2011 TOPICS IN LABORATORY ANIMAL MEDICINE

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Laboratory Rats

Jeff Everitt, DVM
GlaxoSmithKline

THANK YOU!

- Pathology and LAS colleagues at GSK
- University of Missouri/Dr. Craig Franklin
- National Institute of Environmental Health Sciences
- Dr. Mary Grant
- Dr. Iris Bolton

Disclaimers

- This is not an ACLAM sanctioned presentation
- All information is deemed reliable and correct
  - No warranty for accuracy
- No information presented is known to be specifically included in ACLAM Board examinations

Objectives of talk

- Review some basics of rat biology
- Review some basic spontaneous rat models
- Cover a few diseases of rats
- Review some rat pathology
- Review some recent literature
- Not Comprehensive

Rats

- *Rattus norvegicus*
- Not just a big mouse
- Used frequently in toxicological, teratological, carcinogenesis, behavioral, nutritional, endocrinology, aging studies
- Huge physiology database
- Special strains for certain diseases such as diabetes mellitus, diabetes insipidus and hypertension
- GERS just beginning
Rat misc
- Susceptible to light induced retinopathies
- No gallbladder
- Duplex uterus
- Ad lib feeding shortens lifespan
- Coprophagic

What condition is depicted?
- Ringtail
- Lesion of young animals
- Annular constrictions with or without sloughing of tail
- Associated with high temperature and low humidity (<40%?)

Chromodacryorrhea

Coat color
- Albino
- Black-hooded
- Brown-hooded
- Fawn-hooded
- Non-agouti brown
- Rats were domesticated for show
Cardiovascular Models

<table>
<thead>
<tr>
<th>Model</th>
<th>Description</th>
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<tbody>
<tr>
<td>SARDOR</td>
<td>Hypertension Model</td>
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<tr>
<td>Wistar</td>
<td>Hypertension Model</td>
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<tr>
<td>SHR</td>
<td>Hypertension Model</td>
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<td>DOCA</td>
<td>Hypertension Model</td>
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<td>SAD</td>
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<td>Sprague-Dawley</td>
<td>Hypertension Model</td>
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<td>2K1C</td>
<td>Hypertension Model</td>
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<td>2K1C + DOCA</td>
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<td>2K1C + DOCA + Conivaptan</td>
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<td>2K1C + DOCA + Conivaptan + Hydralazine</td>
<td>Hypertension Model</td>
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Metabolic Models

<table>
<thead>
<tr>
<th>Model</th>
<th>Description</th>
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<tbody>
<tr>
<td>ZDF</td>
<td>Obese Model</td>
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<tr>
<td>ZDF + Diabetes</td>
<td>Obese Model</td>
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<tr>
<td>Zucker</td>
<td>Obese Model</td>
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<td>Obese Fagan</td>
<td>Obese Model</td>
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<td>Obese Zucker</td>
<td>Obese Model</td>
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Renal Models

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<tr>
<th>Model</th>
<th>Description</th>
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<tr>
<td>Eker</td>
<td>Familial Cancer Model</td>
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<tr>
<td>TSC2</td>
<td>Familial Cancer Model</td>
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<tr>
<td>Tuberous Sclerosis Complex</td>
<td>Familial Cancer Model</td>
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EKER Rat Model of Familial Cancer

- Mutation in the TSC2 gene (Tuberous Sclerosis Complex)
- 100% develop renal tumors by 12 months
- Autosomal dominant disease
**What type of hooded rat is this?**
- Fawn hooded rat
- What types of abnormalities does this rat have?
  - Abnormal platelet function
  - Hypertension

**Other Hooded Rats…**
- What is the LEC rat?
  - Long Evans Cinnamon
- What abnormality does this rat exhibit?
  - Hepatitis
- Fulminant hepatitis and jaundice at four months of age.
- About 50% of animals die within 1 week of onset of clinical signs.
- Survivors develop hepatocellular carcinoma after 1-1.5 years.
- Model for Wilson’s disease. Also think Bedlington Terrier.

**White/Albino Rats and Their Abnormalities…**
- What albino rat is a model for Type 1 Diabetes?
  - BB
  - Outbred Wistar rats at Bio-Breeding Labs.
  - Abrupt onset of diabetes 60-120d age.
  - Lymphocytic insulitis, destruction of pancreatic beta cells.
  - Males > Females.

**What hooded rat is agouti with a white belly and feet?**
- ACI
  - Genitourinary abnormalities
  - Absent, hypoplastic or cystic kidneys
  - Sometimes associated with an absent or defective uterine horn or atrophic testes on the same side

**Other Hooded Rats…**
- What hooded rat develops retinal degeneration?
  - RCS
  - RCS (Royal College of Surgeons)
  - Carries gene for retinal dystrophy.
  - Overproduction of rhodopsin and progressive loss of photoreceptor cells beginning in third postnatal week.

