### Rat Serology Results

<table>
<thead>
<tr>
<th>Agent/Assay</th>
<th># tested</th>
<th>% positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS-1</td>
<td>63,101</td>
<td>2.3692%</td>
</tr>
<tr>
<td>H-1</td>
<td>81,764</td>
<td>1.6120%</td>
</tr>
<tr>
<td>RPV</td>
<td>88,399</td>
<td>1.6018%</td>
</tr>
<tr>
<td>KRV</td>
<td>88,667</td>
<td>1.5101%</td>
</tr>
<tr>
<td>RMV</td>
<td>44,075</td>
<td>1.4475%</td>
</tr>
<tr>
<td>RTV</td>
<td>34,970</td>
<td>1.2325%</td>
</tr>
<tr>
<td>CARB</td>
<td>25,220</td>
<td>0.2617%</td>
</tr>
<tr>
<td>SDAV</td>
<td>82,375</td>
<td>0.2428%</td>
</tr>
<tr>
<td>MPUL</td>
<td>81,648</td>
<td>0.1727%</td>
</tr>
<tr>
<td>PVM</td>
<td>79,957</td>
<td>0.1438%</td>
</tr>
<tr>
<td>ECUN</td>
<td>22,190</td>
<td>0.1217%</td>
</tr>
<tr>
<td>HANT</td>
<td>22,846</td>
<td>0.0438%</td>
</tr>
<tr>
<td>MAV1,2</td>
<td>34,096</td>
<td>0.0293%</td>
</tr>
<tr>
<td>SEND</td>
<td>80,839</td>
<td>0.0247%</td>
</tr>
<tr>
<td>REO</td>
<td>73,482</td>
<td>0.0082%</td>
</tr>
<tr>
<td>LCMV</td>
<td>36,297</td>
<td>0.0000%</td>
</tr>
</tbody>
</table>
Plus

- “Rat Respiratory Virus”
- *Clostridium piliforme*
- *Corynebacterium kutscheri*

Rat Coronavirus (SDAV/RCV)

- Coronavirus: common in conventional rats (enveloped ss RNA virus) Formerly called SDAV or SDAV/RCV
  - Many strains with varying predilection for salivary gland (most common), to upper respiratory tract, to lower respiratory tract
- Host range: rats only
- The virus has short incubation time and is highly contagious
- Transmitted by aerosol, contact, fomites
- Rapidly reaches high prevalence in infected colonies housed in open-top caging

Rat Coronavirus (SDAV/RCV)

- Very high morbidity: swollen cervical area
  - almost diagnostic, porphyria very nonspecific
- Gross lesions:
  - Swollen edematous salivary glands
  - Cervical lymph node enlargement
  - Rhinitis and possibly interstitial pneumonia
  - Occasional ophthalmologic lesions (keratoconjunctivitis, corneal opacities, megaloglobus, hypopyon, hyphema, etc.)
Rat Coronavirus (SDAV/RCV)

- Histopathology
  - Sialoadenitis (parotid and submaxillary salivary glands) with ductal necrosis and/or squamous metaplasia
  - Dacryoadenitis (Harderian and other lacrimal glands) with lesion patterns similar to the salivary glands
  - Multifocal, interstitial pneumonia associated with necrotizing bronchitis and bronchiolitis; hyperplastic BALT

Rat Coronavirus (SDAV/RCV)

- Histopathology (cont.)
  - Necrotizing laryngitis, tracheitis, and rhinitis with or without epithelial hyperplasia
  - Cervical lymph node reactive hyperplasia (non-specific)
  - Occasional keratoconjunctivitis, anterior synechiae, hypopyon, hyphema, etc.
• Interference with research
  – Reduced food consumption, weight loss, reduced breeding performance
  – Acute and (occasionally) chronic ophthalmologic lesions
  – Occasional respiratory airway lesions
  – Salivary gland is the major source of Epidermal Growth Factor
  – Reduced IL-1 production by alveolar macrophages
  – Exacerbates *Mycoplasma pulmonis* infection

**Rat Coronavirus (SDAV/RCV)**

• Differential diagnoses
  – Iatrogenic salivary enlargement due to jugular catheters
  – Non-specific porphyria
  – Other viral pneumonias (RRV, Sendai virus, PVM)
  – Cytomegalovirus infection (RCMV)
  – Papovaviral Sialoadenitis (athymic nude rats)
  – Hypovitaminosis A (squamous metaplasia of salivary gland ducts)
**Rat Coronavirus (SDAV/RCV)**

- **Diagnosis**
  - Pathology and clinical signs - first week
  - PCR – Early in infection
  - Serology - later (after 7-10 days)
    - Good cross-reaction among all known strains
  - Immunohistochemistry or PCR on paraffin-embedded tissue

