Rabbits

Workshop in Laboratory Animal Medicine

7 -10 May 2009

The Charles Louis Davis, D.V.M. Foundation
College of Veterinary Medicine
North Carolina State University
Raleigh, North Carolina

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Rabbits
Questions, Answers, & Word Slides

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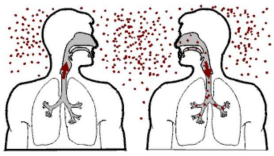
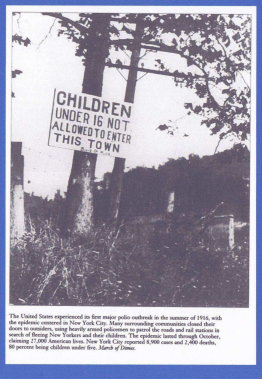
- Clinical Chemistry, Hematology
- Comprehensive Health Monitoring
- Cell line Screening
- Genotyping
- GLP Health Monitoring

Symposium originally referred to a drinking party (the Greek verb *sympotein* means "to drink together") but has since come to refer to any academic conference, whether or not drinking takes place. The sympotic elegies of Theognis of Megara and two Socratic dialogues, Plato's *Symposium* and Xenophon's *Symposium* all describe symposia in the original sense.



Polio Epidemic 1916

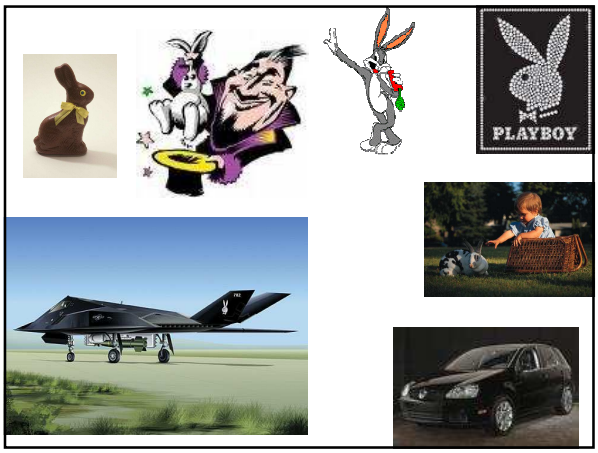


The United States experienced its first major polio outbreak in the summer of 1916, with the epidemic centered in New York City. Many surrounding communities closed their doors to outsiders, using heavily armed policemen to patrol the roads and rail stations in search of fleeing New Yorkers and their children. The epidemic lasted through October, claiming 27,000 American lives. New York City reported 8,900 cases and 2,400 deaths, 80 percent being children under five. *March of Dimes*.

178

Policies developed after the fact often caused more harm than good.

172



Laboratory Rabbit

Phylum

Class

Order

Family

Genus

Species

Phylum Chordata

Class Mammalia

Order Lagomorpha

Family Leporidae

Genus *Oryctolagus*

Species *cuniculus*

only domesticated rabbit

Rabbits are used for

- Exhibition
- Pet
- Hunting
- ?
- ?
- ?
- ?

Rabbits are used for

- Exhibition
- Pet
- Hunting

- Meat
- Fur
- Hair (Angora)
- Laboratory Animals
- Pests

updated 4:08 p.m. ET, Mon., April. 16, 2007
BUDAPEST, Hungary - A **truck carrying 5,000 rabbits to a slaughterhouse** overturned on a major Hungarian highway Monday, sending authorities on a hopping big bunny hunt.
The M1 highway, the main connection between the capitals of Hungary and Austria, was closed for several hours in the morning while authorities tried to recapture them, Highway Patrol spokeswoman Viktoria Galik said. The road reopened in the afternoon.

Number of Rabbits Used in Research

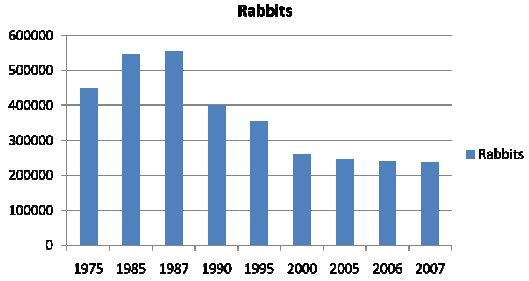
USDA Annual Reports

Going up or going down??

Approximate Numbers used in research per year??

Number of Rabbits Used in Research

USDA Annual Reports



Genus and species – Lab Rabbit

***O*_____ *c*_____**

Rapidly increase in numbers due to its prolific breeding pattern

Breeding all year but primarily happens between January and June

Below TRUE or FALSE

??Rabbits are monogamous

??The males role in upbringing of the young

Rabbit *Oryctolagus cuniculi*

Rapidly increase numbers due to its prolific breeding pattern

Breeding all year but primarily happens between January and June.

Rabbits are not monogamous
the males (bucks) mating with several females (does).

The males have nothing to do with the upbringing of the young.

Social animals live in colonial warrens.
Each warren is comprised of many tunnels providing a means of escape if a predator attacks

Babies born underground and after four weeks they emerge

Rabbit Reproduction

• Male breeding	age ?? mths [wt 4 kg]
• Female breeding	age ?? mths [wt 4 kg]
• **Estrus**	**??**
• **Time of ovulation**	**??**
• Gestation	?? days
• Weaning age	??
• Litter average	??
• Mating system	??
• Hours Light/Dark	??

Rabbit Reproduction

• Male breeding	age 5-7 mths [wt 4 kg]
▪ Female breeding	age 5-6 mths [wt 4 kg]
• Estrus	continuous
▪ Time of ovulation	10-11 hours after copulation • Ovulation induced by mating
• Gestation	30-32 days
▪ Weaning age	8 wks down to 4 wks [1.5kg]
▪ Birth Weight	50-60 g
• Litter average	7-8 [1-18 range]
• First estrus after parturition	35 days
▪ Breeding life span	1-3 years
▪ Mating system	pair mate in male's cage
• Number of females per male	9/1 to 4/1
▪ Hours light / Hours Dark	14 hours light/10 hours dark

Rabbit *Oryctolagus cuniculi*

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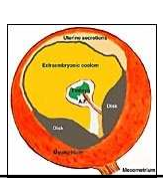
RABBITS

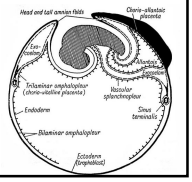
ovulation occurs about 10 hours after coitus -- reflex ovulator (Fox & Laird, 1970)

The tubal transport of fertilized ova and blastocysts was studied by Tsutsumi et al. (1975).

The average placental weight is 4 g (Mårtensson, 1984)
"uterus duplex" (completely separate horns)
placental implantation is superficial with inversion of the yolk sac (Mossman, 1987)
discoid, labyrinthine, chorio-allantoic placenta, with a hemodichorial feto-maternal interface



27 day-old Fetus



Karyotypes of male and female domestic rabbits

44 chromosomes (Nichols et al., 1965; Ray & Williams, 1966; Issa et al., 1968; Hsu & Benirschke, 1967

Hares and other *Lagomorpha* have higher chromosome numbers (Dave et al., 1965; Stock, 1976). Hageltorn & Gustavsson (1979)

"sex chromatin" or "Barr body" is evident in fibroblasts (Melander, 1962; Hulliger et al., 1963)

early sex determination of blastocysts was thus accomplished by Edwards & Gardner (1967).

Spontaneous hybrids between the domestic rabbit and other leporids have not been described. Chang et al. (1964)
Artificial insemination of rabbits with semen of the snowshoe hare (*Lepus americanus*) yielded some fertilized ova, but almost all degenerated before implantation

Rabbit Breeding Notes

Post partum breeding	@ 12 to 14 days
Receptivity	
Breeder female weight	8-10 lbs
	Females are 7 – 8 lbs at 4 months of age.
Females are retired	
Light cycle	16 hours per day light with 8 hours dark.
Pseudopregnancy	**16 to 18 days**
Does will not be receptive during this period.	

Pregnancy can be determined at 8 days using ultrasound versus 14 – 15 days by palpation. Radiographs day 11

•

Rabbit Breeding Notes

Post partum breeding @ 12 to 14 days

Receptivity 85+%

Breeder female weight 8-10 lbs

Females are 7 – 8 lbs at 4 months of age.

Females are retired after 3 *No Pregnancies*

or if reduced litter size [9 versus 5 etc]

Light cycle 16 hours per day light with 8 hours dark. Erratic lighting is a common cause of reproductive difficulties

Pseudopregnancy **16 to 18 days**

Does will not be receptive during this period.

Pseudopregnancy caused by infertile matings and mounting by other females.

Pregnancy can be determined at 8 days using ultrasound versus 14 – 15 days by palpation. Radiographs day 11

•

Rabbits

Adult weight	Lbs	2-12
Birth	Grams	60-65
Eyes open	Days	??
Eat solid food	Days	18-23
Litter size		1-18 (8)
Life span	Years	13 (5)
Daily water	ml/kg	60-250
Daily urine	ml/kg	50-75

Rabbits

Adult weight	Lbs	2-12
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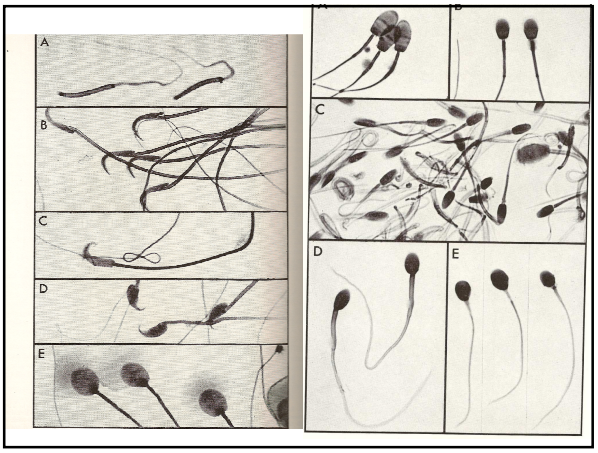


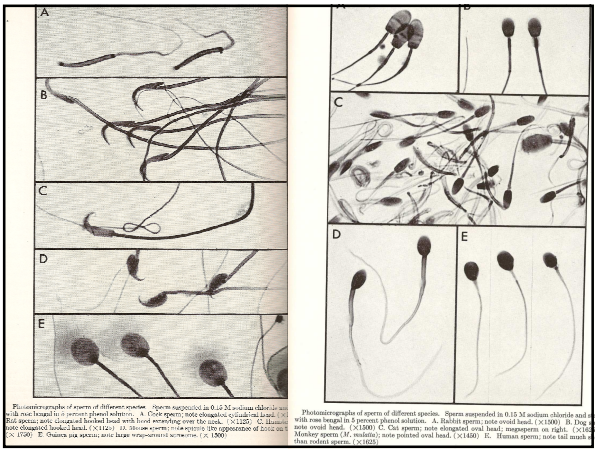
Sperm Identification

- A cock
- B rat
- C hamster
- D mouse
- E guinea pig

- A rabbit
- B dog
- C cat
- D monkey
- E human

Hafez 1970

Reproduction and Breeding Techniques for Laboratory Animals



Rabbit Pest mid 1800s **and also**

hares	foxes	blow flies	house flies
rats	fish	redfin	carp
cats	deer	pigs	horses
donkeys	goats	mice	
sparrows	starlings	Indian mynas	

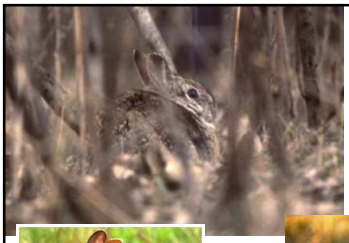
Barwon Park

Rabbits Australia

Thomas Austin imported rabbits in 1859
Responsible, in the eyes of many, for Australia's rabbit problems

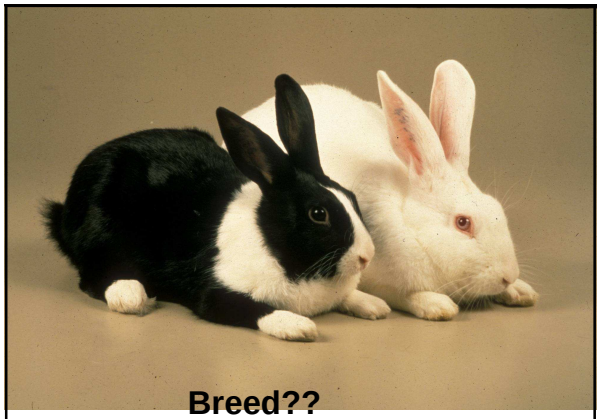
However, rabbits arrived at Port Jackson with the First Fleet in 1788
By the late 1820s they were becoming a pest in Van Diemen's Land

1890 New South Wales – estimate 600,000,000

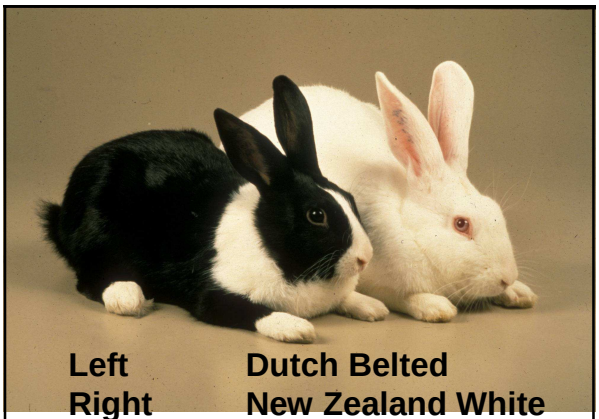


In USA
Genus ??

Genus
Sylvilagus



Breed??



Left **Dutch Belted**
Right **New Zealand White**

NZW Rabbit is Albino

- Inherited disorder of melanin metabolism
- Caused by the lack of the enzyme tyrosinase,
- Impacts melanocytes and neurons.
- Neuronal morphological abnormalities and functional impairments involve:
  - Medial vestibular nucleus
  - Cochlear nuclei
  - Retina

Comparison of albino and pigmented animals have shown differences in pharmacotoxic kinetics

Breed??

How old is this rabbit??

New born – less than 36 hours

Rabbit Milk %

	Water	Protein	Lactose	Fat	Ash
Rabbit *colostrum*	68	?	?	?	1.6
14 days	74	?	?	?	2.2
30 days	63	?	?	?	2.8
Cow	87	**3.5**	4.8	**4.0**	0.7
Goat	87	**3.7**	4.2	**4.1**	0.9
Pig	84	**4.9**	5.3	**5.3**	0.8
Human	87	**1.2**	6.9	**4.6**	0.2

Rabbit Milk %

	Water	Protein	Lactose	Fat	Ash
Rabbit *colostrum*	68	**13.5**	1.6	**14.7**	1.6
14 days	74	**13.4**	1.0	**9.0**	2.2
30 days	63	**16.9**	0.2	**17.5**	2.8
Cow	87	**3.5**	4.8	**4.0**	0.7
Goat	87	**3.7**	4.2	**4.1**	0.9
Pig	84	**4.9**	5.3	**5.3**	0.8
Human	87	**1.2**	6.9	**4.6**	0.2

7 days

3 weeks

Female with newborn

Species? ?

Lepus

Feed Consumption [grams / day]

- Doe
- Buck
- Doe Pregnant
  - 14 days
  - 30 days
- Doe with 21 day old litter
- Future breeding stock

Feed Consumption [grams / day]

- Doe
- Buck
- Doe Pregnant
  - 14 days
  - 30 days
- Doe with 21 day old litter
- Future breeding stock

- 100-120
- 100-120
- 140
- 700
- 1500
- No more than 120

Nutrition Related Diseases Rabbits

- Enteritis / Enterotoxemia
- Enteritis
- Enteritis
- Low Fiber Diets
- Kidney lesions

High energy diets *Clostridium spiroforme*

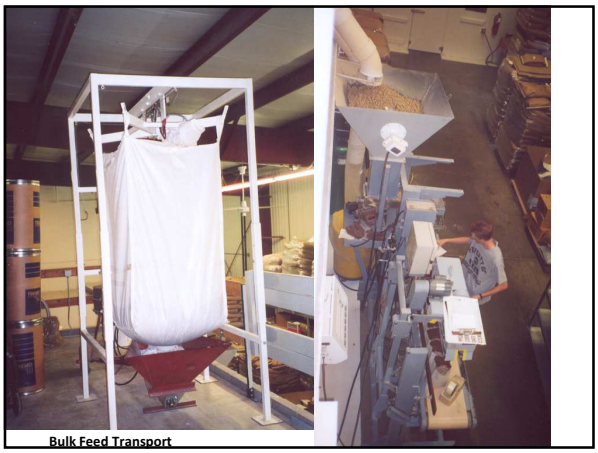
- Dietary protein
- Increased gut pH
- Fur chewing
  Trichobezoars / gut motility
- Calcium
  Alfalfa high in Ca

Nutrition Related Diseases Rabbits

- Vitamin D
- Vitamin A
- Milk fever
- Osteomalacia
- Mycotoxins
- Prevent Obesity
- Require high water intake

- toxicity due to manufacture
- deficiency and toxicity
- low Ca and low P
- low Vitamin D due to high Phosphorus

What is this equipment used for??

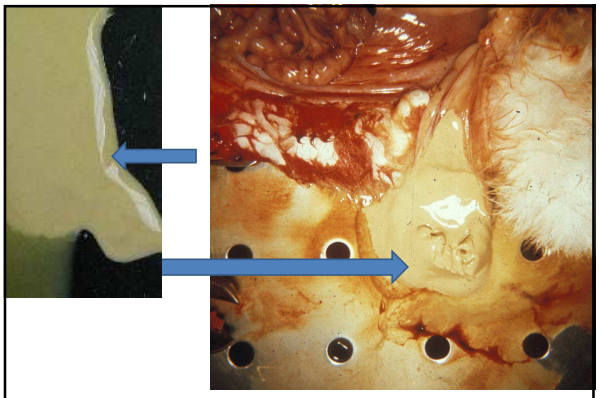


Bulk Feed Transport

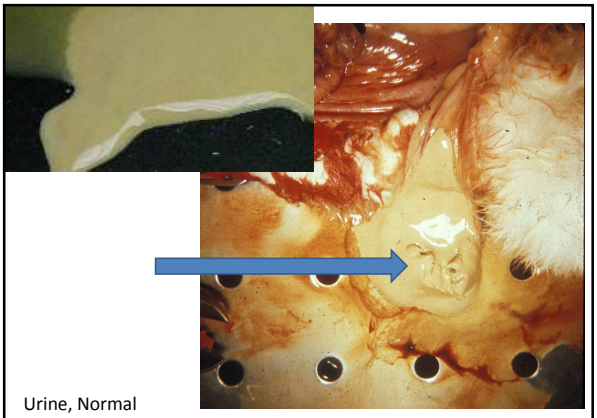
Truths children have learned

You can't trust dogs to watch your food

You can't hide a piece of broccoli in a glass of milk

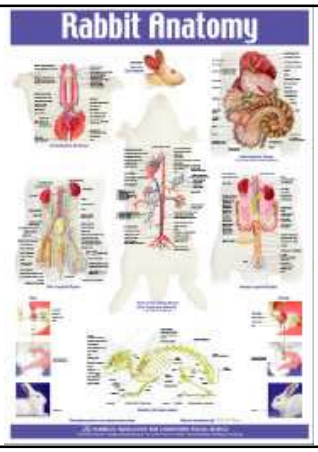


Identify the material? Normal or Abnormal?



Urine, Normal

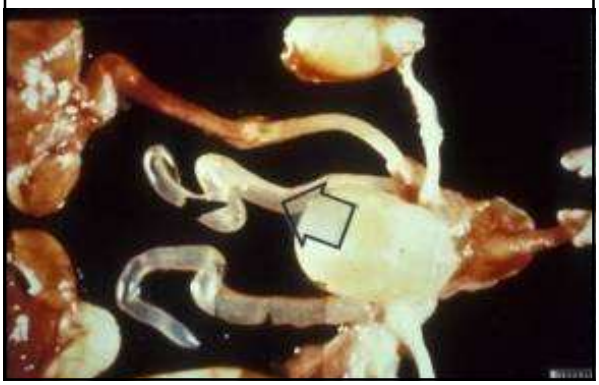
Ammonium magnesium phosphate and calcium carbonate monohydrate



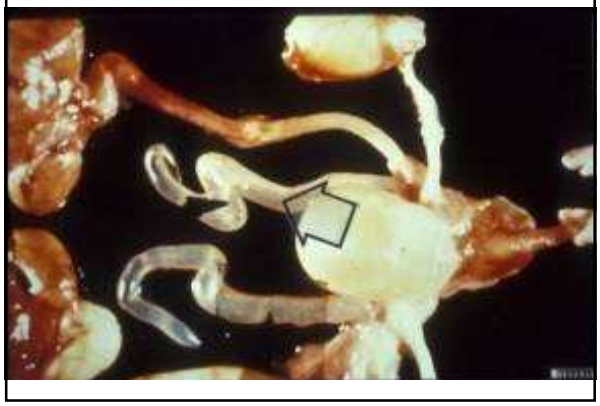
The art of being wise is the art of knowing what to overlook

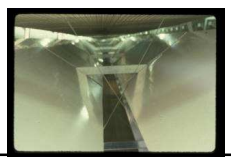
William James

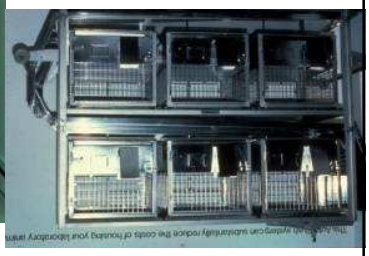
Arrow pointing to ?? Genus? Sex?



Seminal Vesicles Cavia Male







Objects used in the socialization program include:

- Stainless steel rattlers (Bio-Serv, Frenchtown, NJ, USA)
- Wiffle balls (various manufacturers, USA)
- Dumbbells (Bio-Serv, Frenchtown, NJ, USA)
- Plastic balls with stainless steel washers ("Jingle Balls," Bio-Serv, Frenchtown, NJ, USA)
- Fruit-flavored treats ("Fruity-Gems," Bio-Serv, Frenchtown, NJ, USA)
- Latex gloves (various manufacturers, USA)
- Plastic restrainers (Tecniplast, Exton, PA, USA)
- Plastic transport carts (Rubbermaid, USA)
- Scale (American Weight Systems, Mountville, PA, USA)
- Transport boxes (Timbar, Hanover/New Oxford, PA. USA)

Socialization of Dutch Belted Rabbits for Use in Research
Pritt et al Covance Research Products
Tech Talk August 2008

techtalk
The Newsletter for Laboratory Animal Science Technicians

Socialization of Dutch Belted Rabbits for Use in Research

Rabbit Enrichment and Exercise Program
By Cheryl Grieve, Principle Animal Research Technician, Medtronic PRL

During the first month of housing at our facility, the rabbits exhibited agitated behaviors, including circling their cage when someone enters the room and stomping, jumping, and kicking cage walls. We instituted an enrichment and exercise program to condition these single-housed rabbits to their new surroundings. The program allows the rabbits to express natural behaviors such as chewing, foraging, hopping, standing, tossing, and digging.

During quarantine, rabbits receive music as enrichment; they are accustomed to it from the vendor. The music is continued after quarantine, and other enrichment processes are introduced. Palatable treats are offered to express chewing and foraging activities; treats include Jell-O® (the rabbits prefer raspberry), timothy cubes and hay, and sweet treats. The Jell-O is used as a conditioning treat. The timothy hay is incorporated into their daily diet, in addition to a pelleted rabbit food, to mimic natural foraging behavior and promote a healthy gastrointestinal tract.

Our exercise program achieves the natural behaviors of hopping and standing by allowing rabbits to move freely in a portable folding exercise pen (16 sq ft) for a minimum of 2 hours per week. In the wild, rabbits toss sticks and twigs. We offer a variety of hard plastic toys that the rabbits can toss in the exercise pen and their cages. The exercise pen also contains a box filled with aspen shavings, which allows the rabbits to exhibit their natural digging behavior.

After 30 days of the program, our rabbits' agitated behaviors were greatly reduced. The advantages of a well-conducted rabbit enrichment and exercise curriculum are to promote inherent behaviors, alleviate boredom and stress, and allow easier handling. Not only are these positive changes good for the animal care staff, but they are beneficial to the studies.

EDITOR
COMMIT
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Wayne State Uni
Div of Laboratory Animal Re
Samm Bartee, R
University of Ar
at Berm

Novel food items as environmental enrichment for rodents and rabbits

Lab Animal V38, Apr 09

Cyndi Brown, DVM, Dip ABVP Avian

This column discusses the use of novel dietary supplements as environmental enrichment for rodents and rabbits. The purpose of enrichment is to encourage behaviors that are appropriate for a particular species and that satisfy an animal's physical and psychological needs.

The Animal Welfare Act[1] currently contains no environmental enrichment provisions for laboratory-reared rats and mice, nor does it explicitly mandate environmental enrichment standards for other rodents. Science, however, has an ethical responsibility to house animals according to their species-specific needs, which invokes the concept of behavioral and environmental enrichment.