**White/Albino Rats and Their Abnormalities…**
- What abnormality is exhibited by the SHR rat?
  - Hypertension
  - Spontaneously Hypertensive Rat.
  - Develop hypertension spontaneously at 7-15 weeks.
Which rat strain/stock is an accepted genetic control animal for the SHR strain used in hypertension studies?

- BB
- LEW
- WKY
- SHR/SP
- DIO

References:

Godfrey, et al
Clinical Linoleic Acid Deficiency in Dahl Salt-Sensitive (SS/Jr) Rats
Comp Med 55(5): 470-475, 2005

Gunn Rats
- Model for Crigler-Najjar Syndrome
- Abnormality
  - Hyperbilirubinemia (unconjugated)
  - Jaundice
  - Lacks UDP Glucuronyl Transferase in Liver

What rat was developed from Sprague-Dawley stock selected for sodium chloride-induced hypertension?

- Dahl Salt Sensitive (DSS)
- Dahl Salt Resistant (DSR)

WAG/Rij Rat
- Abnormalities exhibited by the WAG/Rij rat
  - Retinal Degeneration
  - Retinitis Pigmentosa
  - Absence Epilepsy
- What albino rat is this strain developed from?
  - Wistar
BUF Rats
- Strain exhibits autoimmune thyroiditis?
- Autoimmune thyroiditis:
  - Spontaneous
  - After ingestion of 3-methylcolanthrene
  - 100% after neonatal thymectomy

Spotting Lethal Mutant
- Model for Hirschsprung Disease?
- Aganglionic Megacolon
- Recessive sl/sl (Chromosome 15)
- White coat with pigmented spot on head

The Zucker rat is a model for which of the following diseases or conditions?
- Obesity
- Type I diabetes
- Diabetes insipidus
- Prostate adenocarcinoma
- Jaundice

Reference:

What is the FBN?
- FBN
- F344
- F1 hybrid of F344 female and Brown Norway male (F1)

How do these animals differ with respect to auditory sensitivity?
- FBN approximately 20dB better hearing at higher frequency
- F344 approximately 20dB better hearing at lower frequency

Comparative Medicine 55(1)Feb2005;12-23
What type of rat is this?
- Brown Norway (BN)
- What type of renal disease is exhibited by BN rats?
  - Brown Norway Hydronephrosis
- What type of urinary tract disease does BN/Rij rats develop?
  - Spontaneous Ureter Tumors

Athymic Nude Rat
- Nude mutation: Spontaneous autosomal recessive mutation in the forkhead box N1 gene (Foxn1<sup>rnu</sup>) on chromosome 10
- mu/mu (homozygous), mu/+ (heterozygous)
- Hairless, failed development of the thymic anlage → lack of thymic T-cell development

What type of rat is this?
- Black Rat
- Genus and Species?
  - Rattus rattus

Tissue from an mu/mu rat with dyspnea

Histo – pulmonary alveoli distended with abundant foamy macrophages.

Causative agent?
- Pneumocystis carinii

What type of rat is this?
- Nude Rat
- What is the gene symbol for the nude rat?
  - mu/mu (Rowett Hooded)
  - mu<sup>nl</sup> (Albino New Zealand)
- mu/mu exhibit cyclic hair growth
- T-cell deficient

Tissue from an mu/mu rat with diarrhea

Describe lesions
- Inflammation of large and small intestine and hyperplasia of mesenteric lymph nodes

Causative agent?
- Always include Helicobacter sp with proliferative disease of large bowel
  - H. bilis
Athymic Nude Rat: Infectious Disease

- **Bacterial agents**
  - *Mycoplasma pulmonis* (bronchopneumonia with bronchiectasis, bacterial/viral coinfections common)
  - *Clostridium piliformis* (Tyzzer’s disease. Necrotizing typhlocolitis with dissemination to liver, lymph nodes, and heart. Intracytoplasmic groups of rod-shaped bacteria)
- **Viral agents**
  - Sendai virus (paramyxovirus. Proliferative interstitial pneumonia leads to mortality in Nude rats. Persistent carrier state)
- **Mycotic agents**
  - *Pneumocystis carinii*

Periarteritis Nodosa

- **Mesentery**
  - Medial hypertrophy
  - Inflammatory infiltrates around affected vessels
  - Inflammatory cells in perivascular fat (pleuritis)
  - Some arteries have fibrinoid degeneration and/or thrombi

What condition is depicted in these slides?

- Polyarteritis Nodosa (Periarteritis Nodosa): Mesenteric
  - Small-Med sized arteries become swollen and damaged
  - Immune-mediated disease
  - Mesenteric, pancreatic, spermatic arteries

What anesthetic combination was most frequently associated with corneal lesions in rats in a recent study?

