INFECTIONIOUS DISEASES OF LABORATORY RATS

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Pathology of Laboratory Animals
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“Virus” prevalence – Rats

Bacterial prevalence - Rats

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.
Experimental SDA @ 8 days pi courtesy of Dr. Dean Percy

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

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
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
  - 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 parvoviruses

<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>
</tbody>
</table>

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) Fungus – *Pneumocystis carinii*

- Immunodeficient – similar to *Pneumocystis* in other species
- Immunocompetent – Discussed here
- 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*

- **Prevalence:** Common (~6% by histologic lesion, ~15% by serology)
- **Epidemiology**
  - Host range - rats are the only known host, all strains susceptible
  - Immunodeficient – similar to *Pneumocystis* in other species
  - Transmitted by contact, dirty bedding, probably aerosol
    - Transmission by additional fomites likely

*Pneumocystis carinii*

- **Epidemiology (cont.)**
  - Epidemic spread (naïve colony)
    - Lesions prevalent (≥ 50%)
  - Endemic spread (colony chronically infected)
    - Lower prevalence (≤ 20%)
    - Lower severity than in naïve colony
**Pneumocystis carinii**

- **Pathogenesis**
  - Naïve rats (Epidemic spread)
    - 1st lesions at about 4-5 weeks post-exposure
    - Lesions reach zenith at 7-8 weeks, then slowly decline
    - Lesions present for at least 13 weeks post-exposure
  - Endemic colony
    - Highest prevalence (best time to screen) 8-12 weeks of age
**Pneumocystis carinii**

- **Research effects**
  - Has caused repeat of inhalation toxicology studies
  - Anecdotal reports of anesthesia problems, *ex vivo* lung problems.

- **Control**
  - Eliminate by Rederivation
  - Persistent infection?
    - Seems eliminated by 10 weeks or so

**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

**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

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

**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
  - 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)

### 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), Vilyuisk human encephalomyelitis virus, Saffold virus.
  - RTV and TMEV are small non-enveloped, RNA viruses.
  - Moderate environmental persistence and resistance to disinfection are expected.

### 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

### 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

### 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

- **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

**Bacterial prevalence - Rats**

<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>9,968</td>
<td>636</td>
<td>7.9810%</td>
</tr>
<tr>
<td>Helicobacter hepaticus</td>
<td>8,031</td>
<td>35</td>
<td>0.4358%</td>
</tr>
<tr>
<td>E. bronchiophila</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>0.0155%</td>
</tr>
<tr>
<td>C. auris</td>
<td>6,452</td>
<td>0</td>
<td>0.0000%</td>
</tr>
<tr>
<td>S. pneumoniaiu</td>
<td>3,554</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>6,357</td>
<td>24</td>
<td>0.3770%</td>
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<tr>
<td>P. aeruginosa</td>
<td>12,931</td>
<td>201</td>
<td>23.8755%</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>
<tr>
<td>Strain pneumoniae</td>
<td>6,484</td>
<td>0</td>
<td>0.0000%</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
  - 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, sniffing/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)

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

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 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 epithelium
  - 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

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

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
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

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.)
  - Fungal pneumonias (i.e., aspergillosis, mucormycosis, etc.)
  - Viral pneumonia (RRV, Sendai virus, PVM, etc.)

- 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)

- 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 do one or more things depending 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

6/1/2011
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

Gross Lesions 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

- 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)
  - Afilatoxicosis
  - 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.** 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
    - Porphyrin 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
- 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

• 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
    • Epitheloid 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*

C. kutscheri infection

- Diagnosis
  - Bacteriology
    • Best 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

- Diagnosis (cont.)
  - 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

Rat Parasitology Results

<table>
<thead>
<tr>
<th>Agent</th>
<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>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