In the *Guide for the Care and Use of Laboratory Animals*[2], natural behaviors are highlighted as a crucial measure of access for an animal program. The *Guide* also suggests that animals should be housed in "natural environments," and an entire section on behavioral management is devoted to consideration of animals' structural, social and activity needs. In addition to social activities, enrichment can be achieved by allowing and promoting

FIGURE 1 | A variety of novel food items used for enrichment with rodents and rabbits. 1, Nutri-Forage cakes in their original form before warming and forming or compressing. 2, Nutri-Forage cakes compressed into holes in a wooden block. 3, Nutri-Forage cake compressed into a pasta shell. 4, Nutriberries. 5, Freeze-dried strawberry pieces. 6, Freeze-dried banana pieces. 7, Timothy Treats. 8, Veggie Treats.

Rats gnaw on wood blocks especially when they are housed without bedding[6,7]. Hamsters and gerbils[8] routinely store food and should be provided with food inside the cage in an attempt to provide them with as natural of an environment as possible for their well-being.

Restrictions on enrichment

Some research studies may have diet restrictions that preclude the use of certain food items as environmental enrichment. Toxicology studies may not permit the use of glues or dyes, which may exist within the novel food items, and behavioral studies often have absolute contraindication to enrichment. Other studies may have a limited scope of permissible enrichment (e.g., food treats are not acceptable for nutrition studies). It is also important to note that GLP

Physiology and Clinical Pathology

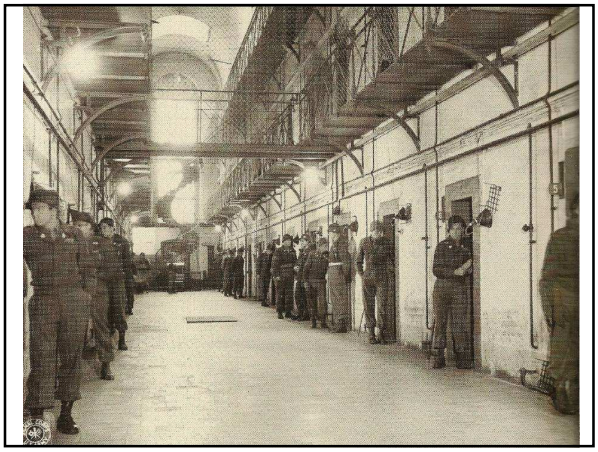
Housed Individually and in Groups

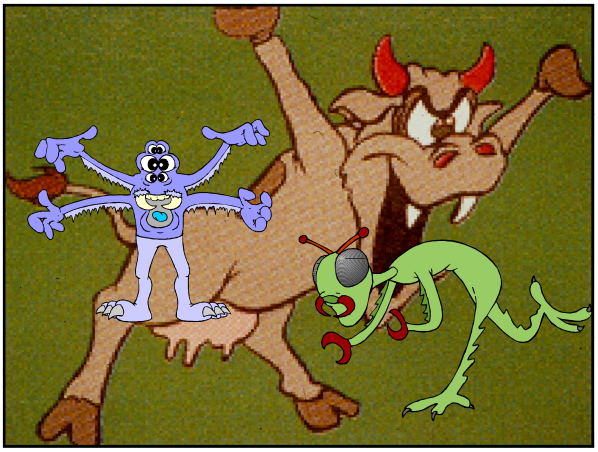
Fuentes, GC.; Newgren, J 2008

- Serum chemistries, antibody production, physiologic plasma cortisol levels, and white blood cell (WBC) counts
- Female New Zealand White rabbits
- Control group was housed individually in stainless steel cages
- Group-housed on aspen shavings
- Plastic crates were placed in the group-housing area to provide opportunities for rabbits to hide, and a litter box was available at all times.
- Both groups received the same food and water and similar environmental enrichment devices.
- **Group-housed animals had lower WBC counts and higher levels of plasma cortisol**
- **Group-housed animals had significantly less weight gain during the first week.**
- **Antibody production did not differ between the 2 groups.**

A rabbit research program was established in 1994 at Texas A&M University-Kingsville.

AMERICAN BRANCH - WORLD RABBIT SCIENCE ASSOCIATION

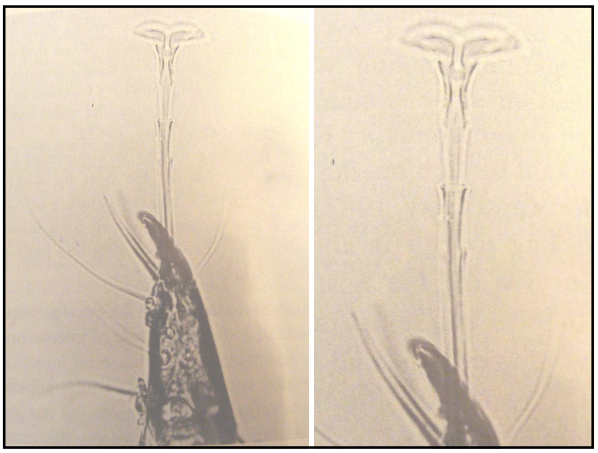


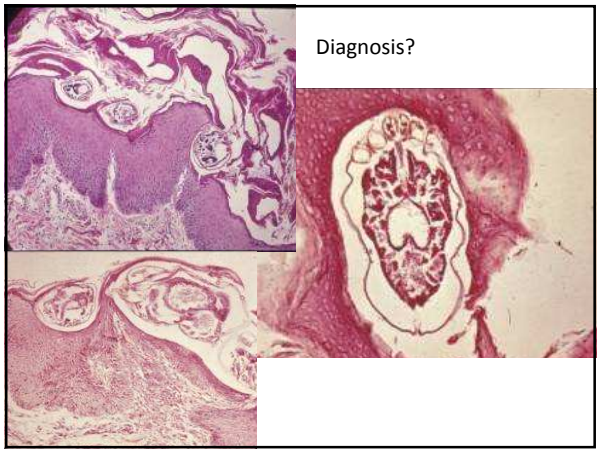


Most likely diagnosis?

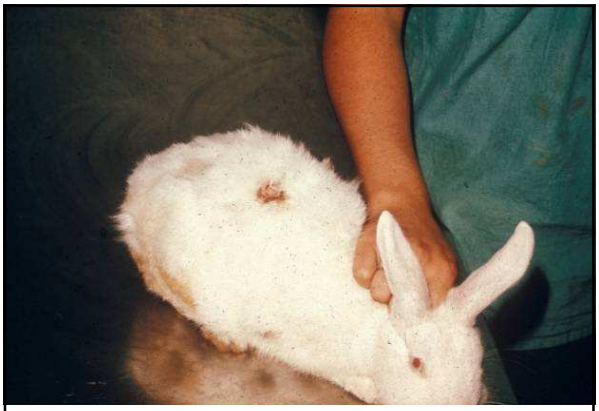
Genus and Species? Sex on right?

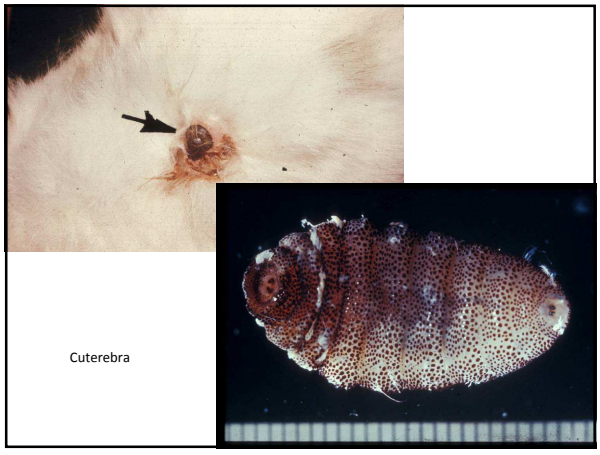
What are the two mites on the left doing?



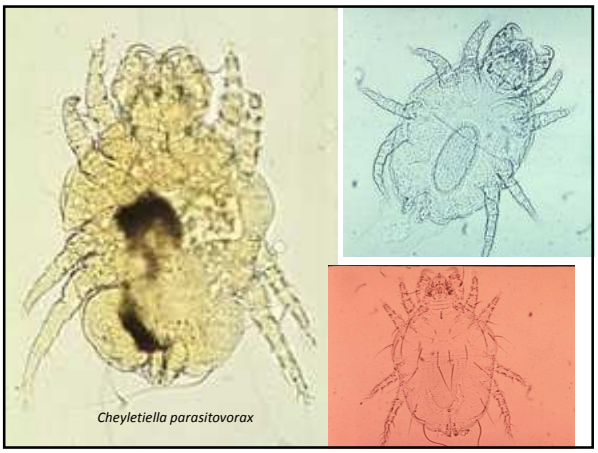


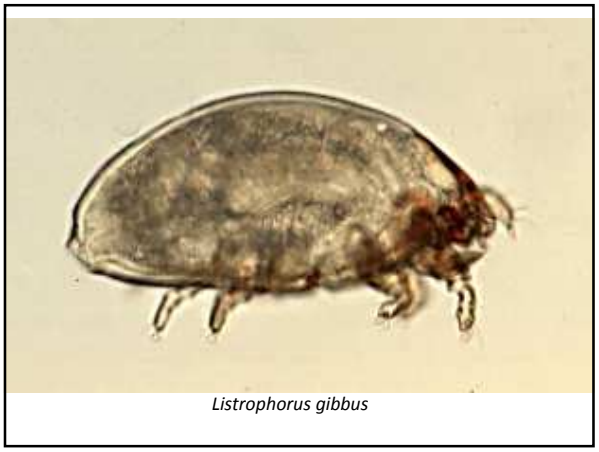
Diagnosis?

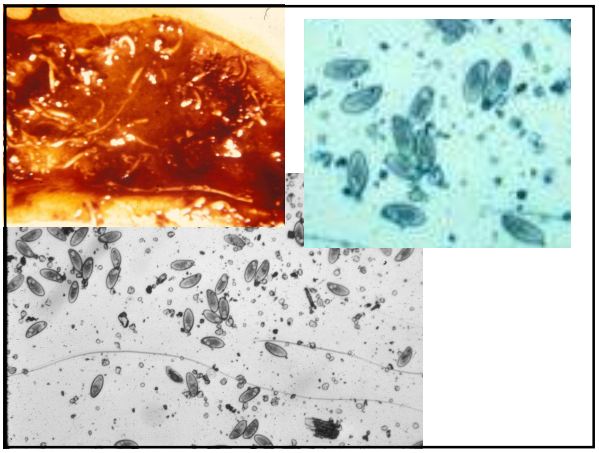




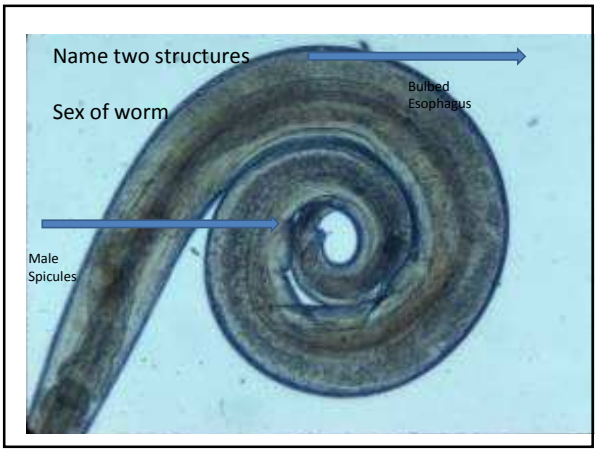
Cuterebra


Cheyletiella parasitovorax


Listrophorus gibbus

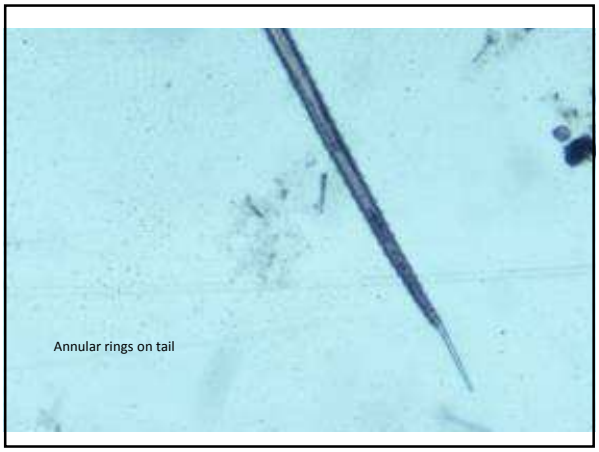



Diagnosis

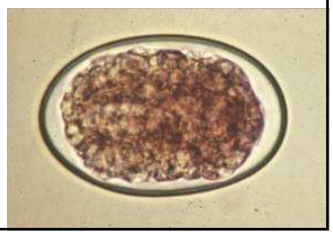

Name two structures
Sex of worm
Bulbed Esophagus
Male Spicules

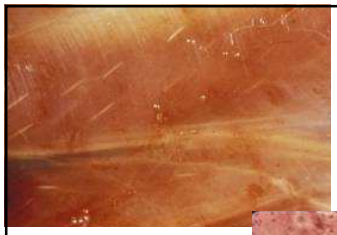
Rabbit Pinworms

- *Passalurus ambiguous*
- *Passalurus nonannulatus*
- *Dermatoxys veligera*

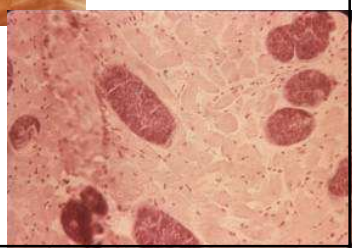


Obeliscoides





Diagnosis

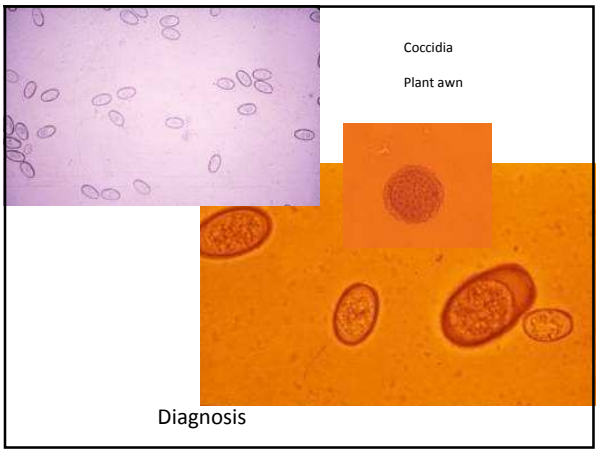


Sarcocystis

- Intermediate stage in rabbits
- Definitive host is the cat
- Transmission by fleas has been suggested
- Usually asymptomatic but lameness has been reported

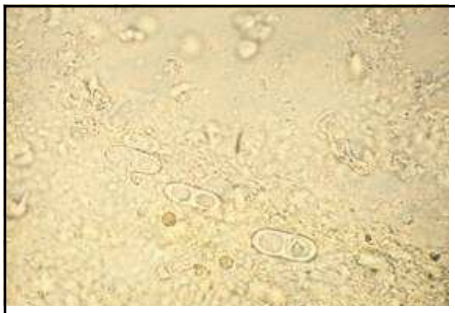
Truths children have learned

No matter how hard you try, you can't baptize cats



Diagnosis

Diagnosis



Cyniclomyces guttulatus

[Saccharomycopsis guttulatus]

Hersey-Bonner Lab Animal Aug 2008

FIGURE 1 | A sample of dark brown-black, semi-solid fetid feces produced by a 2.5-year-old, intact, white female rabbit.

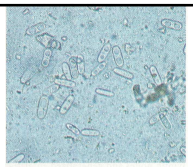


FIGURE 2 | Numerous unicellular organisms obtained from the feces shown in **Figure 1.** The organism was isolated using a Fecalyzer. The flotation sample is unstained and observed with a slight closure of the diaphragm to provide

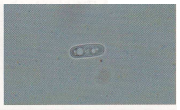


FIGURE 3 | *Cyniclomyces guttulatus* at 400× magnification obtained from the feces of a healthy rabbit.

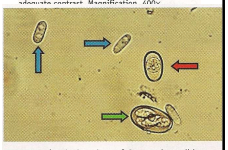
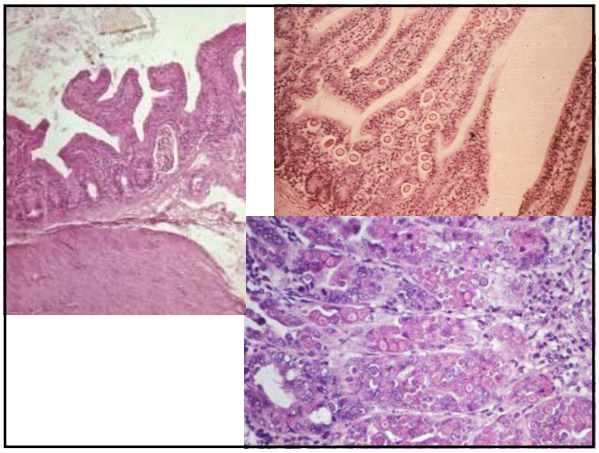
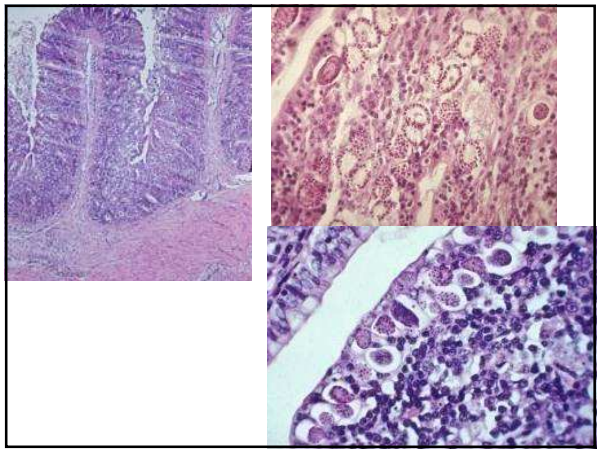
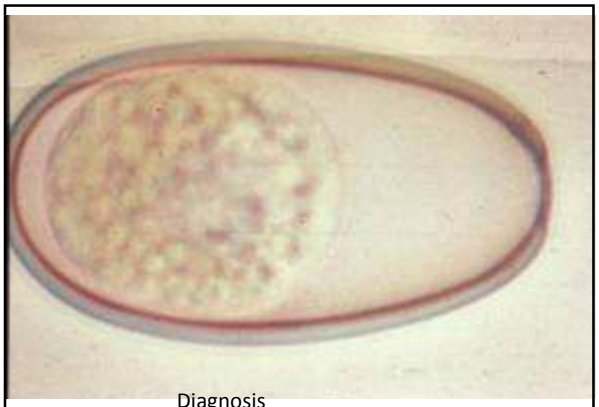


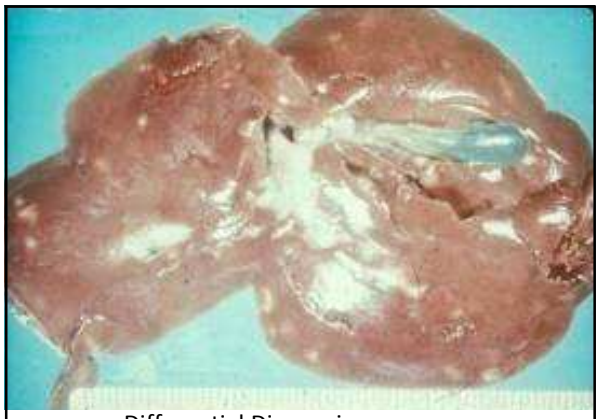
FIGURE 4 | Relative sizes of *C. guttulatus* (blue arrows), a coccidian oocyst (*Eimeria* spp.; red arrow) and a nematode egg (green arrow) obtained from rabbit feces. *C. guttulatus* are smaller than coccidian oocysts and nematode eggs.



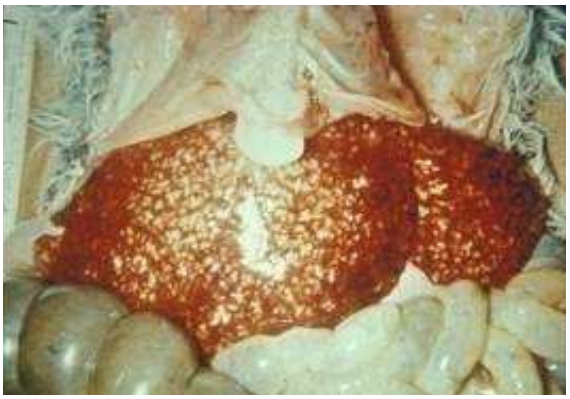


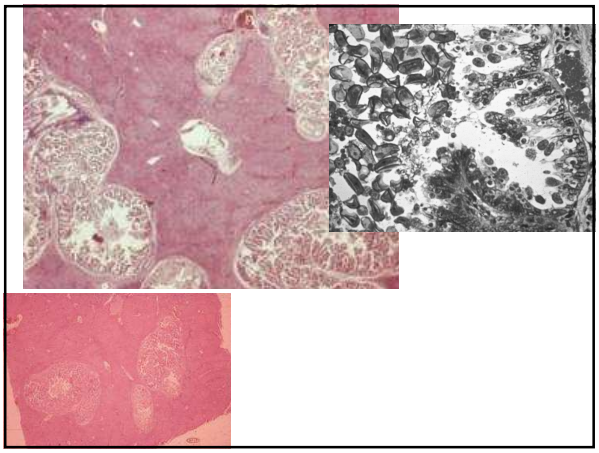


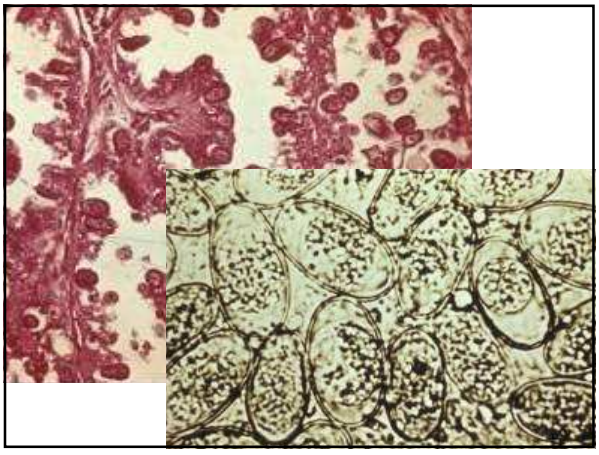
Diagnosis



Differential Diagnosis

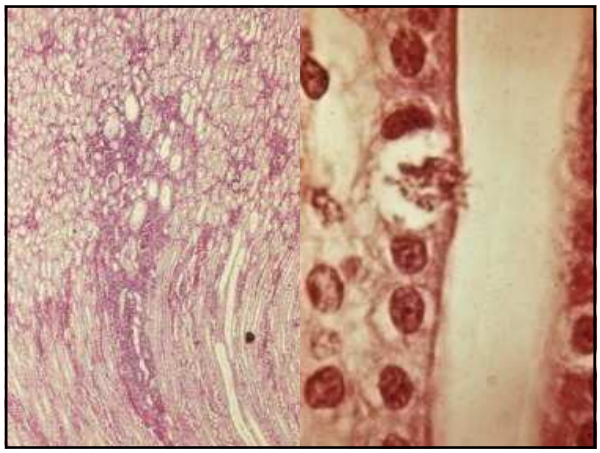


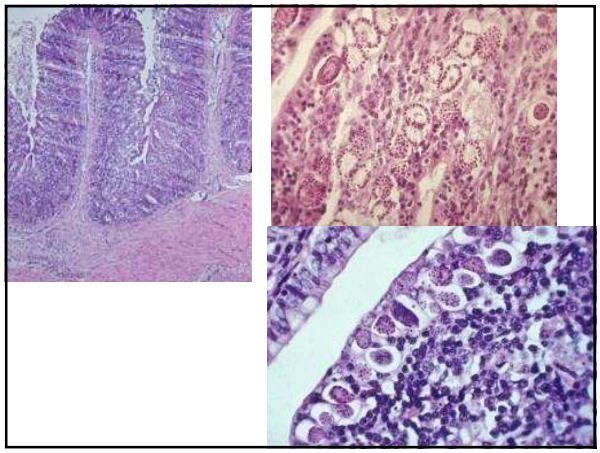


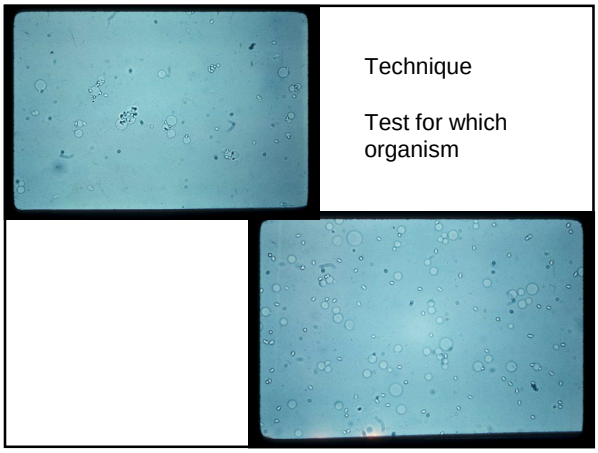


Rabbit Coccidiosis

- Liver
  - *Eimeria steidae*
- Intestinal
  - *E intestinalis*
  - *E flavescens*
  - *E irresidua*
  - *E magna*
  - *E media*
  - *E neoleporis*
  - *E perforans*
  - *E piriformis*

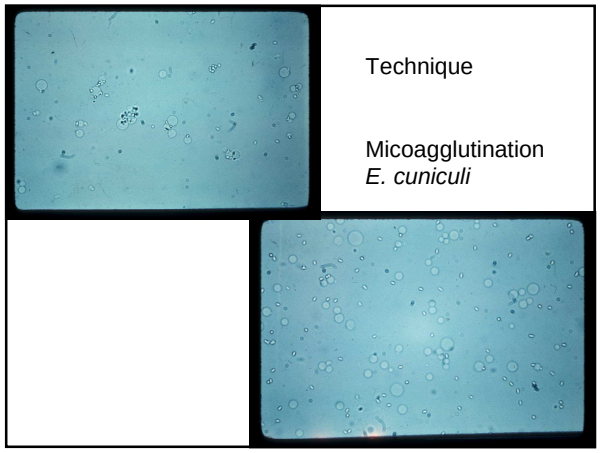






Technique

Test for which organism

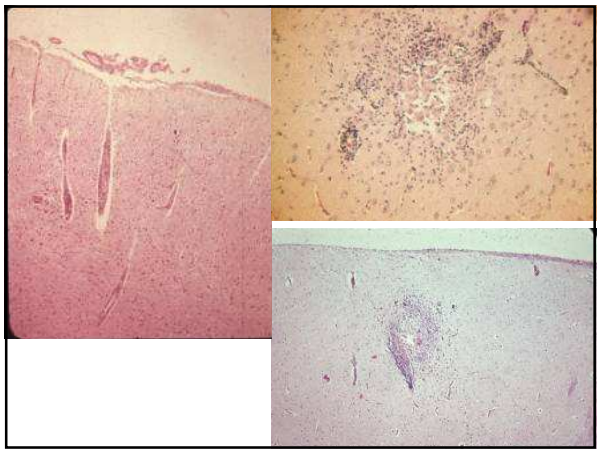


Technique

Micoagglutination
E. cuniculi

Encephalitozoon cuniculi

- > than 50% of colonies
- Horizontal and Transplacental Transmission
- Chronic
- Latent
- Kidney – chronic interstitial nephritis
- Brain - mononuclear cell granulomas
- Diagnosis
  - Histo – Gram Positive, Acid Fast
  - IFA, India Ink, Indirect Micoagglutination
  - Skin test
  - Urine exam
  - PCR



How *Toxoplasma gondii* gets Noticed

R Goldszmid NIH January 19, 09 *J Exp Med,* ALN 15 Feb 09

- *Toxoplasma gondii* triggers an immune response in its host and a strong immune response spares *T. gondii*-infected hosts from deadly infection
- *T. gondii* resides within specialized vesicles inside infected host cells but the process by which peptides from the trapped bugs are processed by infected cells and presented to killer T cells is mysterious
- *T. gondii* infections in mice show how portions of the parasitic proteins escape the vesicle in a process known as cross-presentation
- **Parasite gets noticed by the immune system when the membrane of the bug-containing vesicle fuses to the endoplasmic reticulum**—an organelle normally involved in presenting pathogens to T cells—allowing a swap of parasitic peptides.

