AFIP
Department of
Medical Education

12th Current Laboratory Animal Science Seminar
(CLASS)

Emerging Rodent Diseases

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Charles River

What is an *Emerging Disease*?

- Any of a group of diseases, of various cause, that have newly appeared or are rapidly expanding their range in the human species. (highered.mcgraw-hill.com/sites/0072549238/student_view0/glossary.html)
- An infectious disease that has newly appeared in a population or that has been known for some time but is rapidly increasing in incidence or geographic range. MedicineNet.com
- **BUT** - The rodent diseases we are worried about are **not** new, are **not** increasing in incidence and **not** expanding their range

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- An infectious disease in lab animals that has recently been **discovered**, or is increasing in incidence, or about which **new information** has been discovered that significantly increases awareness of the impact of the disease on research. (Clifford)
  - Note: Many of the diseases we worry about now have only been discovered in the last 15 years or so.

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Epistemology

- To be conscious that you are ignorant is a great step to knowledge
  - Benjamin Disraeli **(1804 - 1881)**, *Sybil, 1845*
- ACLAM boards? – "There is much pleasure to be gained from useless knowledge"
  - **Bertrand Russell (1872 - 1970)**
- The student trap – "You can know the name of a bird in all the languages of the world, but when you're finished, you'll know absolutely nothing whatever about the bird… So let's look at the bird and see what it's doing – that's what counts. I learned very early the difference between knowing the name of something and knowing something."
  - **Richard Feynman (1918 - 1988)**

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Emerging Diseases

- Murine Norovirus
- Parvoviruses
- Helicobacter
- Bordetella hinzii
- Lymphocytic choriomeningitis virus
- "Rat Respiratory Virus"
- Rat Theilovirus
- Ljungan virus

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Mouse serology results

(Pritchett, Cosentino and Clifford, Lab Anim, 2009)

antigen	overall pos*	tested
MNV	32.37%	44,876
MPV	1.96%	555,081
MMV	0.36%	556,309
MHV	1.74%	524,752
ROTA	0.64%	438,932
GD-VII	0.34%	411,375
MCMV	0.04%	143,537
MTLV	0.04%	139,998
ECTRO	0.03%	234,077

•Samples are from non-CRL sources

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Mouse serology results

antigen	overall pos	tested
POLY	0.02%	215,962
MAV1,2	0.02%	219,181
CARB	0.02%	151,527
REO	0.01%	405,797
LCMV	0.01%	229,303
MPUL	**0.01%**	**422,334**
PVM	**0.00%**	**422,908**
HANT	0.00%	141,897
ECUN	0.00%	138,630
SEND	**0.00%**	**434,528**
K	0.00%	215,484

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Rat Serology Results

antigen	# tested *	% positive
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%

* Samples are from non-CRL sources

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Rat Serology Results

antigen	# tested	% positive
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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Murine Norovirus

Human Norovirus Infection
(good model for mouse disease)

- Genus named for Norwalk virus
  - Outbreak in an Ohio elementary school in 1968. Virus discovered in 1972
  - Typical signs in humans - vomiting and diarrhea for ~ two days.
- Noroviruses are major cause of nonbacterial epidemic gastroenteritis worldwide.
  - In US, CDC estimates 23 million cases of noroviral diarrhea each year
  - If similar rate in rest of world, ~ 500 million cases each year. How much diarrhea is that? ~250 - 500 million liters?

Niagara Falls: 5,830m3/sec = 5,830,000 L/sec = 349,800,000 L/min

Human Norovirus Infection

- Two major hurdles to vaccine development.
  1. Myriad strains, grouped into three genetically distinct genogroups (I, II, and IV)
     - Infection with one strain does not result in immunity to others
  2. Human norovirus has not been grown in cell culture *in vitro*, nor has bovine norovirus, Genogroup III, been cultured

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Murine norovirus

- The report of a murine norovirus in *Science* (2003), was important because the authors were able to culture the virus.
  - Discovery was made by a lab screening human material for unknown agents by IC inoculation into RAG2 -/-, STAT1 -/- mice
  - The mice died, and **MNV-1** was isolated. Later, it turned out that non-inoculated RAG STAT mice in the colony also died.
- RAG2 mice experimentally inoculated did not develop clinical signs.

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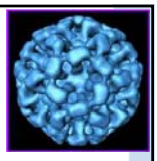
Murine Norovirus (MNV)



- Small, nonenveloped, ssRNA viruses in the *Caliciviridae*.
  - Murine noroviruses (MNV) are Genogroup V.
- Capsid is single protein, with cup-like projections, or calices.
  - Recombinant expression of the capsid protein results in spontaneous formation of virus-like particles (VLP).
- As with human noroviruses, MNV has myriad genetic variants.
  - Not established as to what degree of variation constitutes a strain.
  - CRL Molecular Diagnostics has identified more than 50 different variants.
    - All fall within Genogroup V
    - MNV-1 appears different than other variants

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MNV Epizootiology

- Spread by fecal-oral transmission.
- As a nonenveloped virus, MNV may remain infectious in the environment for long periods of time, possibly weeks.
- Infectious dose for mice is not known, may be small
  - Infectious dose for humans may be only a few virions.
- Infected mice, similarly to infected humans, shed:
  - Massive amounts of virus for a few days after infection
  - Shed small but probably infectious amounts of virus indefinitely
- Prevalence appears very high, ~30% of mice – about 10x any other virus.
- Production colonies of major vendors currently negative.

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MNV Disease

- **Immunocompetent** mice – No clinical signs.
  - Studies in 129 (129S6SvEvTac) mice, inoculated PO with 10^7 PFU MNV-1.CW3
    - Minimal change (13 vs. 8 per high power field) in inflammatory cells in lamina propria of small intestine at 24 hours post infection
    - Increased nuclear staining in red pulp of spleen, but no change cell number
    - Viral nucleic acid found in small intestine, spleen, mesenteric lymph nodes, liver
    - *Possible* decreased "stool contents" at 3 days P.I.