- Ketamine and xylazine (60 mg/kg and 12 mg/kg)

What stocks or strains of rats were more likely to develop corneal lesions after anesthesia with ketamine/xylazine?

- Wistar and Fischer 344 were the most likely (F 344 was the worst). Long Evans were intermediate in likelihood, and SD and Lewis had low likelihood
**What condition is depicted?**

- Rat on the left is normal
- Rat on the right is abnormal
- Amelogenesis imperfecta
  - Underdevelopment of enamel-forming ameloblasts
  - Inherited through autosomal single recessive gene (ami)

**What condition is depicted?**

- Malocclusion

**What are some clinical signs of urolithiasis in rats?**

- Hematuria, red-stained bedding, red-stained or wet pelage, sensitivity to touch in the abdominal area, swollen or palpable kidneys or bladder, unkempt fur, anorexia, reduced urination, reduced water intake, unexpected weight gain or loss

**Diet-induced urolithiasis**

- Rats fed basal diet substituted with choline chloride failed to develop urinary calculi.
- Rats fed basal diet containing FCC grade of choline bitartrate developed urinary calculi.
- Rats fed basal diet containing USP grade choline bitartrate failed to develop urinary calculi.
- P-glycoprotein inhibitor treated rats had significantly reduced risk of developing urinary calculi.

Trichosomoides crassicauda

- Females in urinary bladder, ureters and renal pelvis.
- Small male worms live and are parasitic in the uterus or vagina of the adult female.
- Eggs passed in urine, infection by ingestion of embryonated egg. Eggs hatch in stomach, larvae enter bloodstream then to urinary tract.
- Primary infection from dams to pups prior to weaning.
- Proliferative changes in mucosa – neoplasia?

Pituitary Tumor

- Pituitary Chromophobe Adenoma
  - Vast majority of pituitary tumors in rats
  - May have prominent hemorrhagic areas
  - Generally well circumscribed and compress adjacent brain tissue (head tilt?)
  - Females>Males

What condition is depicted in this slide?

- Renal Aplasia
- What rat strains exhibit renal abnormalities?
  - ACI – often lacks right kidney
  - BN – hydronephrosis
  - BN/BiRij – bladder and ureter tumors

Chronic Progressive Nephropathy

- Gross description
  - Small pale firm kidneys
  - Rough or pitted surface
  - Numerous small foci/cysts
- Interstitial inflammation
- Thickened bm
- Glomerular changes
- Proteinaceous casts

What is depicted?

- Benign fibroadenoma
  - Most common tumor of rats
  - Can occur anywhere there is mammary tissue
  - Hyperplastic fibrous and glandular tissue

Major age-associated lesions in rats

- Neoplasms (mammary, pituitary, adrenal)
- Chronic progressive nephropathy
- Myocardial degeneration
- Polyradiculoneuropathy
Name the tissue and give the histological description…

- **Tissue**
  - Lumbosacral spinal nerve root
- **Histological description**
  - Nerve root on the right appears normal
  - Nerve root on the left severe degeneration
  - Cholesterol clefts

What is the most common testicular tumor in rats?

- **Interstitial Cell Tumor**
  - Although these tumors may become very large, most appear benign
  - What strain has high spontaneous occurrence of this tumor type?
  - F344

What structure and condition are depicted?

- **Structure**
  - Zymbal’s Gland
- **Zymbal’s Gland Tumor**
  - Usually present as an ulcerated mass within or just below the external ear canal

What condition is depicted?

- Cutaneous nodular lesion (chest, back, tail)
- Crusted, thickened surface
- Keratoacanthoma

Zymbal’s Glands

- Modified sebaceous glands
- Surround external ear canal
- Tumors can be induced by a variety of carcinogenic compounds
- Tumors may be either sebaceous or squamous or admixtures

What diagnostic technique is depicted?

- **Nasal Swab**
  - What type of swab is used?
    - Calgi (calcium alginate) swab
  - CAR Bacillus PCR done on nasal swab
  - Advantage – can detect infection in younger rats (>=3wks) before colonization of lung occurs
Differential Diagnosis?
- Mycoplasma pulmonis
- CAR bacillus
- Corynebacterium kutscheri

Mycoplasma pulmonis
- What media is used to culture this organism?
  - Dutch Agar
  - PPLO (Pleuropneumonia-like Organisms) broth
  - Hayflecks, SP4
- In what other sites can this organism be found?
  - Genital tract (uterus, ovary, oviduct)

What type of stain?
- Silver stain
- Bacteria densely clustered at ciliated surface of respiratory epithelium
- Organisms
  - Gram negative
  - Motile
  - Non-spore forming
  - Gliding bacteria

What is this mite?
- Tropical Rat Mite
- Genus and Species?
  - Ornithonyssus bacoti
- What diseases can be carried by this mite?
  - Murine Typhus
  - Rickettsial Diseases
  - Q Fever
  - Plague

What organism is this?
- Hint: Gram + Rods (Chinese letters)
- Corynebacterium kutscheri
- Hematogenous spread
- Where do you culture for this organism?
  - Oral cavity (carry here)
  - Cervical LN
  - Liver ?
  - Brain ?
  - Kidney ?