Parasite twists rats' innate fear

Dangerous game of cat and mouse

Toxoplasma gondii is a puppeteer that can force **a rat to go against its instincts and become attracted to the scent of cat urine.**

Specific part of the rat brain -- amygdala -- is involved in this parasite-imposed death wish.
Toxa can only reproduce in the gut of a cat

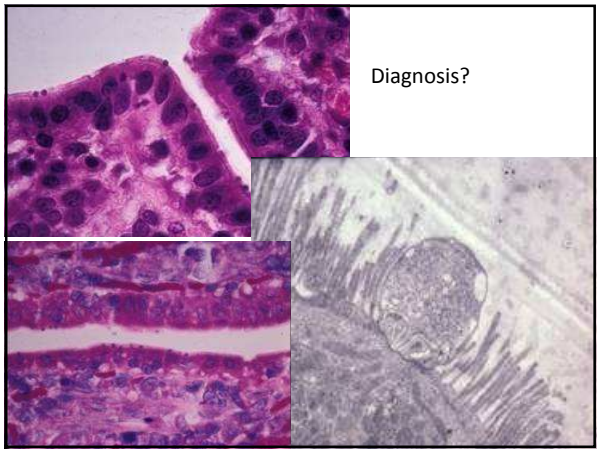
To get into the cat, *Toxo tricks rats into* acting recklessly.

Patrick House at Stanford University has identified two distinct regions of the brain, one important for fear and the other responsible for attraction, that are activated in *Toxo infected* rats after they smell cat odor.

Surprisingly, the attraction region of the rat brain is similarly activated when a male rat encounters a female, suggesting that ***Toxo may fool the rat*** **into mistaking cat urine for a sign of a potential mate.** - *Laura Sanders (i)*

Pneumocystis

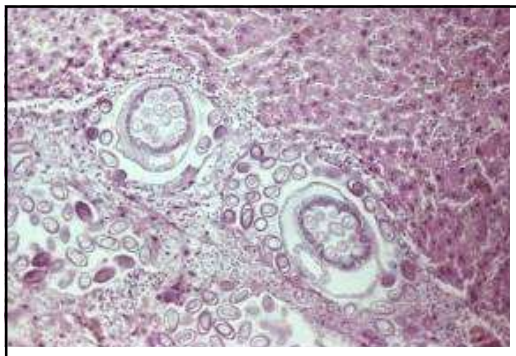
- Usually no clinical signs in rabbits
- One report of pneumonia and animals recovered in 2 weeks
- No clinical signs in infected rabbits after corticosteroid administration



Diagnosis?

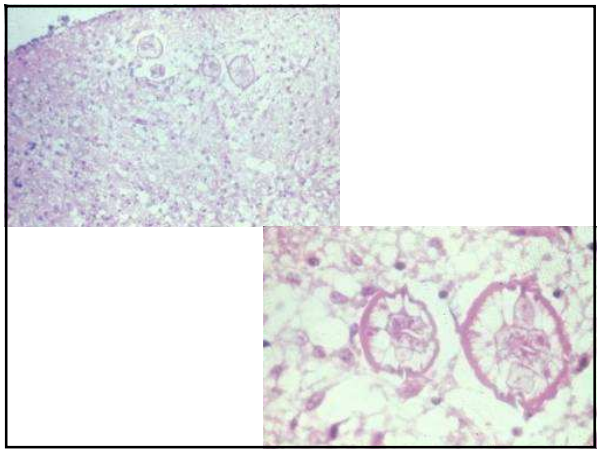
Cryptosporidium parvum - Rabbits

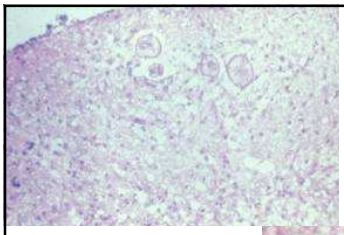
- Ileum
- Jejunum
- **Asymptomatic**
- Architectural change in villi
- Animal Models
  - Fetal rabbits
  - Polyclonal antisera



Tissue? Diagnosis?

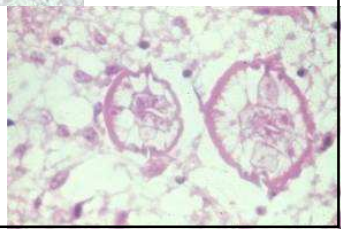
Capillaria hepatica





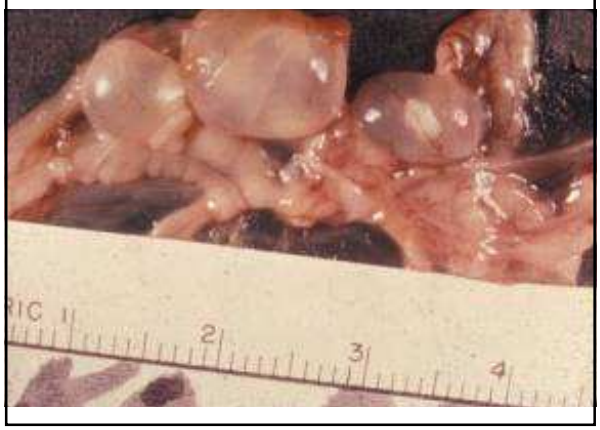
Bayliascaris

Brain



Diagnosis?

Tissue?



Cysticercus pisiformis

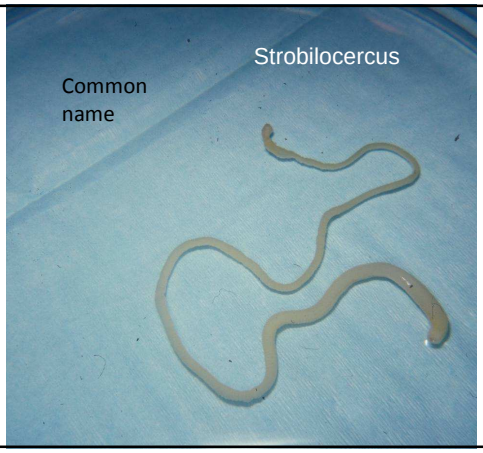
Diagnosis

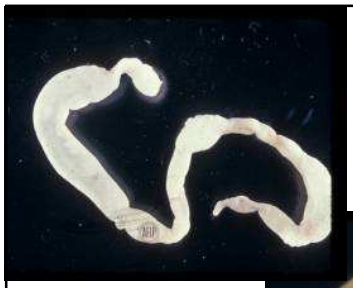
Genus and
Species

Larval form
Adult form

Larval form
Cysticercus fasciolaris

Adult form
Taenia taeniaformis





Genus	Definitive Host	Intermediate Host	Intermediate Stage
Taenia pisiformis			
Taenia taeniaformis			
Multiceps serialis			

Genus	Definitive Host	Intermediate Host	Intermediate Stage
Taenia pisiformis	Dog	Rabbits, Rodents (liver, peritoneum)	*Cysticercus pisiformis*
Taenia taeniaformis	Cat	Rodents (liver, peritoneum)	*C. fasciolaris* (strobilocercus) (miniature tapeworm)
Multiceps serialis	Dog	Rabbits, Nonhuman primates	*C. serialis*

Genus	Definitive Host	Intermediate Host	Intermediate Stage
Taenia hydatigera			
T. ovis			
T. Solium			
T. saginata			
Multiceps multiceps			
Echinococcus			

Genus	Definitive Host	Intermediate Host	Intermediate Stage
Taenia hydatigera	Dog	Goats, Sheep, Rodents [peritoneal cavity]	*Cysticercus tenuicolis* [slender neck bladder worm]
T. ovis	Dog	Sheep, Goats [muscle]	*C. ovis*
T. Solium	Man	Pigs [muscle]	*C. cellulosae*
T. saginata	Man	Cattle [muscle]	*C. bovis*
Multiceps multiceps	Dog	Sheep, Goats, Primate [Brain Gid]	*Coenurus cerebralis*
Echinococcus	Dog	Man and Others [various organs and body cavities	Hydatid cysts

showed "complete concordance" with DNA from one of Nicholas's teeth.

The newly discovered remains are now in a forensic lab in Yekaterinburg. Once the Russian Orthodox Church agrees that they are authentic, they can be buried with the rest of the family in a cathedral in St. Petersburg.

Giant Bug-Eater Descried

Plant explorers have discovered a giant new species of carnivorous pitcher plant on a mountaintop in the Philippines. At 30 cm long and 15 cm wide, the plant's pitchers may set a record for genus *Nepenthes*—the group that includes all 120-odd species of Old World pitcher plants.

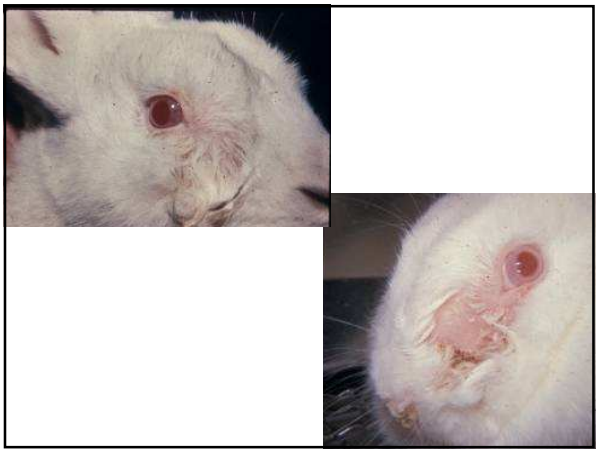
The team spied the plant after scrambling up a 6-m waterfall to reach the summit of Mount Victoria on the island of Palawan, says Alastair Robinson, an independent field botanist formerly at the University of Cambridge, U.K. Dangling from long tendrils attached to a central stem, the trumpet-shaped pitchers are "akin to an open stomach" filled with milky digestive fluids and water, Robinson says. Insects attracted to the pitcher's nectar and color get

green beetles, black beetles, bees, and wasps. Dubbed *N. attenboroughii*, the plant is described in the February *Botanical Journal of the Linnean Society*.

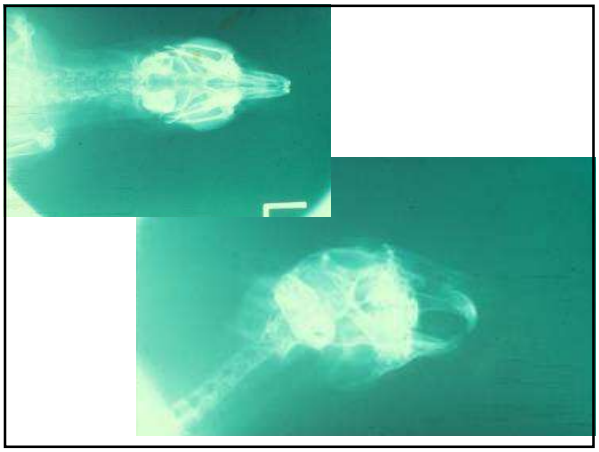
Environmental Factors

- In past 50 years humans take more showers and fewer baths
- Profund impact of aerosolization of microorganisms present in potable water
  - *Legionella pneumophila*
  - *Mycobacterium avium*

Where else do you examine the rabbit for nasal discharge?



Diagnosis??



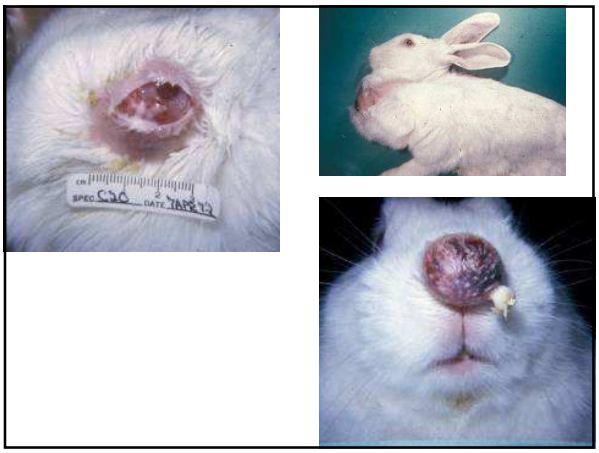
Clinical Diagnosis?

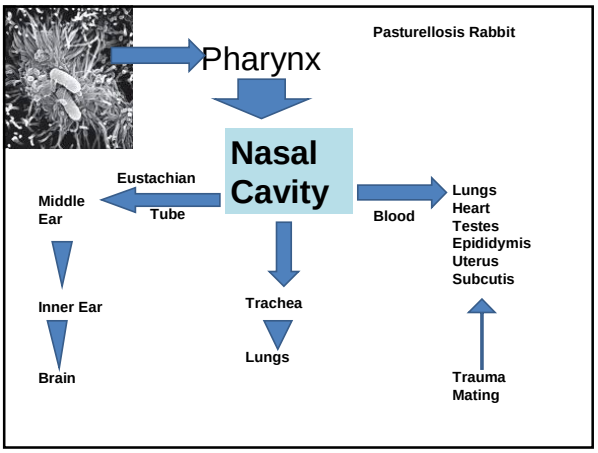
Torticollis

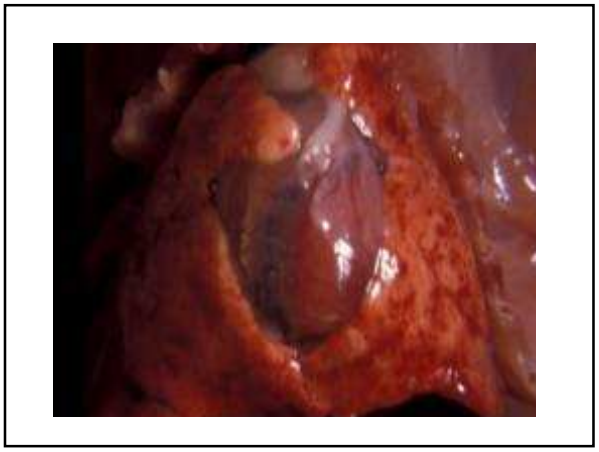
_____

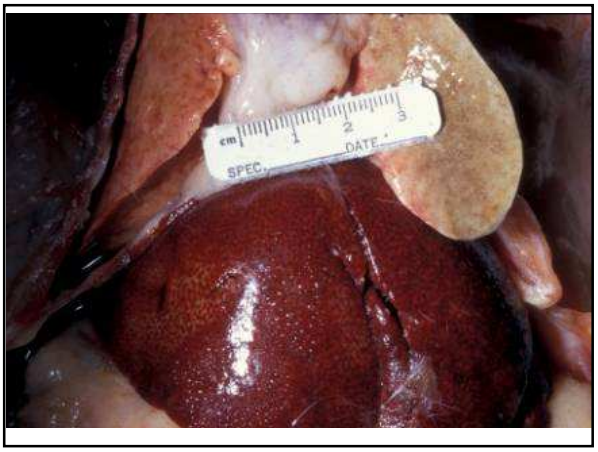
Layman's Term for Condition?

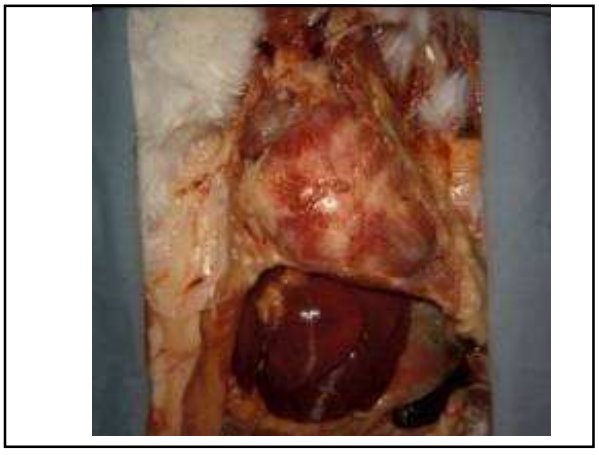
Wry Neck

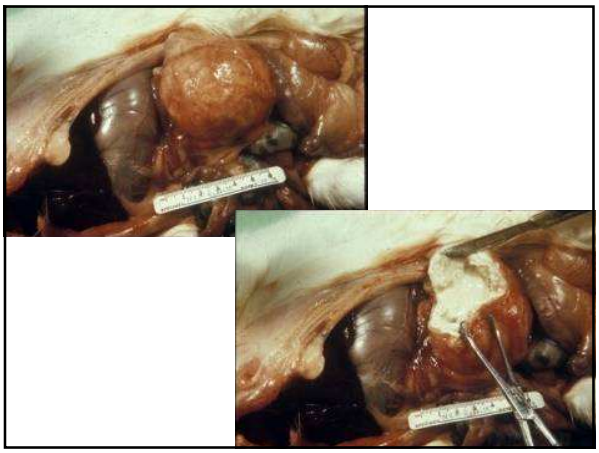


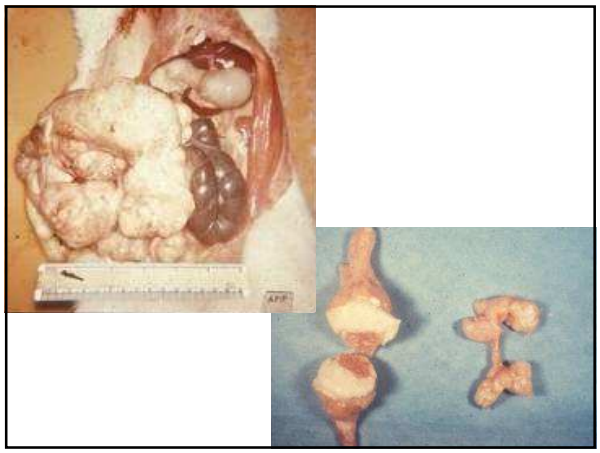

Pasturellosis Rabbit
Pharynx
Nasal Cavity
Eustachian Tube
Middle Ear
Inner Ear
Brain
Trachea
Lungs
Blood
Lungs
Heart
Testes
Epididymis
Uterus
Subcutis
Trauma
Mating

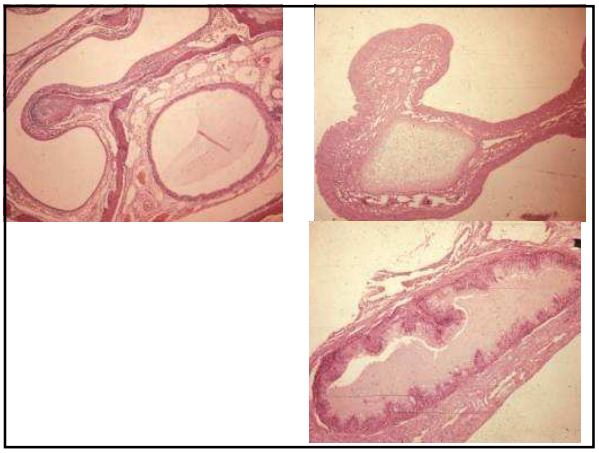


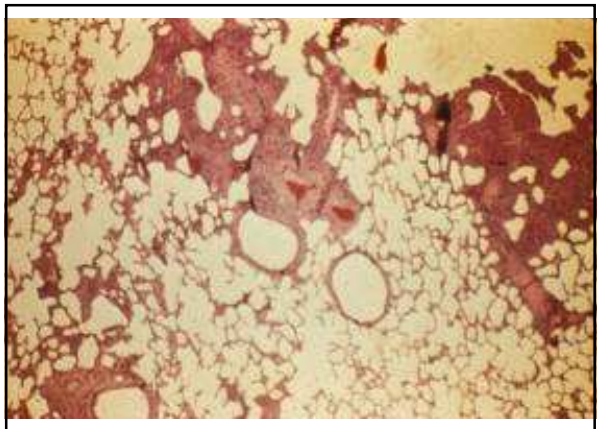


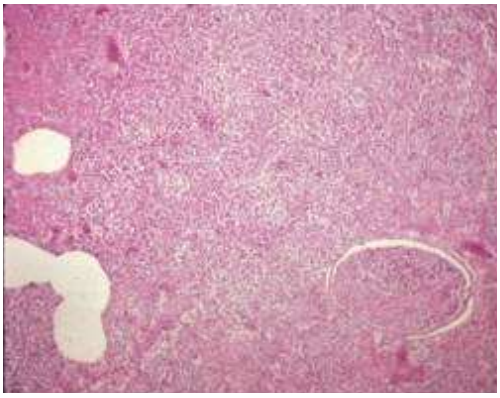












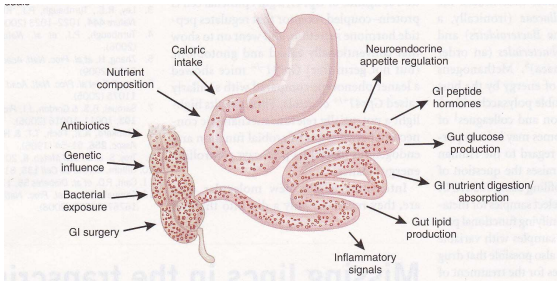
Bordetella bronchiseptica

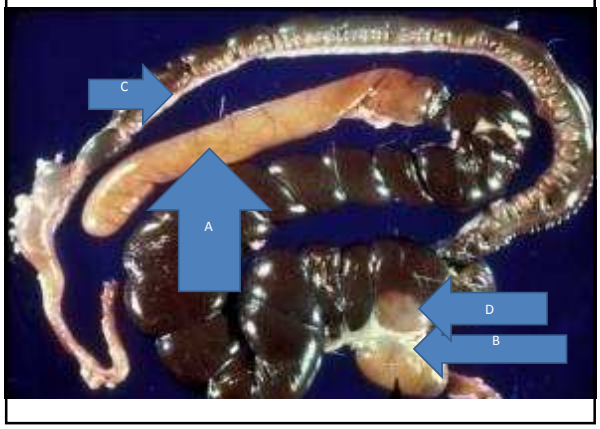
- Usually asymptomatic
- Upper respiratory tract
- Bronchopneumonia [*P multocida??]*

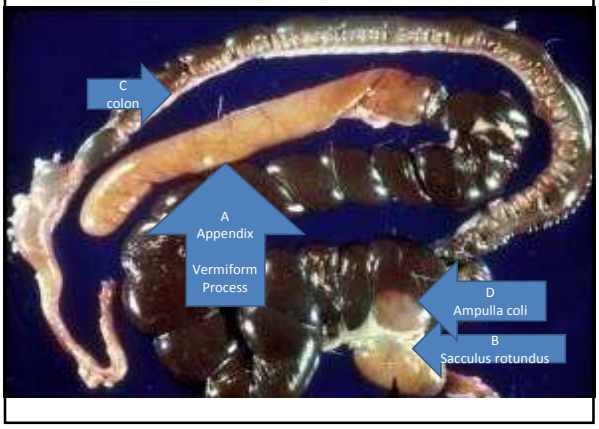
- Abscesses
- Defective macrophage function

Evolution of Symbiotic Bacteria in the Distal Human Intestine.