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MNV Disease

- Mice deficient in **acquired** immunity (RAG, SCID, etc.)
  - MNV antigen and nucleic acid detected in mesenteric lymph nodes – probably in dendritic cells

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MNV Disease

- Mice deficient in innate immunity
  - Lethal infection in STAT1 -/- (with or without RAG2 and PKR), and IFN Rαβγ -/-
  - Hepatitis, interstitial pneumonia
  - Encephalitis only with intracerebral inoculation
  - Virus present in dendritic cells

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MNV Research Effects

- Disease in some (rarely used) strains
- Probable interference with studies of innate immunity and/or dendritic cells
  - MNV-4 infection increased severity of *Helicobacter bilis*-accelerated colitis in *mdr1a -/-* mice.
    - MNV appeared to alter antigen presentation by dendritic cells, to potentiate the *Helicobacter*-induced inflammatory bowel disease.
- No effect observed in immune response to Influenza A or vaccinia virus infection, and no effect on CD8+ T cells. Authors still recommend caution. (Hensley et al., J Virol, 2009)

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MNV Diagnosis

- Colony screening by Serology
  - MFIA, ELISA, IFA (good cross-reaction)
    - Recombinant capsid protein self-assembles into VLP – no need to culture virus
    - **Mice may take up to 8 weeks to seroconvert**
    - Failures of infection transfer by soiled bedding reported
- PCR (Pooled fecal samples, 10:1) Infected mice shed for months.
  - **Release from quarantine**
  - Screening immunodeficient mice
  - Environmental monitoring

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All Relevant Agents Detected by Both PRIA and Bedding Sentinel

Agent	Quarantine* Groups (N)	Sample Source	Assay	Collection Time	Postive #	Postive %
Murine Norovirus	9	Sentinel	MFIA	3 wks	1	11%
				8 wks	3	33%
		Quarantine	PRIA	2-4 days	5	56%
Helicobacter spp.**	7(9)	Sentinel	PCR	3 wks	0	0%
		Quarantine	PRIA	2-4 days	4(6)	56%(77%)
P. pneumotropica (Heyl, Jawetz)	9	Sentinel	Isolation	8 wks	0	0%
		Quarantine	PRIA	2-4 days	5	56%
All		Sentinel	MFIA/PCR	3-8 wks	4	12%
		Quarantine	PRIA	2-4 days	14(16)	56%(59%)

* Total representation > 90 mice for 9 mouse lines

** Bedding sentinels representing 2 positive lines were not tested, parenthesized number includes all detected by PRIA

C. Perkins and K. Henderson, National AALAS, 2009

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MNV Management

- Virus apparently present for a long time
  - Very well adapted to host
  - Many strains
  - Wide geographic distribution
- Consensus seems to be to survey
  - Many facilities waiting to see how attitudes toward MNV evolve in the near future, before taking action
  - Perhaps excluding from new facilities
  - Major vendors in US are negative for MNV
    - Always ask to be sure

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MNV Management

- Rederivation by embryo transfer (or even caesarian section) should be successful
- Early cross-foster seems successful
  - Neonates resistant to MNV infection
- Environmental decontamination may require use of oxidizing disinfectants or heat > 56 C
  - FeCV was used as surrogate, but MNV-1 sensitivity may differ from FeCV
    - Alcohols not effective
    - Ozone, free chlorine, monochloramine is effective

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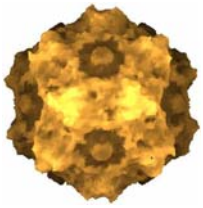

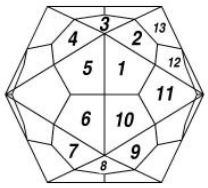

Rodent parvoviruses

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Rodent parvovirus - Discovery

- **General – Most discovered by *in vitro* effects on cell cultures**
- 1959 – Kilham and Olivier describe KRV, now RV
- 1961 – Toolan describes H-1, interested in anti-tumor effects
  - 1965 – injects humans
- 1966 – Crawford describes MVM in cell culture (MVM*p* also described in 1966, MVMi in 1976). Now called MMV (Mice minute virus)
- 1993 – McKisic describes MPV in lymphocyte culture (virus then called orphan parvovirus)

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Parvoviruses

- Small (18-28 nm), nonenveloped ssDNA virus, 2 ORFs
- First ORF - P4 transcripts encode NS proteins - highly conserved
  - NS1 probably responsible for cytopathic effects
    - Site-specific DNA binding and endonuclease, ATPase, Helicase
    - *trans* –regulation of transcription from P4 and P38
  - NS2
    - Required only in host species
    - Probable role in capsid assembly and nuclear egress, interacts with SMN, CRM-1 (exportin-1), and cell cycle regulator 14-3-3
    - May enhance NS1-induced cytotoxicity

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Parvoviruses

- Other ORF, P38 encodes VP1 and VP2
  - VP2 is major coat protein, will self-assemble into VLP
  - VP2 used for strain-specific parvovirus assays
    - Not reliably cross-reactive across serotypes or strains
- Host receptor unknown
- Require host cells (with appropriate receptor) to be in S phase of mitosis to activate P4 promoter and produce NS1 and NS2 which then govern replication

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Parvoviruses

- Late events include massive nuclear reorganization (SMN-associated APAR bodies)
  - Active sites of viral replication
  - Contain SMN, interchromatin granules, Cajal bodies, cyclin A, DNA polymerase, PCNA, et al.)
- Productive infection appears to be cytolytic

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Parvoviruses

- Take-home Lesson? Even simple viruses interact in myriad ways with the cells they infect; each aspect of the interaction carries a potential for interfering with research.
  - The paucity of credible reports of research perturbation by adventitious agents should not be equated with a lack of effects

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Parvovirus Epizootiology

- Prevalence - common
  - Mice ~2%
  - Rats ~3%
  - Most large institutions seem to have some parvo
- Fecal-oral transmission
  - Possible exceptions – Urinary for RV and H-1. RV may also have respiratory spread
- All shed for long times, thought to cause persistent infection
- Parvovirus strains are species-specific
  - Exception #1 – MPV-3 is the same as Hamster Parvovirus (HaPV)
  - Exception #2 - Some can infect cell cultures from multiple species

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Parvovirus Disease

- **Mouse parvovirus** (MPV-1, MPV-2, MPV-3, MPV-4, MPV-5) – No signs or lesions in any strain of mouse, regardless of immune status.
  - **C57BL/6 mice and mice on C57 background require 10-100X infectious dose to become infected and, subsequently, to seroconvert relative to CD-1.**
  - MPV-3 appears to be the same virus as hamster parvovirus. Mice appear to be the natural host.

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Parvovirus Disease

- **MMV(MVM)** – natural infection
  - No disease reported until Besselsen, *et al*. reported MMV*m* strain (the first field strain to be studied) to cause stunting, reduced fertility, premature death in NOD µ-chain KO immunodeficient mice.
  - In contrast to earlier MMV strains (all culture-adapted) MMV*m* caused persistent infection
- MMV*m* is the most prevalent strain

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Parvovirus Disease

- **Rat Virus** (RV)
  - a.k.a., Kilham Rat Virus (KRV)
  - Clinical disease rare, primarily seen in epidemics in large colonies/groups (or experimental situations) where naïve rats exposed to large doses
    - Fetal death
    - Neonatal hemorrhage and necrosis in liver and CNS
      - Possible icterus, ataxia, with cerebellar problems and chronic liver disease possible in survivors
    - Hemorrhagic disease in older rats (very rare)

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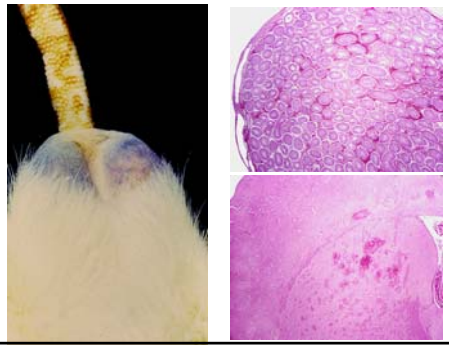
Rat Virus



Parvovirus Disease

- **H-1** – No clinical disease
- **RPV** – No clinical disease
- **RMV** – Genetically and antigenically more similar to KRV than to RPV. *Probably* no clinical disease. May be most prevalent serotype in rats.

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Parvovirus Research Effects

- General – Long-term effects on immune system. Interference with tumor studies. Contaminant of tumor cell lines.
- **MPV** – MPV-1a (cell culture adapted) modulates immune response
  - Suppression of T cell response *in vitro*
  - CD8+ T lymphocyte clones lose function and viability
  - Cytokine- and antigen-induced T cell proliferation *in vitro* suppressed after exposure to MPV-1a
  - Potentiates allograft rejection *in vivo*

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Parvovirus Research Effects

- **MMV** -
  - Can infect many mouse cell lines, as well as some rat embryo lines and transformed human cells (324K, EL-4)
  - *In vitro* reduction of T-cell response by MMVi and *in vivo* late reduction of cytotoxic memory cells by MMVp
  - *In vitro* (A9 cells) dysregulation of gelsolin (↑) and WASP (↓) by MMVp
  - MMVp is oncotropic and oncolytic in some human tumors (hemangiosarcoma) and mouse tumors

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Parvovirus Research Effects

- **RV** –
  - RV infection led to development of diabetes due to immune-mediated islet destruction in the Diabetes Resistant BB rat, probably due to imbalance of Th1 and Th2 responses

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Parvovirus Research Effects

- **H-1** –
  - Oncotropic and oncolytic – being explored as possible treatment for glioblastoma multiforme
- **RPV** –
  - Suppressed *in vivo* growth of LGL leukemia in F344 rats
  - RPV/UT NS protein induced epigenetic modification in a thymic lymphoma line, causing reversion to benignancy
- **RMV** – Nothing reported

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Parvovirus Detection - Serology

- General considerations –
  - ELISA and MFIA primarily detect antibodies to structural proteins (which vary between strains), so must use specific antigens for each serotype.
  - ELISA or MFIA using NS1 protein, which is conserved across serotypes, is more generic.
    - BUT, not all animals will seroconvert to NS1 antigens
  - IFA, which uses virus-infected cells, has both structural and nonstructural proteins so the IFA is more generic. Although sensitive, it is not amenable to automation.

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Parvovirus Detection - Serology

- **Mice**
  - Use panel of all available VP antigens, plus NS1 protein (MPV-1, MPV-2, MMV, NS1).
  - Poor cross-reaction between MPV-1 and MPV-2, but decent cross-reaction with MPV-3. Not so good with MPV-4.
  - C57BL/6 and lines on C57BL/6 background, and DBA/2 mice are partially resistant to infection

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Parvovirus Detection - Serology

- **Rats**
  - Use panel of all available VP antigens, plus NS1 protein (RV, H-1, RPV, RMV, NS1)
  - No known variation in parvovirus susceptibility among rat stocks/strains

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Parvovirus Detection - PCR

- PCR can be generic (NS1) or specific (VP2)
- Mesenteric lymph nodes stay positive indefinitely
  - Can be used to confirm serology
  - Spleen almost as good
- Very high correlation with positive serology
  - Little gain from routinely doing serology and MLN PCR on same animal

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Parvovirus Detection - PCR

- PCR on pooled fecal samples can test for shedding (risk of infectivity)
  - Reduces cost
  - Unknown if shedding can be intermittent
    - Some circumstantial evidence against intermittent shedding
  - Shedding DNA may not always indicate infectivity
  - Must use appropriate controls to detect presence of fecal inhibitors of PCR

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Parvovirus Detection - PCR

- PCR on animals in quarantine (no worries about immune status, can help certify animals as “not dangerous” to facility)
  - Advisable with or without serology
- PCR on cell lines and other biological materials
  - Can contaminate purification columns and contaminate subsequent material
- PCR on environmental swabs as indicator of particle presence, spread, or of disinfection
- Highly sensitive, but does not necessarily indicate the presence of infective virions

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Parvovirus - sentinels

- Advantages
  - Virus should stay infective in soiled bedding for weeks
  - Shedding can persist for long times (but usually only a couple weeks)
  - One sentinel can monitor many cages

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Parvovirus - sentinels

- Problems
  - Infected mice do not always shed enough virus even to infect cagemates
  - Seroconversion may be delayed in older animals exposed to small amounts of virus
    - Many sentinels may fit this description
    - These animals may not seroconvert to NS1 protein.