Which of the following is a characteristic of *Ornithonyssus bacoti*, the tropical rat mite?
- The mite has a life cycle of 28 days from egg to egg production
- The protonymph stage has been shown to survive for 43 days without food
- The principle host is *Mus musculus*
- A single female can lay as many as 1400 eggs during its 90-day lifecycle
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Other Rat Mites...

- Genus and Species of the Spiny Rat Mite?
  - Laelaps echidninus
- What is this mite a vector for?
  - Hepatozoon muris
- Genus and Species for Ear Mange Mite?
  - Notoedres muris

What condition is depicted?

- View of tympanic bullae
- Right and left bullae appear to have whitish material in them.
- Otitis media
- Causative agent?
  - M. pulmonis
  - P. pneumotropica

Which of the following neoplasms represents the majority of mammary tumors described in older rats?

- Endothelioma
- Adenocarcinoma
- Lymphosarcoma
- Fibroadenoma
- Histiocytoma

Double Operculated Eggs

- Dioctophyma renale
- Anatrichosoma
- Capillaria
- Trichuris
- Prosthenorchis
- Trichosomoides
Single Operculated Eggs
- Fasciola
- Paragonimus
- Diphylobothrium
- Athesmia
- Gastrodiscoides

What conditions are depicted?
- Top photo
  - Squinting
  - Photophobia
  - Cervical swelling
- Bottom photo
  - Exophthalmos
  - Cloudy cornea

Name the tissue and give the histological description...
- Tissue
  - Liver
- Histological description
  - Diffuse sinusoidal infiltrate, large mononuclear cells
  - Basophilic polyhedral cells
  - Prominent nucleus

Name the tissue and give the histological description...
- Tissue
  - Glandular tissue
    - Parotid, Harderian, Submandibular, Exorbital Laceral
- Histological description
  - Inflammatory infiltrate
  - Interstitial edema
  - Necrosis of ducts
  - Squamous metaplasia

Diagnosis?
- Large Granular Lymphocytic Leukemia
- Formerly called Mononuclear Cell Leukemia/Fischer Rat Leukemia
- Very common in aged F344
- Can involve virtually any organ in the body (commonly splenomegaly, hepatomegaly, lymphadenopathy)
- Large granule lymphocytes are of NK cell origin

Diagnosis?
- Sialodacryoadenitis virus (SDAV)
- Coronavirus
- Affects serous or mixed glands
- Which salivary gland spared?
- Sublingual salivary gland
- Morbidity high, mortality low
- Animals infected and immune within 3-5 weeks
- Stop breeding for 6-8 wks
Rat bite fever can be caused by *Streptobacillus moniliformis* or what other organism?

- Bartonella henselae
- Spirillum minus
- Staphylococcus aureus
- Brucella rattus
- Pasturella multocida

**Tyzzer’s Disease**

- Clostridium piliforme
- Flaccid dilatation especially ileum
- (“Triad”) - hepatitis, myocarditis, enteritis
- Fecal-oral transmission by spores
- Demonstration of intracellular organism in tissues: Steiner/Warthin-Starry, Giemsa and Methylene Blue.

**Choral Hydrate**

- Hypnotic
- Short acting light anesthesia
- Poor analgesia
- Irritating to peritoneum
- Causes adynamic ileus in rats when given IP

**What condition is depicted?**

- Abdominal swelling
- Rule outs:
  - Ascites
  - Organomegaly
  - Neoplasia
  - Adynamic ileus (megacolon)
- What are possible causes of megacolon?
  - Tyzzer’s Disease
  - Choral hydrate

**Give the gross description...**

- Gross:
  - Fibropurulent pleuritis, pericarditis, and pneumonia
- May also see:
  - Suppurative arthritis
  - Meningitis
  - Splenitis
  - Orchitis
  - Infarcts
  - Spleen
  - Testes
- Diagnosis?
Streptococcus pneumoniae

- Histopathology
  - Characteristic organisms
  - Tissue impression smear gram stain (grams + in pairs)
- Culture:
  - Blood agar (10% CO₂)
  - Optochin inhibition (ethylhydrocupreine)
  - Quellung reaction
  - Capsular types 2, 3, 19

All of the following blood vessels can be used for intravenous injections in the rat EXCEPT?

- Dorsal metatarsal vein
- Sublingual vein
- Brachial vein
- Penile vein
- Lateral tail vein

Reference: