INFECTIONOUS DISEASES OF LABORATORY RATS

Charles B Clifford, DVM, PhD, DACVP

• 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

- **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.
Rat Coronavirus (SDAV/RCV)

- 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

- Differential diagnoses
  - Iatrogenic salivary enlargement due to jugular catheters
  - Non-specific porphyria
  - Other pneumonias (P carinii, Sendai virus, PVM)
  - Cytomegalovirus infection (RCMV)
  - Papovaviral Sialoadenitis (athymic nude rats)
  - Hypovitaminosis A (squamous metaplasia of salivary gland ducts)

- 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
Parvoviruses

- ssDNA, (5.4 kb genome), non-enveloped
  - Virus remains active in environment
    - Resistant to desiccation, non-oxidizing disinfectants
- Four serotypes (RV, H-1, RPV, RMV)
  - 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

Parvoviruses 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)

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
  - RPV 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

**PCR**
- Can be strain-specific (VP2) or generic (NS-1)
- Mesenteric LN stay positive indefinitely (like a library)
- PCR of fecal samples valuable to detect shedding (can pool fecal samples. Beware of fecal inhibitors of PCR)
- Testing biologicals and cell cultures
- Environmental swabs
  - Exhaust plenums, exhaust filters

Rat Serology for paroviruses

<table>
<thead>
<tr>
<th>Agent/Assay</th>
<th># tested</th>
<th>% positive</th>
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<tbody>
<tr>
<td>NS-1</td>
<td>63,101</td>
<td>2.3692%</td>
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<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>
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</tbody>
</table>

Detection of Parvoviruses

**Pneumocystis carinii**

- In immunodeficient rats, disease is similar to Pneumocystosis in other species, maybe a little more inflammation.
- Immunocompetent – Only common cause of pneumonia in laboratory rats
- Idiopathic lymphohistiocytic interstitial pneumonia observed in F-344 rats in early 1990’s
  - Seemed to be a novel lesion presentation
- Reported in:
  - Inhalation Toxicology in 1997, Gilbert, BE, et al.
  - Veterinary Pathology in 2009, Albers, TM, et al.

Pneumocystis carinii

- “Rat Respiratory Virus (RRV)” had been used as a working name since the disease was clearly infectious and the inflammation most resembled viral-induced pneumonia
- *P. carinii* now identified as the etiology of the pneumonia
  - Livingston *et al.*, Comparative Medicine, 2011
  - Henderson *et al.*, Veterinary Pathology, 2012

Pneumocystis carinii

- **Prevalence:** Common
  - ~15% by serology, 18% by serology + PCR
  - ~6% of non-CRL lungs examined at our Dx lab have lesions which meet the criteria for diagnosis

- **Epidemiology**
  - Host range – *P. carinii* is considered species-specific, all strains susceptible
  - Immunodeficient – similar to Pneumocystosis in other species
  - Transmitted by contact, dirty bedding, fomites, probably aerosol
**Pneumocystis carinii**

**Morbidity**
- 50% or more of naïve rats will develop lesions after exposure
- In endemic breeding colony, lesions are milder and may be present in < 20% of rats

**Pathogenesis**
- Cysts inhaled ➔ Doubling time ~4.5 days ➔ When population hits a threshold (3-8 wks), immune response and inflammation ensue ➔ *P. carinii* eventually eliminated, inflammation resolves, antibody levels persist

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**Enzootically Infected Barrier Room**

- Sprague Dawley Rats (N=6/age group)
- Evaluated by Real-time PCR, Histology and IFA

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Log10 PCR Copies/IFA Titers vs Week Post Exposure

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Log10 PCR Copies/IFA Titers vs Weeks of Age

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Pathology images of rat lung showing lesions.
**Pneumocystis carinii**

- **Research effects**
  - Has caused repeat of inhalation toxicology studies
  - Anecdotal reports of anesthesia problems, *ex vivo* lung problems.
- **Control**
  - Eliminate by Rederivation
  - Breeding colonies can be maintained free of *P. carinii*.

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**Pneumocystis carinii**

- **Diagnosis**
  - PCR – positive within a week after exposure
  - Serology (MFIA) – positive ~5-8 weeks after exposure
  - Histopathology – Positive (use published criteria) ~4 – 10 weeks after exposure

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**Comparison of Sample Types for the Detection of *P. carinii* by PCR**

- Contact-exposed CD rats at 3, 4 and 5 weeks post-contact exposure (N = 36)
- Targeted the pre-peak and peak IIP time period when *P. carinii* titers are highest

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**Log10 Copy #**

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Log10 Copy #</th>
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<tbody>
<tr>
<td>Lung Wash</td>
<td>0</td>
</tr>
<tr>
<td>Bronchial Wash</td>
<td>2</td>
</tr>
<tr>
<td>Nasal Wash</td>
<td>4</td>
</tr>
<tr>
<td>Oral Swab</td>
<td>3</td>
</tr>
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</table>
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 (always?) 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: MIFA, ELISA, IFA, HAI
  - Use sentinel mice to screen for cross-reacting antibodies in GP
    - Based on idea that mice would seroconvert to Sendai but not to other PI-1 viruses
- 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)

- Agent
  - Family: Picornaviridae, Genus: Cardiovirus, Species: Theilovirus, Serotype: Rat theilovirus.
  - There are at least four serotypes in the theilovirus species: TMEV, RTV, Vilyuisk human encephalomyelitis virus, Saffold virus.
  - Small, non-enveloped, RNA viruses.
  - Moderate environmental persistence and resistance to disinfection are expected.

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)

- Epizootiology
  - Prevalence – moderate. The CR diagnostic laboratory finds about 2% of rat 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
      - “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

• **Diagnosis**
  – **Serology**
    • MFIA or 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

• **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 bilis</td>
<td>8,031</td>
<td>111</td>
<td>1.3821%</td>
</tr>
<tr>
<td>any Helicobacter</td>
<td>7,968</td>
<td>636</td>
<td>7.9819%</td>
</tr>
<tr>
<td>Helicobacter hepaticus</td>
<td>8,031</td>
<td>35</td>
<td>0.4358%</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 Group B</td>
<td>6,447</td>
<td>221</td>
<td>3.4280%</td>
</tr>
<tr>
<td>Beta Strep Group G</td>
<td>6,447</td>
<td>1</td>
<td>0.0155%</td>
</tr>
<tr>
<td>Strep. pneumoniae</td>
<td>6,484</td>
<td>0</td>
<td>0.0000%</td>
</tr>
<tr>
<td>C. kutscheri</td>
<td>6,492</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>
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<tr>
<td>other Pasteurella</td>
<td>6,357</td>
<td>24</td>
<td>0.3775%</td>
</tr>
<tr>
<td>Ps. 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>Staphylococcus aureus</td>
<td>6,492</td>
<td>1,550</td>
<td>23.8755%</td>
</tr>
</tbody>
</table>

Mycoplasma pulmonis infection

• **Host Range**
  – Rats
  – Mice
  – Guinea pigs, Hamsters and Rabbits (culture evidence but no disease reported)

• **Prevalence** – Infrequent to rare in lab rats
  – Very common in pet rats

• **Clinical signs**
  – Persistent infection, so disease primarily in older rats
  – 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

• *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

Pathogenesis of Mycoplasmosis

• Disease outcome depends on interaction of:
  – 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)

Gross Lesions of Mycoplasmosis

• Upper respiratory tract (young and adults)
  – Chronic 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 Murine Respiratory Mycoplasmosis

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

Histopathology of Murine Respiratory Mycoplasmosis

- Lesions, including 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 as expected from 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

- **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**: Use specific *M. pulmonis assay* (not generic – too many cross-reactions w/other *Mycoplasmataceae*).
    - Pathology – strongly suggestive
    - Immunofluorescence or immunohistochemistry of tissue or exudates

Diagnosis of Mycoplasmosis

- Differential diagnoses
  - **Cilia-Associtated Respiratory (CAR) Bacillus infection**
  - **Iatrogenic pneumonia**
    - Bacterial infections (Pseudotuberculosis, Streptococcosis, *B. hinzii* in mice)
    - Viral infections (Sendai virus, PVM, etc.)
    - Mycotic pneumonia (*P. carinii*, *Aspergillus*, etc.)

*Mycoplasma pulmonis controversy*

Schoeb and McConnell, *Veterinary Pathology* March 2011 vol. 48 no. 2 420-426

- A series of compounds was implicated in causing pulmonary lymphoma in rats, in lifetime studies conducted at a CRO in Europe.
  - Aspartame, methanol, methyl tertiary butyl ether
  - 76.6% found to have bronchitis and 21.4% to have otitis media
    - Compared with 6 NTP bioassays with 0% bronchitis or otitis
  - 328 cases of lymphoma in lung (rare as 1st site)
- **Discard studies? Fate of compounds?**
  - Discussion is ongoing
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
  - Goats
- **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, likely and consistent with morphologic lesions
  - But, not demonstrated

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CAR bacillus

- **Gross lesions of CAR bacillus infection**
  - Resemble those of the primary infections, e.g., Mycoplasmosis, Sendai
  - Rarely, uncomplicated infections may produce bronchectasis, 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*

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CAR bacillus

- **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
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., Pneumocystosis, aspergillosis, mucormycosis, etc.)
  – Viral pneumonia (Sendai virus, PVM, etc.)

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)

Pathogenesis of Tyzzer’s Disease

- **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

Pathogenesis of Tyzzer’s Disease

- **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 (depending on host survival):
    - 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

• 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

• Interference with research
  – Direct effects, especially in immunosuppressed animals
  – Reported to alter hemostatic parameters and cytokines (IL-12, e.g.)

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
    - Usually not ulcerative (helps distinguish from other diseases)

- **Heart** (much less often)
  - 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, typhilitis, 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

• 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 most often found in surviving hepatocytes at the edge or just inside 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. (don’t be fooled)
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*, many others)
  - Adynamic ileus due to chloral hydrate (rat)
  - *Yersinia tuberculosis* (guinea pig)
  - Hepatic coccidiosis (rabbit)
  - *Helicobacter hepaticus* (nude mice)
  - 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

- Provocation tests to provoke latent infections. Efficacy in doubt, but a negative results supports idea that no significant pathogen is present. Must select correct age of animals to immunosuppress
  - Cyclophosphamide
  - Cortisone

- Sentinel animals placed on soiled bedding (not foolproof)
  - Gerbil
  - CBA/N mice
Diagnosis of Tyzzer’s Disease

• **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

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Pseudotuberculosis

• **Etiology:** *Corynebacterium kutscheri*

• **Hosts**
  – Rats
  – Mice
  – Guinea pig, hamster (culture evidence, no disease)

• **Prevalence** – Rare in laboratory animals

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**C. kutscheri infection**

• **Clinical signs**
  – Low morbidity (high mortality in affected)
  – Nonspecific (sick rat) clinical signs may be observed, death in 1 to 7 days
    • Porphyrin and mucopurulent ocular and nasal discharges
    • Respiratory rales and dyspnea
    • Lameness

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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 (reported, but really rare)

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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
Pathogenesis of C. kutscheri infection

- 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

- Histopathology (as expected from gross findings)
  - Lung
    - Abscesses mostly in the interstitium due to hematogenous dissemination
    - Caseous necrosis often
    - 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. (mice), Salmonella spp., etc.
  - Mycoplasmal diseases
  - Mycotic pneumonia (Aspergillosis, Mucormycosis, etc.)
  - Tyzzer's Disease
  - Viral pneumonia
  - Streptobacillus moniliformis (mice)
**C. kutscheri infection**

- **Diagnosis**
  - Bacteriology
    - Best culture site probably submandibular lymph nodes
    - May also be in oral cavity, cecum, colon and rectum
  - PCR
    - Can perform on deparaffinized sections to confirm lesions
  - Pathology
    - May see characteristic configuration of G+ coryneforms in sections or impression smears

- **Diagnosis (cont.)**
  - Cortisone stress (provocation) test - obsolete
    - To activate latent infections
  - Serology
    - May see false positives and false negatives
      - Bacterial serology tends to be problematic, seroconversion may be delayed, not often done.
    - Should be confirmed by PCR or culture

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**Rat Parasitology Results**

<table>
<thead>
<tr>
<th>Agent</th>
<th># tested</th>
<th># pos.</th>
<th>% pos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. tetaptera</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>Spironucleus</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