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Mouse serology for parvoviruses

agent	overall pos	# tested
NS-1	1.81%	540,941
MPV (all)	1.96%	555,081
MVM	0.36%	556,309

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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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Parvovirus - sentinels

- Problems
  - Virus not uniformly distributed in bedding or environment, so exposure of sentinels and principal animals may be sporadic.
  - Prevalence in facility using IVC or filter top caging can be very low, so predictive value of a positive result can also be low
  - With time delays from sentinel monitoring, it's also possible that the index case is gone by the time the sentinel is screened.
  - The sentinel may also be the index case.

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Parvovirus management

- Potential sources – Incoming animals, pests, biologicals, personnel handling infected rodents, fomites (feed, **feed bags**, bedding, water, used equipment, **shared equipment**)
- Disinfection – must use autoclaving or oxidizing disinfectant
- Eliminate all infected animals?
  - Perhaps not necessary if not shedding, but still maybe prudent

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Parvovirus elimination

- Rederivation
  - Embryo transfer
    - MPV reported as detected in sperm and pre-implantation embryos, indicating some risk
    - Charles River experience with many dozens of ET rederivations for parvoviruses has not found transfer
  - C-section - Young females, especially primiparous are more likely to be viremic, so there is a risk of transfer of infected lymphocytes with the uterus. – must quarantine and test offspring and foster dams.
  - Early cross-fostering reported as mostly successful
- Must quarantine and test offspring and recipient dams

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Helicobacter in rats and mice

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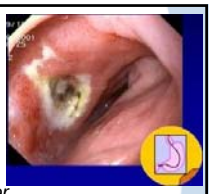
Helicobacter Discovery



- Originally included with *Campylobacter*
  - *Helicobacter* created in 1989
- *H. pylori* probably discovered in 1875,
  - linked to human gastric disease in 1899 (Polish text)
- "Re"discovered in 1979 by Warren
  - 1984 - linked to most gastric ulcers and gastritis (Warren and Marshall, Nobel Prize awarded in 2005)
  - Now linked to gastric carcinoma

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Helicobacter - Discovery

- *H. muridarum* in mice: 1992
- *H. hepaticus:* 1994
  - Liver tumors and hepatitis in A/J mice on carcinogenicity study
  - Lesions resembled aflatoxicosis
  - Spiral organisms discovered in bile canaliculi with Steiner stain
- Currently >40 species of *Helicobacter* described, with many in rodents

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Helicobacter

- Gram-negative bacteria colonizing intestinal tract of warm-blooded animals
  - Gastric (colonization aided by urease)
  - Large intestine
    - Some of these reach liver (enterohepatic)
- Microaerophilic (*H. ganmani* is anaerobic)
- Highly sensitive to desiccation
- Highly adapted to hosts, although many are capable of colonizing multiple host species
- Mechanisms of disease similar among helicobacters
- Animal models useful in studying human disease

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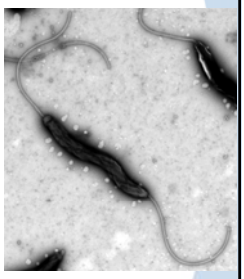
Helicobacter Epizootiology



- Fecal-oral transmission
- Short-term fomites (soiled bedding) possible
- Colonization by weaning, persist for lifetime
  - A few experimental efforts have shown short-term colonization, significance unknown.
- Prevalence: high (~15% in mice, ~8% in rats), especially high in GM mice

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Helicobacter Disease

- Varies tremendously with *Helicobacter* species, rodent strain, immune status, sex, possibly age.
- General – Outcome depends on interaction of *Helicobacter* with gut flora, and on immune response, with most disease being a by-product of the host response.
  - Important components of host response include IL-10, TNF, TH1:TH2 balance, CD4+CD45RB(lo)CD25+ T regulatory cells, and TGF-beta.
- Infection with multiple *Helicobacter* species or with other pathogens, e.g., MHV, can be synergistic

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H. hepaticus and Disease

- Most commonly detected species of *Helicobacter*
- Proliferative colitis in A/J, C3H/HeN, athymic nude, SCID and many other immunodeficient strains, e.g., IL-10 -/-.
- Currently, only common infectious cause of rectal prolapse, especially common in immunodeficient mice
- Chronic hepatitis (necrosis, hepatocytomegaly, biliary proliferation, nonsuppurative inflammation) in A/J, C3H/HeN, and some other immunocompetent strains.
  - Hepatitis may be particularly necrotizing in immunodeficient strains

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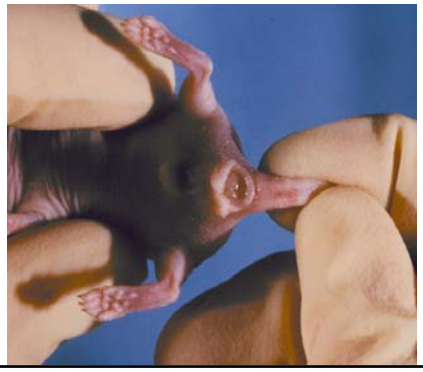
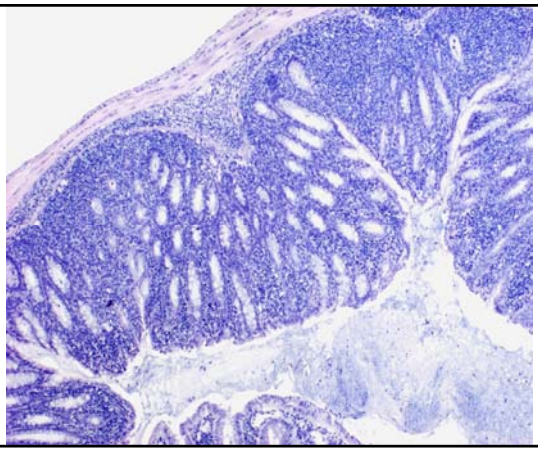
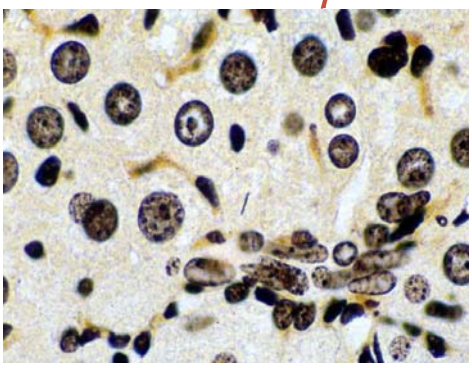
Helicobacter hepaticus



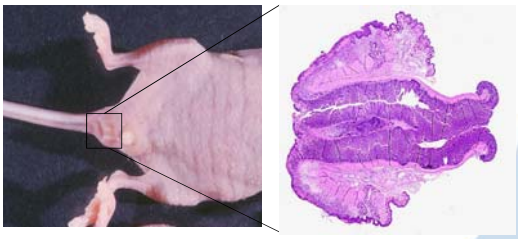


Helicobacter hepaticus



Background Lesions

- Prolapsed rectum in immunodeficient mice can also be non-infectious (sporadic)



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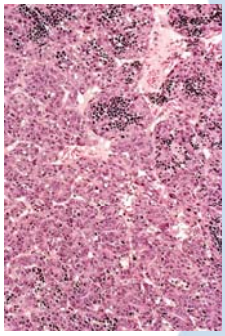
H. hepaticus Disease

- Hepatocellular carcinomas in A/J, C3H/HeN, SCID mice
- Colon carcinoma in SMAD-3 deficient mice
- Promotes colon carcinogenesis in RAG2-/- Apc(min/+)mice
- Increased incidence of mammary carcinoma in RAG2-/- Apc(min/+)mice (secondary to inflammation)
- C57 resistant to disease, but can carry high level of colonization



6-week old AL-ras x AL-myc dual Tg mouse Hepatocarcinoma (not *Helicobacter*-induced)

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H. bilis

- Prevalence about 1/4 that of *H. hepaticus*
- Immunodeficient mice and rats
  - Proliferative typhlocolitis
  - Occasional rectal prolapse
- Immunocompetent mice
  - Mild chronic hepatitis (low incidence)
  - Typhlocolitis in monoassociated outbred Swiss mice

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Helicobacter *Other* Research Effects

- Direct effects, depending on variables above, on large bowel and liver, with broad activation of specific and non-specific aspects of host defense system including development of tertiary lymphoid follicles
- Indirect effects of infection without lesion production are not as well described but may influence remainder of gut flora
  - Attenuates gastric pathology in C57BL/6 mice due to *H. pylori* infection
  - Co-infection with *H. hepaticus* and *H. rodentium* increased bile flow and bile salt flow (C57L/J mice), suggesting potential for enterohepatic helicobacters to alter pharmacokinetic studies

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Helicobacter Research Effects

- IFN-γ deficient (KO) mice on C3H background developed a wasting syndrome with granulomatous peritonitis
  - Co-infection with *H. hepaticus* and **enterotropic** MHV-G reduced mortality and lesion incidence and severity relative to MHV alone during first week
  - In contrast, co-infected mice had more severe hepatitis and meningitis at 28d

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Helicobacter Research Effects

- *H. hepaticus* has been demonstrated to increase cecal expression of IP-10, MIP-1α, IL-10, IFN-γ, and MIG mRNA, in A/JCr mice, with greater increases in females than in males.
- Coinfection with *H. hepaticus* and *H. rodentium* exacerbated the inflammation and expression of inflammatory mediators, but infection with *H. rodentium* alone did not cause hepatitis or enteritis in A/JCr or SCID mice.
- Gene profiling has been used to explore the carcinogenic activity of *H. hepaticus* in A/J mice. Upregulation of putative tumor markers correlated temporally with increasing hepatocellular dysplasia
- Decreased reproduction in IL-10 -/- mice on C57BL6 background when infected with *H. typhlonius* and/or *rodentium*

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Helicobacter Detection

- **Screening**
  - **PCR on pooled fecal pellets** most widely used
  - Culture possible but difficult and lacks sensitivity
  - Serology not widely used
  - Based on epizootiology, frequent in-house monitoring is unnecessary if incoming animals are negative
- Disease investigation
  - Histopathology with silver stains useful in solid tissues
  - PCR shows presence of organism but does not confirm its role

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Helicobacter Management

- Relatively easy to contain within a research facility
  - Anecdotal reports of cages side-by-side without transmission
  - Note reports of **difficulty in transferring by soiled bedding**
- Most or all vendors now negative for it (always good to confirm)
- Mixed reports on success of medicated feed or antibiotic administration by gavage
- Elimination usually successful by **cross-fostering** in 1st 24 hours
- Rederivation by embryo transfer or caesarian transfer seems uniformly successful
  - Note report of PCR positives for *H. typhlonius* in ovary, uterus, testis, epididymis
  - Early abstract report of *H. hepaticus* in a SCID fetus

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Bordetella hinzii infection

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Bordetella hinzii infection

- Agent
  - Gram negative bacillus in *Alcaligenaceae*
  - Not part of *Bordetella pertussis-parapertussis-bronchiseptica* group.
  - Possible pathogen of turkeys (Register, K.B., Kunkle, R.A. 2009. Strain-specific virulence of Bordetella hinzii in poultry. Avian Diseases. 53(1):50-54)
- History
  - *Alcaligenes faecalis* divided into *B avium* and *B avium*-like
  - *B hinzii* (was *B avium*-like) described in 1995 (poultry and humans)
  - First reported in mice in 2008
- Prevalence in mice - unknown

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Bordetella hinzii infection (Hayashimoto et al., Comp Med, 58:440, 2008)

- Mouse (C57BL/6) submitted for sneezing and "chattering"
  - Gross - Pulmonary consolidation (accessory lobe)
  - Histo – Rhinitis, tracheitis, bronchopneumonia
- Inoculation of ICR and NOD-SCID (25 µl w/5 x 10^7 or 5 x 10^3 CFU)
  - Survival – 1 low dose and 2 high dose NOD-SCID died or euthanized
  - Gross – mucus in nasal cavity (no lung lesions)
  - Histo – Rhinitis, bronchopneumonia in all. Interstitial pneumonia in NOD-SCID

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Bordetella hinzii infection

- Diagnostic case at CR in 2002
  - Dyspneic mouse from isolator – immune status unknown.
    - Negative for CAR bacillus and other respiratory agents
    - *B.* "*avium*" isolated from nasopharyngeal lavage cultured onto blood agar
    - Mild bronchitis on H&E – mucosal surface (cilia layer) appeared more basophilic than normal
      - Nasal cavity not examined
    - Warthin-Starry silver stain showed numerous short bacilli

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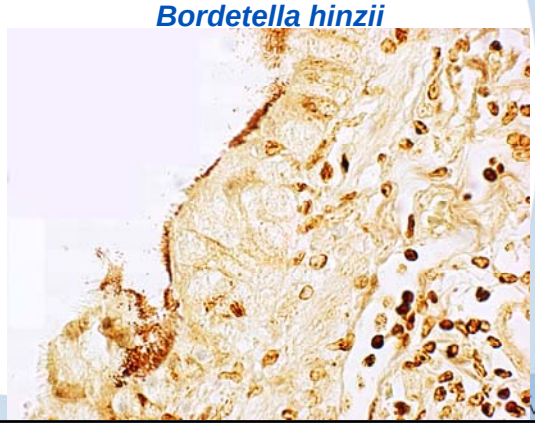
Bordetella hinzii



Bordetella hinzii infection

- Detection
  - Nasopharyngeal lavage onto blood agar
  - Bronchial lavage onto blood agar
    - Culture – produces alkali from malonate
  - PCR? (lavage fluid, nasal swab, feces?)
    - Partial 16s rRNA sequence on GenBank (J Clin Microbiol, 38:789, 2000)
- Research interference – unknown
- Control – Unknown
  - Environmental persistence unknown
    - B bronchiseptica can live long periods in water
    - Persistence in bedding and on surfaces for shorter periods
    - ET should be successful

Lymphocytic choriomeningitis virus

LCMV

- **Discovery**
  - Known for many decades
- Primarily of zoonotic concern
  - Recently caused serious infection in 4 transplant recipients from same donor, 3 of whom died
    - Infection traced to pet hamster
  - Recent report of LCMV-positive, long-term laboratory-housed colony of wild mice
- Most zoonotic infections have been traced to hamsters
  - Very rare in lab hamsters

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LCMV

- Arenavirus, ssRNA
- Enveloped
  - Sensitive to desiccation, disinfection

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LCMV Epizootiology

- **Natural reservoir is wild mice**
- Transmitted *in utero*
- **Only shed (saliva and urine) by:**
  - Immunocompetent mice infected prior to weaning
  - Immunodeficient mice
  - Hamsters
- Nonproductive infections in many mammals including primates, other rodents and canids
  - Do not shed – little or no risk to others
- LCMV is found occasionally in cell lines
  - Has the potential to infect many cell types
  - Found by CRL in 2010 in BHK cells being used at a major teaching hospital (had cell line for long time)

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LCMV Disease

- "Lymphocytic choriomeningitis" lesion is an artifact of experimental IC inoculation
- Mice infected *in utero* or prior to weaning
  - Immune tolerance, with virus in CD8+ cells.
  - Mice may be runted
  - Eventually, perhaps > 1 yr., immune tolerance is overcome and many mice develop lymphocytic infiltrates in many tissues, as well as immune-complex glomerulonephritis
    - Not observed in recent paper from France and Japan
  - Grossly – emaciation and ascites
  - Some may be normal for life
- Mice infected as adults will clear the infection, reported not to shed

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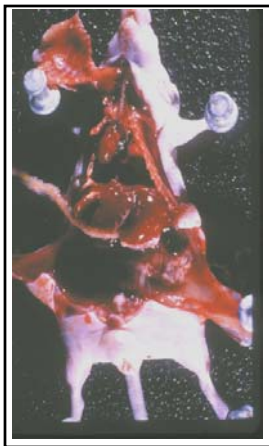

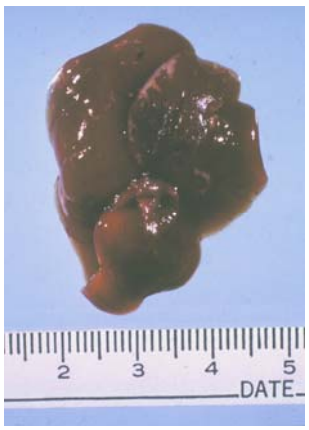

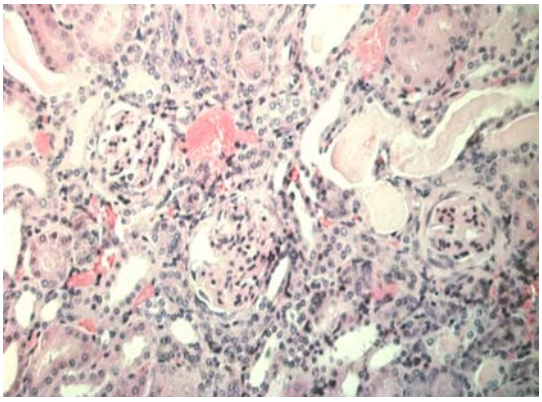

LCMV Research Effects

- Primarily through necessity of eliminating infected groups of mice
- Depresses cellular immunity
- Alters tumor growth
- Hypergammaglobulinemia
- Autoantibodies

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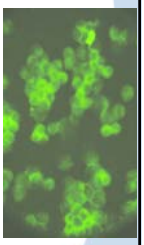
LCMV Detection

- **Serology good on mice infected after weaning**
  - MFIA or ELISA, and IFA
- Immune tolerant animals are likely to be seronegative
  - **Lines of wild murid rodents should be tested by PCR as they may be immunotolerant due to early infection**
  - **LCMV may not transfer well with soiled bedding**
- **PCR on kidney, salivary gland, urine-stained material**
  - Confirmation of serology
  - Cell lines
  - Screening immunodeficient animals and pets
- MAP, mouse inoculation/challenge



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LCMV Management

- Pest control
- Don't cross-breed or co-house with pet or wild rodents
- Test cell lines
- Rederive by embryo transfer
- As an enveloped virus, environmental disinfection is not a problem

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"Rat Respiratory Virus" (RRV)

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RRV

- **Discovery**
  - First noted in early 1990s as a complication to inhalation studies
    - Albers TM, Simon MA, Clifford CB, Histopathology of Naturally Transmitted "Rat Respiratory Virus": Progression of Lesions and Proposed Diagnostic Criteria, Vet Pathol 2009
- **Agent**
  - Uncharacterized agent (Rat Respiratory Agent?)
  - Transmissible
  - Filterable
  - Some IFA evidence suggested possible Hantavirus
    - Not repeated in other labs (Hantavirus IFA prone to false positives)
    - Early Hantavirus reports not substantiated in scientific literature

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RRV Epizootiology

- Widespread, observed in North America, Europe, Asia
- Most prevalent virus (~5%) of rats detected at Charles River Diagnostic Labs (non-CRL rats)
- Experimentally transmitted by soiled bedding – so fomites transmission likely
- Anecdotal reports of transmission to other rooms in a research facility

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RRV Disease

- All strains of rat appear susceptible to infection.
  - Possibility of RRV infection in other species is unresolved
  - Duration of infection unknown
  - Period of shedding unknown
- Clinical signs rare
  - Anecdotal reports of sneezing
- Gross lesions present in majority of naïve rats 6-8 weeks after exposure
  - Non-specific patchy grey-brown areas in lung

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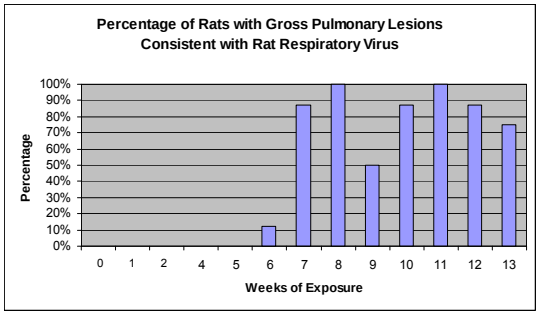
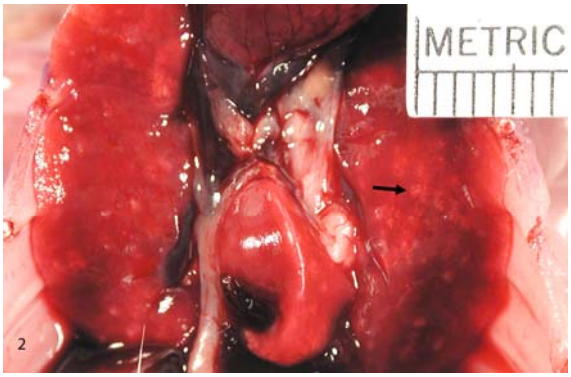
Gross Findings





RRV Disease

- Naïve rats:
  - Histologic lesions evident by 6 weeks p.i.
  - Lymphohistiocytic interstitial pneumonia
    - Progresses to prominent dense lymphocytic perivascular cuffs
    - Essentially no airway involvement
  - Unknown which, if any, other tissues are infected
  - Lesions slowly regress
    - lymphoid cuffs present at least 3-4 months p.i.
- Endemically infected colonies, lesions peak at about 10 weeks of age, i.e., about 7 weeks after weaning

