What Agents to Put on an Institutional Exclusion List(s)

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What makes Sandy Feldman an authority on this?

1. NOT A THING!!
2. I run an animal resource, veterinary care program and diagnostic laboratory like many of you.
3. I used RT-PCR for detection of mouse hepatitis viruses in 1990 and have been running diagnostics by standard and molecular methods for over two decades.
   - That makes me one of the older generation now 😃
4. I’ve identified or further characterized a half dozen diseases of laboratory animals and diseases of wildlife.
5. Someone had to speak to this topic, and Bill White knew I was foolish enough to tackle this.
How did mouse current exclusion lists get to the point where we are now?

1. Let’s just focus on mice.
2. From 1910 until about 1950 rodents began to be used in biomedical science. Most colonies had multiple concurrent infections and disease-free sources of rodents were non-existent.

From 50 Years of Laboratory Animal Science (1999), AALAS pg 131.
The Origins of Mouse Pathogen Exclusion Lists

1. Organization of the Animal Care Panel (1950)
2. Institute for Laboratory Animal Resources (1952).
5. American Society of Laboratory Animal Practitioners (1966)
6. Federation of European Laboratory Animal Science Associations (1978)
7. American Committee on Laboratory Animal Diseases (1979).
Developments in LA Disease Control

1. Gnotobiotic animals and isolators – Dr. Phil Trexler
2. Filter top cages, filtered air change stations – Dr. Lisbeth Kraft.
3. NIH Laboratory Animal Medicine Training Programs
4. Drs. Wallace Rowe and Janet Hartley (NIAID) characterized many mouse viruses; developed the MAP test for cell lines.
5. Dr. Henry Foster at CRL and C. N. Wenworth of Carworth Farms: gnotobiology for commercial production of rodents.
6. 1971 Intramural NIH hysterotomy rederive all mice colonies and initiates disease surveillance: Drs. Tony Allen, David Small, James Ganaway and Thomas Moore, NIH.
Achieving Specific Pathogen-Free Status


2. Diagnostic methods - serology; routine bacteriology, parasitology and histopathology. Recently nucleic acid (NA) sequence detection PCR, RT-PCR and qPCR.
   - Advances - multiplexed fluorometric immunoassays
   - NA detection panels: PCR rodent infectious agent panels (CRL) and infectious microbe PCR amplification tests (RADIL)
What are Our Criteria for Exclusion?

1. May cause clinical disease
2. Confound research results: perturbs cellular processes, physiological responses, behavior, reproduction or alters inflammation.
3. Zooanthroposes
5. Precludes collaboration: intra- or inter-institutional trafficking of mice.
6. Interferes with mouse model development
   - (e.g. *E. hystolitica* infection of mice populated with *E. muris*)

Hantavirus global distribution
# Current Mouse Agent Exclusion List

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Bacteria and Fungi</th>
<th>Parasites</th>
<th>Protozoa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse Hepatitis Virus</td>
<td>Beta hemolytic Streptococci Groups B, C &amp; G</td>
<td>Aspiculuris spp.</td>
<td>Trichomonadaceae</td>
</tr>
<tr>
<td>Mouse Minute Virus</td>
<td><strong>Bordetella</strong> spp.</td>
<td>Hymenolepis spp.</td>
<td>Retortamonas spp.</td>
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<tr>
<td>Mouse Parvovirus 1 &amp; 2</td>
<td><strong>Citrobacter rodentium</strong></td>
<td>Liponyssus spp.</td>
<td>Encephalitozoon cuniculi</td>
</tr>
<tr>
<td>Mouse Rotavirus</td>
<td><strong>Clostridium piliforme</strong></td>
<td>Myobia spp.</td>
<td>Enteromonas spp.</td>
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<tr>
<td>Theiler’s meningoencephalomyelitis Virus</td>
<td><strong>Corynebacterium kutscheri</strong></td>
<td>Myocoptes spp.</td>
<td>Moncercomonoides spp.</td>
</tr>
<tr>
<td>Pneumonia Virus of Mice</td>
<td><strong>Klebsiella oxytoca</strong></td>
<td>Notoedres spp.</td>
<td>Spironucleus spp.</td>
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<tr>
<td>Sendai Virus</td>
<td><strong>Klebsiella pneumoniae</strong></td>
<td>Polyclax spp.</td>
<td>Hexamastix spp.</td>
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<tr>
<td>Lymphocytic Choriomeningitis Virus</td>
<td><strong>Mycoplasma</strong> spp.</td>
<td>Psorergates spp.</td>
<td>Eimeria spp.</td>
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<tr>
<td>Murine Norovirus</td>
<td><strong>Pasteurella pneumotropica</strong></td>
<td>Radfordia spp.</td>
<td>Entamoeba muris</td>
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<tr>
<td>Ectromelia Virus</td>
<td>Other Pasteurella spp.</td>
<td>Rodentolepis spp.</td>
<td>Giardia muris</td>
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<tr>
<td>Hantaan Virus</td>
<td><strong>Pseudomonas aeruginosa</strong></td>
<td>Syphacia spp.</td>
<td>Klossiella muris</td>
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<tr>
<td>Mouse Adenovirus 1 (FL)</td>
<td><strong>Salmonella</strong> spp.</td>
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<tr>
<td>Mouse Adenovirus 2 (K87)</td>
<td><strong>Staphylococcus aureus</strong></td>
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<tr>
<td>Mouse Cytomegalovirus</td>
<td><strong>Spirilium minus</strong></td>
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<tr>
<td>Respiratory Enteric Orphan virus III</td>
<td><strong>Streptococcus pneumoniae</strong></td>
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<tr>
<td>K Virus</td>
<td>Cilia Associated Respiratory Bacillus</td>
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<tr>
<td>Lactic Dehydrogenase Elevating Virus</td>
<td><strong>Corynebacterium bovis</strong></td>
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<tr>
<td>Polyomavirus</td>
<td><strong>Helicobacter hepaticus</strong> &amp; <strong>H. bilis</strong></td>
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<tr>
<td>Mouse Thymic Virus</td>
<td>Other Helicobacter spp.</td>
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<tr>
<td></td>
<td><strong>Pneumocystis jiroveci</strong></td>
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<tr>
<td></td>
<td><strong>Pneumocystis murina</strong></td>
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</table>
Exclude pathogens that cause clinical disease