- PLoS Biol. 2007 Jun 19;5(7):e156. [Epub ahead of print] Links
- Xu J, Mahowald MA, Ley RE, Lozupone CA, Hamady M, Martens EC, Henrissat B, Coutinho PM, Minx P, Latreille P, Cordum H, Van Brunt A, Kim K, Fulton RS, Fulton LA, Clifton SW, Wilson RK, Knight RD, Gordon JI.
- The adult human intestine contains **trillions of bacteria, representing hundreds of species and thousands of subspecies**. Little is known about the selective pressures that have shaped and are shaping this community's component species, which are dominated by members of the Bacteroidetes and Firmicutes divisions. To examine how the intestinal environment affects microbial genome evolution, we have sequenced the genomes of two members of the normal distal human gut microbiota, Bacteroides vulgatus and Bacteroides distasonis, and by comparison with the few other sequenced gut and non-gut Bacteroidetes, analyzed their niche and habitat adaptations. The results show that lateral gene transfer, mobile elements, and gene amplification have played important roles in affecting the ability of gut-dwelling Bacteroidetes to vary their cell surface, sense their environment, and harvest nutrient resources present in the distal intestine. Our findings show that these processes have been a driving force in the adaptation of Bacteroidetes to the distal gut environment, and emphasize the importance of considering the evolution of humans from an additional perspective, namely the evolution of our microbiomes.







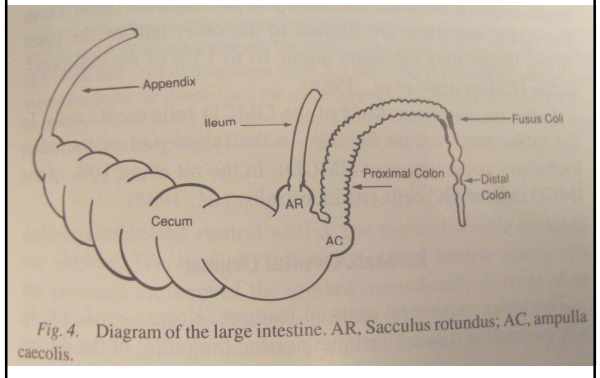
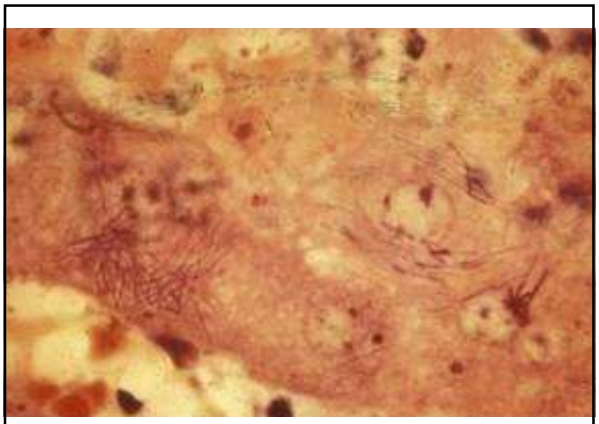


Fig. 4. Diagram of the large intestine. AR, Sacculus rotundus; AC, ampulla caecolis.



Tyzzer's Disease

[Bacillus piliformis] *Clostridium piliforme*

- **3-6 weeks of age**
- **Death 12-72 hours**
- 30-95% mortality
- Ascites
- **Perfuse watery diarrhea**
- Dehydration
- Gut edema
- Necrosis

  liver ileum cecum colon
  myocardium

Tyzzer's Disease Rabbits

- **Associated with stress**
  - High temperature
  - Poor sanitation
  - Overcrowding
  - Weaning
  - Transportation
  - Irradiation
  - Cortisone

Tyzzer's Rabbit

Scharmann 1983

- Diet control

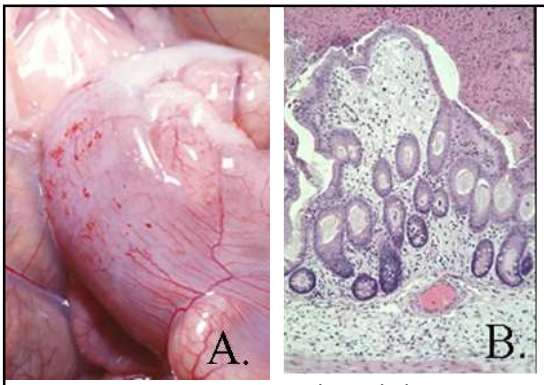
  20% crude protein / 10% fiber

  Changed to

  15% crude protein / 14% fiber

  Death rate

  27-50% down to 1.6% within 6 months



Enterotoxemia Clostridial species
principally *C. spiroforme* but also *C. difficile*

Enterotoxemia

- All ages
- Death 72 hours
- Profuse diarrhea
- Rough hair coat
- Anorexia
- Listless
- **Cecum -- hemorrhage, edema**
- Cecum, Colon, Ileum -- fluid, gas
- Cecal contents -- toxic

Clostridium spiroforme

- **Enterotoxemia**
- **Spontaneously or in association with use of antibiotics**
- **Stress**
  - Weaning
  - Antibiotics
  - Diet – change, high energy
- Hemorrhage
- **Cytotoxin similar to *Clostridium perfringens***
- ***Type E iota toxin***
- Toxin neutralized by antiserum *C perfringens* Type E Iota Toxin

Clostridium spiroforme

Experimental infection Carman 1984

- **Weanlings -- diarrhea**
  weaning stress
- **Adults -- no diarrhea**
  unless pretreated with Clindamycin

Clostridium spiroforme

- Anaerobic
- Gram Positive
- **Spore bearing helically coiled bacillus**
- Ferments
  - glucose
  - fructose
  - mannose
  - sucrose
- Type E Iota Toxin

Clostridium spiroforme

- The pathogenicity *in rabbit in 1982 (Carman and Boriello, 1982)*
- Disease experimentally reproduced by oral administration of lincomycin (Yonushonis *et al., 1987).*
- *The antibiotic-associated origin of mostly of C. spiroforme outbreaks can have an indirect evidence in sporadic outbreaks* ***due to accidental rabbit*** **feeding with feed cross contaminated with small amounts of amoxicillin**: rabbits developed symptoms of serious intoxication associated with imbalance in caecal microbiota and abnormal proliferation of *C. spiroforme thanks to destruction of antagonist bacteria due to the β-lactam unintentionally* swallowed.
- A **similar mechanism is known in the "antibiotic associated diarrhoea" due to *C. difficile*** and affecting several animal species and in humans (Voth and Ballard, 2005).

CLOSTRIDIUM SPIROFORME DRUG SUSCEPTIBILITY

Agnoletti F.*, Ferro T., Guolo A., Cocchi M., Drigo I., Bacchin C., Marcon B., Bano L.

- Istituto Zooprofilattico Sperimentale delle Venezie, Viale Brigata Treviso 13/a, 31100 Treviso, Italy
- *Corresponding author: fagnoletti@izsvenezie.it

- Among all antimicrobials tested, **only doxycycline** showed MICs supposed to be of therapeutic efficacy. Results support the field hypothesis of an extensive acquired resistance of *C. spiroforme to antimicrobials and the presence of intrinsic resistances of importance.*
- *Both findings* suggest the necessity to **give greater priority to prophylaxis of enteritis caused by *C. spiroforme by* attempting to reduce risk factors rather than controlling outbreaks by therapy**
- *9th World Rabbit Congress – June 10-13, 2008 – Verona – Italy*

Clostridium spiroforme

- In addition to sporadic and accidentally intoxication, **the increased use of antimicrobial agents to control the enzootic rabbit enteropathy** (ERE, a disease causing important losses in rabbit breeding during the last ten years) is a factor increasing *C. spiroforme clostridiosis that affects industrial farms. 9th World Rabbit Congress – June 10-13, 2008 – Verona – Italy*
- *Other trigger* factors may include **hyper-protein diets** that induce an increase trypsin secretion which through the enzymatic scission of the sub-units "Sa" and "Sb" is a potent **activator of *C. spiroforme binary toxin*** (Ellis *et al., 1991).*
- **High carbohydrate and low fibre diets** *can also induce clostridial overload (Percy et al., 1993).*
- The seriousness of *C. spiroforme pathology is heightened by difficulties in therapeutic control.*
- *Field* evidences suggest *C. spiroforme has high drug resistance, differently to C. perfringens which* diplays good susceptibility to several antimicrobials

Clostridium difficile–associated disease

- Among the animals commonly used for laboratory research, Syrian hamsters **(*Mesocricetus auratus*) are the most sensitive** to naturally acquired disease.[84,137]
- Lesions attributed to spontaneous or experimentally induced *C. difficile* infection have also been documented in guinea pigs (*Cavia porcellus*),[87] mice (*Mus musculus*),[105] rats (*Rattus norvegicus* and *R. rattus*),[31] and **rabbits (*Oryctolagus cuniculus*);**[24]
- Disease develops spontaneously in a variety of other species including horses (*Equus caballus*),[93] hares (*Lepus* spp.),[32] pigs (*Sus scrofa*),[156] nonhuman primates (*Macaca* spp., *Pongo pygmaeus*, and *Saguinus oedipus*),[128,150] domestic dogs (*Canis familiaris*),[161] domestic cats (*Felis catus*),[162] ostriches (*Struthio camellus*)[42] and black-tailed prairie dogs (*Cynomus ludovicianus*).[104]

Clostridium difficile

- gram-positive, anaerobic, spore-forming bacillus commonly associated with diarrhea and colitis in humans and other mammals.[140]
- It was first isolated from feces and meconium of asymptomatic newborn infants, and was originally named *Bacillus difficilis* because of its morphology and the difficulties encountered in cultivating it.[35]
- The initial report described production of a potent toxin, but the organism was not considered an important pathogen due to the high carrier rate among asymptomatic human infants.
- In the past 30 years, however, *C. difficile* has been implicated as the principal infectious cause of antibiotic-associated diarrhea in adult humans, and similar clinical conditions in a variety of other mammals.[65,85,141]
- **It is now recognized as one of the most important nosocomial pathogens of humans**. Clinically affected humans often experience diarrhea without appreciable lesions, but the disease can progress, resulting in the severe tissue changes associated with pseudomembranous colitis.[65]
- More serious sequelae, including prolonged ileus (toxic megacolon), intestinal perforation, and peritonitis develop, but are less common.
- The **β-lactams represented by amoxicillin are considered the antimicrobials of preference in the treatment of human clostridiosis and show extremely high efficacy *in vitro even against C. spiroforme.***

Clostridium difficile

- Isolated on selective media that are commercially available or made fresh according to published techniques.[47,47,165]
- The contents of the large intestine or fecal samples --anaerobic transport medium. If samples cannot be delivered to the laboratory within 24 hours they should be sent refrigerated to be received as soon as possible.
- Toxin is detected by ELISA or by assessing the cytopathic effect of fecal filtrate on cell lines. The ELISA is rapid, sensitive, and specific, and is commercially available.[116,135]
- The cytotoxin assay is specific and more sensitive, but requires cell culture facilities and takes considerably more time than the ELISA.[116,144]
- **Toxin production** by isolates is determined by use of **polymerase chain reaction assays,** using primers specific for TcdA and TcdB[23,145].
- **Toxin is also identified in culture filtrate by ELISA or cell culture assays.**[89]
- It is important to determine the potential of isolates to produce toxin, as some isolates lack one or both toxin genes or essential elements of the genes.[11,95]

Clostridium dificile

Newborn rabbits appear resistant

A prominent hypothesis to explain the resistance of such neonates is that they lack the proper toxin receptors until later in life.[18] Binding of TcdA to ileal brush borders is decreased in neonatal rabbits, but maximal binding is observed in 90-day-old rabbits.[35]

However, in apparent contradiction to these findings, the binding kinetic variables of TcdA to intestinal brush borders of hamsters were similar for adults and infants and could not account for the age-related susceptibility to CDAD.[125]

In contrast to neonatal rabbits, **newborn hares are sensitive to the enteric effects of *C. difficile*.** Inoculation of young hares resulted in inflammation and necrosis, principally of the duodenum, cecum, and colon.[32]

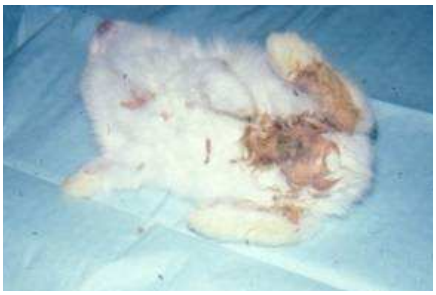
Additionally, **severity of the disease was greater for co-infections with *C. perfringens*** or *C. tertium*, or both, even though these species failed to cause disease in monoassociated hares.

Clostridium perfringens

- Type E
- Alpha & Iota Toxin
- Enterotoxemia?

Clostridial Diarrhea

- Normal Digestion
- Maldigestion
- Substrate changes
- Colonization resistance decreased
- **Toxin** → 8-18 hours



Colibacillosis

- **5-8 weeks of age**
- **70% mortality**
- Watery brown diarrhea
- Gas in cecum
- Paintbrush hemorrhages cecum
- Transparent cecal wall
- **Nonenterotoxigenic**

Escherichia coli

- Commensal inhabitant of intestinal tract
- Bacterial pathogen
- Highly variable mutation rates
- **Enteropathogenic**
- **Attaching**
- **Effacing**
- **Enterohaemorrhagic *Escherichia coli* (EHEC)**

Escherichia coli

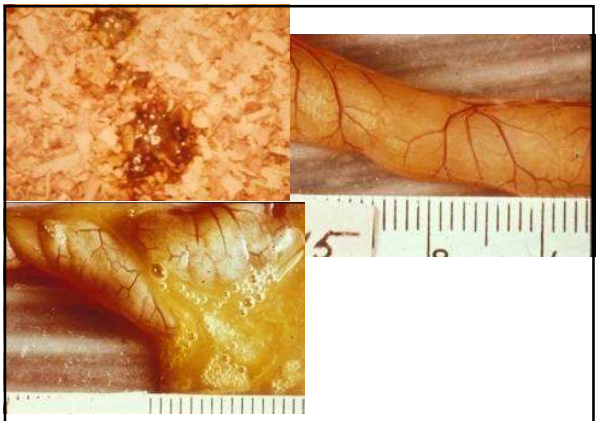
- Facultative anaerobic Gram Negative bacillus
- Motile with flagellae or nonmotile
- Ferments lactose
- Pili – adhesive organelles
- Classification
  - Serogroups — Somatic (O), Flagellar (H), and Capsular (K)
  - Biotypes — Carbohydrate fermenting patterns [Rhamnose]
- Some strains have plasmid – or bacteriophage-acquired virulence factors
- Pathogenic *E. coli* cause Disease
  - Human – enterotoxigenic, enteroinvasive, enteropathogenic
  - **Rabbit - only enteropathogenic**

- **Fatal epizootics in commercial rabbitries**
- **RDEC-1** [Cantey and Blake 1977]
- 150 organisms caused severe disease
- Did not invade mucosa
- **Nontoxigenic** **Noninvasive**

Colibacillosis Clinical Disease

DeLong Manning 1994

- **Neonatal 1-2 week old sucklings**
  - Severe yellow diarrhea, high mortality 0109:H2
- **Weanling 4-6 week old**
  - Profuse liquid diarrhea, dehydration, weight loss
  - High mortality in 5-14 days
- **Highly virulent strains**
  0103:H2 Rhamnose Negative 015:H2
  Rhamnose Positive [RDEC1 like]
  - Mild diarrhea with no dehydration, transient growth retardation, minimal mortality
- **Less virulent strains** 0123, 0128
  0132 Rhamnose Positive
- USA 015:H



Mucoid Enteropathy

- **4-8 weeks of age**
- **Die in 12 hours**
- **50-80% mortality**
- Primarily in winter
- Anorexia
- Polydipsia
- Listless
- Rough hair coat
- Bloated abdomen
- Teeth grinding
- **Mucoid to gelatinous diarrhea**
- **Enterotoxin induced secretory diarrhea**
- No gross or microscopic lesions

Some cases of apparent mucoid enteropathy are due to a dysautonomia

Whitwell (Vet Record, 28 Sept 1996

Caecal impaction, anorexia, depression and death in weanling rabbits

Dysautonomia
broad term that describes any disease or malfunction of the autonomic nervous system

Mesenteric autonomic ganglia
chromatolysis-like degenerative changes
neuronal vacuolation

Similar to equine grass sickness.

Mucoid Enteropathy Prevention

- High quality feed
- Fiber 12-14%
- Reduced carbohydrate
- Improved cecocolonic motility
- Limit environmental stressors

Rabbit Diarrhea

- Stress
- Weather
- Diet
- Physiological
- Genetics
- Antiobiotic use
- Protozoa
- Viruses
- Bacteria

Rabbit Diarrhea / Enteritis

- *Clostridium piliforme*
- *Clostridium spiroforme*
- *Clostridium dificile*
- *Clostridium perfringens*
- *Escherichia coli*
- *Lawsonia intracellularis*
- *Salmonella sp.*
- *Campylobacter*
- *Vibrio sp.*
- Rabbit Enteric Adenovirus
- Rabbit Enteric Coronavirus
- Lapine Parvovirus
- Rotavirus
- *Cryptosporidium parvum*
- Eimeria – hepatic and intestinal coccidia
- Mucoid Enteropathy

Antibiotics / Diarrhea

- Clindamycin
- Lincomycin
- Penicillin
- Ampicillin
- Amoxicillin

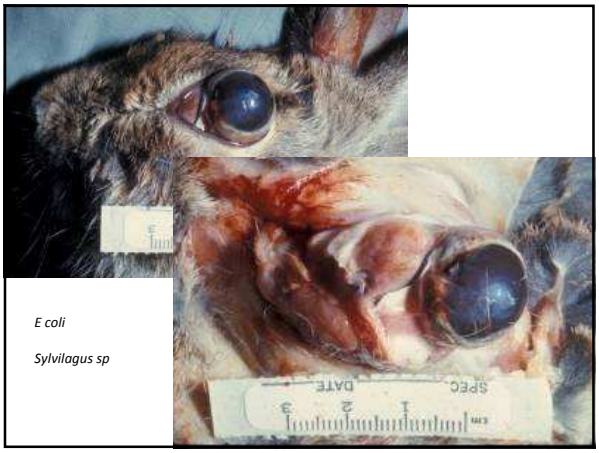
Enteritis Prevention

- High fiber diet
- 20% alfalfa
- Copper sulfate 250ppm
- Oxytetracycline
- Sulfaquinoxalone
- Improve sanitation and environment

Rx Diarrhea

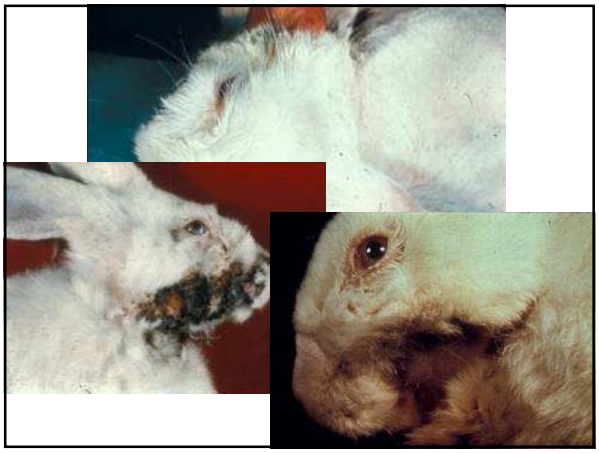
- Salt water
- Fluids
- Electrolytes
- B12
- Pineapple juice
- Fiber diet

Pseudomonas sp



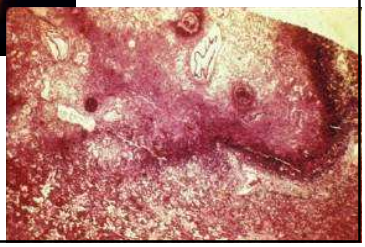
E coli

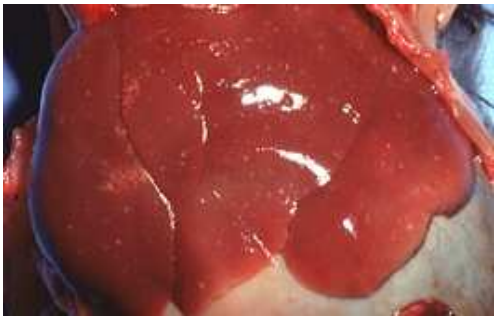
Sylvilagus sp



Sphaerophorus necrophorus

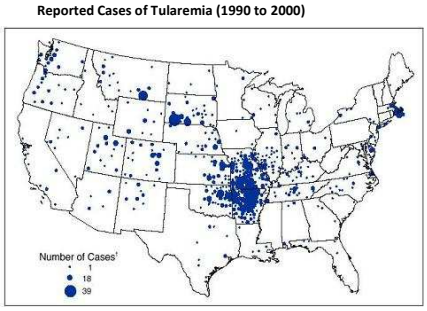
Schmorl's Disease





Franciscella tularensis

- ***Gram negative*** coccobacillus
- acute septicemic disease (tularemia)in a wide range of mammalian hosts, including humans.
- **Infection is common in wild rabbits but rare in laboratory rabbits.**
- Two biovars infect rabbits, with *F. tularensis* bv. tularensis (found only in North America) being the more pathogenic
- Transmission is via multiple routes, most commonly arthropod vectors and direct contact
- Clinical signs may consist of anorexia, depression, and ataxia, or sudden death without premonitory signs.
- Pathologic changes include focal coagulative necrosis and congestion of the liver, spleen, and bone marrow



Tularemia Symptom

3-Agent **Detect. Identify. Move on.**

The Cepheid® GeneXpert 3-Agent Biothreat Assay is designed for the surveillance of the etiologic agents of anthrax, plague and rabbit fever (or tularemia) in environmental samples.

Screening and identification in one kit.
Cepheid's 3-Agent Biothreat Assay consists of a simple two-cartridge system. Either cartridge can be used for screening, or to rule out a substance in question. If one of the three targets is detected during a single cartridge test, the second cartridge is used to identify the specific agent in question.

Fig.1. Thickening of the ileal wall (small arrow); open segments of the ileum with a finely corrugated aspect (black arrow) and reticulated aspect (white arrow).

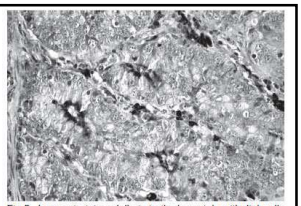


Fig.5. *Lawsonia intracellularis* in ileal cryptal epithelial cells (arrows) and in macrophages, detected by *Lawsonia intracellularis* polyclonal antibody by the avidin-biotin immunohistochemistry method. Obj.40x.

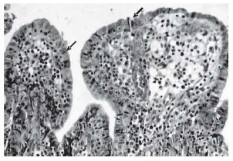


Fig.3. Poorly differentiated epithelial cells (arrows) overlaying the apexes of flattened ileal villi; mixed inflammation in the submucosa. HE, obj.16x.

Lawsonia intracellularis

- 1988 in Rio de Janeiro state, Brazil
- acute 24-48 hours – death sometimes in 24 hours
- Clinical signs characterized by brownish or green diarrhea and dehydration
- Proliferative enteropathy
- **Silver impregnation** revealed large numbers of bacteria with morphology of the genus *Lawsonia* in the apical pole of cryptal enterocytes.
- Bacteria reacted **positively to a *Lawsonia intracellularis* polyclonal antibody** using the avidin-biotin immunohistochemistry method.

Lawsonia intracellularis
obligate, intracellular, curved rod-shaped, argyrophilic bacterium

located within the the apical cytoplasm of infected crypt epithelial cells.

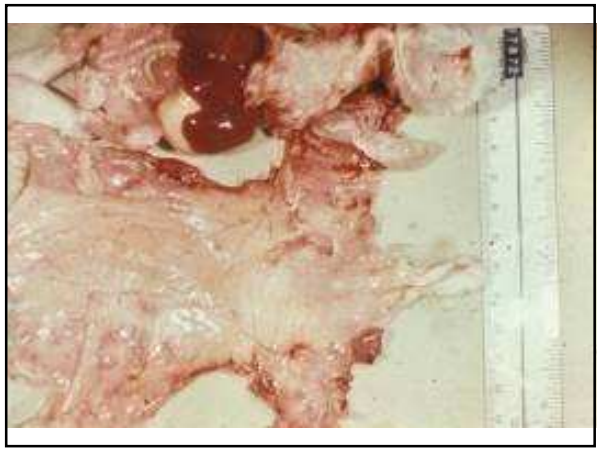
Yersinia pseudotuberculosis

CAR Bacillus

- Gram negative, filamentous, rod-shaped bacterium
- Colonizes ciliated epithelial cells of the respiratory tract

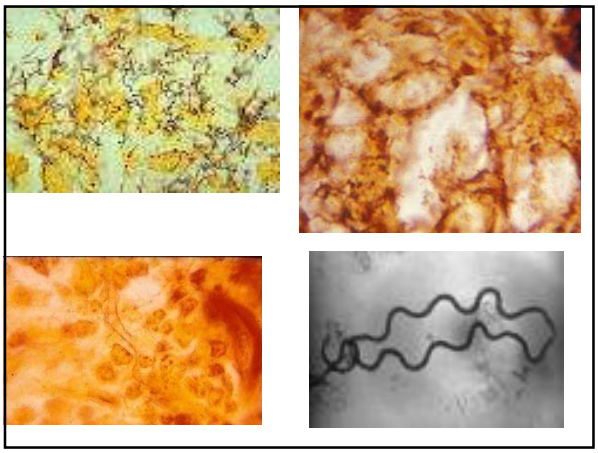
- No clinical disease in rabbits

Staphylococcus aureus



Listeria monocytogenes

- Gram-positive, rod-shaped, intracellular bacterium
- **Uncommon disease in rabbits**
- **Acquired with contaminated feed**
- Clinical signs are generally absent or may be nonspecific, including anorexia, ascites, depression, weight loss, and sudden death
- **Pregnant does may abort and are more susceptible to infection**, either because of physiological stress or because of a uterine microenvironment more conducive to survival of the organism
- Pathologic findings multifocal hepatic necrosis and microabscesses may be seen in the spleen and adrenal glands
- Septicemic spread is facilitated by phagocytosis and transport by macrophages
- Pregnant does may develop acute necrotizing suppurative metritis.
- Abortion may also be related to the ability of pathogenic strains of *L. monocytogenes*to cause myometrial contraction
- Hematologic changes include a marked monocytic reaction



Treponema paraluis-cuniculi

- ***gram-negative*** spirochete
- Rabbit Syphilis
- Transmission is primarily sexual via penetration of mucous membranes but may also occur by other routes
- Lesions develop 3 to 6 weeks following exposure and are most apparent on and around mucocutaneous junctions of the face and genitalia
- Lesions begin as areas of erythema and edema, with or without vesicles, and progress to ulcers and crusts.
- Lesions generally resolve after several weeks
- Histologically, epidermal ulceration, hyperkeratosis, hyperplasia, and acanthosis overlain by crusts

Treponema paraluis-cuniculi

- **Serologic responses are also slow to develop, requiring 2 to 3**
- **months from the time of infection**
- carrier state, possibly in regional lymph nodes
- treponemes cannot be grown in vitro
- *T. pallidum, the causative agent of human syphilis, i*nfects rabbits
- laboratory rabbit has been extensively used as a **model of human syphilis**

Treponema Diagnosis

- Microscopic
  - Darkfield scrapings
  - Preputial washing
  - Silver stain
- Florescent monoclonal Antibody MFI
- VDRL Elisa
  - Wassermann Test (CF)
- Rapid plasma reagin test kit 104
  - Hynson, Wescott and Dunning – Baltimore
  - Venereal Disease Research, Laboratory (VDRL) Slide Test
- Sera-Tek Treponemal Antibody test
  - Microhemagglution test
  - Miles Laboratories, Elkhart, IN

Treponema Treatment

- Benzathine Penicillin G
  - 200,000 Units
  - IM
  - Weekly
  - For 2 weeks

Dermatophytes

- **most commonly *Trichophyton mentagrophytes but also Microsporum gypseum* and *M. canis***
- Infection and clinical disease (dermatophytosis, ringworm, favus) are low in well-managed animal facilities
- Young or immunocompromised rabbits are most susceptible
- Dermatophytes infect the epidermis and adnexal structures,
- including hair follicles and shafts, usually on or around
- the head, and cause pruritus, patchy alopecia, erythema, and crusting
- Histopathologic changes in the underlying skin include neutrophilic and lymphoplasmacytic dermatitis, hyperkeratosis, folliculitis, and acanthosis. Abscess formation in hair follicles may occur secondarily

Zoonotic agents

Encephalitozoon cuniculi

Dermatophytosis

Microsporum spp

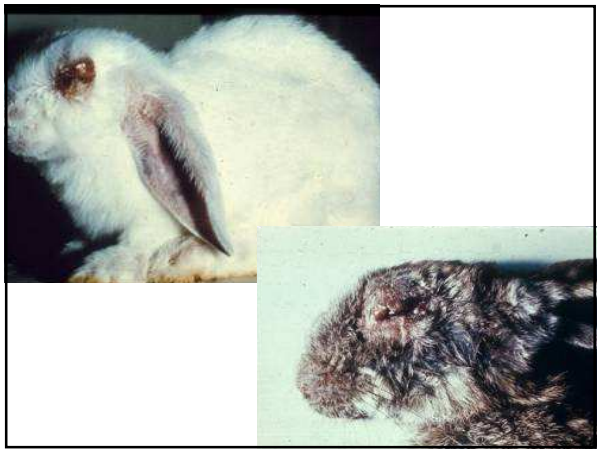
Trichophyton spp

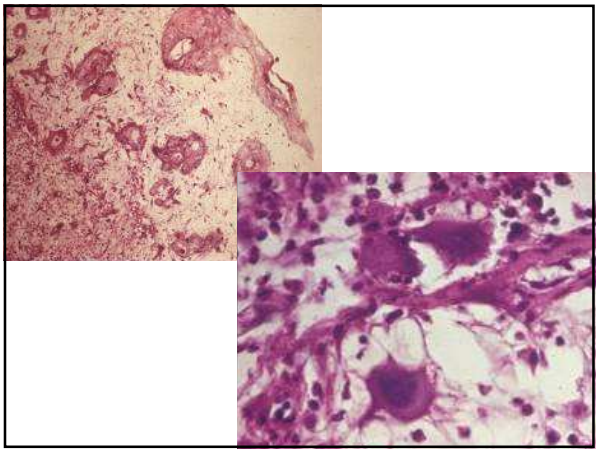
Salmonella spp

Franciscella tularensis

Leptospirosis spp

You are the only person on this earth who can use your ability





Myxomatosis

- 1896 Uruguay
- 1930 California Oregon
- 1950 Australia
- 1952 France
- 1953 Belgium, Holland, Germany, Luxemburg, Spain, England
- Several strains of DNA virus
- Different virulence
- Mortality in *Oryctolagus* – 20-90%
- Vaccine – Shope Fibroma

Myxomatosis

Slow painful death

Gelatinous subcutaneous swelling

Eyelids conjunctiva anogenital

Hemorhage Necrosis

gut heart lymph nodes lung liver spleen

No mortality in *Sylvilagus sp*

Spread primarily by arthropods

mosquitoes fleas flies gnats mites lice

Protection from vaccination is not very long lasting

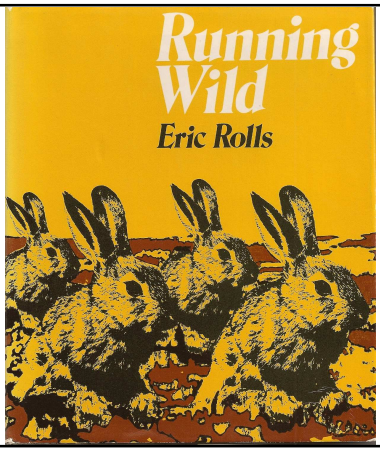
Rabbit Myxomatosis

- Australia $50,000 prize
- 1888 **Pasteur** – chicken cholera – disease did not spread
- Dame Jean **Macnamara** – Melbourne pediatrician
- USA in 1933 to study Poliomyelitis and met Dr Richard **Shope** in California
- Myxomatosis – South American disease
- 1950 Myxo introduced as biologic control agent
- December 1950 – disease spread 1100 miles long and 1000 miles wide in 3 months
- At first, the virus killed virtually every rabbit it infected (99.8% lethality), reducing the rabbit population by 85%, to a mere 100,000,000
- Essentially free of rabbits in 3 years

Rabbit Myxomatosis Australia

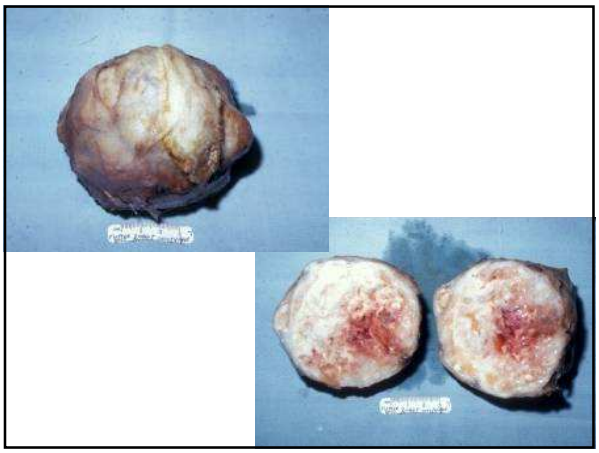
- Sir Macfarlane **Burne**, Dr Ian Clunies **Ross** and Professor Frank **Fenner** [smallpox, myxoma virus, malaria, and Nobel Prize]

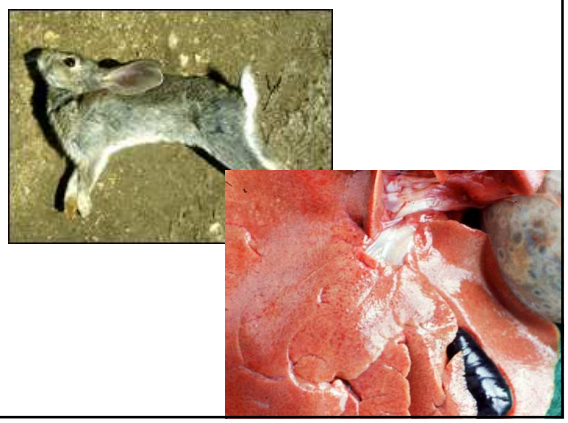
  Inoculated themselves to prove human safety
- After some 20 years of adaptation, most rabbits survived infection, and the population rebounded
- Virus evolved to a point where it killed about 70% of rabbits it infected
- Additionally, the virulent virus killed the rabbits so fast that not many mosquitos got a chance to bite them. Hence, an evolution to reduced, but still very severe, virulence.

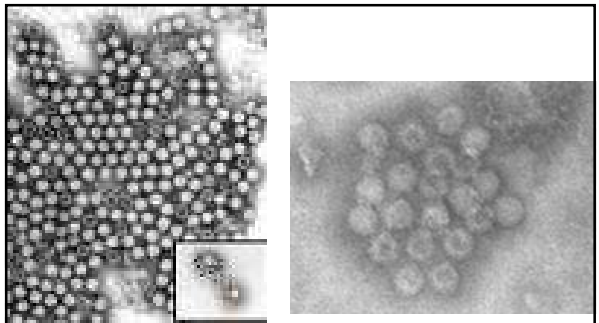


Shope Fibroma

- *Sylvilagus sp*
- *Oryctolagus cuniculus* -- one natural outbreak reported
- Antigenically related to myxoma virus
- Spread - Arthropods – mosquito
- Subcutaneous
  - Flat
  - Diffuse
  - Loose
  - Rubbery







Rabbit Viral Hemmorhagic Disease

Eugster 1989

- China 1984
- Mexico 1989
- Italy – 32 million deaths
- Initially suspected to be a Parvovirus or a Picornavirus on electron microscopy
- 90-100% mortality
- Peracute form of the disease -- no outward signs
- Acute form – 3 days
- Massive internal hemorraging of at least one major organ
- Usually liver lesions - necrotizing

Rabbit Hemorrhagic Disease

- Calicivirus
- Several routes and vectors
- Diseminated intravascular coagulation-fibrinogen antigens
- Nonpathogenic strains of virus
- Virion size variation
- No human disease

Rabbit Hemorrhagic Disease

- Virus isolation / propagation -- None
- HAI
- ELISA
- Viral antigen in liver lung spleen kidney
- Reverse Transcriptase PCR [RT-PCR]
- Fresh and Formalin fixed tissue

RHD

Discovered among farmed rabbits in China in 1984

USA in April 2000, when 25 pet rabbits in an Iowa backyard rabbitry died from the disease.

Outbreaks in Utah, Idaho, Montana, and Illinois, more than 4,000 rabbits died or killed

Society's Queens Zoo/Wildlife Center in Flushing, New York about a half-dozen rabbits

Cottontails and jackrabbits are not susceptible to the disease

Highly contagious

No vaccine in the United States and no cure

U.S. Department of Agriculture **does not quarantine or require health certificates** for imports of rabbits, wool, yarn, or other products—even from countries where the disease is present.

Rabbit hemorrhagic disease (RHD) in backyard rabbitry in Vanderburgh County, Indiana, on May 27, 2005.

The outbreak was confined to the index premises where 104 rabbits were euthanized on June 8.

Foreign Animal Disease (FAD) investigation initiated

Many of the **200 rabbits on the premises suddenly died during the previous 10 days.**

The investigation revealed that about a dozen rabbits from Kentucky had recently been introduced into the herd.

Investigations closed June 23, 2005.

The investigation did not reveal a possible origin of the infection

CEI impact worksheet, World Organization of Animal Health (OIE), ProMedMail
http://www.aphis.usda.gov/vs/ceah/cei/worksheets.htm

RHD

Survivors who become carriers

Transmission can be oral or airborne

Remains active on dry cloth for 105 days if kept at a temperature of 68 degrees

Proper disinfection of rabbit cages, bowls, and other items is imperative.

1 part bleach to 32 parts water, and letting the solution sit for at least 10 minutes before thoroughly rinsing

Rabbit Hemmorhagic Disease

The morbidity rate among rabbits who contract the virus is estimated at 90 percent (though rabbits four weeks old or younger are not as susceptible to infection).

Those who contract the peracute form of the disease succumb to it without showing any outward signs or after a short period of intense illness.

Typical symptoms of the acute form include high temperature (2 to 4 degrees above the normal temperature of 103 degrees); difficulty in breathing; lethargy; lack of appetite; spasms; blue color on the lips and mucous membranes; and bleeding from the nose, rectum, and mouth.

Rabbits generally die within 48 hours, but those with chronic forms of the disease may live up to two weeks, displaying symptoms that include jaundice, lethargy, weight loss, diarrhea, and mucous in the feces.

Cause of death involves massive internal hemorraghing of at least one major organ, and necropsies have shown that all rabbits who die of RHD have lesions on the liver

Rabbit Hemmorhagic Disease

As with some diseases that affect cats and dogs, RHD does have some survivors—rabbits who get sick, recover, develop immunity to future infection, and then, unfortunately, become carriers of the virus and pass it along to other rabbits.

Means of transmission can be oral or airborne; the virus can also spread through scrapes, abrasions, or contact with feces from an infected rabbit.

The virus has been shown to remain active on dry cloth for 105 days if kept at a temperature of 68 degrees, making proper disinfection of rabbit cages, bowls, and other items imperative.

In the shelter, this means scrubbing to remove any residues, applying a solution of 1 part bleach to 32 parts water, and letting the solution sit for at least 10 minutes before thoroughly rinsing.

Care should also be taken to either don a new pair of gloves or disinfect hands between handlings of different rabbits.

On January 21, 2005, the OIE received a report of an outbreak of rabbit hemorrhagic disease (RHD) in Havana City and Havana province of Cuba.

First outbreak of RHD in Cuba since 2001.

Animals affected totaled 14,450, with 2,362 deaths.

Cuba issued a disease alert, enhanced surveillance and banned movement of rabbits in and around the outbreak.
http://www.aphis.usda.gov/vs/ceah/cei/worksheets.htm.

Rabbit Haemorrhagic Disease

Vaccine – recombinant

Cylap HVD and is made by the company Cyanamid in Spain and registered for distribution in New Zealand if the disease arrives

Standard routine is two vaccinations two weeks apart

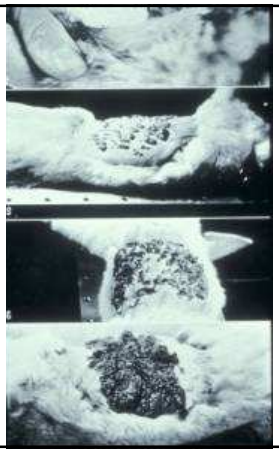
First against rabbit haemorrhagic disease

Second against myxomatosis.

Rabbits can be vaccinated from 10 weeks of age and need an annual re-vaccination.

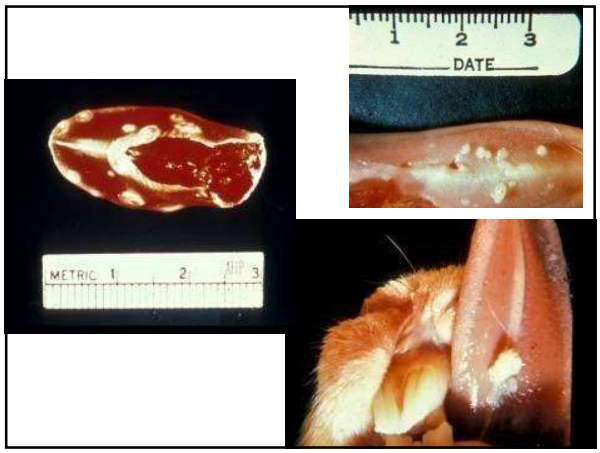
European Brown Hare Syndrome

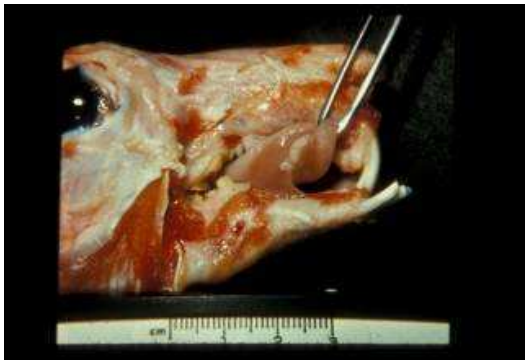
- 1980s
- *Lepus europaeus* *Lepus timidus*
- Severe necrotizing hepatitis and hemorrhage



Shope Papilloma

- Keratinized irregular projections on skin of neck, shoulders, abdomen
- Most regress
- Squamous Cell Carcinoma in 75% over 6 months





Oral Papillomatosis

- New York, Massachusetts, United Kingdom, Holland
- Direct contact
- 2-18 months of age
- Sessile Rugose Ulcerate
- 145 day maximum
- Papovavirus
- Shope Oral Papilloma

Immune Responses during Regression of Rabbit Oral Papillomavirus Wilgenburg, BJ. Et al 2005

Mucosatropic papillomavirus

Small benign discrete papillomas within the oral cavity

L1 and L2 viral capsid proteins were lost rapidly at a time that coincided with an inflammatory response

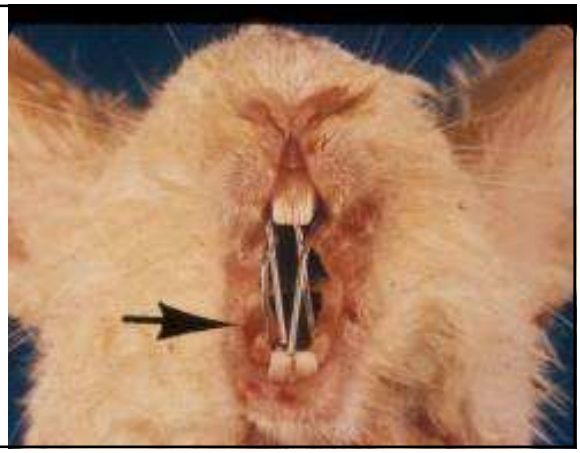
Inflammatory response began with a rapid rise in numbers of $CD11c^+$ cells at early regression and continued to increase in frequency through mid-regression and remained the most-represented cell through late regression.

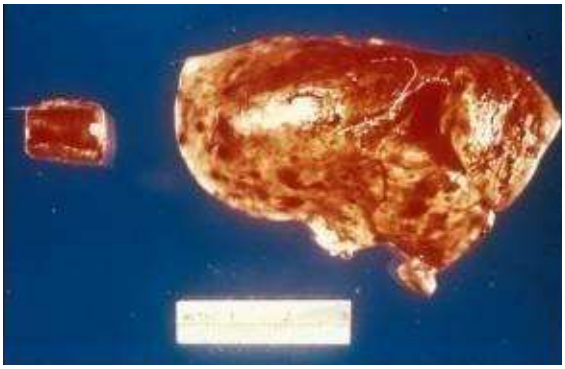
The initial rise in **$CD11c^+$ cells was followed by an infiltrate containing increased numbers of activated T cells, including $CD4^+$ and $CD25^+$ cells, during mid-regression.**

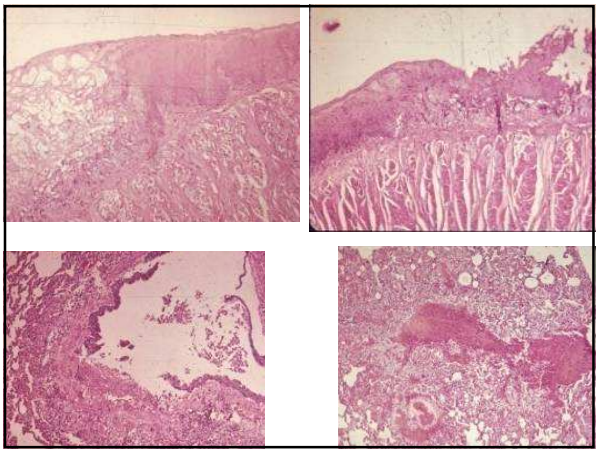
Mid-regression coincided spatially with a loss of viral capsid stain, suggesting that immune cells or cytokines or both were playing a key role in clearance of the papillomas.

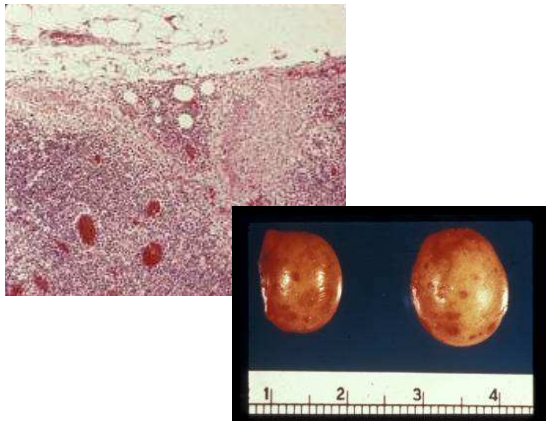
$CD8^+$ cells increased at the lowest rate

$CD11c^+$ and major histocompatibility class II^+ cells were the last populations of cells to decrease in number.









Rabbit Pox

- 6 outbreaks
- Initially isolated at Rockefeller University
- **Antigenically related to Vaccinia Virus and may be a laboratory variant of vaccinia**
- Up ot 95% mortality in 5 days
- Hemorrhage
- Edema
- Skin – rash, papules, crusts, edema, ulceration, hemorrhage
- Necrosis – mouth, nasal passages, spleen, liver, testes, lymph nodes
- Tooth loss
- Keratitis
- CNS signs
- Vaccination with Vaccinia Virus protects uninfected rabbits

Rabbit Enteric Coronavirus

- Only one natural outbreak – Germany
- Serology positives – Canada, Germany, USA
- **Two distinct forms of Coronavirus**
  - **rabbit enteric coronavirus**
  - **pleural effusion disease/cardiomyopathy virus**
- Clinical signs in 3- to 8-week old rabbits included lethargy, diarrhea, abdominal distension, and 100% mortality
- Cecum was distended with watery fluid
- In experimental infections, clinical signs are limited
- Diffuse inflammation and mucosal edema throughout the intestinal tract
- Interference with polyclonal anti-mammalian coronavirus serum produced in rabbits

Rabbit enteric coronavirus

- **Two distinct forms of coronavirus** infection have been reported in rabbits. These include rabbit enteric coronavirus and pleural effusion disease/cardiomyopathy virus
- Rabbit enteric coronavirus, an ssRNA virus, has been detected in the feces of young rabbits with diarrhea in Canada and Europe
- Serologic surveys have extended knowledge of the range of infected rabbitries to the United States
- Only one natural outbreak of disease has been reported, in Germany
- clinical signs in 3- to 8-week old rabbits included lethargy,
- diarrhea, abdominal distension, and 100% mortality
- Cecum was distended with watery fluid, and diffuse inflammationand mucosal edema were found throughout the intestinal tract

Rabbit enteric coronavirus

- In experimental infections, clinical signs are limited to variable
- fecal water content without mortality
- small intestines were congested, with transient evidence of villus tip and M cell necrosis, atrophy, and crypt hyperplasia.
- The cecal contents were watery
- There is a high level of serologic cross-reactivity between rabbit enteric coronavirus and other mammalian group 1 viruses.

Pleural Effusion Disease

- Generalized infection and mortality in laboratory rabbits
- First described in the late 1960s
- In rabbits used for the propagation of Nichols' pathogenic *Treponema pallidum* in Scandinavian laboratories
- Using the *T. pallidum* immobilization (TPI) test for the serological diagnosis of syphilis
- **Found as a contaminant in treponemal suspensions in laboratories in Europe, the USA, and Japan.**
- Virulent and avirulent isolates
- Purified by passage in a hamster model
- Baby rabbits surviving infection developed viremia persisting for at least six months
- Clinical Signs
  - Fever Anorexia Lymphocytopenia Leucocytosis
  - Anemia Iridocyclitis Hypergammaglobulinemia
- PED virus is a Coronavirus. Human or Rabbit Source?

Rotavirus

- Isolated from rabbits with diarrhea and normal rabbits
- Initially in England, and since in Japan, Europe, USA
- Serologic surveys indicate that virus is widespread

Rotavirus

dsRNA viruses of the family *Reoviridae*

isolate that infects rabbits ----- group A serotype 3

also infects humans and other animals

Infection is common in both wild and laboratory rabbits

Clinical signs vary depending on presence of other synergistic organismsin

In most outbreaks, attempts to demonstrate the presence of other pathogens have not been made

In endemically infected colonies,outbreaks are most common in recently weaned rabbits at time of diet change and microbial flora changes

Clinical signs include severe diarrhea, anorexia, dehydration, and high mortality

Mostly mild infections

Rotavirus Rabbits

Thouless 1988

- Diarrhea
- Enteritis
- Aerosol?
- CoFactors?

- Weanlings
  - 1-3 week old
    - High Morbidity
    - Mortality
  - 3-6 week old
    - Low morbidity

Rotavirus

Lesions are limited to

- fluid-filled cecum
- swollen mesenteric lymph nodes
- small intestinal villous atrophy most pronounced in the ileum
- small intestinal distension with mucosal hemorrhage
- increased crypt depth
- lymphocytic infiltrates in the lamina propria
- petechiation of the colon

Rotavirus

- Synergistic effect *Escherichia coli*
- **weanling rabbits developed more severe diarrheal disease than that resulting from either pathogen alone**
- Infection is self-limiting, and immunity is long-lasting
- Natural infection of laboratory rabbits with rotavirus would have at least temporary adverse effects on research involving intestinal physiology

Adenovirus Rabbits

- Uncommon in rabbits
- **Spontaneous disease reported only in Hungary [diarrhea]**
- 4-10 week old
- 15% mortality
- Virus found in
  - Intestinal contents
  - Intestinal wall
  - Spleen
  - Kidney
  - Lung
- **Associated with**
  E coli ***Eimeria sp***

Adenovirus

- **dsDNA viruses**
- uncommon in rabbits and have been
- reported only in Europe.
- spleen, kidney, lungs, and intestines of 6- to 8-week-old rabbits with diarrhea.
- persistent viral infection of lymphoid tissues following experimental infection of rabbits with human adenovirus type 5
- Recombinant adenoviruses have successfully infected rabbit hepatocytes , autologous rabbit vascular interposition grafts, and cultured rabbit corneal epithelial cells .
- In vivo rabbit model system used to test the efficacy of novel antiviral drugs against human adenovirus type 5 infections
- endogenous infections with rabbit adenovirus would interfere with such studies as well as with research on rabbit intestinal physiology or with adenovirus vaccine studies conducted in rabbits

Rabbit Adenovirus experimental

- **dsDNA viruses**
- Persistent viral infection of lymphoid tissues following experimental infection of rabbits with human adenovirus type 5
- Recombinant adenoviruses have successfully infected rabbit hepatocytes , autologous rabbit vascular interposition grafts, and cultured rabbit corneal epithelial cells
- **Adenovirus rabbit models**
  - **Human adenovirus type 2 and type 5**
  - **Vaccine studies**
  - **Antiviral drugs**
  - **Recombinant adenoviruses**

Endogenous infections would interfere with above studies as well as research on rabbit intestinal physiology or with adenovirus vaccine studies conducted in rabbits

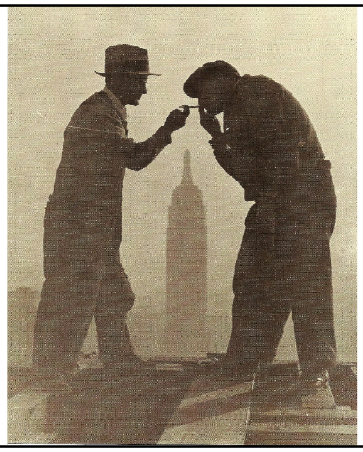
Lapine Parvovirus

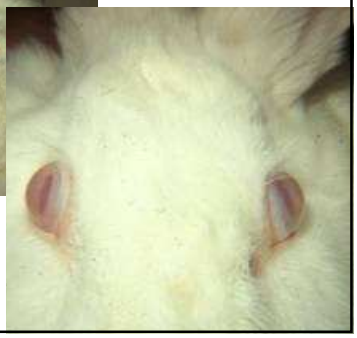
- **ssDNA virus.**
- **Identified serologically in commercial rabbitries in the United States, Europe, and Japan**
- Clinical signs in neonatal rabbits consist of anorexia and listlessness.
- Pathologic changes
- catarrhal enteritis
- hyperemia of the small intestine
- hypersecretion of intestinal mucus
- exfoliation of small intestinal epithelial cells
- Virus can be detected in most visceral organs
- Natural infection of laboratory rabbits could interfere with research in which **rabbit cell cultures or in vitro immunologic assays**

Rabbit Herpesviruses

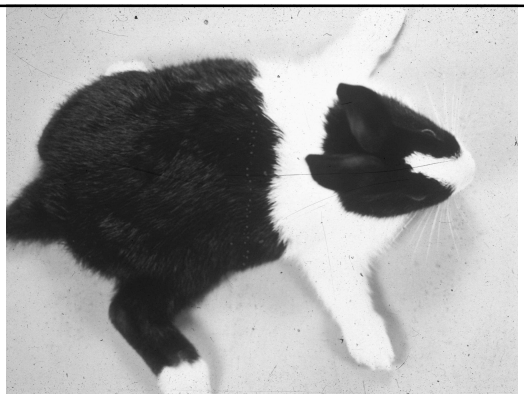
- Two viruses isolated from rabbit kidney cultures
- No naturally occurring disease
- *Herpesvirus sylvilagus in cottontails*
  - *Gammaherpesviridae*
  - *Replicates in nucleus*
  - *Nonfatal lymphoid hyperplasia and lymphoma that resembles mononucleosis*

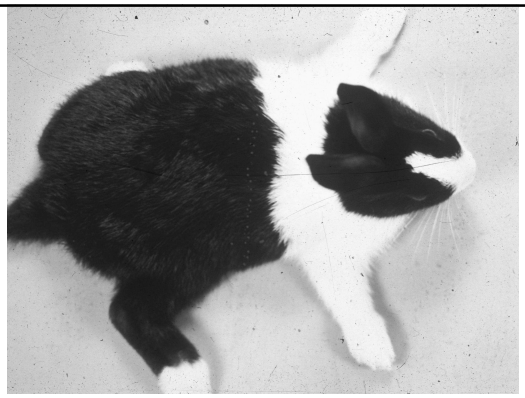
RELATIVE RISK



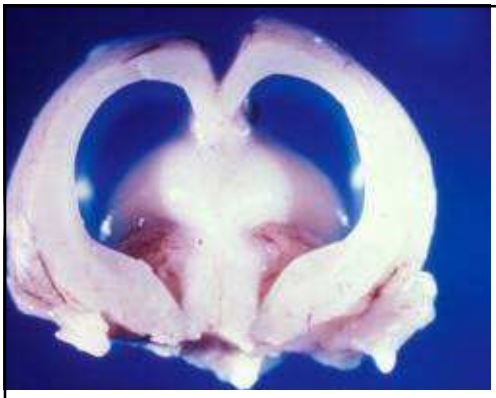


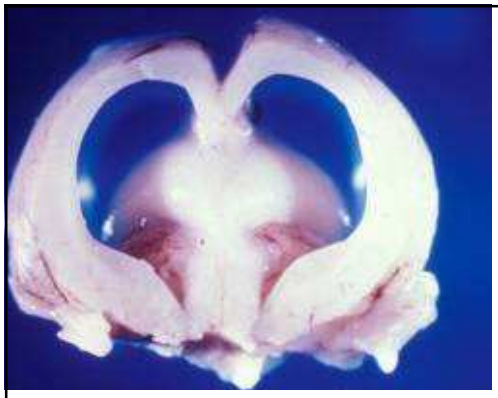
Bupthalmia
Glacoma
Ox eye





Splay Leg [Swimmer]

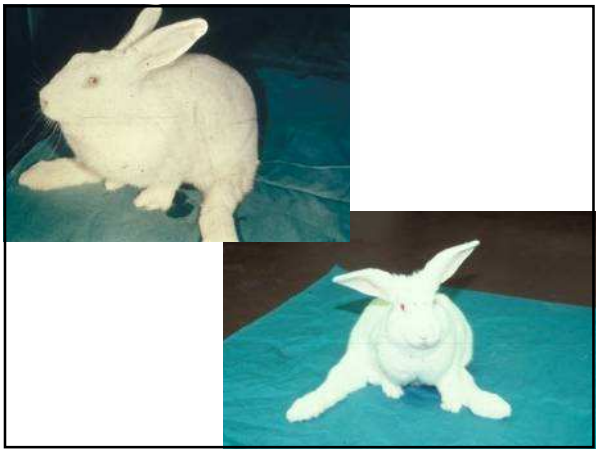


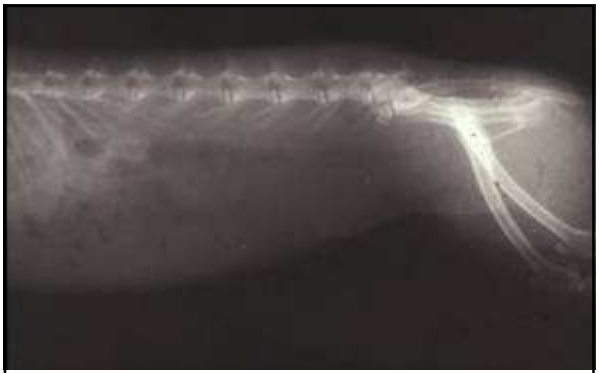


Hypovitaminosis A

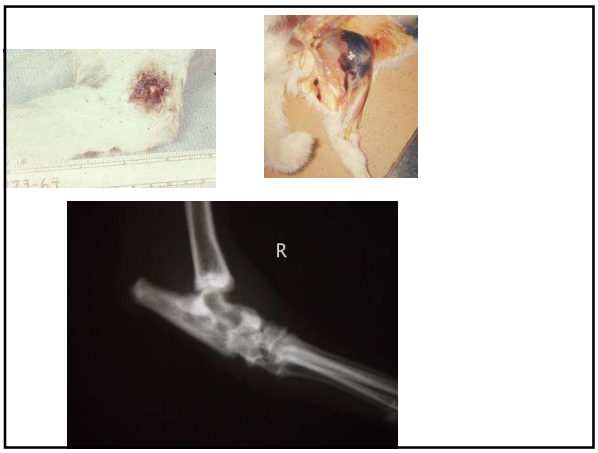
Ulcerative pododermatitis

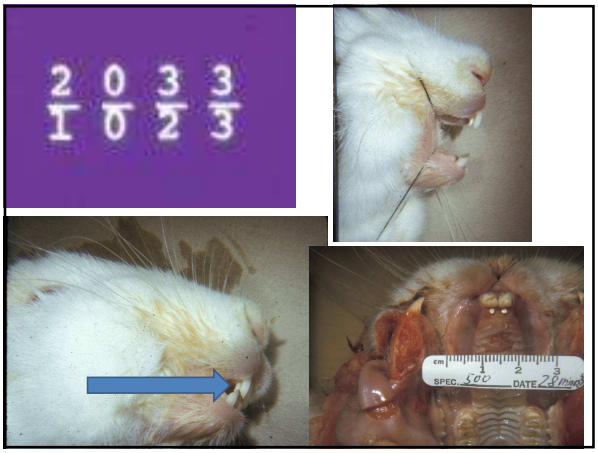
Hutch Burn

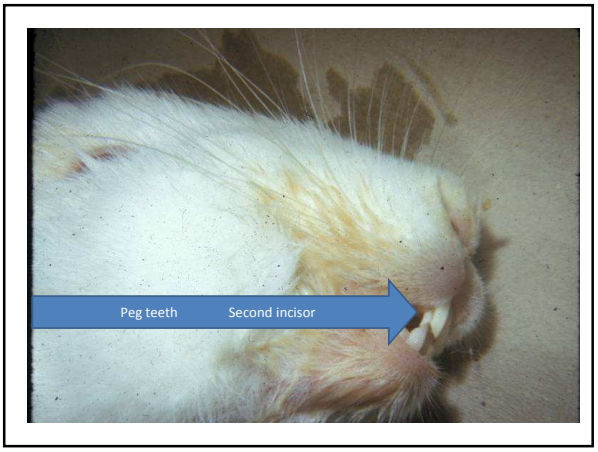


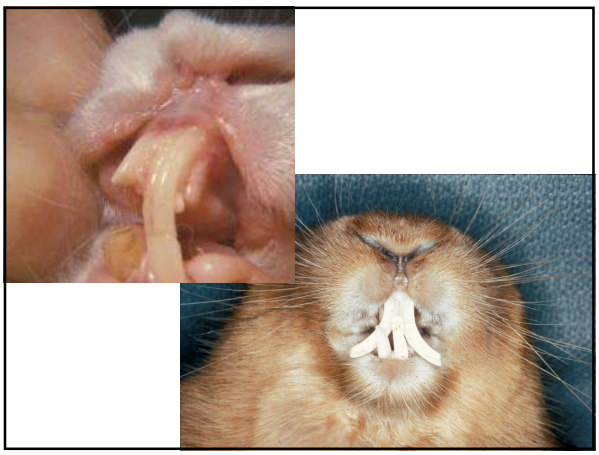


Traumatic Vertebral Separation
Frequently Lumbar 6 / 7 / Sacral 1

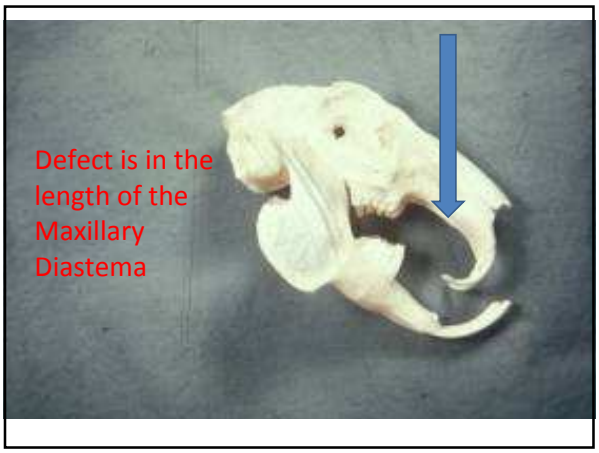


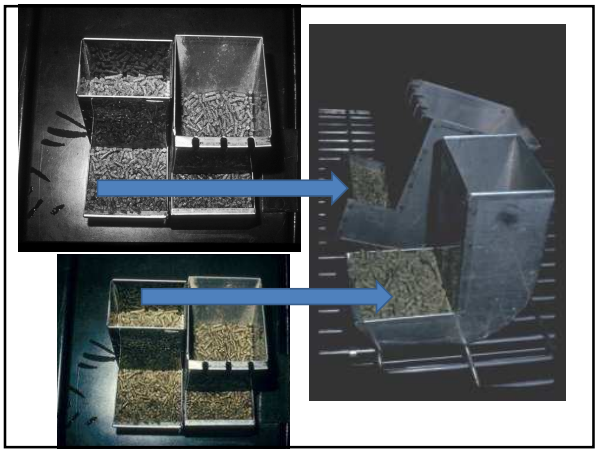


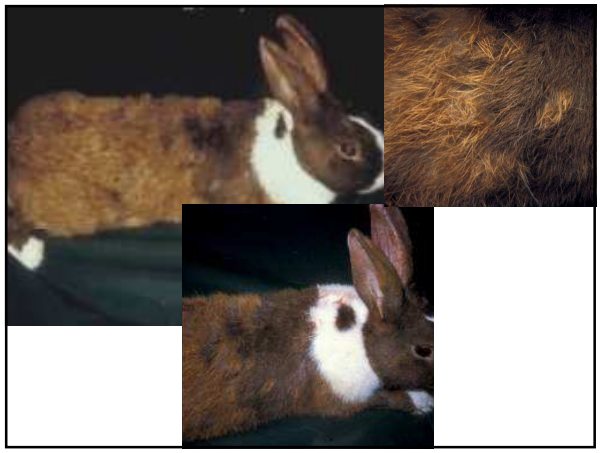




Mandibular prognathism
Overgrown Teeth

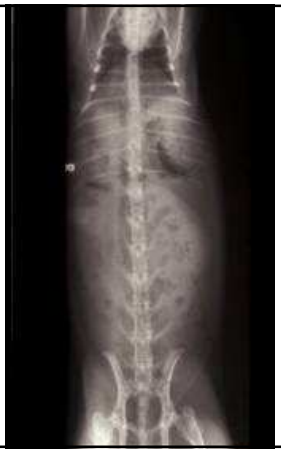

Defect is in the
length of the
Maxillary
Diastema

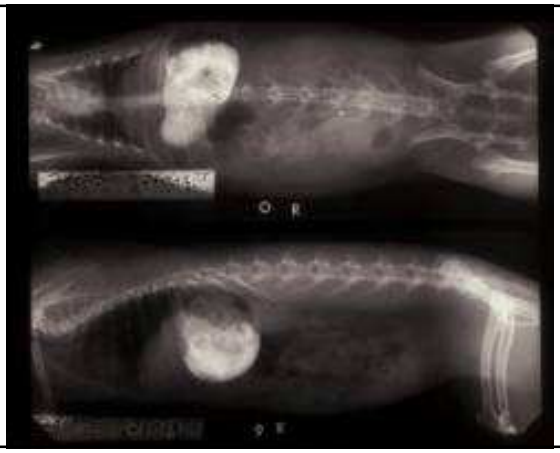





SATURDAY
NIGHT FEVER


R



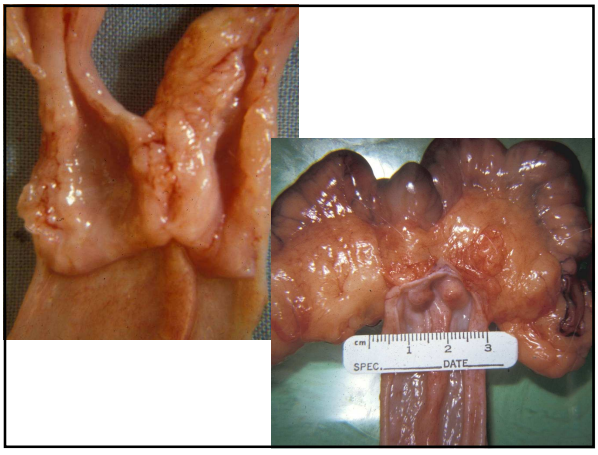


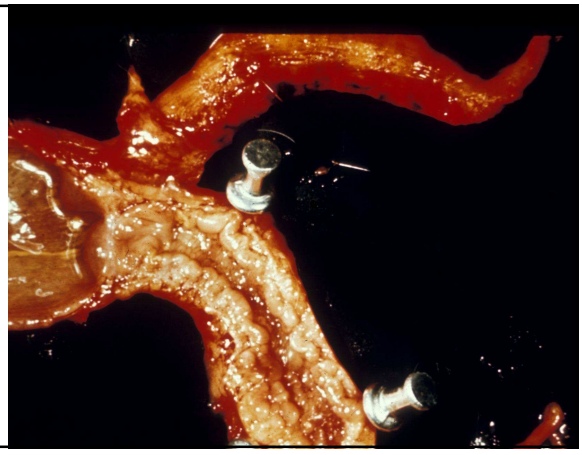
Gastric trichobezoars

Bromelin –pineapple **Papain-papaya**

Gastric Ulceration

- 73 of 1000
- Mostly 6-9 months of age
- Ischemia
- Autodigestion
- **Associated with**
  - **Late pregnancy**
  - **Parturition**
  - **Post parturition**





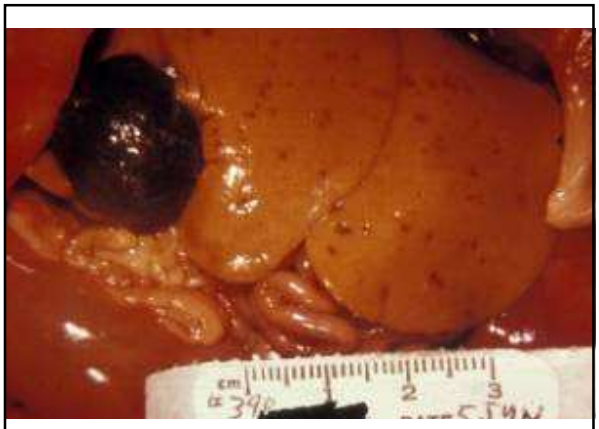
Pseudopregnancy

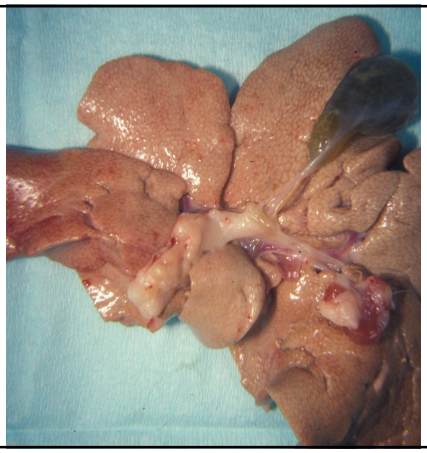
- Can follow mounting by other does, sterile matings by bucks, administration of luteinizing hormone, or the presence of nearby bucks
- Ovulation occurs and is followed by a **persistent corpus luteum [secreting progesterone] that lasts 15-17 days**
- Uterus and mammae enlarge
- Does will begin to pull hair

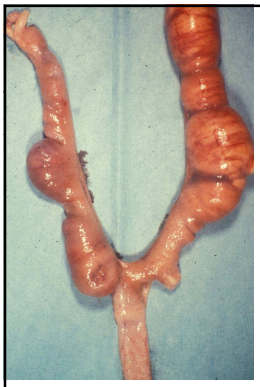
Pregnancy Toxemia

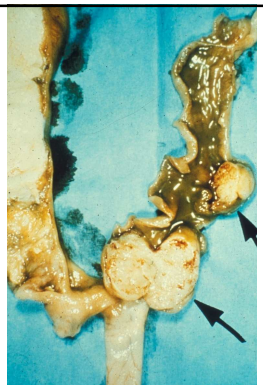
- Dyspnea
- Agitation
- Acetone breath
- Convulsions

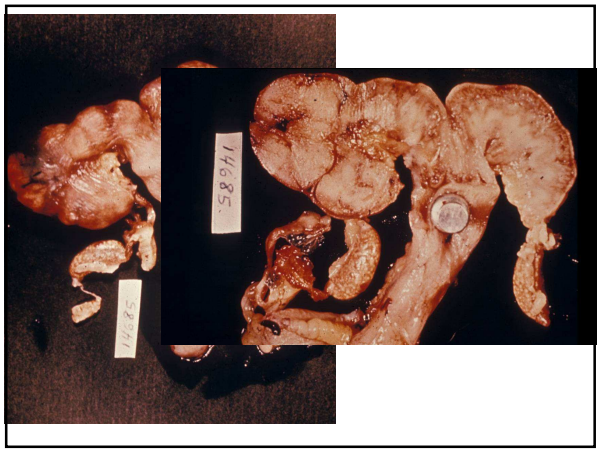
- Rx
  Glucose
  Prevent obesity
  Increase CHO last two weeks of pregnancy

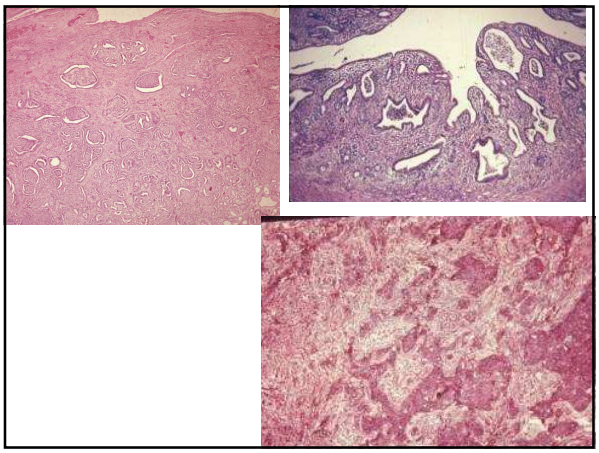


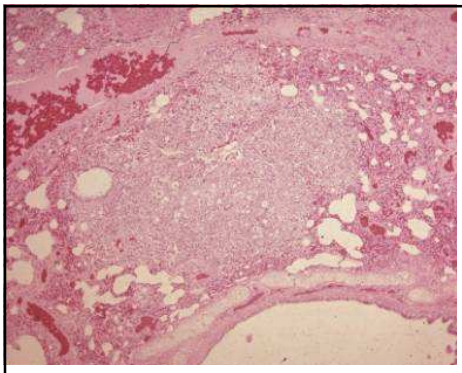






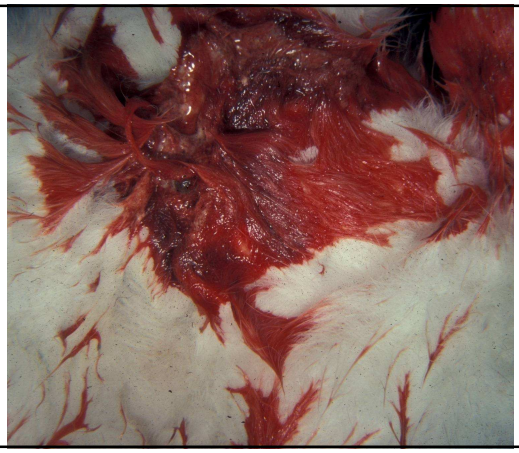


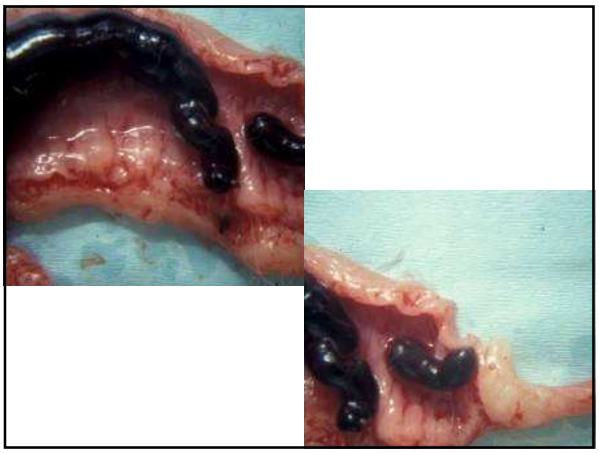


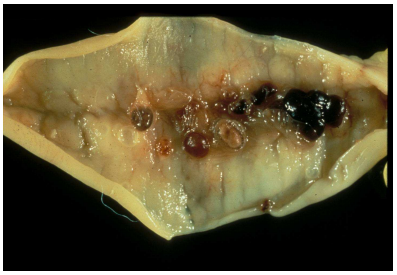


Uterine Adenocarcinoma

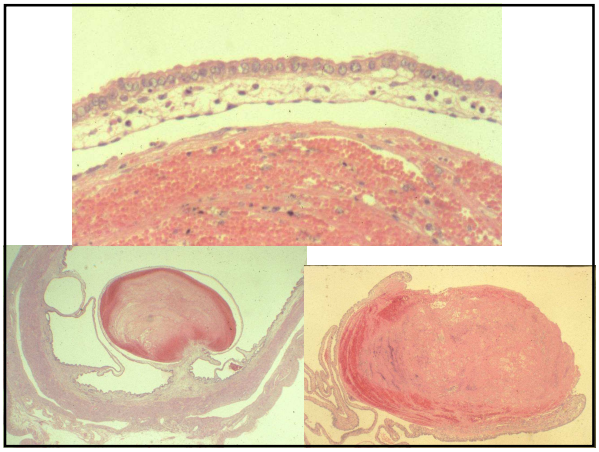
- 10-20 months of age
- Reduced litter size
- Increased stillborn
- Doe desertion
- Metastases

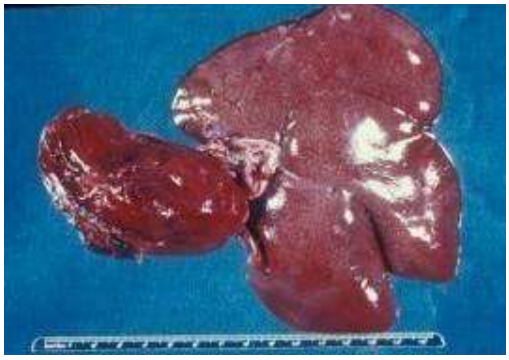






Endometrial venous aneurysms



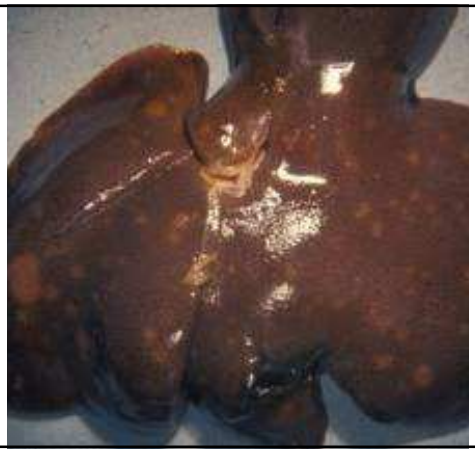


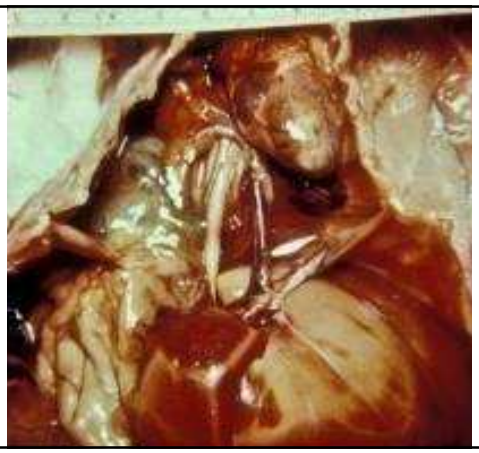
Diagnosis

Post mortem degener-ation

and

probably Hepatic coccidiosis





Arterial Mineralization

- Metastaic calcification
- Hypervitaminosis D
- Mineralization also occurs in
  - other soft tissues
  - kidney basement membranes

Rabbit – Antibody Assays

- *Bordetella bronchiseptica*
- Cilia-Associated Respiratory Bacillus [CARB]
- *Clostridium piliforme* [Tyzzer's Disease]
- Rotavirus
- Rabbit Hemorrhagic Disease Virus
- *Encephalitozoon cuniculi*
- *Toxoplasma gondii*
- *Treponema cuniculi* [microhemagglutination assay]

Rabbit – PCR Assays

• CARB	• Trachea, Lung
• *C piliforme*	• Cecum
• *E cuniculi*	• Urine, Kidney, Brain
• *Helicobacter*	• Feces, Cecum
• *Pneumocystis*	• Lung
• *Staphylococcus aureus*	• Nasopharyx
• *Mycoplasma*	• Cells

harlan™ Health Monitoring Report

Latest Quarterly Update: 7 November 2008

Most Recent Results
N - Negative P - Positive
NS - Not Significant

Location: Oxford, MI | Barrier: 240 | Species: Rabbit

	Test Frequency	Quarterly Results	Test Method	Historical Results*	Recent Results	Test Date
Viruses						
Rabbit Hemorrhagic Disease Virus	Semi-annually	0/6	ELISA	0/18	N	31-Jul-08
Myxomatosis	Semi-annually	0/6	ELISA	0/18	N	31-Jul-08
Bacteria, Mycoplasma and Fungi						
Bordetella bronchiseptica	Bimonthly	0/12	Culture	0/54	N	31-Jul-08
CAR Bacillus	Bimonthly	0/12	MFI	0/54	N	31-Jul-08
Clostridium piliforme	Bimonthly	0/12	MFI	0/54	N	31-Jul-08
Helicobacter bilis	Semi-annually	0/6	PCR	0/18	N	31-Jul-08
Helicobacter hepaticus	Semi-annually	0/6	PCR	0/18	N	31-Jul-08
Helicobacter spp	Semi-annually	0/6	PCR	0/18	N	31-Jul-08
Pasteurella multocida	Bimonthly	0/12	Culture	0/54	N	31-Jul-08
Pasteurella pneumotropica	Bimonthly	0/12	Culture	0/54	N	31-Jul-08
Salmonella spp	Bimonthly	0/12	Culture	0/54	N	31-Jul-08
Toxoplasma spp^	Semi-annually	0/6	MFI	0/6	N	31-Jul-08
Treponema cuniculi	Bimonthly	0/12	MFI	0/54	N	31-Jul-08
Parasites						
Ectoparasites	Bimonthly	0/12	Microscopy	0/56	N	31-Jul-08
Endoparasites	Bimonthly	0/12	Microscopy	0/56	N	31-Jul-08
Enteric Protozoan	Bimonthly	0/12	Microscopy	0/56	N	31-Jul-08
Encephalitozoon cuniculi	Bimonthly	0/12	MFI	0/54	N	31-Jul-08
Pathological Lesions						
Gross Exam	Bimonthly	0/12	Pathology	0/62	N	31-Jul-08

charles river — HEALTH REPORT

printed on : 13/12/2008
Folio : 1

CHARLES RIVER LABORATORIES*France*

Unit n°: ZE2 Species : RABBIT Health status : SPF STATUS
Housed strains NZW
Last control references : 2008002636,2008002631

	HISTORICAL RESULTS	RESULTS AND DATES OF LAST CONTROLS	LABORATORIES	METHODS
SEROLOGY				
RAB HEMORAG DISEASE (RHDV) (a,e)	0 / 48	0 / 8 28/10/2008	CRLF	ELISA
RABBIT ROTAVIRUS (a,e)	0 / 48	0 / 8 28/10/2008	CRLF	IF.,MFIA
BACTERIOLOGY				
TYZZER DISEASE (C.PIL) (a,e)	0 / 48	0 / 8 28/10/2008	CRLF	CLINICAL EXAM AND NECROPSY
DERMATOPHYTE (a,e)	0 / 40	0 / 8 28/10/2008	CRLF	CLINICAL EXAM AND NECROPSY
BORDETELLA BRONCHISEPTICA (b,e)	0 / 48	0 / 8 28/10/2008	CRLF	CULTURE
PASTEURELLA MULTOCIDA (a,e)	0 / 48	0 / 8 28/10/2008	CRLF	CULTURE
PASTEURELLA PNEUMOTROPICA (b,e)	0 / 48	0 / 8 28/10/2008	CRLF	CULTURE
SALMONELLA SPP (a,e)	0 / 48	0 / 8 28/10/2008	CRLF	CULTURE
CARBACILLUS (SEROLOGY)(a,e)	0 / 48	0 / 8 28/10/2008	CRLF	IF.,MFIA
PARASITOLOGY				
ARTHROPODS (a,e)	0 / 48	0 / 8 28/10/2008	CRLF	CLINICAL EXAM AND NECROPSY
HELMINTH (a,e)	0 / 184	0 / 8 24/11/2008	CRLF	DIRECT EXAM + FLOTATION TEST
EIMERIA SP (a or b or c, e)	0 / 184	0 / 8 24/11/2008	CRLF	DIRECT EXAM + FLOTATION TEST
GIARDIA SP (b,e)	0 / 184	0 / 8 24/11/2008	CRLF	DIRECT EXAM + FLOTATION TEST
SPIRONUCLEUS SP (b,e)	0 / 184	0 / 8 24/11/2008	CRLF	DIRECT EXAM + FLOTATION TEST
E. CUNICULI (SERO) (a,e)	0 / 48	0 / 8 28/10/2008	CRLF	IF.,MFIA
LESIONS OBSERVED				
GROSS PATHOLOGY	NO GROSS LESION	28/10/2008	CRLF	CLINICAL EXAM AND NECROPSY
HISTOPATHOLOGICAL EXAMINATION	NOT PERFORMED because of the absence of gross lesion			

COMMENTS

All these results, as well bacteriological and parasitological as serological, are satisfactory.
Parasitology testing is also performed every 4 weeks on feces samples.

LEGEND :
CRLF = CHARLES RIVER LABORATORIES France LABORATORY OF HEALTH AND GENETIC TESTING
CRL = CHARLES RIVER LABORATORIES US
NT = NOT TESTED

FOR INFORMATION, CRL BREEDING COLONY POLICY FOR POSITIVE RESULT
a = immediate termination
b = planned future recycled of the colony
c = no action except if presence of clinical sign and / or lesion

TESTING SCHEDULE
d = screened every 4 weeks
e = screened quarterly
f = screened annually

A. GIROD
DVM,
Veterinary Activities Manager

LABORATORY RABBIT HEALTH MONITORING SUMMARY

<u>SEROLOGY</u> <u>METHOD</u>

AGENT	PRIMARY	ALTER-NATIVE
Cilia-Associated respiratory bacillus (CARB)~	IFA	PCR, Histo
Encephalitozoon cuniculi (ECUN)~	IFA	PCR, Histo
Treponema cuniculi (TREP)~	RPR	ELISA, Histo
Clostridium piliforme (CPIL)**	IFA	PCR, Histo
Lymphocytic choriomeningitis virus (LCMV)	IFA	PCR
Parainfluenzavirus 1 (PI-1)**	IFA	PCR
Parainfluenzavirus 2 (PI-2)**	IFA	PCR
Pneumonia virus of mice (PVM)**	IFA	PCR
Reovirus type 3 (REO)**	IFA	PCR
Rotavirus (ROTA)**	IFA	NONE

METHODS: ELISA = Enzyme-Linked Immunosorbent Assay
IFA = Indirect Fluorescent Antibody test
PCR = Polymerase Chain Reaction
Histo = Histopathology

SPECIES: RB = Rabbit

~ Screened twice each quarter (2x/13 weeks), ** Screened annually

<u>MICROBIOLOGY AND PARASITOLOGY</u> (Twice each quarter)

Some of the agents that we screen for are listed on the attached table.
Screening procedures are also capable of detecting a wide variety of nonpathogenic and opportunistic organisms whose presence is recorded. This information is available upon request. Some of the agents are listed on our standard health reports while findings on other less common agents can be obtained upon request.

<u>GROSS PATHOLOGY</u> (Every 13 weeks)

The major internal organs are examined grossly during necropsies performed as a part of the comprehensive HM program. If gross lesions are present, affected organs/tissues are histologically evaluated. In addition, brain, lung and kidney are examined annually by histopathology as a supplemental screen for *Encephalitozoon cuniculi* and respiratory pathogens.

Revised: February 3, 2007

Supplier Health Reports
Bacteria – Culture except as noted below

- *Bordetella bronchiseptica*
- *CAR Bacillus* MFI
- *Clostridium piliforme* MFI
- *Helicobacter bilis* *H hepaticus* *H spp* PCR
- *Klebsiella oxytoca* *K pneumoniae*
- *Pasteurella multocida*
- *Pseudomonas aeruginosa*
- *Salmonella sp*
- *Staphylococcus aureus*
- *Streptobacillus moniliformis*
- *Streptococcus pneumoniae*
- *Streptococcus spp Group B Beta*
- *Streptococcus zooepidemicus*
- *Treponema cuniculi* MFI

White Spots on the Liver

- Hepatic Coccidiosis
- *Capillaria hepatica*
- Abscesses
  - *Pasteurella multocida*
  - *Franciscella tularensis*
  - *Yersinia pseudotuberculosis*
  - *Staphylococcus aureus*
- Neoplasia

nytimes.c

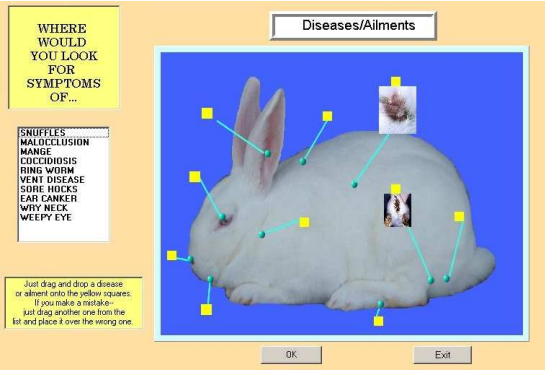
Pet Ferret Hit by an Arrow? Here's a Book for You


Merck/Merial Manual for Pet Health: Home Edition (Merck & Company, $22.95)

chapters on sugar gliders and pot-bellied pigs.

Published: December 18, 2007

Does your gerbil seem depressed?
Is its coat rough, its appetite flagging, its posture hunched?
Does its exercise wheel stand sadly silent?
If so, it might be suffering from Tyzzer's disease, a bacterial infection commonly seen in gerbils, especially when stressed.
Then again, the little rodent might just have a bad case of pinworms.
Not knowing which diagnosis applies — or more likely, never having heard of either ailment — is often frustrating for pet owners, who are unsure how serious their animal's illness is or what to do about it.
The new Merck/Merial Manual for Pet Health: Home Edition (Merck & Company, $22.95), an exhaustive guide to the illnesses and care of many of the species that humans, sensibly or not, have turned to for companionship, aims to help out.

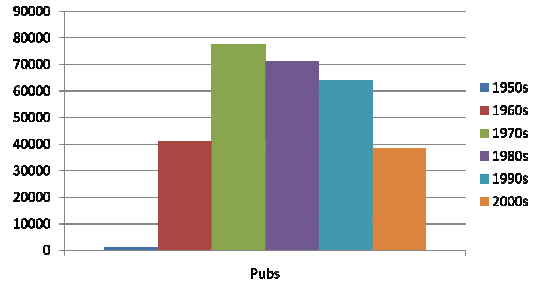


Rabbit Research Use

- Atherosclerosis
- Infectious disease
- Ophthalmology
- Vision research
- Polyclonal antibody testing
- Toxicity and safety testing

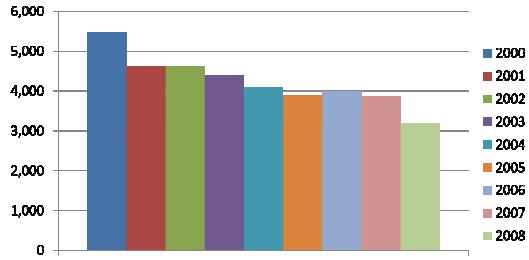
Rabbit Publications

Paper Chase



Rabbit Publications

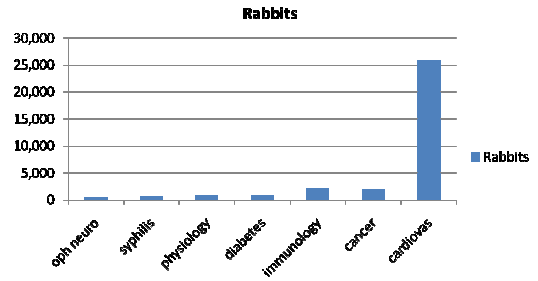
Paper Chase



(700 total as of 5 May 09}

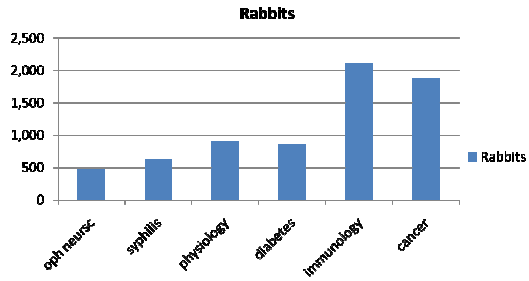
Publications

Paper Chase



Publications

Paper Chase



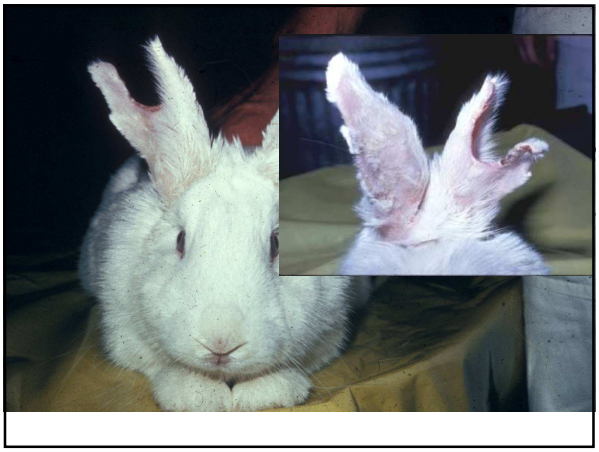
Rabbit Advantages

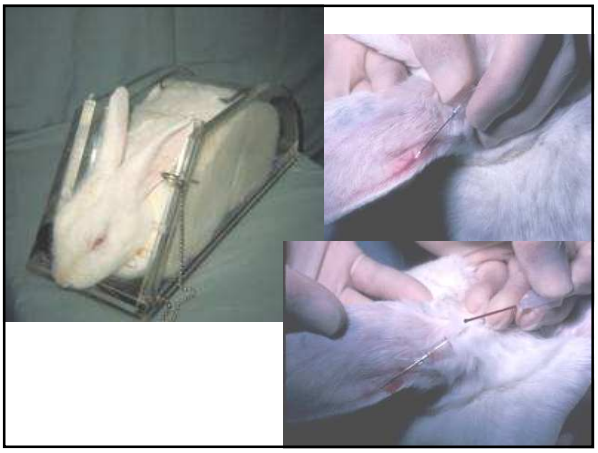
- Small size
- Genetic diversity
- High growth rate
- High feed efficiency (3 lb feed / lb gain)
- Noncompetitive food sources
- Constant reproduction
- High quality meat (7% fat)

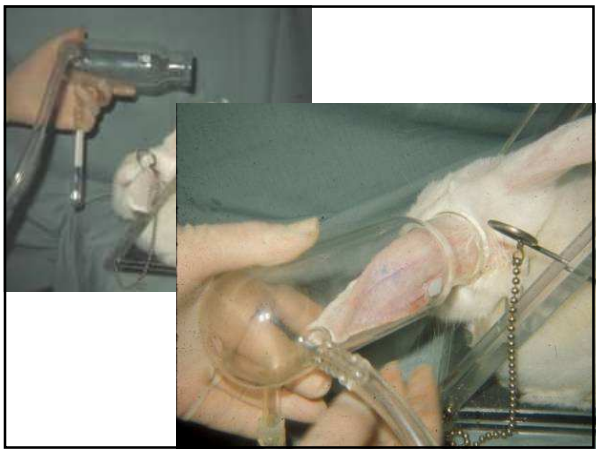
Rabbit Disadvantages

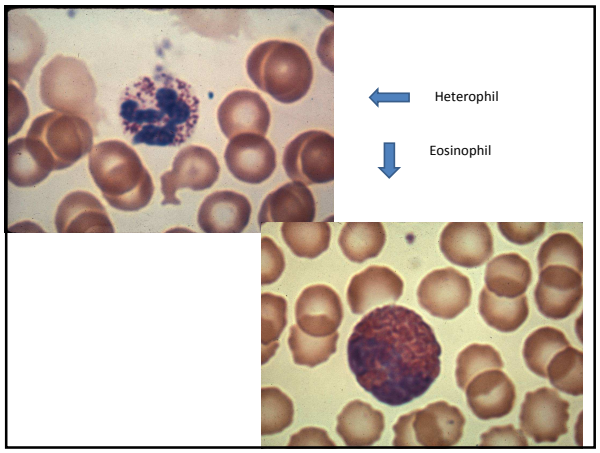
- Disease
- Labor

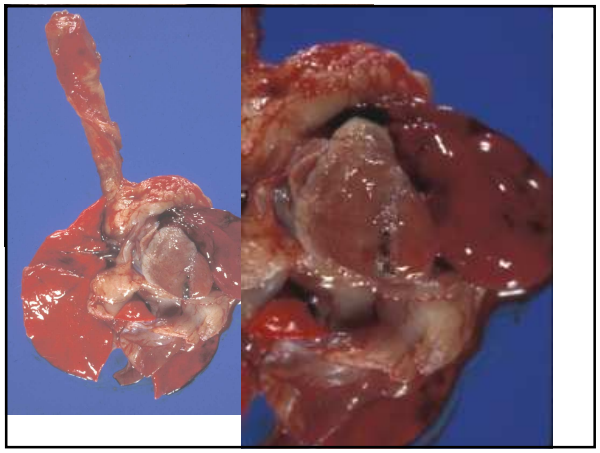
  1 cow = 13 ewes = 211 does
- Limited basic biology research programs



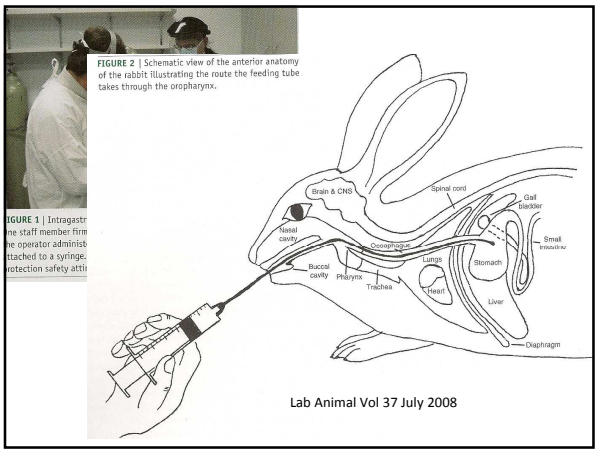




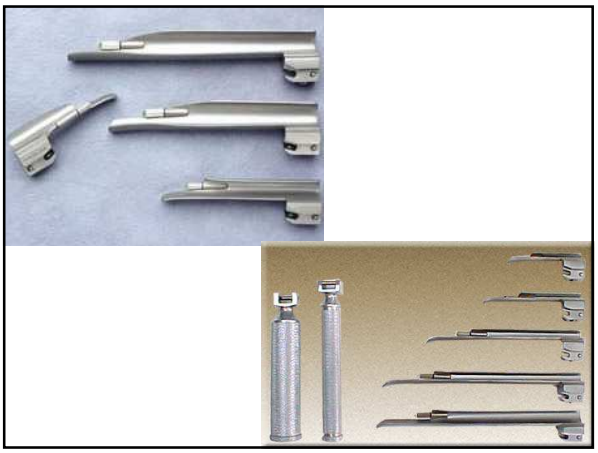




Sudden death in rabbit. Found soon after investigators were in the room. Diagnosis?



Lab Animal Vol 37 July 2008



Wisconsin laryngoscope

Miller laryngoscope

In 1941, *Robert Arden **Miller*** reported on a new blade for direct laryngoscopy (Anesthesiology 2:317-320, 1941).

The ***Miller laryngoscope blade*** is a straight blade curved near the distal end specifically designed to lift the epiglottis. The blade has a shallow vertical portion (suitable for patients with limited mouth opening) with a small flange facing to the right. Later, he developed a similar blade specifically designed for use in infants and pediatric patients .

The Miller laryngoscope blade is commercially available for standard laryngoscope handles in various adult and pediatric versions and modifications. This blade was initially manufactured by the *Foregger Company* (New York City, New York, USA).

Rabbit tracheal intubation Dr. susan kelleher. 2007

- **Rabbit intubation can be accomplished by visualizing the glottis w/ a Miller -0- laryngoscope blade.**
- The key is to hold the laryngoscope in your right hand and put it into the mouth sideways w/ the bulb side towards the roof of the mouth. Extend the blade to the back of the mouth before pushing down on the tongue. Otherwise you cause the back of the tongue to buldge up and obscure the larynx.
- Other factors that help is to pre - med the rabbit w/ 100 ug domitor/kg.
- The holder makes a big difference . I like to have a tech behind the rabbit holding the mouth open w/ tie-tie straps around the upper and lower incisors. It helps if the holder extends the maxilla slightly out more rostral (towards you) than the mandible.
- I like to use a 1/16th IM pin w/ the sharp tip nipped off as a stylet. Bend the tip of the stylet approx 30 degrees to help turn the curve to get down into the trachea.
- I like to spritz the larynx w/ about 0.1cc lidocaine then mask them down some more before introducing the tube.
- The technique can be difficult to master at first, but once you've done a few you can do them in a matter of seconds.
-

Intranasal Intubation?

JMS DeValle
JAALAS Mar 09

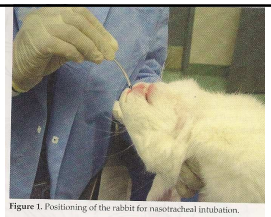


Figure 1. Positioning of the rabbit for nasotracheal intubation.

Figure 2. Tube secured and sutured to the rabbit. Gauze was used to stabilize and secure the head position.

JMS DeValle
JAALAS Mar 09

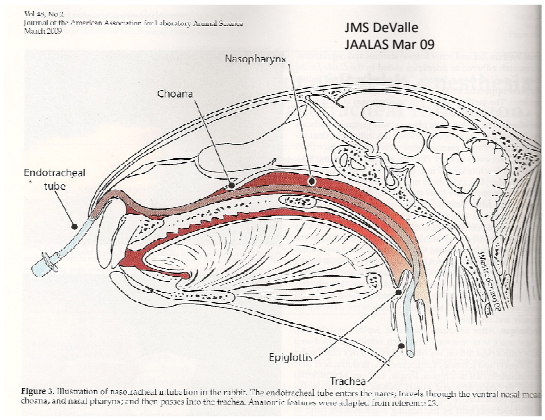


Figure 3. Illustration of naso-tracheal intubation in the rabbit. The endotracheal tube enters the nares, travels through the ventral nasal meatus, choana, and nasal pharynx, and then passes into the trachea. Anatomic features were adapted from reference 25.

Video examination of the mouth of rabbits, hamsters and guinea pigs has allowed us to more accurately diagnose and treat overgrown teeth. The 27X magnification allows viewing problems up close. These teeth are overgrown with sharp points that poke into the tongue and cheek wall.

Intraoral examination in rabbits

Dental examination should be included in all physical examinations of rabbits because they have continuously growing teeth and are predisposed to dental disease. This column describes the procedure and equipment used for oral examination in rabbits.

Brown Lab Animal 37 Sept 2008

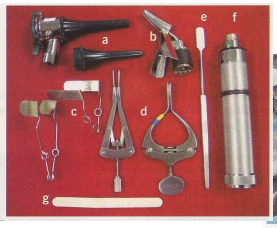


FIGURE 1 | Instruments used for intraoral examination of the rabbit. (a) Otoscope head with plastic cones (sizes 5 mm and 7 mm). (b) Bivalve nasal speculum with transilluminator. (c) Short- and long-blade cheek dilators. (d) Manual mouth gags. (e) Metal spatula (tongue depressor). (f) Wooden speculum (popsicle stick) for tongue and cheek manipulation. (g) Base for otoscope and nasal speculum attachment (interchangeable).

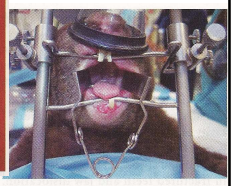
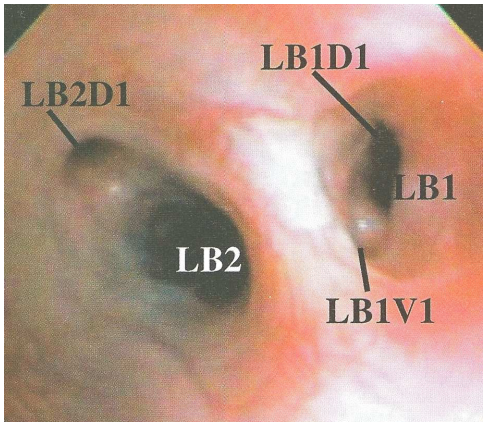


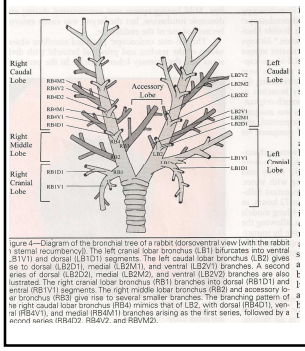
FIGURE 2 | An anesthetized rabbit restrained with a tabletop gag for intraoral examination. Inhaled anesthesia is being delivered via nose mask. Note the fractured lower right incisor and abnormal curvature of the upper incisors. Long-blade cheek dilators are in place to aid visualization of cheek teeth.



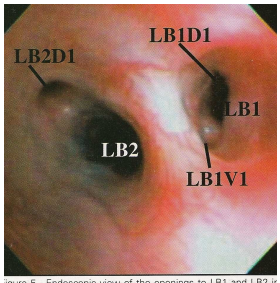
Endoscopic evaluation of bronchial morphology in rabbits

Lynelle R. Johnson, DVM, PhD; Tracy L. Drazenovich, DVM; Michelle G. Hawkins, DVM

AJVR September 2007



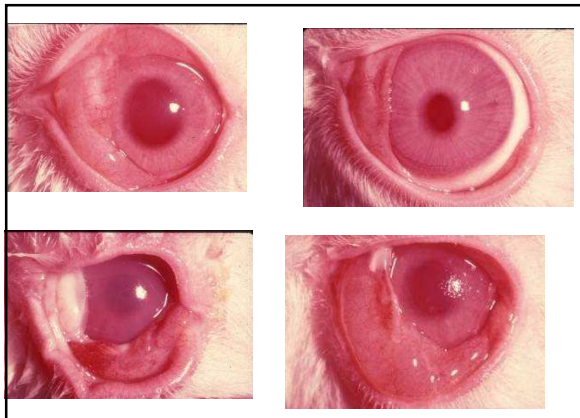
igure 4—Diagram of the bronchial tree of a rabbit (dorsoventral view [with the rabbit sternal recumbency]). The left cranial lobar bronchus (LB1) bifurcates into ventral B1V1) and dorsal (LB1D1) segments. The left caudal lobar bronchus (LB2) gives se to dorsal (LB2D1), medial (LB2M1), and ventral (LB2V1) branches. A second eries of dorsal (LB2D2), medial (LB2M2), and ventral (LB2V2) branches are also lustrated. The right cranial lobar bronchus (RB1) branches into dorsal (RB1D1) and entral (RB1V1) segments. The right middle lobar bronchus (RB2) and accessory lo- ar bronchus (RB3) give rise to several smaller branches. The branching pattern of he right caudal lobar bronchus (RB4) mimics that of LB2, with dorsal (RB4D1), ventral (RB4V1), and medial (RB4M1) branches arising as the first series, followed by a econd series (RB4D2, RB4V2, and RB4M2).



igure 5—Endoscopic view of the openings to LB1 and LB2 ir rabbit. Notice that LB1 immediately branches into LB1D1 anc B1V1. The LB2D1 is also evident. *See* Figure 4 for key.

Atropine Esterase

- Inactivates atropine
- Rabbits can consume diets containing belladonna leaves without adverse effects



Draize Test

Draize Test

Holden 1988

- Test developed 1944
- Coalition 1978
  - Redundant
  - Unnecessary
- Tests down by over 50%
- Three rabbits instead of six
- Regulation - FCA, EPA, CPSC

- A 2004 study by the U.S. Scientific Advisory Committee on Alternative Toxicological Methods analyzed the modern Draize skin test. They found that the test would:

- Misidentify a serious irritant as safe: 0-0.01%
- Misidentify a mild irritant as safe: 3.7%-5.5%
- Misidentify a serious irritant as a mild irritant: 10.3%-38.7% [5]

- Significant differences between rabbits' eyes and human eyes: [11]
- The rabbit epithelial (surface) layer is 10 times more permeable to hydrophilic solutes than the human eye.
- Bowman's membrane (the next layer) is six times thicker in man.
- The rabbit's threshold of pain in the eye is much higher than that of humans, so irritating substances are not washed away as readily.
- Rabbits have a less efficient tearing system than humans.
- Unlike people, rabbits have a nictitating (winking) membrane (third eyelid), which has an unclear effect on elimination of foreign materials.
- Humans develop corneal epithelial vacuoles in response to some toxic substances, but rabbits do not.
- The rabbit mean corneal thickness is .37 mm, while that of man is .51 mm.
- The cornea represents 25% of the rabbit eye surface area, but only 7% of the surface area in man.
- However, a recent review in the journal *Alternatives To Laboratory Animals* concluded, "despite extensive efforts ... there is still no *in vitro* method that is fully validated as a regulatory replacement" (Curren and Harbell 2002).

- According to the British Research Defence Society, a group representing 5,000 animal researchers and institutions in the UK, the Draize eye test is now a "very mild test," [7] in which small amounts of substances are used and are washed out of the eye at the first sign of irritation.
- The UK Home Office has published guidance and minimum severity protocols for the procedure. [8]
- In a letter on January 12, 2006 to the science journal *Nature*, written to refute an article saying that the Draize test had not changed much since the 1940's, Nobel prize winner Professor Sir Andrew Huxley described the test as follows:
- "A substance expected from its chemical nature to be seriously painful must not be tested in this way; the test is permissible only if the substance has already been shown not to cause pain when applied to skin, and *in vitro* pre-screening tests are recommended, such as a test on an isolated and perfused eye. Permission to carry out the test on several animals is given only if the test has been performed on a single animal and a period of 24 hours has been allowed for injury to become evident." [9]

- The **Draize Test** is an acute toxicity test devised in 1944 by Food and Drug Administration (FDA) toxicologist John H. Draize.
- Initially used for testing cosmetics, the procedure involves applying 0.5mL or 0.5g of a test substance to an animal's eye or skin for four hours.
- The animals are observed for up to 14 days, for signs of erythema and edema in the skin test, and redness, swelling, discharge, ulceration, hemorrhaging, cloudiness, or blindness in the tested eye.
- The test subject is commonly an albino rabbit.
- The tests are controversial. They are viewed as cruel by critics, as well as unscientific because of the differences between rabbit and human eyes, and the subjective nature of the visual evaluations.
- Sometimes modified so that anaesthetics are administered and lower doses of the test substances used. [3]
- Chemicals already shown to have adverse effects *in vitro* are not currently used in a Draize test, [4] thereby reducing the number and severity of tests carried out.

Draize Test Alternatives

- Chorionic allantoic membranes
- Whole eyes – mice, rabbits, cows
- Corneal cells – rabbits, mice
- Mammalian skin cell
- Tetrahymena – multicellular, aquatic

The Draize Skin Test

The Draize skin test is performed to test the potential for skin irritancy of a substance.

rabbits and guinea pigs.

The substance being tested is applied to shaved and abraded skin of the animals which is then covered with plastic sheeting or cast so that the animal does not attempt to scratch or lick the substance off.

The skin of the animal is abraded by pressing adhesive tape and quickly ripping it off. This process is repeated so that many layers of skin are removed prior to testing.

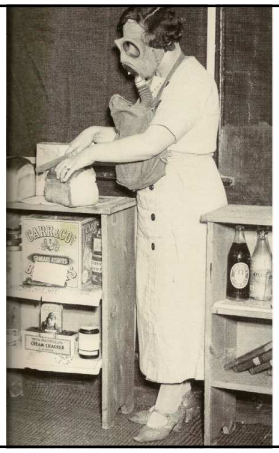
The animals are then observed for signs of edema and erythema at regular intervals.

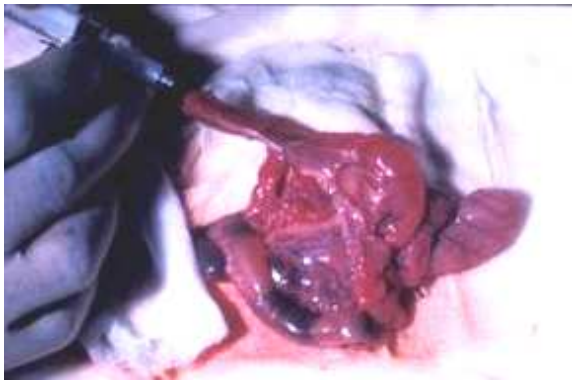
CFA

Pyrogen Testing

Pyrogen Testing

- Possible febrile response of patient to parenteral test agent
- USP 1990
- Three healthy rabbits
- Temperature monitoring probe into rectum
- Baseline, 1, 2, and 3 hour temperatures post dosing
- Product meets requirement standards if individual body temperatures do not exceed 0.6C or the sum does not exceed 1.4C





Ileal Loop -- Test for Endotoxin

J Bacteriol. 1969 Nov; 100(2): 985–993.
Cholera Toxins: Immunogenicity of the Rabbit Ileal Loop Toxin and Related Antigens. J Kaur, W Burrows, L Cercavski Dept Microbiology, U of Chicago

World J Gastroenterol 1999; June 5(3):245-248
Pathogenic effects of Opolysaccharide from Shigella flexneri strain
Qi-Ping Zhong

Rabbit ileal loop assay

Rabbits were fasted for 24 hours.

The rabbit ligated ileal loops (5cm) were prepared in rabbits (weight 2kg) anesthesized with procaine hydrochloride by local infiltration.

Twenty μg O-polysaccharides in 0.5mL saline was injected into the loop.

Rabbits were sacrificed 24 hours later.

Portions of tested loops were taken and fixed in 10% buffered formalin immediately.

The pathologic slices of the specimen were prepared with standard procedures.

Everest et al P**athological changes in the rabbit ileal loop model caused by Campylobacter jejuni from human colitis.**
J Medical Microbiology 1993;38(5):316-21.

- **Four strains of *Campylobacter jejuni* isolated from children** with inflammatory diarrhoea were assayed in the rabbit ileal loop model of infectious diarrhoea.
- All caused **inflammatory reactions with severe macroscopic and microscopic damage in infected rabbit ileal tissue similar to that observed in the patients** by endoscopy and histological analysis of colonic biopsies.
- **Haemoglobin and other proteins were observed in loop fluids**, consistent with leakage of serum from damaged mucosa. Loop fluids also contained significant bicarbonate concentrations, indicative of an **active secretory component similar to that in control loops inoculated with cholera toxin.**
- We propose that host-derived mediators of secretion may be important in pathogenesis.
- A mutant strain of *C. jejuni* with impaired motility, obtained from the National Collection of Type Cultures, did not induce tissue damage or fluid secretion in rabbit ileal loops.

1976: Johnson D E; Calia F M **False-positive rabbit ileal loop reactions attributed to Vibrio parahaemolyticus broth filtrates.** J Infectious Diseases 1976;133(4):436-40.

Vibrio parahaemolyticus broth filtrates have previously been shown to produce positive reactions in rabbit ileal loops only if concentrated 10-fold by lyophilization.

This method of concentration produces solutions that contain greater than 20% NaCl.

In the present study, however, **concentrations of NaCl of greater than or equal to 4% induced positive responses in ileal loops, and desalting rendered previously reactive, concentrated broth filtrates negative.**

Therefore, enterotoxin was not demonstrated in our broth filtrates of V. parahaemolyticus, a finding which suggests that previous studies require further evaluation.

Since most culture media contain 0.5% NaCl, it is important to determine and to control the NaCl content and the osmolality of all lyophilized concentrates tested in the ligated rabbit ileum.

DETECTION OF BACILLUS CEREUS DIARRHEAGENIC TOXIN **USING A RAT LIGATED INTESTINAL LOOP ASSAY**

WEI-TSYI TING [1] GEORGE J. BANWART [1] [1] Department of Microbiology, The Ohio State University.

A study was performed to determine if the rat ligated intestinal loop assay could detect *Bacillus cereus* diarrheagenic toxin.

The results obtained indicated that this assay system could be used for such a purpose but it **was not as sensitive as the young rabbit ligated ileal loop assay.**

When rats were used, concentrating the cell free culture filtrate of *B. cereus* 30- to 40-fold was necessary to detect diarrheagenic toxin activity with the intestinal loop assay

Watanabe

- LDL Receptor Deficiency
- Lipid deposits arterial media, intima
- Skin / Cornea - xanthomas

Watanabe Heritable Hyperlipidemic Rabbit [WHHL]
J Arteriosclerosis 3:87-101, 1983

- Watanabe heritable hyperlipidemic rabbit. **Animal model for familial hypercholesterolemia**. Havel et al Arteriosclerosis (1989 Jan-Feb) 9(1 Suppl):I33-8
- The mutant **low density lipoprotein (LDL) receptor in the WHHL rabbit lacks four amino acids** in the third repeat unit of the receptor- binding domain.
- Rabbits develop fatty intimal streaks and later complicated atherosclerotic lesions, as do cholesterol-fed rabbits.
- The lipoproteins accumulating in the blood include not only LDL but also remnants of very low density lipoproteins (VLDL)
- VLDL remnants are metabolized sluggishly, and a much larger fraction than normal is converted to LDL, which, therefore, accumulate not only because of impaired removal, but also as a result of increased formation from VLDL precursors.

Transgenic Rabbits

Enhanced transmural fiber rotation and connexin 43 heterogeneity are associated with an increased upper limit of vulnerability in a **transgenic rabbit model of human hypertrophic cardiomyopathy.**
Ripplinger et al Circ Res (2007 Nov 9) 101(10):1049-57

- Some **endocrine traits of transgenic rabbits. I. Changes in plasma and milk hormones** Sirotkin et al Physiol Res (2008) 57(5):735-43
- **Expression of human interferon beta in the mammary gland of transgenic rabbits** Khodarovich et al Bioorg Khim (2008 Mar-Apr) 34(2):185-93

Belgian town of Geel, a rabbit is strapped into a canvas sling. The research assistant attaches rubber teats to the animal and switches on the milking machine.

Genetically engineered rabbit to produce enzyme. Dutch company Pharming suggest this protein could provide the cure for sufferers of the rare muscle-wasting disease called Pompe's.

Enzyme therapy for Pompe disease with recombinant human α-glucosidase from rabbit milk

Van den Hout et al J Inherited Metabolic Disease Vol 24, April, 2001 Pages266-274

- Pompe disease is a metabolic myopathy caused by deficiency of lysosomal acid -glucosidase.
- Review of first 36 weeks of a clinical study on the safety and efficacy of enzyme therapy aimed at correcting the deficiency.
- Four patients with infantile Pompe disease were enrolled. **They received recombinant human -glucosidase from transgenic rabbit milk.** The product is generally well tolerated and reaches the primary target tissues. Normalization of -glucosidase activity in skeletal muscle was obtained and degradation of PAS-positive material was seen in tissue sections.
- **The clinical condition of all patients improved.**
- **The effect on heart was most significant, with an impressive reduction of the left ventricular mass index (LVMI).**
- Motor function improved. The positive preliminary results stimulate continuation and extension of efforts towards the realization of enzyme therapy for Pompe disease.

F.D.A. Approves Drug From Gene-Altered Goats

ANDREW POLLACK NY Times February 6, 2009

- **GTC Biotherapeutics,**
- human anticlotting protein is produced by a herd of 200 bioengineered goats living under carefully controlled conditions on a farm in central Massachusetts.
- The drug was approved to prevent blood clots in people born with a rare hereditary deficiency of antithrombin while they undergo surgery or childbirth. At other times such people can reduce their clotting risks by taking blood thinners like warfarin, but during surgery or childbirth blood thinners are typically avoided because of the risk of excessive bleeding.
- ATryn will be sold in the United States by Ovation Pharmaceuticals, which said it had not yet set the price.

- **Pharming,** which is based in the Netherlands, plans to apply this year for approval of a drug**, produced in the milk of transgenic rabbits**, to treat hereditary angioedema, a protein deficiency that can lead to dangerous swelling of tissues.

GENE THERAPY

Effect of cell-based **VEGF gene therapy on healing of a segmental bone defect** Li R et al J Orthop Res (2009 Jan) 27(1):8-14

- **Gene therapy in heart failure** Vinge et al Circ Res (2008 Jun 20) 102(12):1458-70
- Local adiponectin treatment **reduces atherosclerotic plaque size in rabbits** Li et al J Endocrinol (2007 Apr) 193(1):137-45
- Ex vivo gene delivery of ephrin-B2 induces development of **functional collateral vessels in a rabbit model of hind limb ischemia** Katsu et al J Vasc Surg (2009 Jan) 49(1):192-8

Pitt scientists on mission to grow teeth from scratch
By Allison M. HeinrichsTRIBUNE-REVIEW
Sunday, February 8, 2009

- Dr. Charles Sfeir, director of the Center for Craniofacial Regeneration in Pitt's School of Dental Medicine
- one method draws on recent success in growing bone.
- It removed slightly more than half-inch segments of arm bone from **rabbits,** and filled the gap with a "scaffolding" laced with proteins that encourage bone to grow.
- Bone replaced the scaffold.
- Using the same methods, the team plans to build tooth scaffolds pumped full of stem cells and implant them in lab animals, next to organs where blood vessels are plentiful.
- In a few weeks they hope to open the animal back up and pull out a tooth that could be implanted in the animal's jaw.

- Snuffles
- Mange
- Vent Disease
- Ear Canker
- Wry Neck
- Weepy Eye
- Hutch Burn
- Warbles
- Dewlap
- Bumblefoot
- Schmorl's disease
- Coprophagy
- Night feces
- Kitt
- Wolf teeth
- Splay leg
- Swimmers
- Sore hocks
- Fur block
- Slobbers
- Kindling
- Wet dewlap
- Laurices
- Ox Eye
- Blue Bag
- Cecotrophy
- Rabbit fever
- Kittling

Reducing suffering - Rabbit welfare
rabbits@rspca.org.uk

UFAW/RSPCA Rabbit Behaviour and Welfare Group

Refining Rabbit Care: A Resource for Those Working With Rabbits in Research sets out the welfare needs of the rabbit, based on the current laboratory animal science and welfare literature, and explains how these needs can be fulfilled.

The 26-page report is intended for animal technologists, facility managers, veterinarians and scientists.

Reducing suffering
Refinement
Rabbit welfare

- The RSPCA is a charity registered in England & Wales no. 219099

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If opportunity doesn't knock, build a door

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