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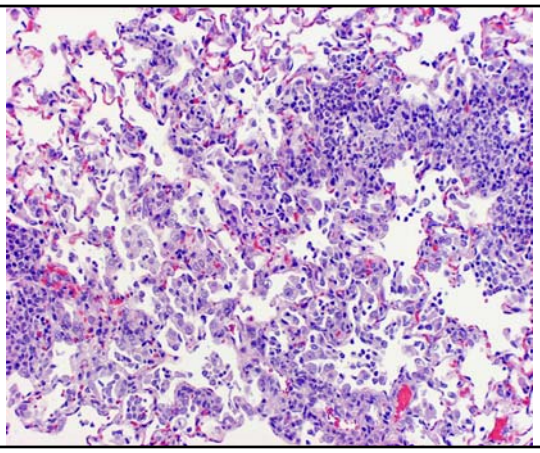
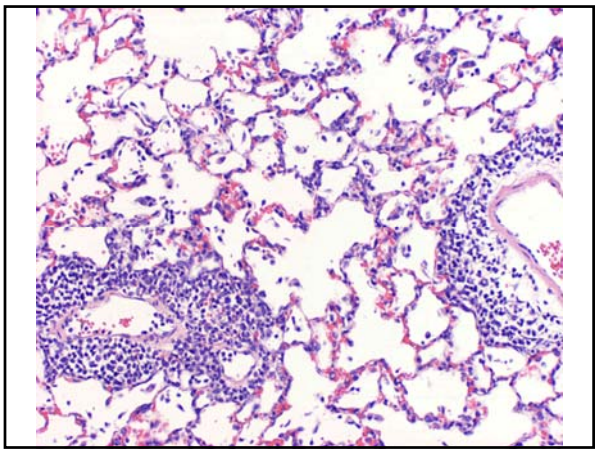
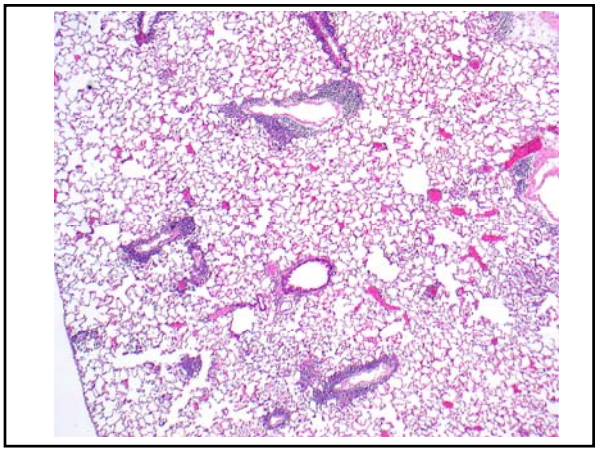
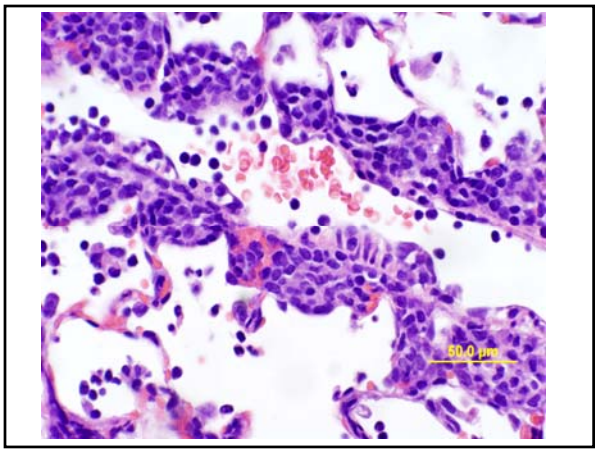
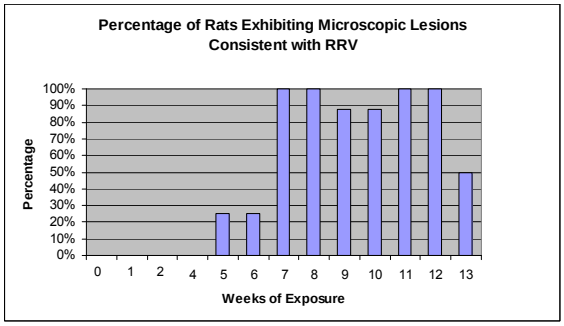







50.0 µm

Microscopic Findings



RRV Research Effects

- Unknown.
  - Anecdotal reports, although controversial, include increased problems with anesthesia and isolated perfused lung preparations.
- RRV has also interfered with interpretation of some inhalation studies

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RRV Detection

- Histopathology
  - Recommend formalin-inflated lungs.
  - Sentinels should be examined 2-3 months after exposure
  - Best age to screen endemic (or unknown) colonies is 8-12 weeks of age, although most rats of any age will develop lesions 6-8 weeks after 1st exposure
  - Quarantine? – best to receive additional 10-12 week old rats for pulmonary histopathology
- No serology or PCR available

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RRV Management

- Eliminate with rederivation
- Some early evidence suggested enveloped virus, but use of an oxidizing disinfectant is recommended as agent status is uncertain

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Rat Theilovirus

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Rat Theilovirus (RTV)

- **Discovery**
  - Serologic titers have long been detected in rats using antigen 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.

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Rat Theilovirus (RTV)

- **Discovery**
  - As no disease was observed, it received relatively little attention, except an occasional worry that it could be related to EMCV, another cardiovirus.
  - 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)
    - The sequence of RTV-1 has been filed on GenBank (by both CR)

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Rat Theilovirus (RTV)

- **Agent**
  - Family: *Picornaviradae*, Genus: *Cardiovirus*, Species: *Theilovirus*, Serotype: Rat theilovirus
    - There are four 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
    - Titers may be low
  - 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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Ljungan Virus

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Ljungan Virus

- **Discovery**
  - 1st reported in 1999 in bank voles in Sweden
    - Found same virus in voles in Denmark and US
    - Initially speculated on possible role in human myocarditis, diabetes, Guillain-Barré syndrome
  - Associated with diabetes in bank vole

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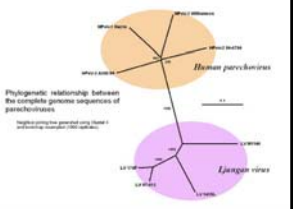
Ljungan Virus

- Picornavirus, most closely related to parechoviruses
  - 1 of 5 genera in picornaviridae
  - Some members cause GI and respiratory disease in humans, occasional flaccid paralysis
  - nonenveloped



Phylogenetic relationship between the complete genome sequences of parechoviruses

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Ljungan Virus Epizootiology

- Mode of transmission – unknown, presumably fecal-oral. *In utero* transmission has been demonstrated.
- Host range unknown
  - Voles – *Clethrionomys* and *Microtus*
  - Mice – Experimentally transmitted to CD-1 mice (IC or IP)

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Ljungan Virus Epizootiology

- Host range (continued)
  - Rats – Reported in BB rats
    - 16/16 from Sweden and 10/10 from UW Seattle – by PCR and IHC
      - Found in islets and brain
      - Suggested as the cause of diabetes in BB rats
    - 10/10 Wistar and 5/5 SD rats from Sweden also tested positive by IHC – tissue not stated

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Ljungan Virus Disease

- Autoimmune destruction of β cells of islets in voles, speculated in BB rats
  - Stress is co-factor
- Diabetes and fetal malformations in CD-1 mice (experimental inoculation of 1,000 ID50)
- Also found in 5/5 cases of human intrauterine fetal death in Sweden, but in only 1/18 cases of trisomy 21

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Ljungan Virus

- **Prevalence in Lab rodents**
  - Unknown
  - **All CR colonies have tested negative**
- **Research Effects**
  - Unknown, *could* be an important factor in diabetes research
- **Diagnosis**
  - CR has PCR assay
    - But, since we get all negative results, is the assay good enough?
- **Management**
  - No action recommended at this time
- **Conclusion**
  - *Probably* a false alarm, but it serves to remind that there are always new (old) viruses being discovered

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Thanks for your attention

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