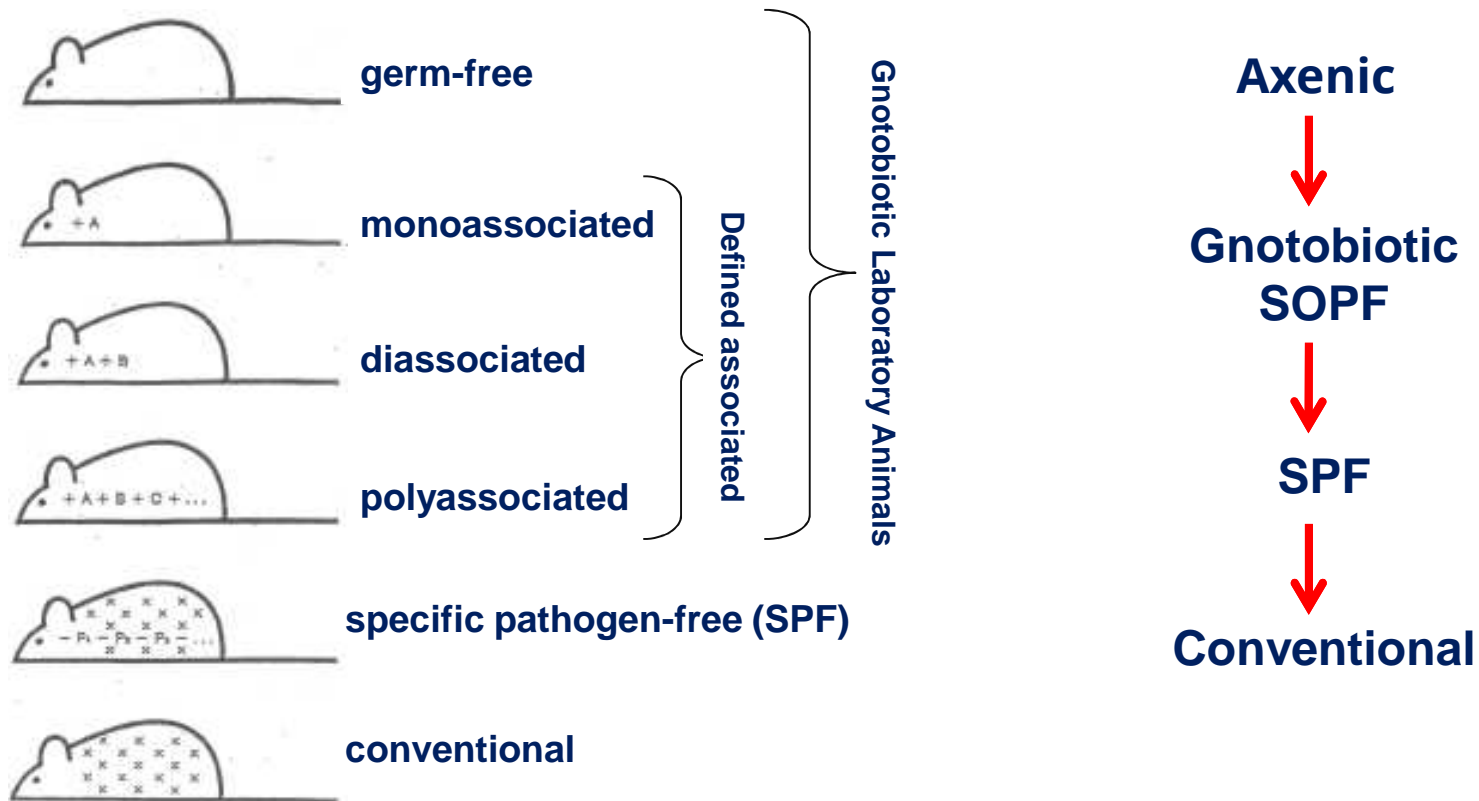
Pathogens influence welfare of the animals.

Risk of Zoonosis : some (rare) mouse pathogens like Ectromelia, Hantaan and Salmonella can be dangerous for men.

**A HEALTHY MOUSE IS ONE WHOSE BIOLOGICAL RESPONSE TO
EXPERIMENTAL MANIPULATION IS NOT DISTORTED BY
ENVIRONMENTAL OR MICROBIAL INFLUENCES**



Microbiological category of laboratory animals



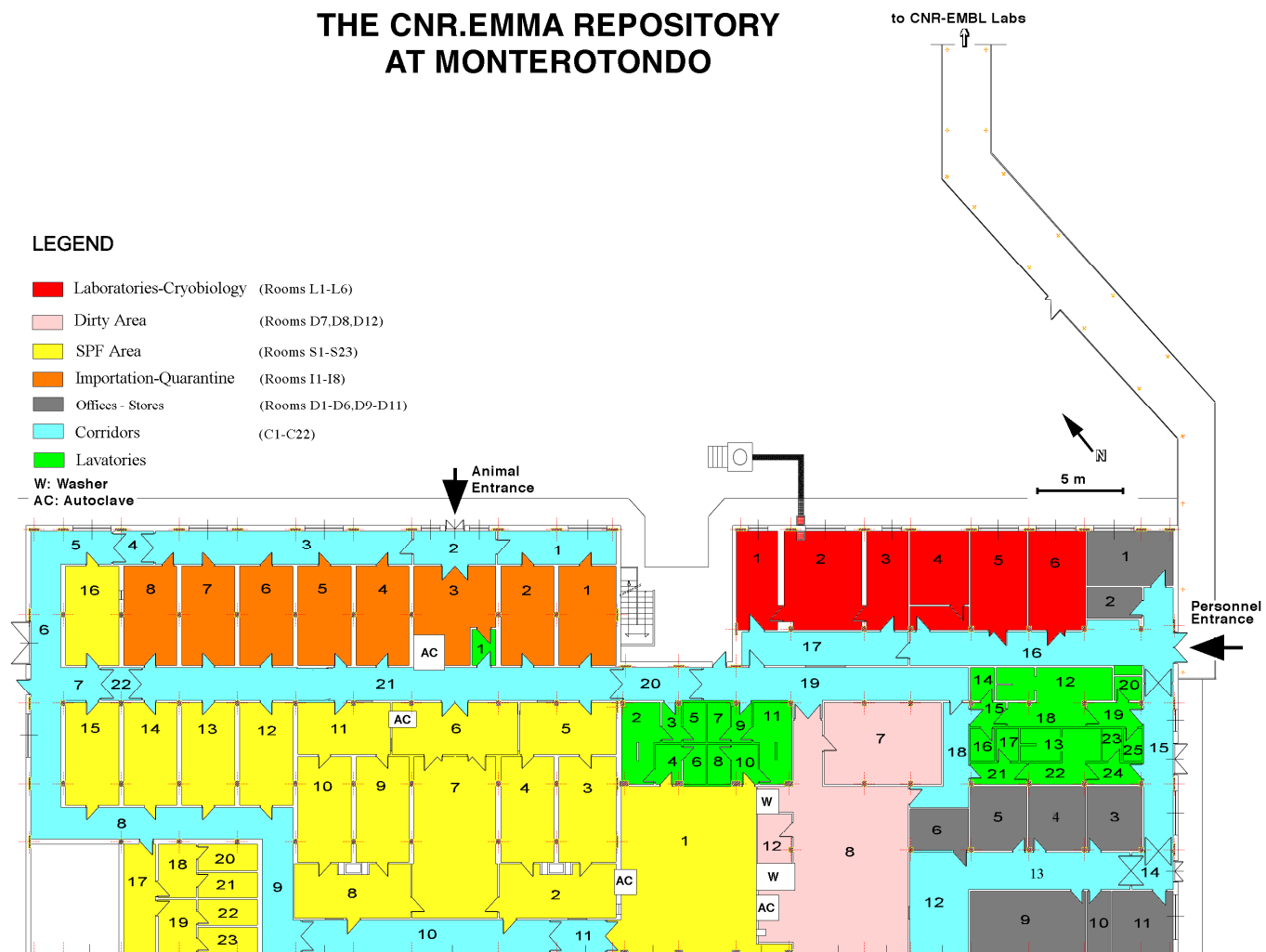


THE CNR.EMMA REPOSITORY AT MONTEROTONDO

LEGEND

- Laboratories-Cryobiology (Rooms L1-L6)
- Dirty Area (Rooms D7,D8,D12)
- SPF Area (Rooms S1-S23)
- Importation-Quarantine (Rooms I1-I8)
- Offices - Stores (Rooms D1-D6,D9-D11)
- Corridors (C1-C22)
- Lavatories

W: Washer
AC: Autoclave





-
- **STERILIZING PROCEDURE**
 - **SANITIZING PROCEDURE**
 - **ENVIRONMENTAL CONTROL**
 - **ANIMAL HEALTH MONITORING**



STERILIZING PROCEDURES

Sterilization : Autoclave cycle, Irradiation

Could be monitored with Bacillus spores inside the material

Sterilization with Gas : Peroxide Fumigation or other gas

Could be monitored with Bacillus spores outside, this process sterilize by gas contact only accessible surfaces



SANITIZING PROCEDURES

Sanitization of drinking water: Filter and/or Chlorine/Acidify

Monitor bacteriology every time in liquid growth medium

Cage/Bottle washer

Monitor collecting water or swab after washing

Cleaning with detergents : rotation of active principles

Ammonium quaternary salts, Chlorine, Oxygen, Alkaline.
Monitor by swab culture before and after cleaning



ENVIRONMENTAL MONITORING

Monthly monitor each room and working bench by swab

This allows control of cleaning procedures and of efficacy of detergents

Periodical Surface Air System (SAS)

Monitor 500 liters of air, gives information on number and quality of bacterial flora of each room.
Changes indicate structural problems



Table 3. Recommended infectious agents to monitor and frequencies of monitoring for laboratory mice (*Mus musculus*).

| | Every 3 months | Annually |
|--|----------------|----------|
| Viruses | | |
| Mouse hepatitis virus | x | |
| Mouse rotavirus | x | |
| Murine norovirus | x | |
| Parvoviruses: | | |
| Minute virus of mice | x | |
| Mouse parvovirus | x | |
| Theiler's murine encephalomyelitis virus | x | |
| Lymphocytic choriomeningitis virus | | x |
| Mouse adenovirus type 1 (FL) | | x |
| Mouse adenovirus type 2 (K87) | | x |
| Mousepox (ectromelia) virus | | x |
| Pneumonia virus of mice | | x |
| Reovirus type 3 | | x |
| Sendai virus | | x |

Table 3. Recommended infectious agents to monitor and frequencies of monitoring for laboratory mice (*Mus musculus*).

| | Every 3 months | Annually |
|---|----------------|----------|
| Bacteria | | |
| <i>Helicobacter</i> spp. | x | |
| If positive, speciation for <i>H. hepaticus</i> , <i>H. bilis</i> and <i>H. typhlonius</i> is recommended | | |
| <i>Pasteurella pneumotropica</i> | x | |
| Streptococci β -haemolytic (not group D) | x | |
| <i>Streptococcus pneumoniae</i> | x | |
| <i>Citrobacter rodentium</i> | | x |
| <i>Clostridium piliforme</i> | | x |
| <i>Corynebacterium kutscheri</i> | | x |
| <i>Mycoplasma pulmonis</i> | | x |
| <i>Salmonella</i> spp. | | x |
| <i>Streptobacillus moniliformis</i> | | x |
| Parasites | | |
| Endo- and ectoparasites (reported to the genus level) | x | |

Table 3. Recommended infectious agents to monitor and frequencies of monitoring for laboratory mice (*Mus musculus*).

Additional agents*

Viruses:

- Hantaviruses
- Herpesviruses (mouse cytomegalovirus, mouse thymic virus)
- Lactate-dehydrogenase elevating virus
- Polyomaviruses (mouse polyomavirus, K virus)

Bacteria and fungi:

- Cilia-associated respiratory bacillus
- Klebsiella oxytoca*, *Klebsiella pneumoniae*
- Other *Pasteurellaceae*[†]
- Pneumocystis murina*
- Pseudomonas aeruginosa*
- Staphylococcus aureus*

Others as necessary

All agents listed should be reported if found in diagnostic examinations irrespective of when they are found.

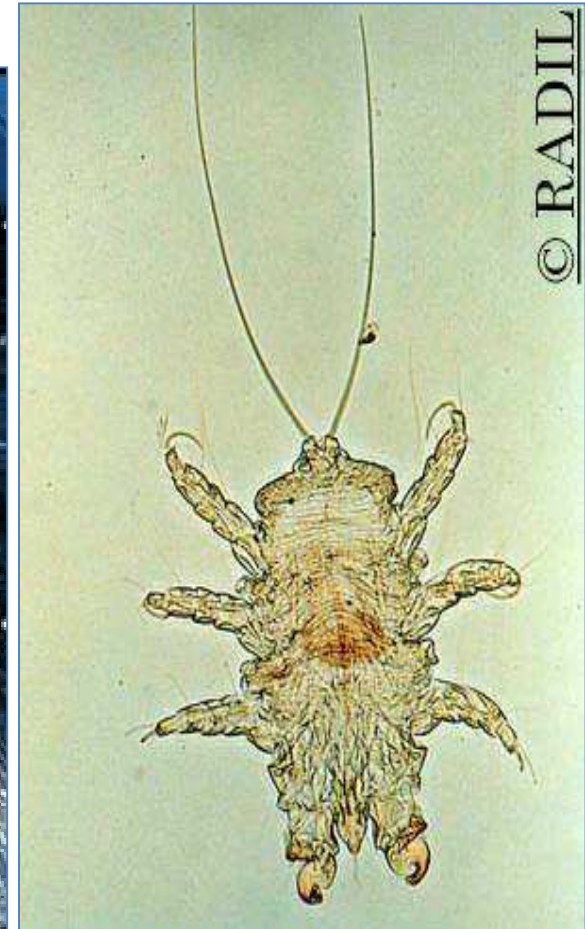
*Testing for these agents is optional and should be pursued if there is a specific need. Frequency of testing will depend on local circumstances.

†We acknowledge that the inclusion of the *Pasteurellaceae* family is controversial. Screening for the family can be conducted should the facility wish, and the difficulty of some commercial kits to correctly identify *Pasteurella pneumotropica*, as well as the fluidity of the correct phenotypic classification, should also be acknowledged.



ECTOPARASITE

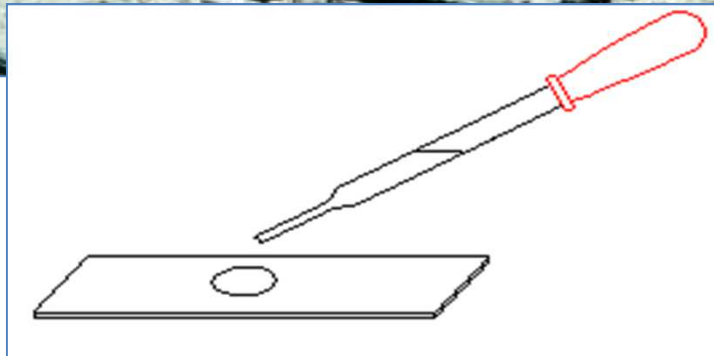
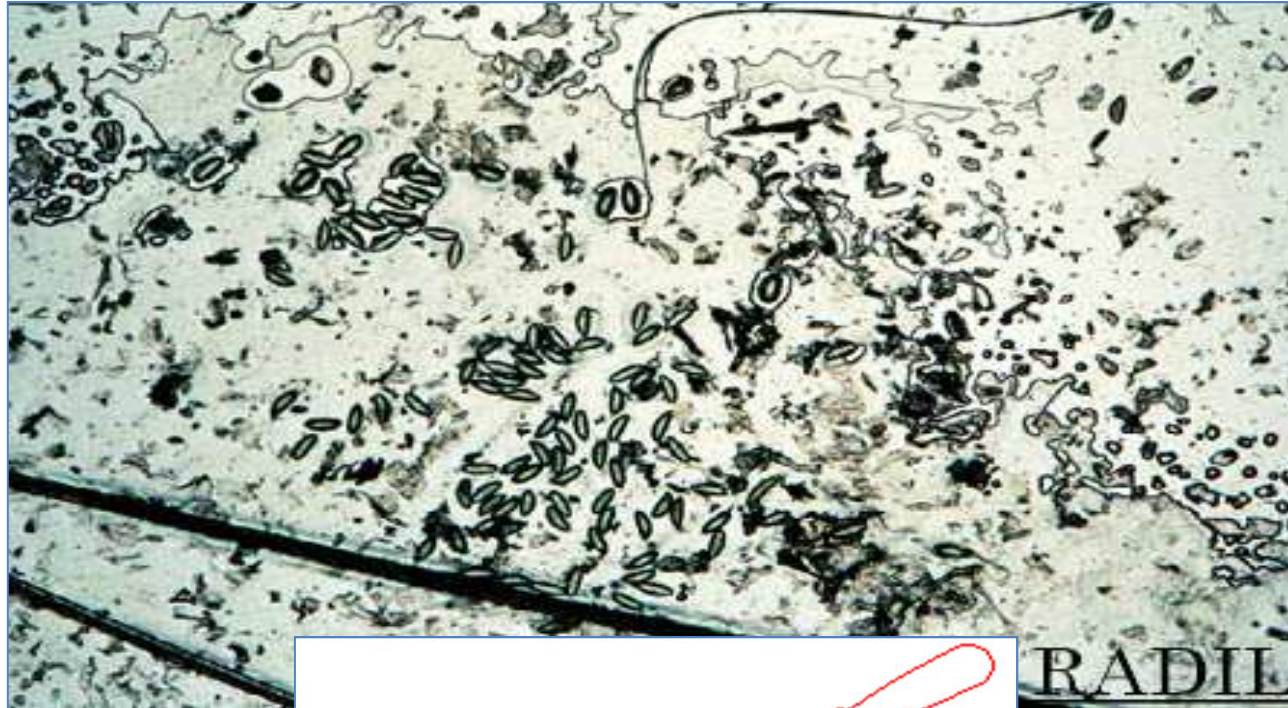
Myobia, Myocoptes, Radfordia, Detection by Microscopy or PCR



- FUR MITES: IRRITATION, ALOPECIA, ULCERATION
- Possible treatment with several cycles of Ivermectin

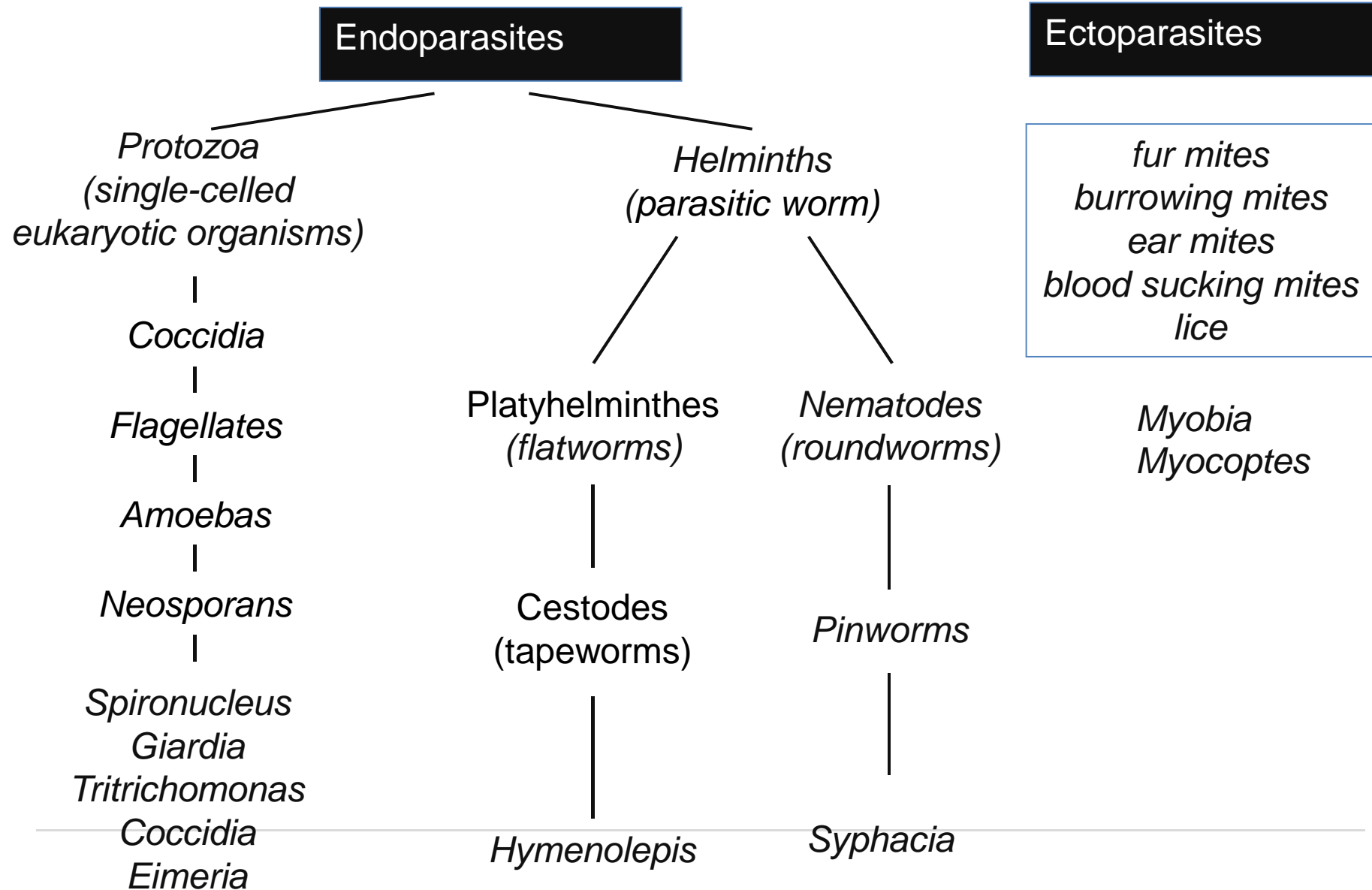


Endoparasite: Microscopy of cecum content





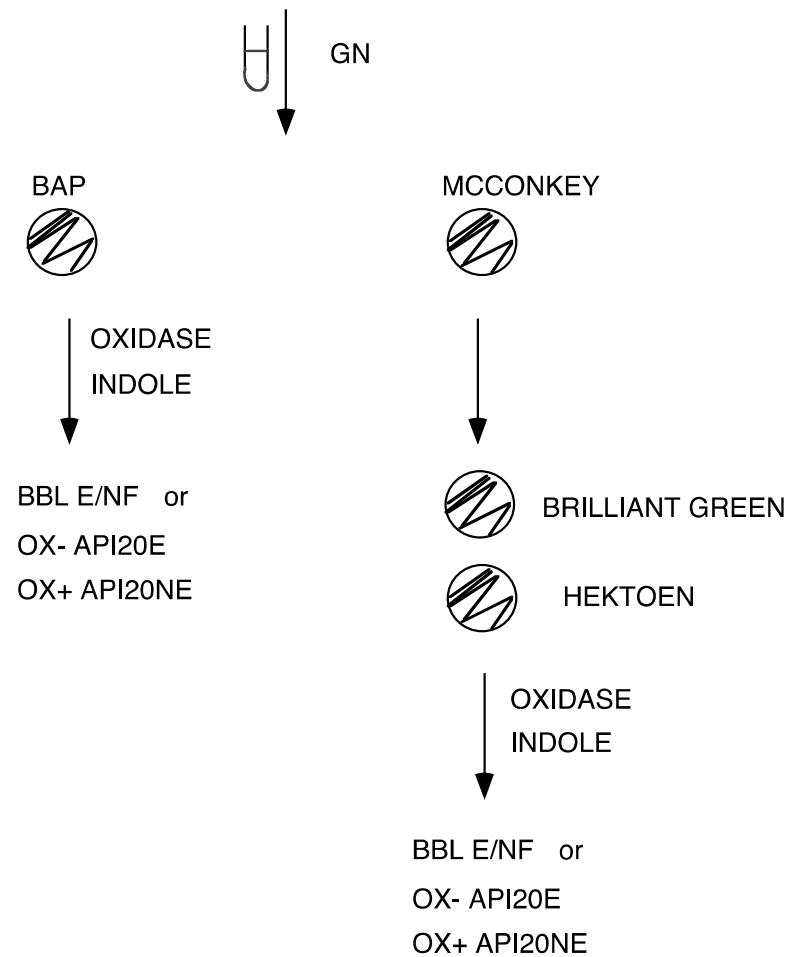
Classification of Parasite (Microscopic observation. PCR)





Bacteriology of intestinal apparatus (classical microbiology or culture)

SMALL INTESTINE



HELICOBACTER

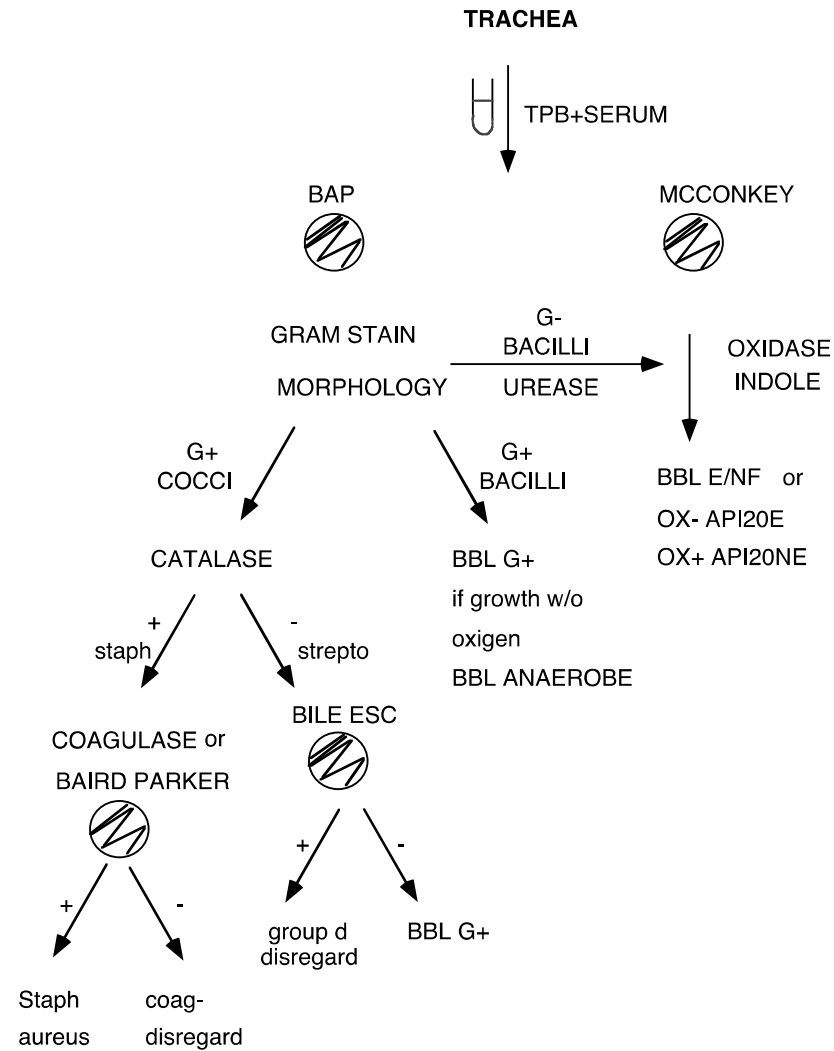




Bacteriology of respiratory apparatus (classical microbiology or culture)

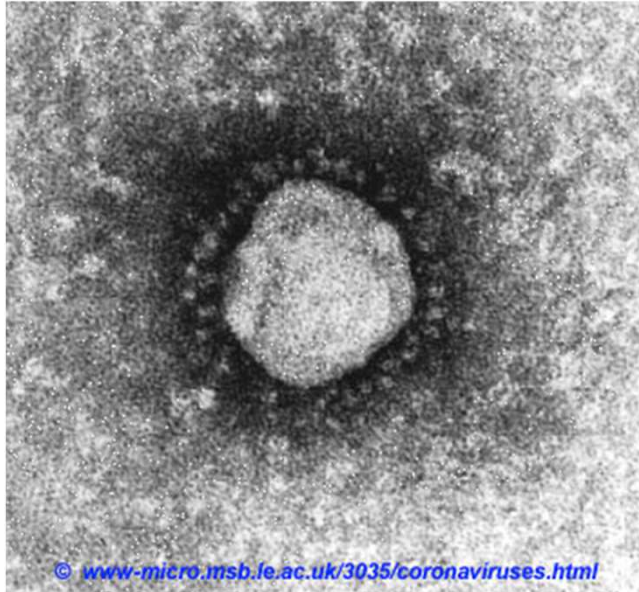


PASTEURELLA





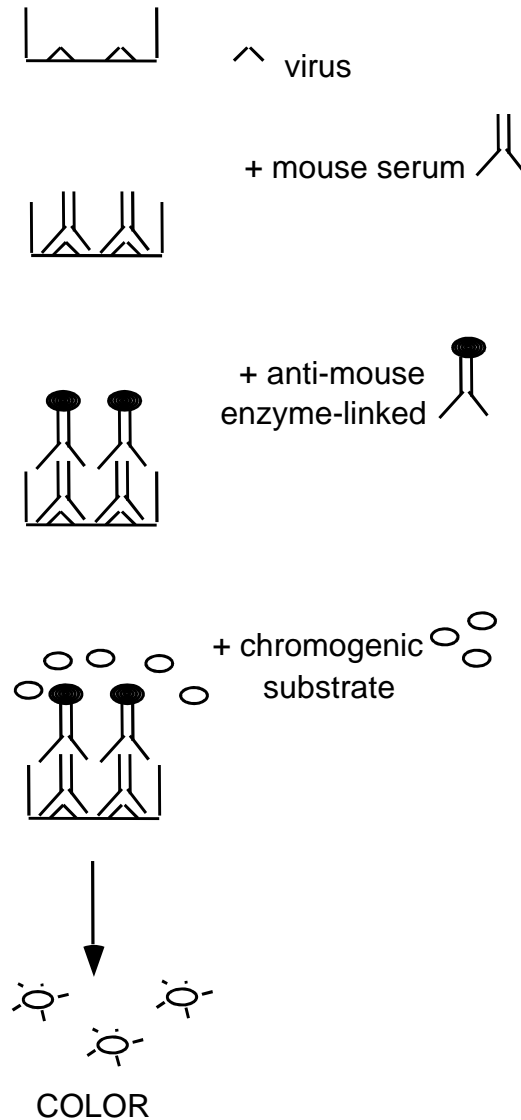
Virology (Serology es. ELISA: Enzyme Linked Immunosorbent Assay)



- Corona Virus, RNA

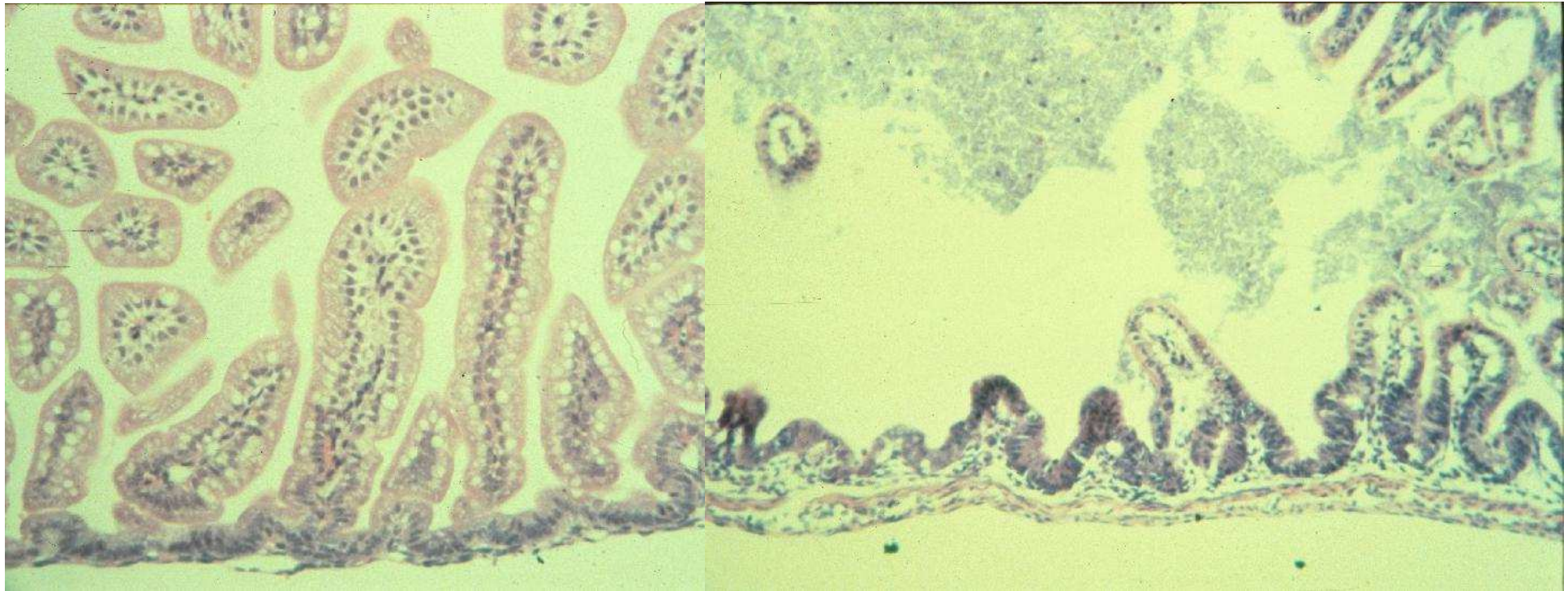
- serotypes

- MHV-1
 - MHV-2
 - MHV-3
 - A59





MHV : INTERFERENCES WITH RESEARCH/ Gut and Metabolic disorders



- **in immune-competent animals:**
 - anemia, leucopenia, thrombocytopenia
- **mortality after**
 - thymectomy
 - irradiation
 - chemotherapy
 - halothane anesthesia
 - corticosteroids treatment



MHV : INTERFERENCES WITH RESEARCH



- **subclinical infection MHV**
 - adult immune-competent mice
- **epidemic clinical MHV**
 - young animals
 - nu/nu, NSG, RAG
- **Wasting syndrom**
 - nu/nu
- **Detection by Serology or PCR**



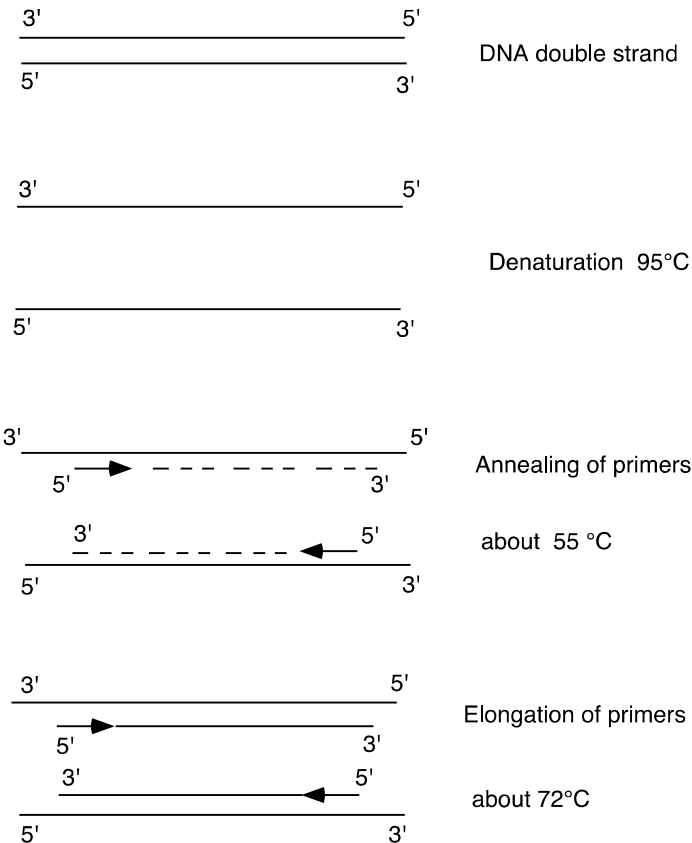
Molecular detection of DNA/RNA of Virus, Bacteria and Parasite (PCR)



NOROVIRUS DETECTION by RT-PCR

Enteric virus discovered in 2003
Lethal in a very immunodeficient KO.
MNV is used as a model to study human
Norovirus that is the cause of gastroenteric
Disease in men.

Polymerase chain reaction



Cycle is repeated 30-40 times with an exponential amplification of the DNA product that can be visualised on an agarose gel



INFRAFRONTIER



EMMA



Consiglio Nazionale delle Ricerche

Infrafrontier – EMMA – CNR Monterotondo Campus International Development

HEALTH MONITORING REPORTS

(species: mouse)

Date of issue: 14-12-2016

Unit No.: S7

Report No.: S7-16-01

LAB ID n°: HS16-42, HS16-43, HS16-44, HS16-45, HS16-46, HS16-47, HS16-48, HS16-49, HS16-141, HS16-142, HS16-143

HS16-144, HS16-145, HS16-146, HS16-147, HS16-148, HS16-302, HS16-303, HS16-304, HS16-305, HS16-306

HS16-307, HS16-414, HS16-415, HS16-416, HS16-417, HS16-418, HS16-419, HS16-420, HS16-421

| | Method | Time | Histor.15 | 1°Quar.16 | 2°Quar.16 | 3°Quar.16 | 4°Quar.16 |
|---|--------|------|-----------|-----------|-----------|-----------|-----------|
| Viruses | | | | | | | |
| Mouse hepatitis virus (MHV) | E | Q | 0/31 | 0/8 | 0/8 | 0/6 | 0/8 |
| Mouse rotavirus (EDIM) | E | Q | 0/31 | 0/8 | 0/8 | 0/6 | 0/8 |
| Mouse norovirus | E | Q | 0/31 | 0/8 | 0/8 | 0/6 | 0/8 |
| Minute virus of mice (MVM) | E | Q | 0/31 | 0/8 | 0/8 | 0/6 | 0/8 |
| Mouse parvovirus (NS1) | E | Q | 0/31 | 0/8 | 0/8 | 0/6 | 0/8 |
| Theiler's encephalomyelitis virus (GDVII) | E | Q | 0/31 | 0/8 | 0/8 | 0/6 | 0/8 |
| Lymphocytic choriomeningitis virus (LCV) | E | A | 0/8 | 0/8 | / | / | / |
| Mouse adenovirus type 1 (FL) | E | A | 0/8 | 0/8 | / | / | / |
| Mouse adenovirus type 2 (K87) | E | A | 0/8 | 0/8 | / | / | / |
| Ectromelia virus | E | A | 0/8 | 0/8 | / | / | / |
| Pneumonia virus of mice (PVM) | E | A | 0/8 | 0/8 | / | / | / |
| Reovirus type3 (REO3) | E | A | 0/8 | 0/8 | / | / | / |
| Sendai virus | E | A | 0/8 | 0/8 | / | / | / |
| Bacteria and mycoplasma | | | | | | | |
| <i>Helicobacter</i> spp. | PCR | Q | 0/31 | 0/8 | 0/8 | 0/6 | 0/8 |
| <i>Pasteurellaceae</i> | C | Q | 0/31 | 0/8 | 0/8 | 0/6 | 0/8 |
| <i>Streptococci</i> β -hemolytic | C | Q | 0/31 | 0/8 | 0/8 | 0/6 | 0/8 |
| <i>Streptococcus pneumoniae</i> | C | Q | 0/31 | 0/8 | 0/8 | 0/6 | 0/8 |
| <i>Citrobacter rodentium</i> | C | A | 0/8 | 0/8 | / | / | / |
| <i>Clostridium piliforme</i> | E | A | 0/8 | 0/8 | / | / | / |
| <i>Corynebacterium kutscheri</i> | C | A | 0/8 | 0/8 | / | / | / |
| <i>Mycoplasma</i> spp. | E | A | 0/8 | 0/8 | / | / | / |
| <i>Salmonella</i> spp. | C | A | 0/8 | 0/8 | / | / | / |
| <i>Streptobacillus moniliformis</i> | C | A | 0/8 | 0/8 | / | / | / |
| Parasites and Protozoa | | | | | | | |
| Ectoparasites: | M | Q | 0/31 | 0/8 | 0/8 | 0/6 | 0/8 |
| Species designation | | | | | | | |
| Endoparasites: | M | Q | 0/31 | 0/8 | 0/8 | 0/6 | 0/8 |
| Species designation | | | | | | | |
| Pathological lesions observed | Path | Q | 0/31 | 0/8 | 0/8 | 0/6 | 0/8 |

NOTES:

Methods: C = culture, E =ELISA, M = microscopic examination, PCR= polymerase chain reaction.

A = annual, Q = quarterly

Health monitoring programme complies with the Federation of European Laboratory Animal Science Associations (FELASA)

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FELASA recommended information for health monitoring (HM) report

Unità microbiologica (Barriera, Convenzionale, IVC, Isolatore)

Identificazione delle specie presenti all'interno dell'unità

Data del report attuale e del precedente

Frequenza dei test per ogni agente esaminato e metodo usato

Nome dei patogeni analizzati (Virus, Batteri e Ecto ed Endo-Parassiti)

Risultati del test e risultati storici recenti almeno dell'ultimo anno (positivi/totale)

Risultati dell'analisi patologica e autoptica (lesioni osservate)

Commenti sugli eventuali trattamenti (es. antibiotico)

Nome del responsabile del programma di analisi sanitaria e del HM

Descrizione del programma sanitario (link ad un website, es:
www.infrafrontier.eu/procedures)



MOST COMMON PATHOGENS

- **MOUSE HEPATITIS VIRUS (MHV)**
- **HELICOBACTER**
- **MOUSE PARVOVIRUS (MVM, MPV)**
- **RODENT PARASITES (MITES, PROTOZOA, PINWORMS)**
- **MOUSE NOROVIRUS (MNV)**

- **THESE PATHOGEN ARE EXTREMELY CONTAGIOUS, RESISTANT OR DIFFICULT TO DIAGNOSE** (because they usually give subclinic infections)

- **CAN BE ERADICATED BY EMBRYO DERIVATION / CESAREAN DERIVATION, /CROSS-FOSTERING, / BREEDING STOP, / CHEMICAL TREATMENT**(Ivermectin, Metronidazole, Fenbendazole, Specific Antibiotic)



Treatment

Mites

with IVERMECTIN

Protozoa

with METRONIDAZOLE

Round and Tape Worms

with FENBENDAZOLE

Bacteria

with Specific Antibiotic (Antibiogram)

**Viruses and difficult agents
(Helicobacter, Pneumocystis,
Mycoplasma etc)**

**ALL with Embryo Derivation
Most with Cesarean Derivation
Some with Cross Fostering
Some with Stop of Breeding**

All treatment must be controlled during time with observation and health monitoring



ANIMAL HEALTH MONITORING WITHOUT SENTINELS

EXHAUST AIR DUST MONITORING :

A filter placed at the exhaust of an IVC rack or at the exhaust of the ventilation system of an animal room can collect micro-organisms that can be analysed by culture or PCR.

This is another complementary approach to the sanitary animal And environmental control of the facility and of the procedures.

SAMPLE MONITORING OF EXPERIMENTAL ANIMALS :

With the advent of molecular detection, we can detect pathogens from feces, oral swabs, skin swab directly from the experimental colony without killing animals.



ELEMENTS OF ROUTINE HEALTH MONITORING

- **FREQUENCY OF SAMPLING (quarterly or annually)**
- **DETERMINATION OF SAMPLE SIZE (3-5% of population)**
- **SAMPLING STRATEGY (sentinels or colony animals)**
- **TEST ALL STRAINS (immuno- competent and deficient)**
- **TEST ALL ANIMALS SHOWING PATHOLOGICAL SIGNS**
- **METHODS OF SAMPLE TESTING (have more methods)**
- **IN-HOUSE RISK ASSESSMENT (depend on the facility)**
- **MICROBIOLOGICAL UNIT (SPF, clean conventional, quarantine)**



ROUTINE HEALTH MONITORING PLAN

It is important to have a routine health monitoring plan to prevent (even subclinical) infections of the animals.

This plan should take in consideration that dirty bedding sentinels are necessary but not enough to represent a colony.

Animals of the colony of different sex, age, strain, immune status should be tested quarterly

The best way is to have a comprehensive approach testing procedures, environment and animals with different methods

Table 4. Recommended infectious agents to monitor and frequencies of monitoring for rats (*Rattus norvegicus*).

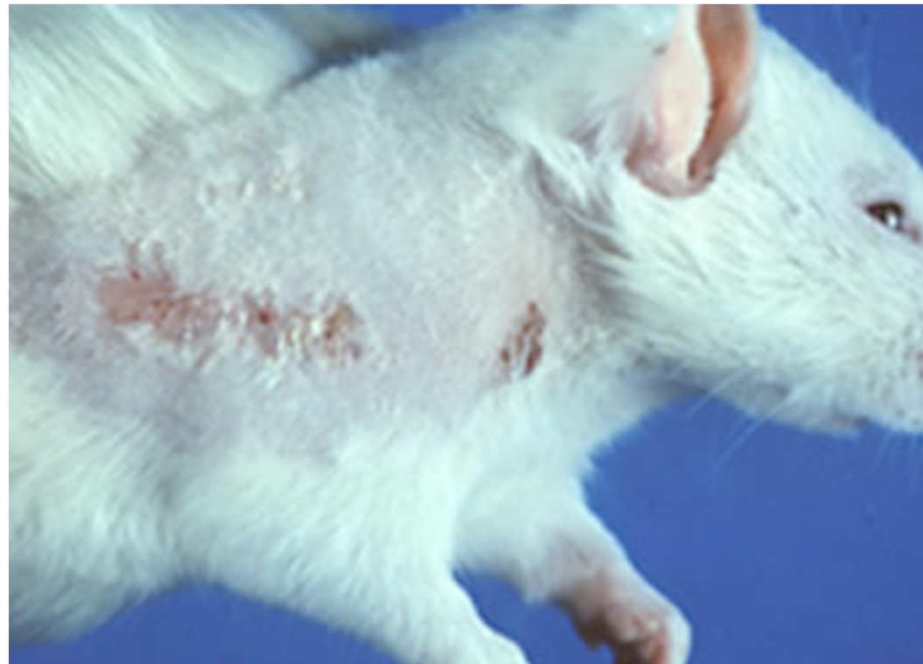
| | Every 3 months | Annually |
|--|----------------|----------|
| Viruses | | |
| Parvoviruses: | | |
| Kilham rat virus | x | |
| Rat minute virus | x | |
| Rat parvovirus | x | |
| Toolan's H-1 virus | x | |
| Pneumonia virus of mice | x | |
| Rat coronavirus/Sialodacryoadenitis virus | x | |
| Rat theilovirus | x | |
| Hantaviruses | | x |
| Mouse adenovirus type 1 (FL) | | x |
| Mouse adenovirus type 2 (K87) | | x |
| Reovirus type 3 | | x |
| Sendai virus | | x |
| Bacteria and fungi | | |
| <i>Clostridium piliforme</i> | x | |
| <i>Helicobacter</i> spp. | x | |
| If positive, speciation for <i>H. bilis</i> is recommended | | |
| <i>Mycoplasma pulmonis</i> | x | |
| <i>Pasteurella pneumotropica</i> | x | |
| Streptococci β -haemolytic (not group D) | x | |
| <i>Streptococcus pneumoniae</i> | x | |
| Cilia-associated respiratory bacillus | | x |
| <i>Pneumocystis</i> spp. | | x |
| <i>Salmonella</i> spp. | | x |
| <i>Streptobacillus moniliformis</i> | | x |
| Parasites | | |
| Endo- and ectoparasites (reported to the genus level) | x | |

