53rd Pathology of Laboratory Animals

Virginia Beach, VA

June, 2010

INFECTIOUS DISEASES OF LABORATORY RATS

Charles B. Clifford, DVM, PhD, DACVP

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Rat Serology Results

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

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Plus

- "Rat Respiratory Virus"
- *Clostridium piliforme*
- *Corynebacterium kutscheri*

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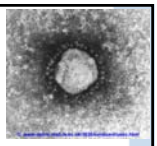
Rat Coronavirus (SDAV/RCV)



- 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

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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.)

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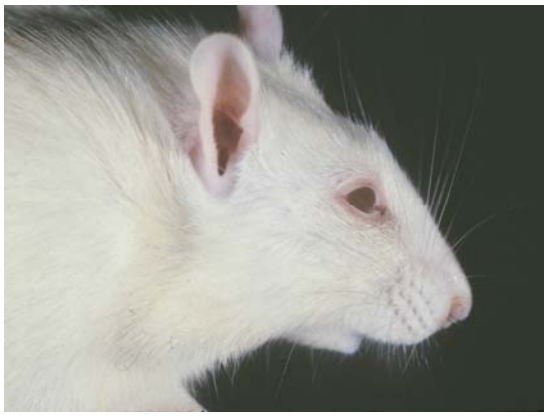
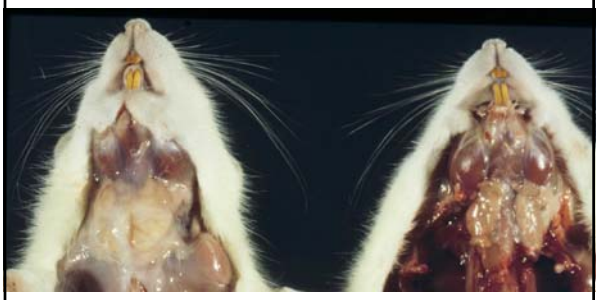
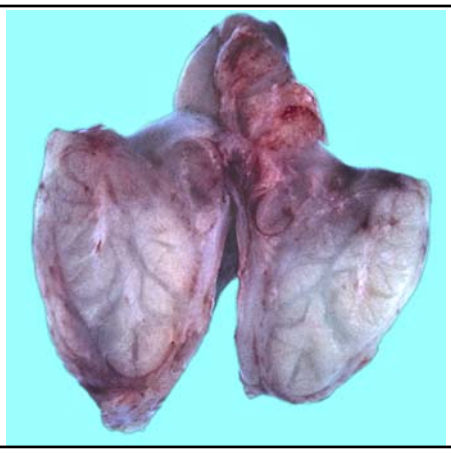




courtesy of Dr. Dean Percy



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

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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.

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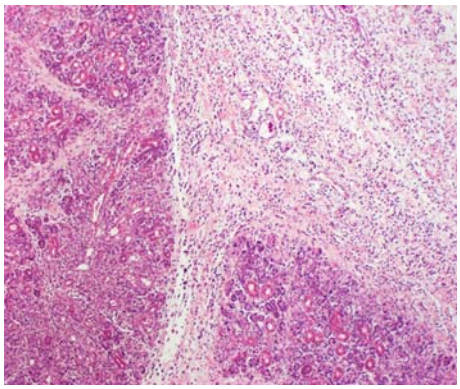
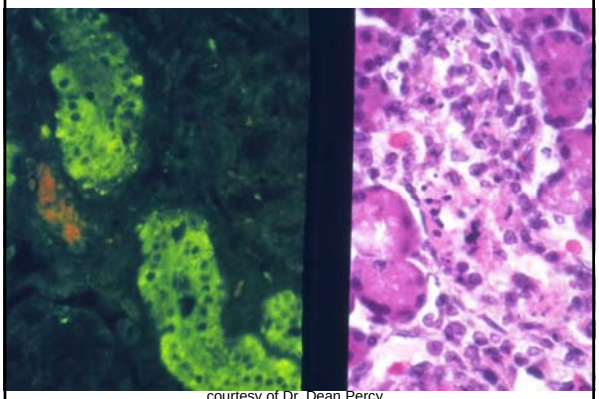
RCV





