Basic Mouse Diseases and Their Possible Effects on Research

Joshua Kramer, DVM Dipl. ACLAM and ACVP and Gillian Braden, VMD MS Dipl. ACLAM
Leidos Biomedical Research/NCI
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LASP Rodent Health Monitoring – Sentinel Testing

• One cage/rack receives dirty bedding from all other cages
• Clean mice get 12 weeks exposure to dirty bedding
  – Serum antibodies to mouse diseases
  – Fecal samples for bacteria/parasite testing
  – Hair samples for parasite samples
• After obtain results
  – Confirmatory testing
  – Full necropsy
    • Histopathology
    • GI and Respiratory contents for culture
    • Mesenteric LN PCR for MPV
Agents Monitored

**Serum**
- Ectromelia (Mousepox)
- EDIM (Rotavirus)
- C. piliforme
- CAR bacillus
- GDV II
- Hantavirus
- MPV
- MHV
- Mouse adenovirus
- Mouse CMV
- LCMV
- MNV
- Theiler’s Murine Enceph. Virus
- Polyomavirus
- Pneumonia Virus of Mice
- Reovirus 3
- Sendai Virus
- Mycoplasma pulmonis

**Feces/Hair**
- Helicobacter
- Pinworms (Aspicularis, Syphacia)
- Fur mites (Myobia, Myocoptes, Radfordia)
- Parasites – Giardia, Spironucleus, others
- Bacteriology

**Lymph node**
- MPV
LASP Rodent Health Monitoring – Direct Animal Testing

- Direct testing of research/colony animals
- Noninvasive sampling of a cross-section of animals quarterly
  - PCR for fur mites, pinworms, Helicobacter, Spironucleus
- Bethesda only
  - Send the sentinel cage lid for PCR testing for a longer list of pathogens
Why do we do this?

• Colony health

• Human health

• Research interference
Protect Colony Health
Colony Health - Prevent the Catastrophic

• Ectromelia (Mousepox)
  – Outbreaks in 1995 at Naval Medical Center, 1999 at Weill Cornell Medical College, 2003 at UC Berkeley
  – 1995 → Depopulation of ~5000 mice in facility
  – 1999 → Depopulation of room
  – 2003 → Depopulation of room. Saved some valuable transgenic mice in same room

• In all cases the cause was *commercially procured contaminated mouse serum* inoculated into colony animals
Ectromelia

• Mouse disease
  – High morbidity and mortality
  – Rash, loss of digits, facial swelling
  – Necrotizing hepatitis, splenitis, enteritis, dermatitis

• Diagnosis
  – Serology, histology, PCR

• Eradication
  – Depopulation
  – Can save strains with rederivation or vaccination potentially
Ectromelia - Mousepox
Colony Health - Prevent the Catastrophic

- 2012 Report of Human outbreak of LCMV in workers from a rodent feeder breeding facility
  - 4 cases of aseptic meningitis in employees of breeder operation
  - 31% of employees (~30 people) had LCMV antibodies
- Mice from one facility were tested and 21% were LCMV antibody-positive
- CDC mandated depopulation of 400k mice from Facility 1
- 380k mice from a second facility were also euthanized
- 800k frozen mice were destroyed
- Total = 1,600,000 mice
Lymphocytic Choriomeningitis Virus (LCMV)

• Mouse disease → Relatively minimal
  – In utero infection → runting, persistent subclinical disease
  – Adult infection → minimal signs, acute immune mediated disease, LCM in aged adults, glomerulonephritis

• Human disease → flu like symptoms to aseptic meningitis, fatal disease if immunosuppressed, birth defects

• Research effects
  – Human disease risk
  – Depressed cellular immunity

• Eradication
  – Depopulation
  – Embryo transfer may not be successful
LCMV
Colony Health – Prevent the Troublesome

• Some effect on colony health but big risk for research goals
  – Pinworms
  – C. bovis
  – Helicobacter
  – Basically everything else…. 
Protect Human Health
Zoonotic Diseases of Rodents

- Zoonotic disease → Disease transmitted between animals and humans
  - Bacteria
    - Rat bite fever (*Strep moniliformis, Spirillum minus*)
    - Tularemia
    - Salmonella
    - Leptospirosis
  - Virus
    - Hantavirus
    - LCMV
  - Parasites
    - Ringworm
    - Sarcoptic mange
    - Tropical rat mite
Hantavirus

Spread

Hantavirus Pulmonary Syndrome

38% fatality rate
Rat Bite Fever (*Strep moniliformis* or *Spirillum minus*)

- Multifocal petechial hemorrhage
- Vesicular dermatitis

10% fatality rate
Tularemia

Ulcers at bite site

Oculoglandular

Also oral lesions, pulmonary lesions - ~2% fatality rate
Category A priority bioterror pathogen
Effects on Research
Disease Adverse Effects on Research

• Overt disease
• Shortened lifespan
• Effects on breeding
• Immune-suppression
• Immune-activation
Mouse Hepatitis Virus

- **Mouse disease**
  - General no clinical signs in immunocompetent mice; wasting in immunodeficient mice
  - Polytropic strains (respiratory) – spread hematogenously, necrotic spots on liver classic, multiorgan disease in immunodeficient mice
  - Enterotropic strains – lesions in gut

- **Diagnosis**
  - Fecal PCR, histology, sentinel serology
Mouse Hepatitis Virus

• Research effects
  – Infects lymphoid tissue (monocytes, macrophages, dendritic cells) effecting immune system
    • Immunosuppression or stimulation
    • B/T cell dysfunction
    • Activates NK Cell activity
  – Alters liver enzyme levels
  – Bone marrow infection → anemia, thrombocytopenia, leukopenia
  – Tumor effects
    • Spontaneous regression of normally stable tumor lines
    • Abnormal invasion patterns or other tumor behavior
    • Enhances pristane-induced plasmacytomas
    • Rejection of xenografts by nude mice
  – Alters course of concurrent infections
Mouse Hepatitis Virus

• Eradication
  – Depopulation option
  – Burnout – Cease all breeding and import for ~8 weeks (immunocompetent mice only)
    • Competent mice clear infection
    • Also easy to clean and not persistent in environment, so this is possible
Mouse Parvovirus/Minute Virus of Mice

• Mouse Disease
  – Essentially none
  – MVM causes some disease in SCIDs and some other strains
  – MVM can cause disease or death in neonates
  – Both cause **persistent infections**

• Diagnosis
  – Serology, PCR (mesenteric lymph nodes, spleen, feces)
Mouse Parvovirus/Minute Virus of Mice

• Research effects
  – Cancer studies
    • Some strains are oncotropic and oncolytic; cause viral induce apoptosis in tumors/cell lines
    • Activate anticancer immune response
    • Tumor cell lines show increased susceptibility
  – Immune system
    • Suppressed T cell function on proliferation

• Eradication
  – Consider depopulation and rederivation
  – Environment:
    • Extremely resistant to heat, time
    • Some chemical disinfectants work (Clidox, ozone, etc)
**Helicobacter**

- **Mouse Disease**
  - Infects cecum, colon, liver/GB, +/- stomach
  - Different strain susceptibilities
  - In susceptible strains, rectal prolapse, diarrhea, colon cancer, gastric cancer, hepatocellular carcinoma,

- **Diagnosis**
  - Fecal PCR

- **Eradication**
  - Can use antibiotic treatment
  - Bacteria are highly sensitive to desiccation so doesn’t spread super easily
  - Can rederive mice → Most common reason for rederivation in quarantine
Helicobacter

- Research effects
  - Immunity
    - Alter immune responses to vaccines
    - Promotes system allergic response
  - Cancer
    - Effect breast cancer progression
      - promote breast cancer in APCmin mice
    - Promotes colon tumorigenesis in some strains (pictured)
  - General
    - May reduce reproductive performance
    - Cause overt disease and intestinal inflammation in multiple strains
Mycoplasma pulmonis

• Mouse Disease
  – Subclinical to severe acute respiratory disease if concomitant factors exist
  – Genital tract infection = reduced fertility
  – Arthritis
  – Otitis media

• Diagnosis
  – Serology +/- culture or PCR of lavage fluid

• Eradication
  – Depopulation and repopulation
  – Embryo transfer rederivation
Mycoplasma pulmonis

• Research effects
  – Immunity
    • Mitogenic effect on B and T cells
    • Stimulates NK cells
    • Effects on cytokines
  – Infertility or fetal loss
  – Cancer
    • Infects cell lines
**Mycoplasma pulmonis**

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**ENVIRONMENTAL PATHOBIOLOGY**

Vet Pathol **46**:952–959 (2009)
DOI: 10.1354/vp.08-VP-0240-S-COM

**COMMENTARY**

*Mycoplasma pulmonis* and Lymphoma in Bioassays in Rats


Department of Genetics (TRS, MM) and Emeritus (JRL) University of Alabama at Birmingham, Birmingham, AL; ToxPath, Inc., Raleigh, NC (EEM); Department of Comparative Pathobiology, Purdue University, West Lafayette, IN (JKD); and US Food and Drug Administration, Silver Spring, MD (MKD)

**Abstract.** Lymphomas were reported to be induced in rats in bioassays of aspartame, methyl tertiary-butyl ether (MTBE), and other chemicals conducted by a nonprofit cancer research organization. European regulatory authorities concluded that lymphomas in the aspartame study were caused by *Mycoplasma pulmonis* and suggested that this also was the case for the MTBE bioassay. To assess the role of *M. pulmonis* in these bioassays, we reviewed the tumor data for the aspartame and MTBE bioassays and, additionally, the organization’s bioassay of methanol. For all 3 studies, the most frequently reported hematopoietic neoplasm was lympho-immunoblastic lymphoma, the most frequently affected organ was the lung, and, in almost half of the rats with this diagnosis, the lung was the only affected organ. Lesions diagnosed as lymphoma in published illustrations had pleomorphic cellular morphology and appeared to contain neutrophils. Information from these reports and other sources indicated that lesions typical of *M. pulmonis* disease were prevalent among the aspartame and MTBE study rats and that the rats were not specific-pathogen-free. Because the lymphoma type, cellular morphology, and organ distribution reported in these studies are atypical of lymphoma in rats, because lymphocyte and plasma cell accumulation in the lung is characteristic of *M. pulmonis* disease; and because *M. pulmonis* disease can be exacerbated by experimental manipulations, including chemical treatment, we suggest that a plausible alternative explanation for the reported results of these bioassays is that the studies were confounded by *M. pulmonis* disease and that lesions of the disease were interpreted as lymphoma.

*Key words:* Bioassay; lung; lymphoma; *Mycoplasma pulmonis*; rats.
Potential Sources of Disease
Source of Diseases

- Infected Biologics
  - Rodent tumors/cell lines, serum, plasma, etc.
- Infected Mice
  - Approved vendors
    - Review health reports
    - Commercial breeders
  - Quarantine for other sources
    - Direct testing of animals
  - Health Monitoring Program (Sentinels)
- Infected fomite
  - Instruments, supplies, PEOPLE, etc. from other buildings
MAP Testing
- Ectromelia
- LCMV
- LDHV
- MVM
- MHV
- Polyomavirus
- Pneumonia Virus
- Sendai
- Reovirus 3
- Theiler’s Murine Encephalomyelitis Virus

MTBM
- MHV
- MPV
- MVM
- Polyoma Virus
- Sendai
- Pneumonia Virus
- Reovirus 3
- LCMV
- Ectromelia
- LDHV
- Mycoplasma
- Norovirus
- EDIM
- Adenovirus
- CMV
- Theiler's Murine Encephalomyelitis Virus
### Infected Biologics - MTBM Test Cell Lines

<table>
<thead>
<tr>
<th>MTBM-M</th>
<th>MTBM-R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse hepatitis virus (MHV)</td>
<td>Rat coronavirus (RCV)</td>
</tr>
<tr>
<td>Polyoma virus (POLY)</td>
<td>Sialodacryoadenitis virus (SDAV)</td>
</tr>
<tr>
<td>Sendai virus (SEN)</td>
<td>Sendai virus (SEN)</td>
</tr>
<tr>
<td>Pneumonia virus of mice (PVM)</td>
<td>Pneumonia virus of mice (PVM)</td>
</tr>
<tr>
<td>Reovirus 3 (REO3)</td>
<td>Reovirus 3 (REO3)</td>
</tr>
<tr>
<td>Minute virus of mice (MVM)</td>
<td>Kilham virus (KRV)</td>
</tr>
<tr>
<td>Theiler's murine encephalomyelitis virus (GDVII or TMEV)</td>
<td>Toolan's H-1 virus (H-1)</td>
</tr>
<tr>
<td>Lymphocytic coriomeningitis virus (LCMV)</td>
<td>Rat theilovirus</td>
</tr>
<tr>
<td>Ectromelia virus (ECT)</td>
<td>Mycoplasma spp. (MYCO)</td>
</tr>
<tr>
<td>Lactic dehydrogenase-elevating virus (LDHV)</td>
<td>Rat parovirus (RPV)</td>
</tr>
<tr>
<td>Mycoplasma spp. (MYCO)</td>
<td>Rat murine virus (RMV)</td>
</tr>
<tr>
<td>Mouse parvovirus (MPV)</td>
<td>Lymphocytic coriomeningitis virus (LCMV)</td>
</tr>
<tr>
<td>Mouse norovirus (MNV)</td>
<td>Rat cytomegalovirus (RCMV)</td>
</tr>
<tr>
<td>Mouse rotavirus (EDIM or MROTA)</td>
<td>Seoul virus (SEO)</td>
</tr>
<tr>
<td>Mouse adenovirus (MAD)</td>
<td>Mouse adenovirus (MAD)</td>
</tr>
<tr>
<td>Mouse cytomegalovirus (MCMV)</td>
<td>Mouse adenovirus (MAD)</td>
</tr>
</tbody>
</table>

- All tissue/cell lines with some exceptions
- All biologics produced in presence of mouse products
- All serum
- Matrigel
# Infected Mice - Use approved vendors!

| Approved Source                          | • Charles River Laboratories  
|                                         | • Taconic                     
|                                         | • Jackson Laboratories – PRODUCTION ONLY  
|                                         | • ENVIGO (formerly Harlan)     |
| Modified Approved Source                | • NIH Building 37              
|                                         | • NIH Building 41              
|                                         | • NIH 10/CRC                   
|                                         | • ACRF 12                     |
| Non-Approved Source                     | • Jackson Laboratories – JAX Faculty Strains  
<p>|                                         | • All other sources            |</p>
<table>
<thead>
<tr>
<th>Source Category</th>
<th>Arrival</th>
<th>Testing</th>
<th>Release</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modified Approved Source</td>
<td>A percentage of animals are tested for common murine pathogens using endo- and ectoparasitology, bacteriology, as well as tested for <em>Helicobacter</em> spp.</td>
<td>Three weeks after arrival a percentage of animals will be bled for basic serological testing.</td>
<td>Dependent on diagnostic results* but typically 7-10 days after last set of tests are submitted.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Appx 4.5 weeks after arrival</strong></td>
</tr>
</tbody>
</table>
# Timelines

<table>
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<tr>
<th>Source Category</th>
<th>Arrival</th>
<th>Testing</th>
<th>Release</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Approved Source</td>
<td>A percentage of animals are tested for common murine pathogens using serology, endo- and ectoparasitology, bacteriology, as well as tested for <em>Helicobacter</em> spp.</td>
<td><strong>Two weeks</strong> after arrival a percentage of animals are re-tested using endo- and ectoparasitology, bacteriology, as well as tested for <em>Helicobacter</em> sp. <strong>Three weeks</strong> after arrival a percentage of animals are bred for basic serological testing. <strong>Six weeks</strong> after arrival a percentage of animals are tested using serology, endo- and ectoparasitology, bacteriology, as well as tested for Helicobacter spp.</td>
<td>Dependent on diagnostic results but typically 10-14 days after last set of tests are submitted. <strong>Appx 7.5 weeks after arrival</strong></td>
</tr>
</tbody>
</table>
Quarantine Process

• Receiving and Quarantine (R&Q)
  – NCI-Frederick, Bldg. 429

• Request for Importation
Request for Importation Form

Animal Study Proposal Number

- Proposal Number:
  - ACUC assigned Animal Study Proposal (ASP) number
  - E.g. – 17-037, LB-094

- Project ID:
  - Cost center/project billing information
  - E.g. – 001.001.0001
  - Used for per diems, shipping costs, technical services, etc.
Request for Importation Form

• **Contact for Health Science Report**
  – Contact at SENDING institution for health reports for requested importation
  – E.g. – facility veterinarian, shipping coordinator, facility manager, etc.
  – If sending from another NIH institute, DVR contacts are listed on their website

• **Contact for Shipment**
  – Contact at SENDING institution to arrange shipment
  – E.g. – shipping coordinator, facility manager
• Breeding animals in quarantine
  – Discouraged, but allowed in certain circumstances
  – In general, allow 3 weeks prior to arranging breeding pairs/trios
  – Provide details in “Special Notes/Instructions” section or contact R&Q staff directly
  – Genotyping (tail or ear samples), ear tagging/notching can be requested for additional technical charge
Request for Importation Form

Do the animals have a functional immune system?

- Yes
- No
- Unknown

• Please complete accurately!
  – Immunocompromised mice may be unable to generate appropriate antibody response to pathogens
  – Serology will not be performed on immunocompromised animals
  – Sentinel animals will be used instead
Animals to be released to non-NCI, NIH facilities will need an Online Rodent Import Application (ORIA) permit number.

http://dvrapps.ors.od.nih.gov/RodentImport/

If you are unsure of how to complete the ORIA form, please contact R&Q staff and we will complete for you.
<table>
<thead>
<tr>
<th>Name</th>
<th>Title</th>
<th>Contact</th>
</tr>
</thead>
</table>
| Gillian Braden     | Animal Program Veterinarian Manager, Receiving and Quarantine | Phone: 301-846-5195  
Fax: 301-846-6024  
Email: gillian.braden-weiss@nih.gov |
| Sandra Warfield    | Departmental Secretary                                    | Phone: 301-846-1133  
Fax: 301-846-6031  
Email: warfieldsl@nih.gov |
| Byron Bowie III    | Laboratory Animal Technician                              | Phone: 301-846-1133  
Email: Byron.bowie2@nih.gov                                           |
| Rick Moxley        | Manager, Technical Operations – 429                       | Phone: 301-846-5792  
Email: Richard.moxley@nih.gov                                          |
## NCI-Bethesda Contact Information

<table>
<thead>
<tr>
<th>Name</th>
<th>Title</th>
<th>Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melody Roelke-Parker</td>
<td>Animal Program Veterinarian Rodent Import Officer</td>
<td>Phone: 301-827-4230 Email: <a href="mailto:Melody.Roelke-Parker@nih.gov">Melody.Roelke-Parker@nih.gov</a></td>
</tr>
</tbody>
</table>

For imports into DVR facilities, please contact DVR in addition to the NCI staff listed above. Contact information can be found at: [https://www.ors.od.nih.gov/sr/dvr/Pages/default.aspx](https://www.ors.od.nih.gov/sr/dvr/Pages/default.aspx)

Joanne Smith is the NIH Rodent Import Officer and works for DVR. She can be reached at [smithjo@mail.nih.gov](mailto:smithjo@mail.nih.gov)
Fomites/People – Wash your hands, don’t go between facilities

![CDC Clean Hands Poster](https://www.cdc.gov/handhygiene)
Summary

• Mouse diseases can affect mouse health, human health and research

• LASP staff uses considerable resources to protect your mice
  – We want to protect our staff, research staff, mice, and your research

• The effort to protect your mice also includes you
  – Understand risks and follow facility rules
  – Know what’s in your colony in NCI buildings and other places
  – Understand possible effects on research

• Ask questions of the facility staff, vets, etc
  – We want to help and work with you
References

• Implications of Infectious Agents on Results of Animal Experiments
  – http://www.utexas.edu/research/arc/misc/GVSOLAS.pdf

• Old Enemies, Still With us After All These Years
  – http://ilarjournal.oxfordjournals.org/content/49/3/291.full

• Of Mice and Microflora: Considerations for Genetically Engineered Mice
  – http://vet.sagepub.com/content/49/1/44.full.pdf

• University of Missouri Disease of Research Animals
  – http://dora.missouri.edu/

• Charles River Infectious Agent Technical Sheets