Rat Pathogens similarly to Mouse Pathogens usually give subclinical infections in immunocompetent animals

- OBSERVATION IS IMPORTANT, SICK ANIMALS MUST BE CHECKED
- ANY ABNORMAL FEATURE MUST BE REPORTED
- INTERFERENCE WITH RESEARCH HAPPENS EVEN IF NO PATHOLOGICAL SIGN IS EVIDENT
- ROUTINE HEALTH MONITORING HELPS TO DETECT INFECTIOUS DISEASE AGENTS BEFORE IT'S TOO LATE



Rat/Mouse *Staphylococcus aureus* dermatitis





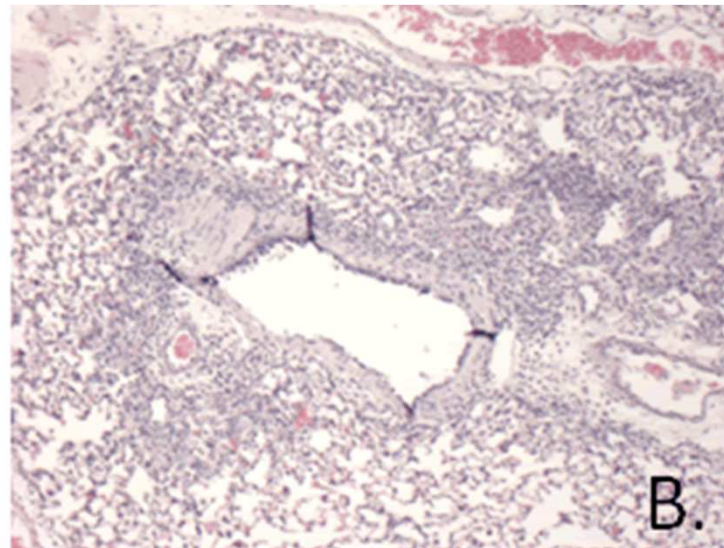
Rat Respiratory Virus now *Pneumocystis carini* (Fungus)



**Common pathogen give gross polmonary lesions in immunodeficient rats.
Pneumocystis can be treated with antibiotic (Trimethoprim/Sulfamethoxazole)
or embryo-cesarean derivation, cross fostering .**



MOUSE/RAT SENDAI



Now rare mouse/rat pathogen give respiratory disease and pulmonary lesions

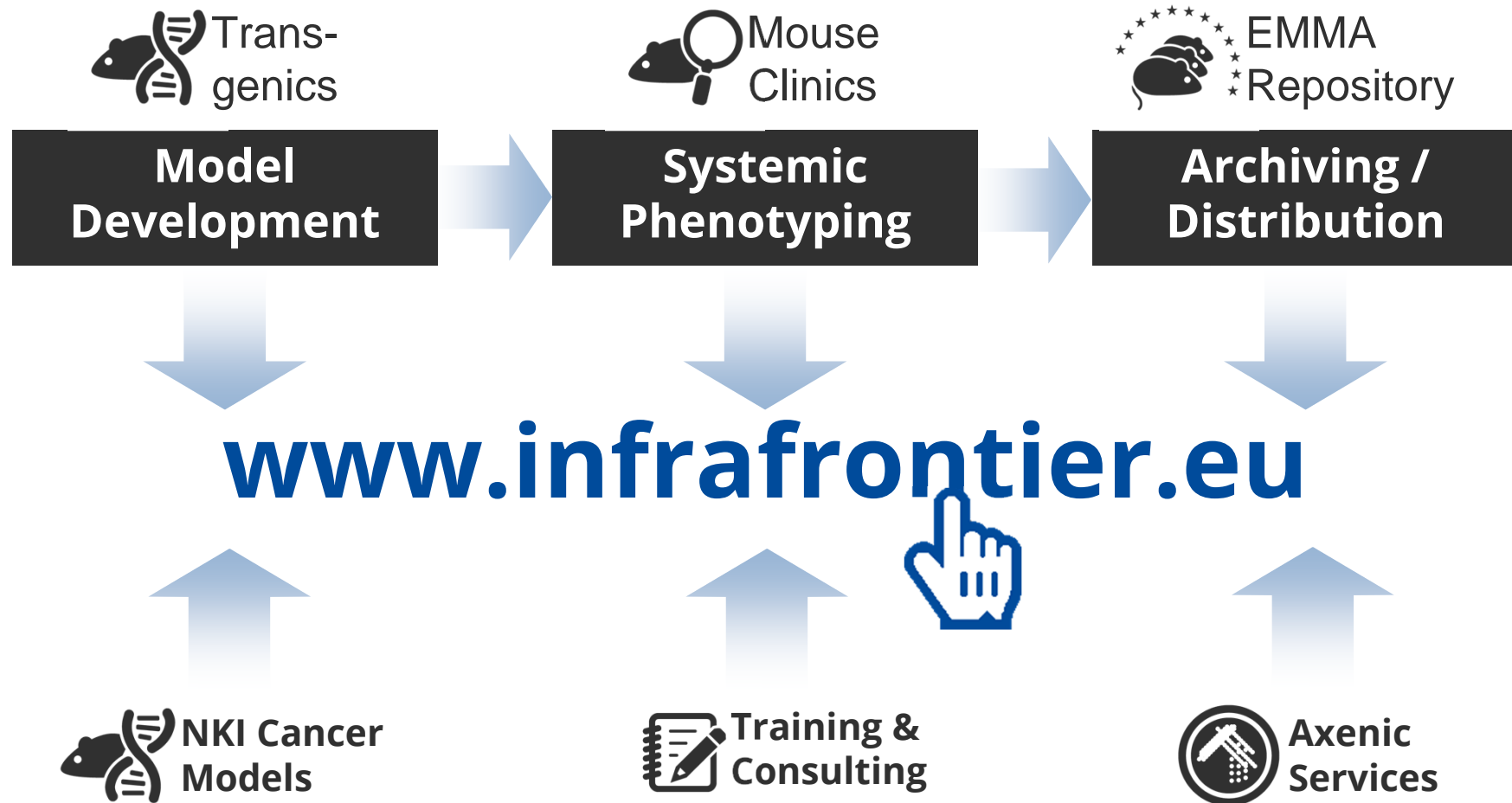


Rat/Mouse *Mycoplasma pulmonis*



Respiratory disease, purulent lungs

INFRAFRONTIER Resources and Services



Distributed research infrastructure – one face to the customer