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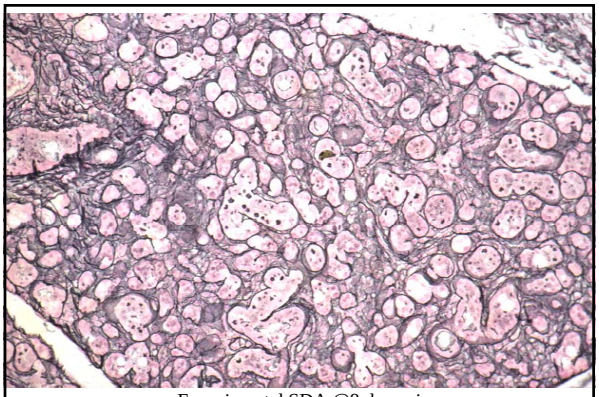
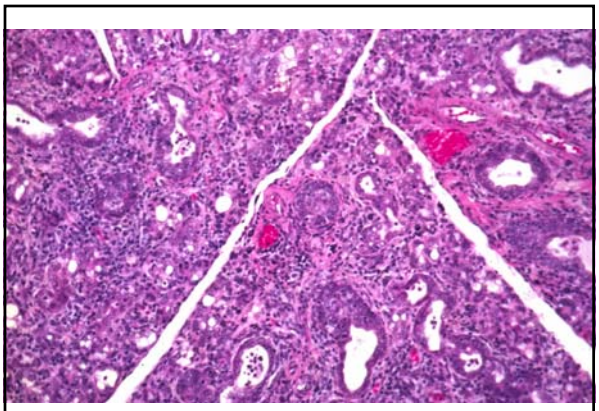
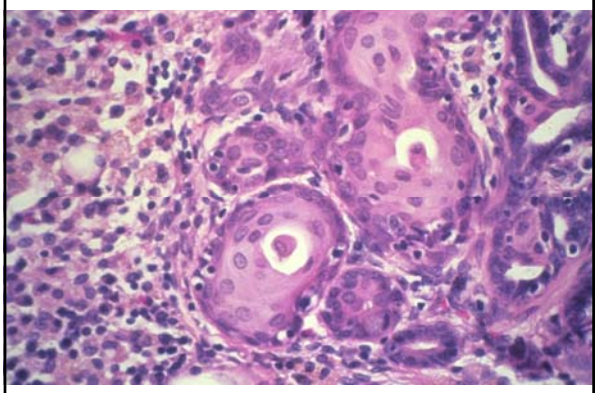


Experimental SDA @8 days pi

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

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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)

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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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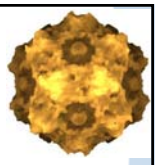
Parvoviruses



- 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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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

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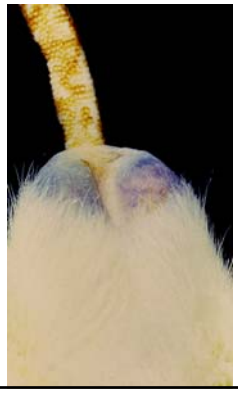
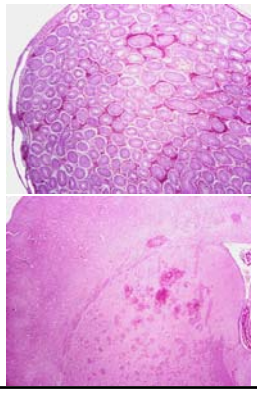
Rat Virus





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)

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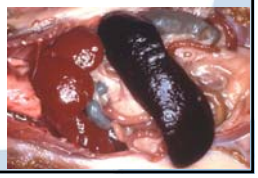
Rat Parvovirus

- 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

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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

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Rat Serology for parvoviruses

Agent/Assay	# tested	% positive
NS-1	63,101	2.3692%
H-1	81,764	1.6120%
RPV	88,399	1.6018%
KRV	88,667	1.5101%
RMV	44,075	1.4475%

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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

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"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.
  - Toxicologic Pathology in 1997, Elwell, MR, et al.
  - Toxicologic Pathology in 1998, Slaoui, M, et al.
  - Veterinary Pathology in 2009, Albers, TM, et al.

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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.

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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)

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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

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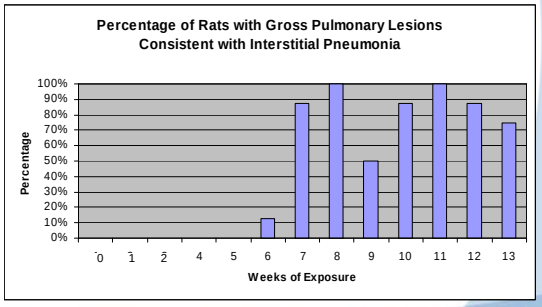
Gross Findings



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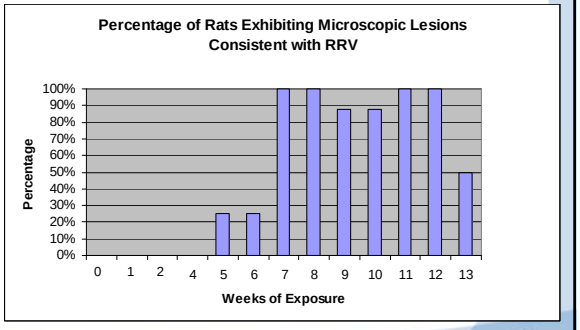
Microscopic Findings



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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

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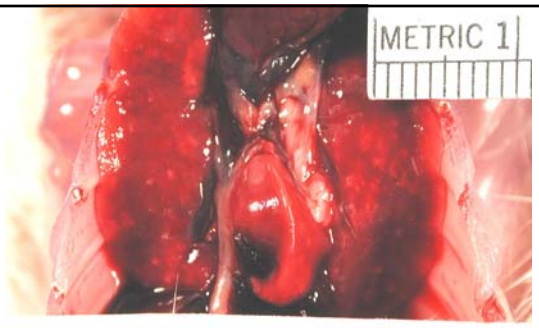