Exclude pathogens that preclude free mouse exchange between institutions

Exclude pathogens that confound research

Exclude pathogens that cause disease, confound research or reduce fertility

- Strongly agree
- Agree
- Agree with reservations
- Disagree
Difficulty eradicating disease in microisolator caging:
- Impossible: 24%
- Difficult: 72%
- Simple: 4%
- Depends on the microorganism: 1%

Difficulty eradicating disease in open top caging:
- Impossible: 0%
- Difficult: 51%
- Simple: 14%
- Depends on the microorganism: 34%

Predominant mode of disease transmission:
- Infected rodents: 28%
- Fomites carrying infectious agents such as clothing, equipment, etc.: 40%
- Poor barrier practices by research staff: 7%
- Contaminated food, water or bedding: 23%
- Insect Vectors: 2%
Exclusion based on Select Microorganisms

- Staphylococcus aureus
- Bordetella species
- Pasteurella species (any)
- Helicobacter species (any)
- B-hemolytic Streptococci Group D or G
- Any of the following protozoans - Entamoeba
- Pneumocystis murina
- Burkholderia gladioli
- MHV-68 (murine gamma-herpesvirus)

- Not exclude
- Exclude in immune deficient mice only
- Exclude
Why include these commensal protozoa?

Protozoa

*Trichomonadaceae*
- *Retortamonas spp.*
- *Enteromonas spp.*
- *Moncercomonoides sp.*
- *Hexamastix spp.*
- *Entamoeba muris*

As indicators of uncontrolled microbiota expansion?
Trichomonas muris in ICK-/-
Will you continue testing for these low prevalence agents?
Bacteria Isolated from GEM Clinical Cases at UVA

1. Pyometra – *Enterobacter intermedius*

2. Heart blood post surgery – *Staphylococcus intermedius, Enterococcus faecium*

3. Subcutaneous abscess or vibrissae furunculosis – *Staphylococcus constellatus, Staphylococcus xylosus, Staphylococcus aureus, Enterococcus intermedius, Proteus mirabilis, E. coli, Brevundimonas vesicularis*

4. Otitis media – *Leminorella richardii (1X), Sphingomonas sanguinis (3X), Bordetella hirthi (1X), Bordetella avium (1X), Bordetella trematum (1X), Haemophilus paraphrophilus (1X), Micrococcus luteus (1X), Acinetobacter calcoaceticus (1X), Staphylococcus xylosus (3X), Staphylococcus cohnii (2X), Streptococcus pneumoniae (1X), Staphylococcus gallinarum (1X), Deinococcus proteolyticus (1X), Streptococcus macacae (3X) and Raoultella terrigena (1X)*

SHOULD THESE BE ON MY EXCLUSION LIST?
Bacteriology and Molecular Testing Currently Designed to Show What is Not Present.
Can Mice be too Clean?

1. When we re-derive mice to eradicate pathogens we reduce other microbiota.
   – Loose phenotype of some transgenic mice (e.g. TNF-ARE and IBD)
   – Altered time course in development of inflammatory based lesions (atherosclerosis, type I diabetes, IBD)
   – Loose beneficial flora potentiating novel opportunistic infections.
Adverse Impacts of Exclusion

1. Additional cost of testing and rederivation.
2. Increase difficulty maintaining specific pathogen-free status as the lists grows larger.
3. Interference with on-going research
4. Inhibit the intra- and inter-institutional collaborative movement transgenic mice.
5. Potential loss of credibility with Principal Investigators if we cannot justify excluding agents based on good science.
Conclusions

• We continue to test and exclude known pathogens that cause disease, confound research or inhibit collaboration if they are extremely low prevalence.

• Our opinion splits into thirds whether to exclude new low level pathogens, examine them on a case-by-case basis, or to not exclude them.

• We seem to exclude some agents as indicators of uncontrolled microbiota expansion.

• We are wary of disease introduction from these sources: rodents, fomites and poor barrier techniques.