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**Paroviruses**

- ssDNA, (5.4 kb genome), non-enveloped
  - Virus remains active in environment
    - Resistant to desiccation, non-oxidizing disinfectants
  - Most are common in lab rats and mice
  - Require cells in S phase of mitosis
    - Triggers production of nonstructural proteins, NS1 and NS2, which direct viral replication and assembly and are responsible for cytotoxicity.
  - Very low or no morbidity
  - Cause persistent infection
  - Different serotypes not very cross-reactive on ELISA/MFIA

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**Paroviruses of Rats**

- **RV** - Rat Virus (previously KRV, Kilham Rat Virus)
  - Natural infections usually asymptomatic, but persistent
  - Infects rapidly growing cells: Vascular endothelium, lymphoreticular and hematopoietic tissues, developing cerebellum and liver
  - Rare epizootic disease in fetal/neonatal rats: Cerebellar hypoplasia, anemia, thrombocytopenia
  - Very rare disease in older rats: Hemorrhagic disease
Rat Virus

- Long-term infection, especially if infected as young rats.
  - May cause persistent infection (6 months or more)
  - May have prolonged shedding (10 weeks or more)
- Research Effects:
  - RV induced diabetes in DR BB rats (Guberski et al., 1991)
    - Possibly due to imbalance in Th1 and Th2 responses (Jun and Yoon, 2001)
  - Multiple strains exist
  - No clinical disease reported
  - Research effects: Suppression of LGL lymphoid tumor growth in vivo in F344 rats: RPV-1a
  - RV NS protein induced epigenetic modification in thymic lymphoma line, causing reversion to benignancy (Iseki H., 2005)
  - RPV does not infect mice

Rat Parvovirus

- Few studies in literature, very difficult to isolate
  - Multiple strains exist
  - No clinical disease reported
  - Research effects: Suppression of LGL lymphoid tumor growth in vivo in F344 rats: RPV-1a
  - RV NS protein induced epigenetic modification in thymic lymphoma line, causing reversion to benignancy (Iseki H., 2005)
  - RPV does not infect mice
Parvoviruses of Rats

- H-1 (Toolan’s H-1)- no natural disease
  - Significance through research interference: liver
  - Current interest (and historic) in possible use treating human tumors
- Rat Minute Virus (RMV)
  - Almost nothing in literature
  - Serologically and genetically more similar to RV than to RPV

Detection of Parvoviruses

- Serology – Best for screening
  - MFIA or ELISA
  - Use panel of antigens for each serotype, plus the generic NS-1 antigen
    - Rats - RV, H-1, RPV, RMV and NS-1
  - IFA – Good follow-up assay for positive/equivocal MFIA/ELISA

Rat Serology for paroviruses

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Detection of Parvoviruses

- PCR
  - Can be strain-specific (VP2) or generic (NS-1)
  - Mesenteric LN stay positive indefinitely
  - PCR of fecal samples valuable to detect shedding (can pool fecal samples. Beware of fecal inhibitors of PCR)
  - Valuable for testing biologicals and cell cultures
  - Applicable to environmental swabs

“Rat Respiratory Virus (RRV)”

- Pneumonia first observed in F-344 rats mid-1990s
  - Idiopathic pneumonitis
- Reported in:
  - Inhalation Toxicology in 1997, Gilbert, B.E, et al.
  - Veterinary Pathology in 2009, Albers, TM, et al.

Rat Respiratory Virus (RRV)

- Prevalence: Common (~5-6% by histopathology in external sample stream at Charles River)
  - True prevalence likely higher if serology or PCR were available
- Biology
  - Agent not identified. Discussed as probably viral, but keep an open mind. Rat Respiratory “Agent”?
  - Published abstracts implicating a hantavirus are not widely accepted and (in presenter’s opinion) are erroneous.
    - Based on cross-reactions seen by one diagnostic lab using a Hantaviral IFA, which is allegedly more prone to false positives than many tests. No confirmation of these reports by culture or molecular techniques in several years since initial suggestion.
Rat Respiratory Virus (RRV)

- Epidemiology
  - Host range - rats are the only known host, all strains susceptible
  - Cases detected in North America, Europe, Asia
  - Transmitted by aerosol and/or dirty bedding
  - Additional fomite transmission likely
  - Lesions most prevalent (=>50%) and most pronounced in naïve colony (epizootic form)
  - Lesions have low prevalence (<=20%) and low severity in endemic colony (enzootic form)

Rat Respiratory Virus (RRV)

- Pathogenesis
  - Naïve rats
    - 1st nonspecific lesions at about 3-4 weeks post-exposure
    - Lesions reach zenith at 7 weeks, then decline
    - Lesions present for at least 13 weeks post-exposure
  - Endemic colony (young rats exposed while they still have some maternal antibodies)
    - Highest prevalence (best time to screen) 10-12 weeks of age

Gross Findings

<table>
<thead>
<tr>
<th>Percentage of Rats with Gross Pulmonary Lesions Consistent with Interstitial Pneumonia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weeks of Exposure</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
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<tr>
<td>7</td>
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<tr>
<td>8</td>
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<tr>
<td>9</td>
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<tr>
<td>10</td>
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<tr>
<td>11</td>
</tr>
<tr>
<td>12</td>
</tr>
<tr>
<td>13</td>
</tr>
</tbody>
</table>
Microscopic Findings

Percentage of Rats Exhibiting Microscopic Lesions Consistent with RRV

Rat Respiratory Virus (RRV)

• Diagnosis
  - Gross Lesions: Scattered brown to grey areas on pleural surface, suggestive of interstitial pneumonia.
    - RRV is currently the only common cause of interstitial pneumonia in rats
  - Histopathology is diagnostic
    - Prominent perivascular cuffs distributed in lungs
    - Interstitial pneumonia (lymphohistiocytic)
    - Syncytial cells (occasional)
    - Lesions graded minimal to moderate
  - No Serology or PCR available
RRV

- Control
  - Eliminate by Rederivation
  - Duration of shedding? - No definite answer
  - Disinfection - Unknown
RRV

- Research interference
  - Nothing demonstrated.
  - Anecdotal reports suggest increased mortality under anesthesia, failed ex vivo lung studies, and confounded pulmonary histopathology assessment of inhalation studies.

Sendai Virus Infection

- Etiology: Sendai virus, Parainfluenza virus type I (PI-1)
  - Sendai is not the only PI-1 virus. Rats may also be susceptible to other PI viruses, such as PI-3.
- Host range
  - Mice
  - Rats
  - Hamsters
  - Guinea pigs: usually non-specific serological reactions with other parainfluenza viruses
- Prevalence – rare in lab rodents (0.003% in mice, 0.024% in rats)
Sendai Virus Infection

- Histopathology
  - Reparative stage: Proliferation and regeneration of target epithelium
    - Epithelial hyperplasia and dysplasia in upper and lower airways and alveolar septa
    - May see squamous metaplasia, polypoid masses in bronchiolar lumina

Sendai Virus Infection

- Histopathology
  - Recovery stage: Either a return to normal or persistent scars
    - Fibrosis
    - Cholesterol clefts
    - Dilated airways containing inspissated secretions
    - Peribronchial, peribronchiolar, and perivascular mononuclear cell cuffs and aggregates

Diagnosis of Sendai Virus Infection

- Serology: MFIA, ELISA, IFA, HAI
  - Use sentinel mice to screen for cross-reacting antibodies in GP
- PCR
- Pathology
  - Lesions not specific, but inclusions in airway cells and syncytia are very suggestive of Sendai virus infection
- Virus isolation
- Immunohistochemistry and immunofluorescence of tissues
Rat Theilovirus (RTV)

• Discovery
  – Serologic titers have long been detected in rats using antigen from the GD-VII strain of TMEV
    • Some colonies were positive, others negative, suggesting the presence of a virus related to TMEV.
    • Since the rat virus did not appear to transfer to mice, and vice versa, the rat virus was thought probably distinct from TMEV.
  – The virus in rats has been now sequenced, the taxonomy of picornaviruses has been adjusted, and the virus is now referred to as rat theilovirus (RTV).

Rat Theilovirus (RTV)

• Agent
  – Family: Picornaviridae, Genus: Cardiovirus, Species: Theilovirus, Serotype: Rat theilovirus.
    • There are three serotypes in the theilovirus species: TMEV, RTV (or Theiler’s-like virus of rats), Viluisk human encephalomyelitis virus, Saffold virus.
  – RTV and TMEV are small non-enveloped, RNA viruses.
    • Moderate environmental persistence and resistance to disinfection are expected.

Rat Theilovirus (RTV)

• Epizootiology
  – Prevalence – moderate. The CR diagnostic laboratory finds about 2% of rats serum samples from external sources are positive for RTV
  – The host species range is unknown, but there is evidence against natural spread to mice
  – Infected rats have been reported to shed RTV for at least 13.5 weeks
Rat Theilovirus (RTV)

• Disease
– No disease resulting from natural infection has been reported
– Experimental Disease (IC inoculation of sucklings with material from rat intestine)
  • Ohsawa, et al. – no disease
  • Rodrigues, et al. – flaccid paralysis, tremor, death
    – No histopathology. Demonstrated virus in brain. No HM on “donor” rats, and did not check for other agents in affected sucklings
  • Henderson, et al. – No neurologic disease.
    “Possible” wasting in nude rats after oral gavage
– Conclusion – at this time potential pathogenicity, or variation in virulence among strains is not known

Rat Theilovirus (RTV)

• Research Effects
– None reported

Rat Theilovirus (RTV)

• Diagnosis
  – Serology
    • MFIA of ELISA
    • IFA
  – PCR
    – virus shed for long periods, PCR may be the preferred method to screen animals in quarantine
  – Soiled bedding should be adequate exposure for sentinels
### Rat Theilovirus (RTV)

**Management**
- Rederivation by embryo transfer or caesarian section should be successful
- Success at early cross-fostering not reported
  - Reported as successful for most litters for TMEV
- Pest control. TMEV reported from wild mice. RTV status of wild rats is not known.
- Environmental disinfection should be as for other nonenveloped viruses, e.g., parvoviruses
  - Oxidizing disinfectants

### Rat Bacteriology Results

<table>
<thead>
<tr>
<th>Agent</th>
<th># tested</th>
<th># pos.</th>
<th>% pos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helicobacter bila</td>
<td>8,031</td>
<td>111</td>
<td>1.3821%</td>
</tr>
<tr>
<td>Helicobacter hepaticus</td>
<td>8,531</td>
<td>35</td>
<td>0.4308%</td>
</tr>
<tr>
<td>B. bronchiseptica</td>
<td>6,477</td>
<td>0</td>
<td>0.0000%</td>
</tr>
<tr>
<td>Beta Strep sp</td>
<td>6,505</td>
<td>1</td>
<td>0.0154%</td>
</tr>
<tr>
<td>Beta Strep Grp B</td>
<td>6,447</td>
<td>221</td>
<td>3.4280%</td>
</tr>
<tr>
<td>Beta Strep Grp G</td>
<td>6,447</td>
<td>1</td>
<td>0.0150%</td>
</tr>
<tr>
<td>C. Autololerti</td>
<td>6,432</td>
<td>0</td>
<td>0.0000%</td>
</tr>
<tr>
<td>M. pulmonis</td>
<td>3,594</td>
<td>2</td>
<td>0.0556%</td>
</tr>
<tr>
<td>P. multocida</td>
<td>6,409</td>
<td>0</td>
<td>0.0000%</td>
</tr>
<tr>
<td>P. pneumotropica</td>
<td>6,409</td>
<td>340</td>
<td>5.3050%</td>
</tr>
<tr>
<td>other Pasteurella</td>
<td>6,557</td>
<td>24</td>
<td>0.3770%</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>12,931</td>
<td>301</td>
<td>2.3277%</td>
</tr>
<tr>
<td>Salmonella</td>
<td>6,430</td>
<td>0</td>
<td>0.0000%</td>
</tr>
<tr>
<td>Other Staphylococcus</td>
<td>6,494</td>
<td>24</td>
<td>0.3770%</td>
</tr>
<tr>
<td>Streptococcus pneumonia</td>
<td>6,484</td>
<td>0</td>
<td>0.0000%</td>
</tr>
</tbody>
</table>

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<thead>
<tr>
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<th># tested</th>
<th># pos.</th>
<th>% pos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. tetraptera</td>
<td>8,350</td>
<td>4</td>
<td>0.0479%</td>
</tr>
<tr>
<td>S. muris</td>
<td>8,350</td>
<td>139</td>
<td>1.6647%</td>
</tr>
<tr>
<td>S. obvelata</td>
<td>8,350</td>
<td>1</td>
<td>0.0120%</td>
</tr>
<tr>
<td>All pinworms</td>
<td>8,350</td>
<td>144</td>
<td>1.7246%</td>
</tr>
<tr>
<td>Lice</td>
<td>7,307</td>
<td>0</td>
<td>0.0000%</td>
</tr>
<tr>
<td>Mites*</td>
<td>7,310</td>
<td>0</td>
<td>0.0000%</td>
</tr>
<tr>
<td>Giardia</td>
<td>6,957</td>
<td>0</td>
<td>0.0000%</td>
</tr>
<tr>
<td>Spirochrous</td>
<td>6,957</td>
<td>15</td>
<td>0.2156%</td>
</tr>
<tr>
<td>&quot;other&quot; flagellates</td>
<td>6,957</td>
<td>500</td>
<td>7.1870%</td>
</tr>
<tr>
<td>Entamoeba</td>
<td>6,957</td>
<td>191</td>
<td>2.7454%</td>
</tr>
</tbody>
</table>

* - Outbreaks of Ornithonyssus bacoti reported in some facilities in southern, southwestern, and eastern US
Mycoplasma pulmonis infection

- Host Range
  - Rats
  - Mice
  - Guinea pigs, Hamsters and Rabbits (culture evidence but no disease reported)
- Prevalence – Infrequent to rare
  - Very common in pet rats

Mycoplasma pulmonis infection

- Clinical signs (disease of older animals)
  - Usually clinically silent in young, non-specific in older
    - Rales and dyspnea, snuffling/chattering
    - Ocular and nasal discharge as well as chromodacryorrhea
      - Rubbing of eyes
      - Head tilt
      - Rats spin when held up by tail
      - Decreased reproductive efficiency (rats)

Pathogenesis of Mycoplasmosis

- Transmission
  - Horizontal transmission (aerosol or in utero exposure, rats only)
  - Venereal transmission (?)
- Note: Mycoplasmas that can commonly infect cell cultures are not M. pulmonis. Many can be eliminated by passaging the cell lines through rodents. However, M. arginini has been found in cell cultures and can cause arthritis in mice.
Pathogenesis of Mycoplasmosis

• Disease outcome depends on interaction of:
  – Host factors
    • Age
    • Strain (BALB/c more susceptible than C57BL/6, SD > Lewis, F344)
    • Immune status, concurrent infections, nutritional status (e.g., vitamin A and E deficiencies)

• M. pulmonis possibly damages host cells by:
  – “Ciliostasis and ciliolysis”
    • Probably responsible for exudate accumulation, opportunistic bacterial infections, and impaired transport of ova (infertility).
  – Competing for the host cells’ metabolites
  – Toxic metabolites (e.g., peroxides)
  – Production of nonspecific mitogens >> autoreactive clones of lymphocytes >> immune-mediated damage
  – M. pulmonis may also cause damage indirectly through bystander effect from host leukocytes
• Infection persists – Disease primarily in older rats

Gross Lesions of Mycoplasmosis

• Upper respiratory tract (young and adults)
  – Suppurative: rhinitis, otitis media, laryngitis, tracheitis
• Lung
  – “Cobblestone” lung (older adults primarily, rare)
    • Suppurative bronchopneumonia with or without abscesses
    • Atelectasis
    • Bronchiectasis and/or bronchiolectasis
Gross Lesions of MRM

- Arthritis (occasionally)
- Genital tract
  - Usually no lesion observed
  - Female rat
    - Partially resorbed fetuses
    - Suppurative salpingitis

Histopathology of MRM

- Airway lesions in the respiratory tract are usually characterized by
  - Suppurative exudate
  - Hyperplasia of the mucosal epithelium
  - Hyperplasia of the bronchial associated lymphoid tissue
Histopathology of Mycoplasmosis

• Other respiratory tract lesions related to gross lesions
  – Squamous metaplasia of airway epithelia
  – Pseudoglandular hyperplasia of nasal epithelium (chronic)
  – Peribronchial alveolar type-II pneumocyte hyperplasia
  – CAR bacillus and/or secondary bacterial pneumonias
  – Syncytia may be observed on the surface of nasal and bronchial mucosa (mice)
  – Loss of cilia

Histopathology of Mycoplasmosis

• Lesions in the female genital tract (rats)
  – Suppurative oophoritis
  – Hydrosalpingitis or suppurative salpingitis
  – Suppurative endometritis or pyometra; maybe epithelial hyperplasia and squamous metaplasia

Diagnosis of Mycoplasmosis

• Differential diagnoses
  – Cilia-Associated Respiratory (CAR) Bacillus infection
  – Iatrogenic pneumonia
  – Bacterial infections (Pseudotuberculosis, Streptococcosis, B. hinzii in mice)
  – Viral infections (RRV, Sendai virus, PVM, etc.)
  – Mycotic pneumonia
**Mycoplasma pulmonis infection**

- **Diagnosis**
  - **Culture**: Especially exudates in the upper respiratory tract and middle ears. More sensitive than serology for early infections. Culture takes 2 weeks.
  - **Serology**: Best for screening large, freely-mixing populations
  - **PCR**: Specific (not generic – cross-reactions).
- **Pathology**
  - Immunofluorescence or immunohistochemistry of tissue or exudates

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**Cilia-associated (CAR) bacillus**

- **Cause**: Gliding bacterium, similar to *Flavobacterium* and *Flexibacter*
- **Prevalence**: Rare (< 0.2% rats, 0.0% mice)
- **Natural lab animal host range of CAR bacillus**
  - Rats
  - Mice
  - Rabbits
- **Clinical signs of CAR bacillus infection**
  - Sometimes nonspecific respiratory signs (dyspnea)
  - Sometimes weight loss

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**CAR bacillus**

- **Pathogenesis of CAR bacillus infection**
  - Transmission probably via direct contact with infected animals, contaminated fomites (soiled bedding) and aerosol not important
  - CAR bacillus may act in synergy with other respiratory agents to produce chronic respiratory disease
- **Interference with research (unknown)**
  - Effects on mucociliary clearance and immune function speculated, not demonstrated
CAR bacillus

- Gross lesions of CAR bacillus infection
  - Resemble those of the primary infections, e.g., Mycoplasmosis, Sendai
  - Rarely, uncomplicated infections may produce bronchiectasis, mucus accumulation in bronchioles, and lymphoid hyperplasia
    - Inflammation can be neutrophilic, but less suppurative than with mycoplasmosis
    - Bronchial epithelium is preserved, or hyperplastic
    - Cilia prominent, not lost as with M. pulmonis

- Histopathology of CAR bacillus infection
  - Cilia on respiratory epithelium may appear slightly basophilic with H&E
  - Long, slender bacilli among the cilia at any level of respiratory epithelium (nasal cavity to bronchioles) - observed in silver stained sections
  - Hyperplastic BALT
  - Rarely, there may also be suppurative bronchopneumonia
Bordetella hinzii

CAR bacillus

• Differential diagnoses for CAR bacillus infection
  – Mycoplasma pulmonis (very often co-infection)
  – Other bacteria (i.e., Bordetella hinzii, S. pneumoniae, C. kutscheri, etc.)
    • Mycotic pneumonias (i.e., aspergillosis, mucormycosis, etc.)
  – Viral pneumonia (RRV, Sendai virus, PVM, etc.)

CAR bacillus

• Diagnosis of CAR bacillus infection
  – Serology – MFIA or ELISA
  – PCR – Lung wash, lung tissue, feces
  – Histopathology
    • Warthin-Starry silver stain
    • Grocott’s methenamine silver stain
  – Isolation in embryonated eggs or tissue culture
  – Electron microscopy
  – Immunofluorescence (tissue)
Tyzzer's Disease

- Etiology: *Clostridium piliforme*
- Hosts (some evidence of partial species-specificity of strains)
  - Rodents (virtually all, Mongolian gerbil very susceptible)
  - Rabbits
  - Carnivores (cat, dog)
  - Horses
  - Non-human primates
  - Humans (infection has been reported in one HIV+ patient to date, but seroconversion, always suspect, has been reported in many)

Tyzzer's Disease

- Prevalence: Tyzzer's Disease is infrequent, although the organism may be widespread
- Clinical signs
  - Usually absent
  - Overt disease mostly in young recently weaned animals
    - Acute death with or without clinical signs
    - Diarrhea with or without mucus and blood
    - Distended abdomen (rat)
    - Anorexia, Lethargy, Emaciation, Ruffled fur

Pathogenesis of Tyzzer's Disease

- May be widespread in nature
- Vegetative form survives only inside of cells
  - Epithelium (small and large intestine, gall bladder, bile duct)
  - Hepatocytes
  - Myocardial fibers
  - Smooth muscle of small and large intestine
Pathogenesis of Tyzzer’s Disease

• Transmission
  – Horizontal transmission
    • Ingestion of spores in
      – Feces
      – Contaminated feed and bedding
      – Carcasses (cannibalism)

• Proposed sequence of infection
  – Spores ingested >> produce the vegetative form, actively phagocytosed by epithelial cells overlying the GALT >> vegetative form escapes phagosome >> multiples in intestinal mucosal epithelial cells and possibly RE cells in Peyer's patches

• Proposed sequence of infection (cont.)
  – Most infections appear to be cleared at this point, and animals stop shedding spores within about 2 weeks.
  – If infection extends past GI tract - Vegetative form reaches liver by one or more routes
    • Portal circulation (most likely)
    • Lymphatics
    • Common bile duct (the vegetative form is motile)
Pathogenesis of Tyzzer’s Disease

• Proposed sequence of infection (cont.)
  – Vegetative form infects and multiples in the hepatocytes, then may do one or more things depending on how long the animal survives
    • Enter into the blood stream or lymphatics to colonize the myocardium
    • Possibly enter into epithelium of biliary tree to multiply and eventually be shed into bile to re-infect intestine and liver (auto-infection)

Pathogenesis of Tyzzer’s Disease

• Factors which influence infection and outcome
  – Host factors
    • Age (recently weaned most susceptible)
    • Genotype (CBA/N mice supposedly very susceptible, C57BL/6 more resistant than DBA/2)
  – Immune function
    • Latent infection may be activated by:
      » Stress, Drugs (cortisone, cyclophosphamide, etc.), Leukocyte injection
      » Nutritional status (Fasted mice resistant to overt disease)
      » Gnotobiotic status
      » Escherichia coli reportedly potentiates C. piliforme in rabbits

Pathogenesis of Tyzzer’s Disease

• Factors which influence infection and outcome
  – Bacterial factors
    • Strain
      » Some species-specificity
      » Some strains produce a high-molecular weight, cytotoxic protein. Pathogenicity seems dependent on this. Some strains may be non-pathogenic.
    • Dose
Pathogenesis of Tyzzer’s Disease

- Factors which influence infection and outcome
  - Environmental factors
    - Increased environmental temperatures and humidity
      - May precipitate a latent infection (stress)
      - May increase number or viability of spores >> increasing exposure
    - Damp feed and poor husbandry
      - May also increase number of spores in environment
    - Overcrowding
      - Stress and increased spores in environment

Pathogenesis of Tyzzer’s Disease

- Interference with research
  - Direct effects, especially in immunosuppressed animals
  - Reported to alter hemostatic parameters and cytokines

Gross Lesions of Tyzzer’s Disease

- Perianal fecal staining may be present
- Liver
  - Multiple, disseminated, pinpoint or larger, pale foci (necrosis) within and on the surface of the liver
  - The liver may only be swollen and mottled
Gross Lesions of Tyzzer's Disease

- Intestine
  - Megaloileitis (rat)
  - Greatly dilated, fairly flaccid, hyperemic small intestines (ileum)
  - Hyperemia, edema, hemorrhage, and possibly ulceration of any part of the intestines, but especially the terminal ileum, cecum, and colon
Gross Lesions of Tyzzer’s Disease

- Heart
  - Pale, circumscribed, sometimes raised foci may be present on the surface
  - Pale linear streaks near the apex of the heart
- Enlarged, hyperemic and edematous mesenteric lymph nodes
Histopathology of Tyzzer’s Disease

- Intestine
  - May see nothing even if lesions in liver and heart
  - Necrotizing enteritis, typhlitis, and colitis with or without
    - Edema (common)
    - Blunted and fused villi
    - Crypt epithelial hyperplasia
    - Ulceration
    - Hemorrhage
    - Cellular debris in crypts and lymphatics

Histopathology of Tyzzer’s Disease

- Liver
  - Coagulative necrosis (frequently periportal) with or without
    - Inflammation (neutrophils, mononuclear cells, histiocytes, and rare multinucleated giant cells)
  - Hemorrhage
  - Dystrophic calcification
  - Fibrosis
Histopathology of Tyzzer’s Disease

• Heart
  – Myocardial degeneration with or without
    • Necrosis
    • Mixed inflammatory cells
    • Dystrophic calcification

Histopathology of Tyzzer’s Disease

• Diagnostic if characteristic bacilli seen
  – Sometimes visible with H&E, but usually need special stains
    • Warthin-Starry silver stain (best)
    • Immunoperoxidase stain
      – Probably excellent, but not commercially available
    • Giemsa and methylene blue stains
      – Tissues or smears
    • Brown & Brenn stain
      – Organism is gram-negative but stains very poorly
Histopathology of Tyzzer’s Disease

• Liver
  – Organisms are most often observed in surviving hepatocytes at the periphery or within lesions
  – May be in hepatocytes not associated with a lesion
• Intestine
  – Normal gut flora within mucosal crypts and superimposed upon the mucosal epithelial cells may complicate evaluation.

Histopathology of Tyzzer’s Disease

• Vegetative form of C. piliforme is 8.0 to 20.0 x 0.3 to 0.5 microns bacillus. (long and thin, piliform)
  – One or usually more bacilli are present in cells in either a jumbled array (pickup stick) or parallel arrangement depending on the shape of the cell
  • Hepatocytes, epithelial cells,
  • neurons: Pickup-stick arrangement
  • Smooth muscle and myocardial fibers: Parallel arrangement
Tyzzer's Disease

• Differential diagnoses
  – Bacteremia (*Streptococcus*, others)
  – Adynamic ileus due to chloral hydrate (rat)
  – *Yersinia tuberculosis* (guinea pig)
  – Hepatic coccidiosis (rabbit)
  – Alflatoxicosis
  – Others

Diagnosis of Tyzzer's Disease

• Pathology
  – Cytology or histopathology with the identification of intracellular long bacilli is diagnostic
  – Warthin-Starry silver stain (tissue)
  – Giemsa or methylene blue stain (smear or tissue)
  – PCR on paraffin-embedded tissue
  – Immunohistochemistry (tissue)
  – Immunofluorescent staining of tissues
Diagnosis of Tyzzer's Disease

• Provocation tests to provoke latent infections. Some doubt as to efficacy, but may distinguish infections with potentially pathogenic strains. Must select correct animals to immunosuppress.
  • Cyclophosphamide
  • Cortisone
  • Sentinel animals placed on soiled bedding (not foolproof)
  • Gerbil
  • CBA/N mice

• Serology (does not distinguish between pathogenic and non-pathogenic strains)
  – MFIA, ELISA, IFA
  – Positive finding should be confirmed by pathology

• PCR
  – Feces (if shedding) can be hard to extract DNA from spores
  – Tissue - should be positive if lesions are due to Tyzzer's

• Isolation of the organism (not practical)
  – Cell culture
  – Embryonated eggs

Pseudotuberculosis

• Etiology: Corynebacterium kutscheri
• Hosts
  – Rats
  – Mice
  – Guinea pig, hamster (culture evidence, no disease)
• Prevalence - Rare
C. kutscheri infection

- Clinical signs
  - Infections are frequently clinically silent
  - Nonspecific (sick rat) clinical signs may be observed, death in 1 to 7 days
    - Purpura and mucopurulent ocular and nasal discharges
    - Respiratory rales and dyspnea
    - Lameness

Pathogenesis of C. kutscheri infection

- Latent infections are currently rare in laboratory rats and mice. However, infected animals are usually clinically normal. In these, the organism may be cultured from:
  - Submaxillary (cervical) lymph nodes
  - Oral cavity
  - Nasal cavity
  - Middle ears
  - Preputial gland abscesses

Pathogenesis of C. kutscheri infection

- Factors which may precipitate latent infections include age and conditions which immunosuppress the host
  - Stress (poor husbandry, overcrowding, shipping, etc.)
  - Concurrent infections
  - Irradiation
  - Immunosuppressive drugs (steroids, cyclophosphamide, etc.)
  - Malnutrition (e.g., pantothenic acid and biotin deficiencies)
Pathogenesis of *C. kutscheri* infection

- Transmission is probably through direct contact and/or oronasal exposure.
- Septic emboli become trapped in organs or tissues with either a large capillary network (lung, liver, and kidney) and/or responsible for filtering blood (synovia and glomeruli). This accounts for the distribution of the lesions.

- Although any or all organs and tissues may be involved, the frequency of lesion distribution varies with the species:
  - Rat: pulmonary involvement
  - Mouse: hepatic and renal involvement

*C. kutscheri* infection

- Gross:
  - Lung: 1 or more randomly distributed abscesses +/- hemorrhage and pleuritis (fibrinous or fibrous)
  - Liver: Solitary or multiple abscesses and/or necrosis
  - Kidney: Solitary or multiple abscesses and/or pyelonephritis
  - Preputial gland: Abscess
  - Joints: Suppurative arthritis
  - Skin: Abscess(es), ulcerations, fistulous tracts, pododermatitis
  - Middle ear: Suppurative otitis media
C. kutscheri infection

- Histopathology (related to gross findings)
  - Lung
    - Abscesses predominately in the interstitium due to the hematogenous seeding of the lung with bacteria
    - May see caseous necrosis
    - Epithelioid macrophages and multinucleated giant cells may be present in the abscesses
    - Bronchi and bronchioles may contain suppurative exudate
C. kutscheri infection

- Histopathology (cont.)
  - Liver
    - May see caseous necrosis
  - Kidney
    - Septic embolic glomerulitis
    - Abscesses with or without pyelonephritis
  - May see lesions in any tissue (e.g., brain, skin, joints)
C. kutscheri infection

- Differential diagnoses
  - Localized or disseminated opportunistic bacterial infections: *Staphylococcus* spp., *Streptococcus* spp., *Salmonella* spp., etc.
  - Mycoplasmal diseases
  - Mycotic pneumonia (Aspergillosis, Mucormycosis, etc.)
  - Tyzzer’s Disease
  - Viral pneumonia
  - *Streptobacillus moniliformis*

- Diagnosis
  - Bacteriology
    - May culture site probably submandibular lymph nodes
    - May also be in oral cavity, cecum, colon and rectum
  - PCR
  - Pathology
    - May see characteristic configuration of G+ coryneforms in sections or impression smears

C. kutscheri infection (cont.)

- Diagnosis
  - Cortisone stress (provocation) test - obsolete
    - To activate latent infections and also possibly *Pneumocystis carinii* and Tyzzer’s disease
  - Serology
    - May see false positives and false negatives
    - Should be confirmed by PCR, culture