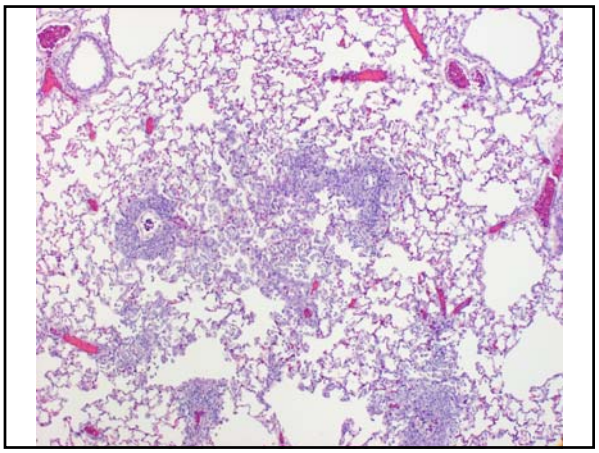

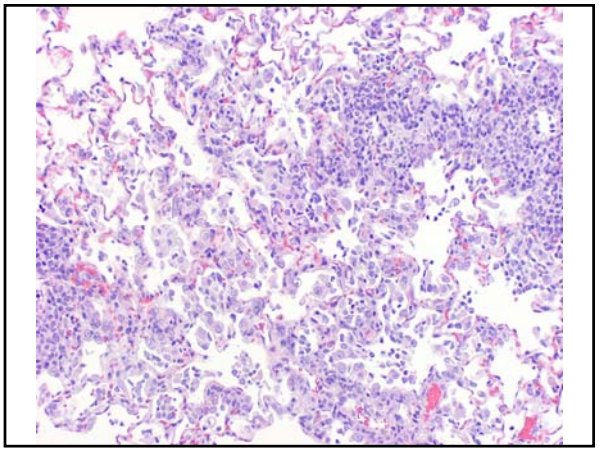

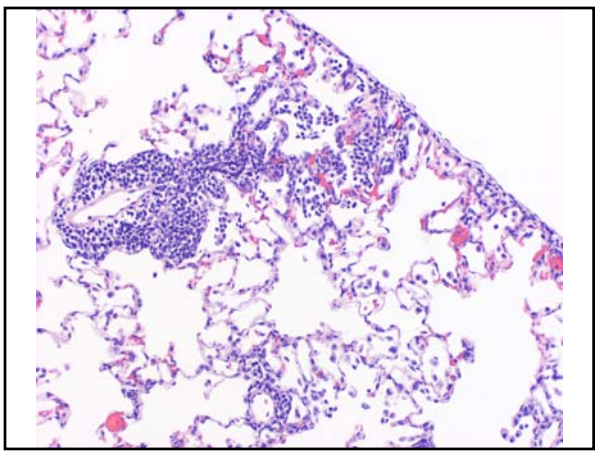

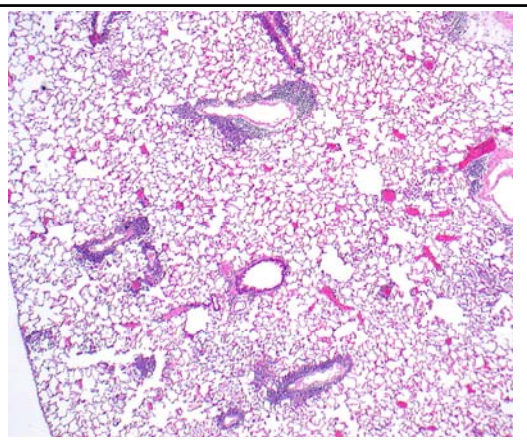

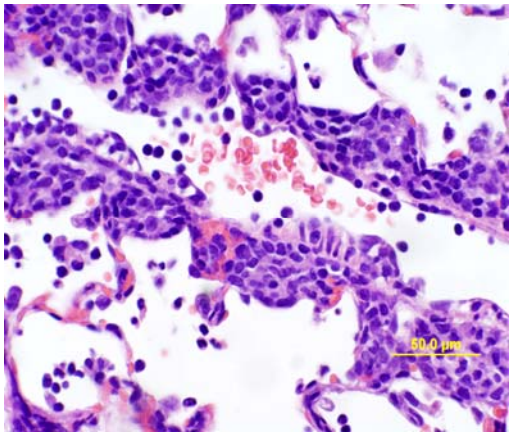

RRV

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

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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.

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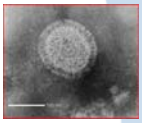
Sendai Virus Infection



- **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)

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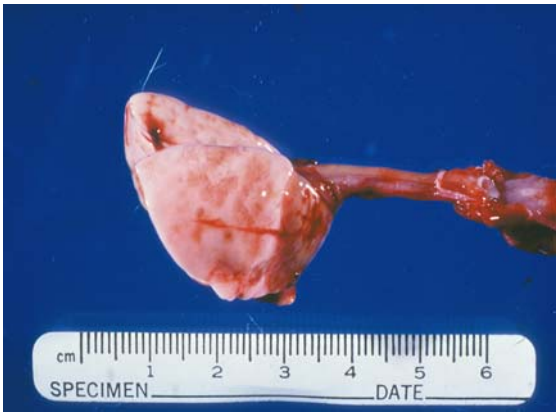

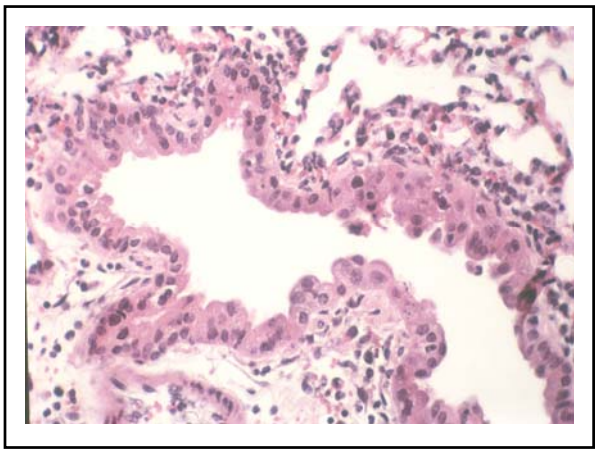

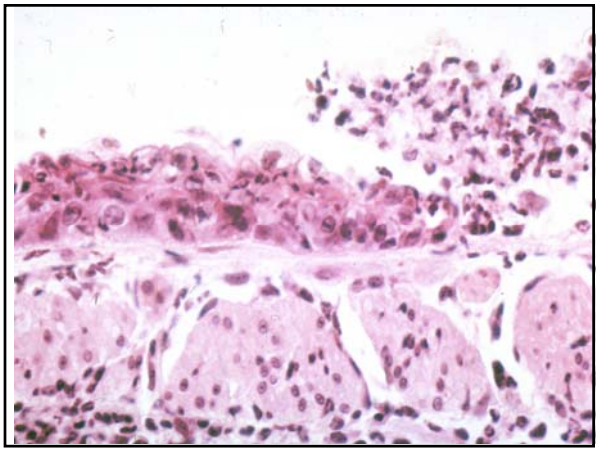

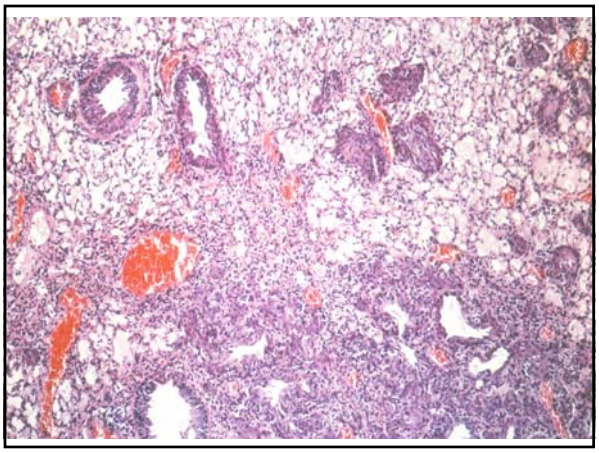

Sendai Virus Infection

- 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

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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

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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

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Rat Theilovirus (RTV)

http://phene.cpmc.columbia.edu/Wintkey/Images/RENDER/1tmf.jpg

- **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)

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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.

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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

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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

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Rat Theilovirus (RTV)

- **Research Effects**
  - None reported

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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

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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

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Rat Bacteriology Results

Agent	# tested	# pos.	% pos.
Helicobacter bilis	8,031	111	1.3821%
any *Helicobacter*	7,968	636	7.9819%
Helicobacter hepaticus	8,031	35	0.4358%
B. bronchiseptica	6,477	0	0.0000%
Beta Strep sp	6,505	1	0.0154%
Beta Strep Grp B	6,447	221	3.4280%
Beta Strep Grp G	6,447	1	0.0155%
C. kutscheri	6,492	0	0.0000%
M pulmonis	3,594	2	0.0556%
P. multocida	6,409	0	0.0000%
P. pneumotropica	6,409	340	5.3050%
other *Pasteurella*	6,357	24	0.3775%
Ps aeruginosa	12,931	301	2.3277%
Salmonella	6,430	0	0.0000%
Staphylococcus aureus	6,492	1,550	23.8755%
Strep. pneumoniae	6,484	0	0.0000%

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Rat Parasitology Results

Agent	# tested	# pos.	% pos.
A. tetraptera	**8,350**	**4**	**0.0479%**
S. muris	**8,350**	**139**	**1.6647%**
S. obvelata	**8,350**	**1**	**0.0120%**
All pinworms	**8,350**	**144**	**1.7246%**
Lice	7,307	0	0.0000%
Mites*	7,310	0	0.0000%
Giardia	6,957	0	0.0000%
Spironucleus	6,957	15	0.2156%
"other" flagellates	**6,957**	**500**	**7.1870%**
Entamoeba	**6,957**	**191**	**2.7454%**

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

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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

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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)

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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.

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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)

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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

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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

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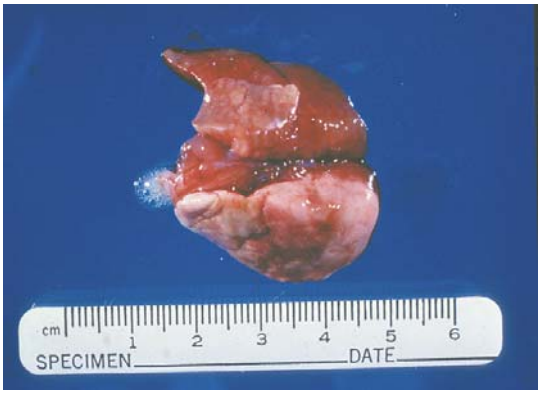
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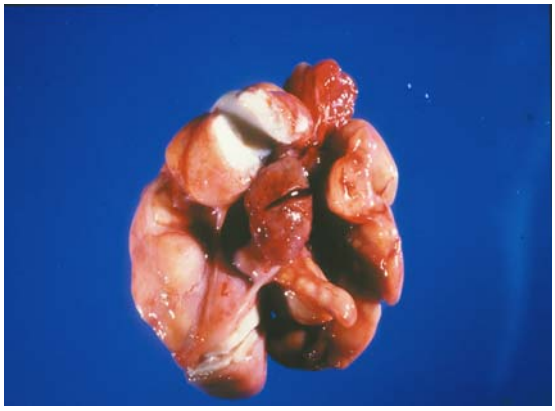


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Gross Lesions of MRM

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

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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

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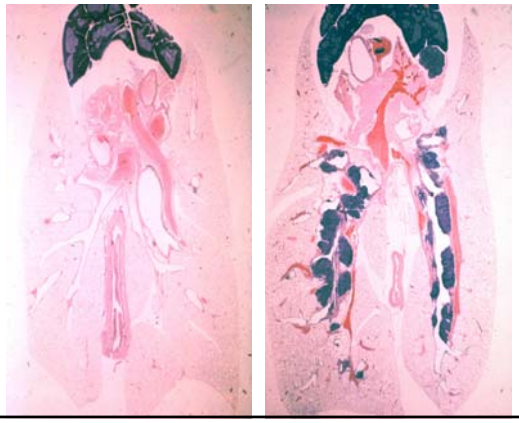

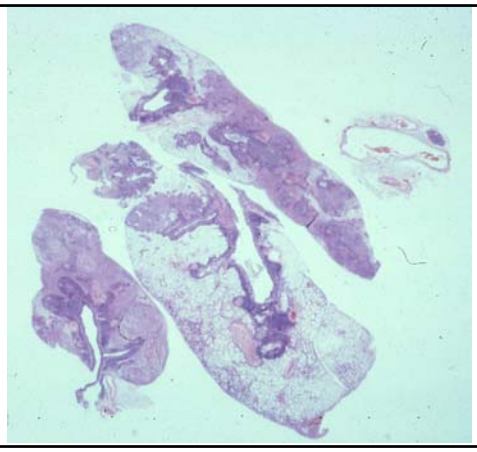

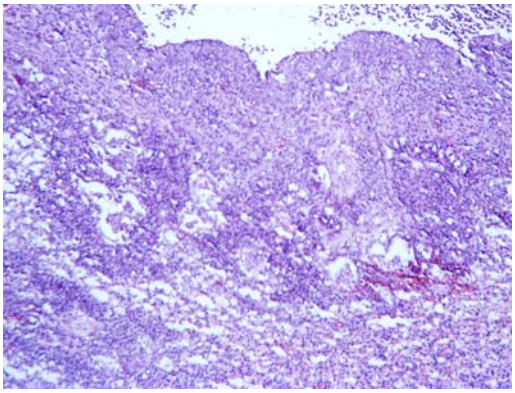

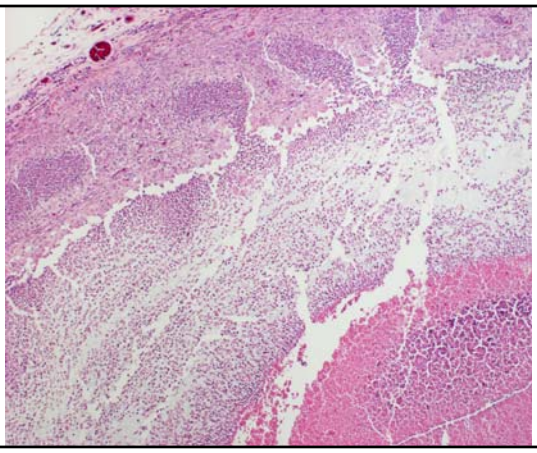

Histopathology of Mycoplasmosis

- 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

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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

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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

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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

http://www.zerberus-online.de/Problematik/Flexibacter-2.jpg

- **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

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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 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*

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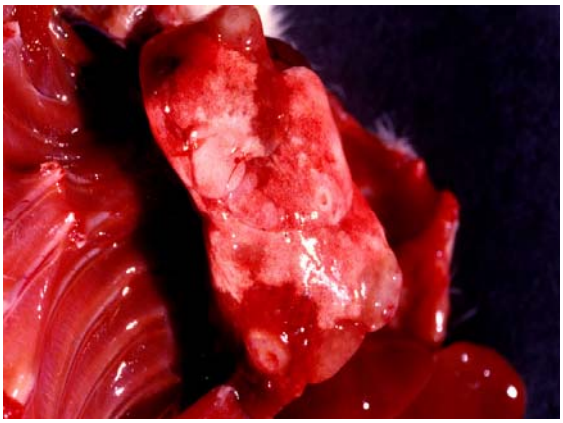

CAR bacillus

- **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

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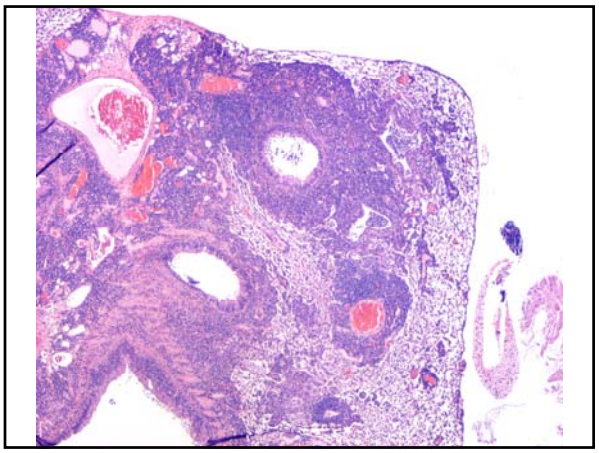
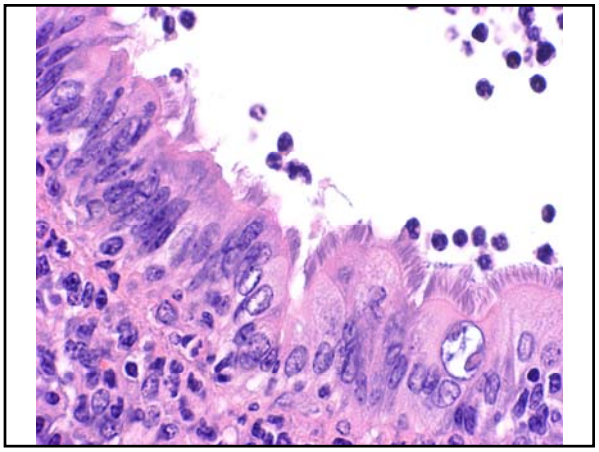
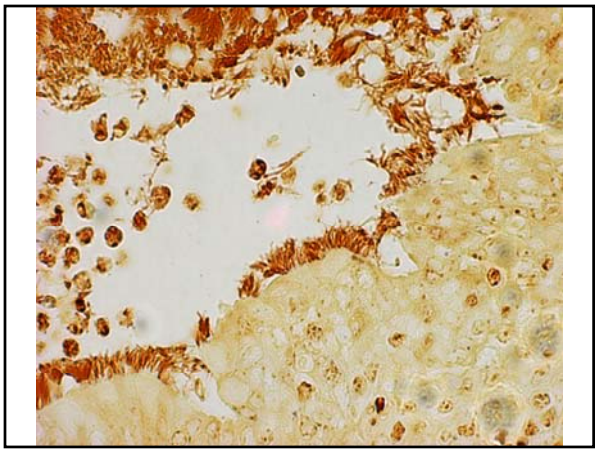
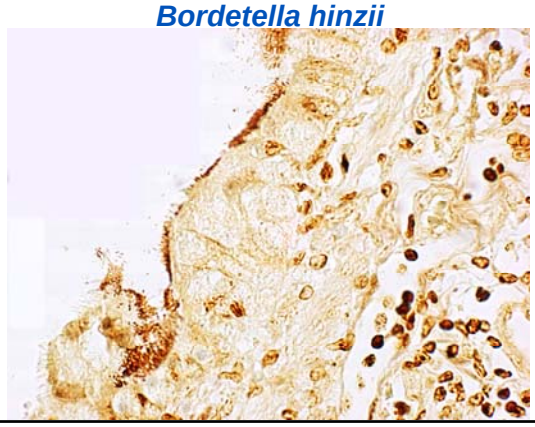






Bordetella hinzii



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.)

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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)

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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)

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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

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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

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Pathogenesis of Tyzzer's Disease

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

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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

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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)

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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)

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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

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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

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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

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Pathogenesis of Tyzzer's Disease

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

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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

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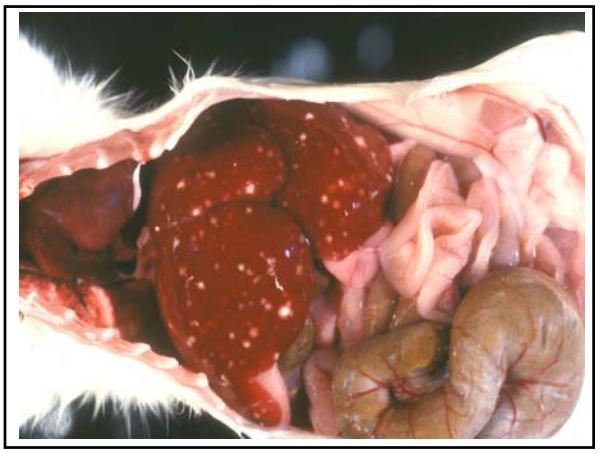

Gross Lesions of Tyzzer's Disease

- 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

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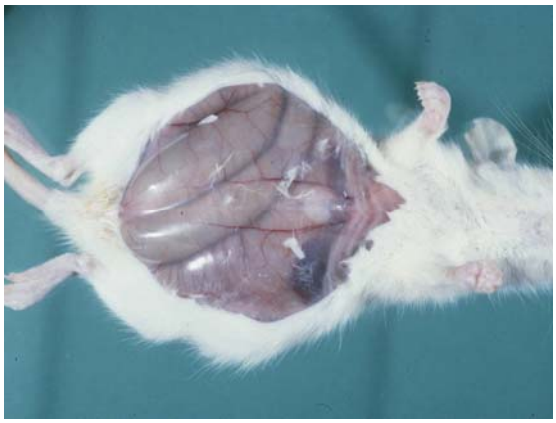

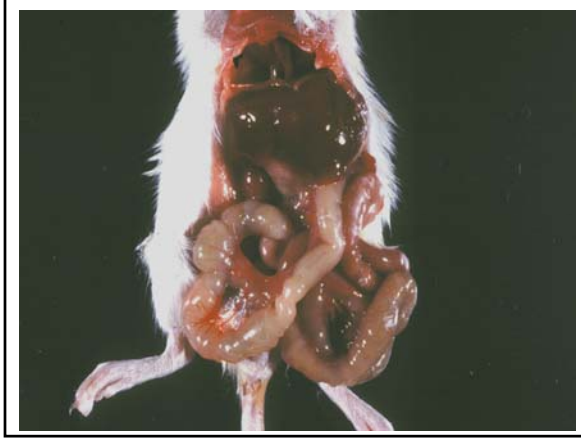

Gross Lesions of Tyzzer's Disease

- 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

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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

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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

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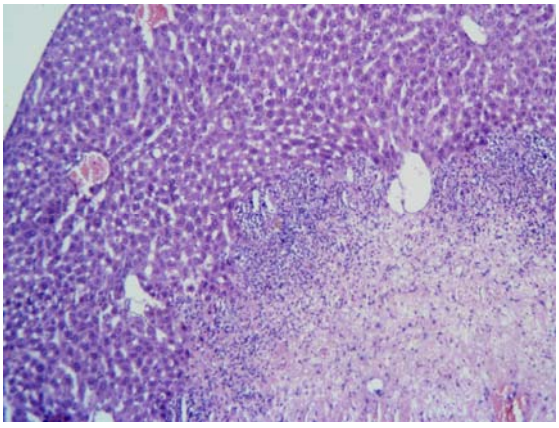

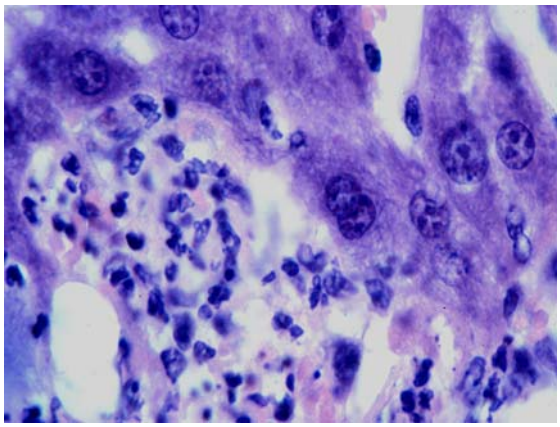

Histopathology of Tyzzer's Disease

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

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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

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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.

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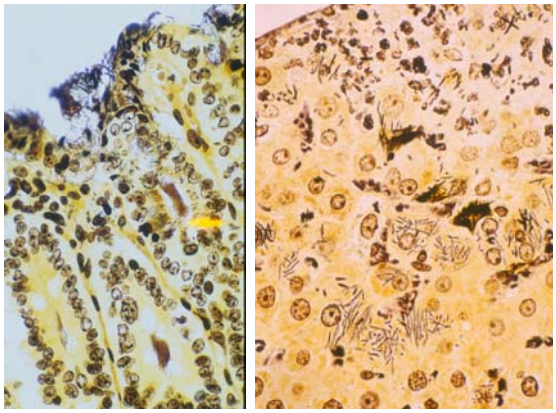

Histopathology of Tyzzer's Disease

- Vegetative form of *C. pilforme* 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

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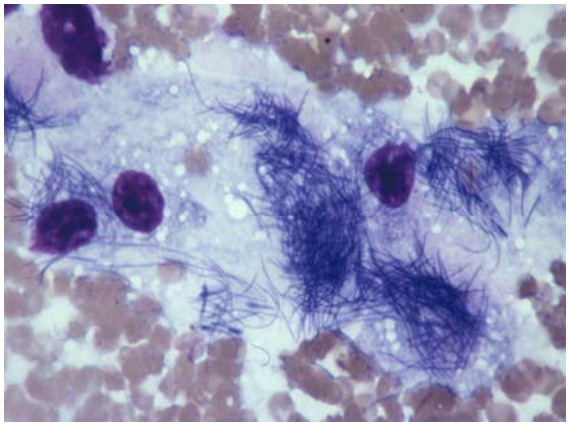

Tyzzer's Disease

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

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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

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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

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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 form 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

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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

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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

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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)

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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

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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

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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

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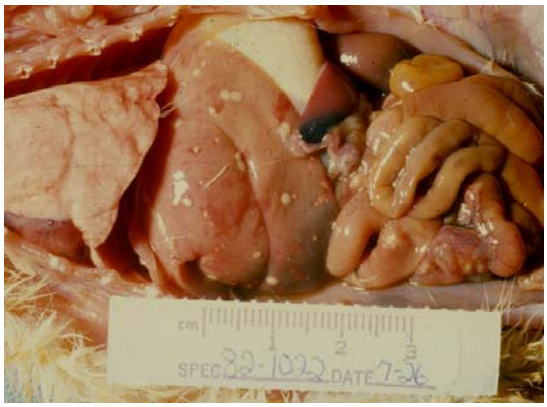
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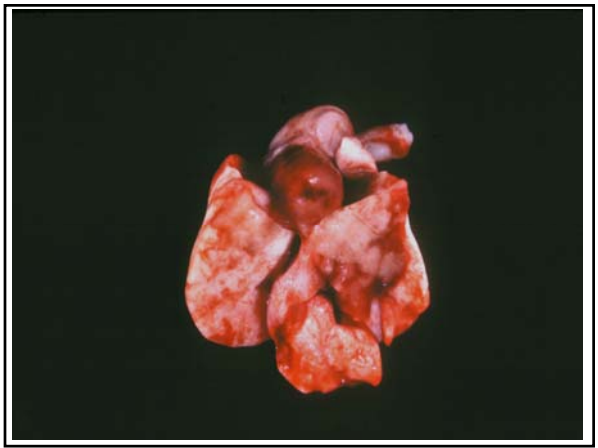

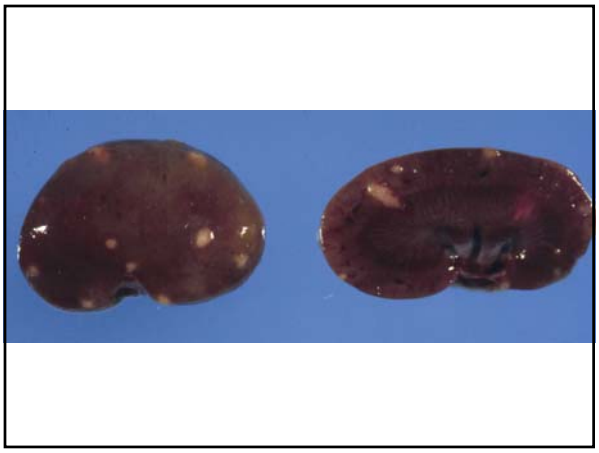

C. kutscheri infection

- 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

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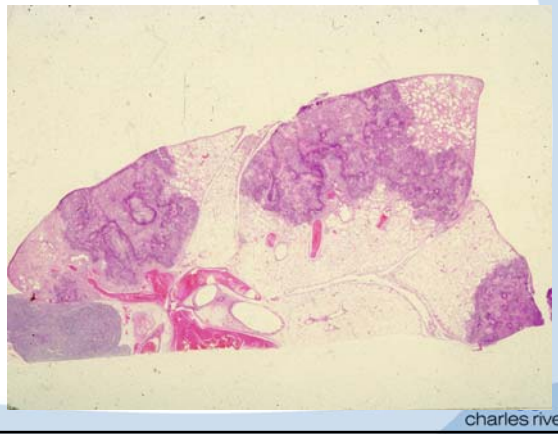

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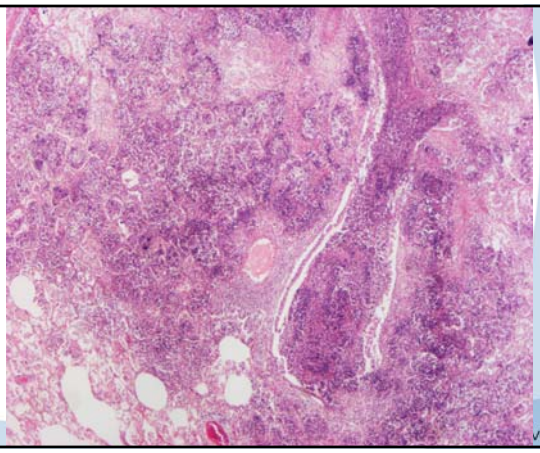

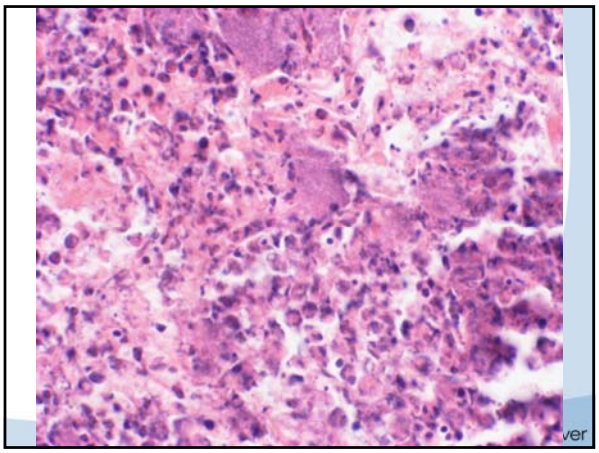

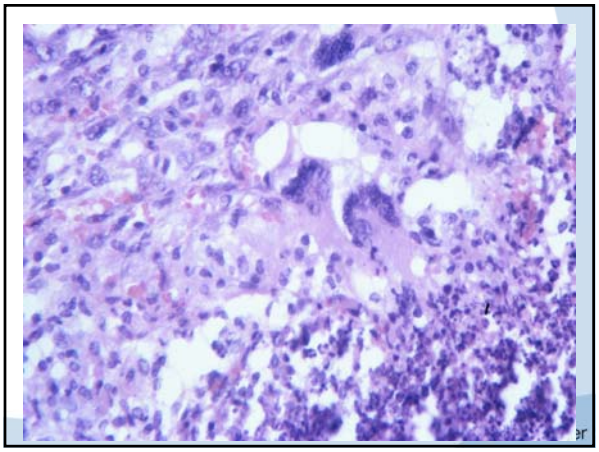

C. kutscheri infection

- 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*

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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

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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

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