

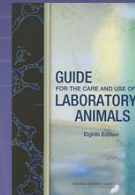
## WORKSHOP IN LABORATORY ANIMAL MEDICINE

### RUMINANTS

May 20, 2011  
Melaney K. Gee, MS, DVM  
Wake Forest University Health Sciences

USDA Animal Care Policy # 29 has adopted the following guidelines for ruminants used in biomedical research

- Guide for Care and Use of Agricultural Animals in Agricultural Research and Teaching (Federation of Animal Science Societies)
- Guide for Care and Use of Laboratory Animals (Institute for Laboratory Animal Research)



## Disclaimers

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



## Unique Anatomic and Physiologic features

- Digestive System
  - 4 compartments
  - Main source of energy is Volatile Fatty Acid(VFA) as opposed to glucose
  - Eructation of large amounts of CO<sub>2</sub> and Methane
- Hemal Nodes
- BUN is not a good indicator of renal function due to GI production of urea nitrogen
- ALT cannot be used to evaluate liver disease in goats
- Small RBCs (especially sheep)
- Chromosome number ?
  - Goats 54
  - Sheep and Cattle 60



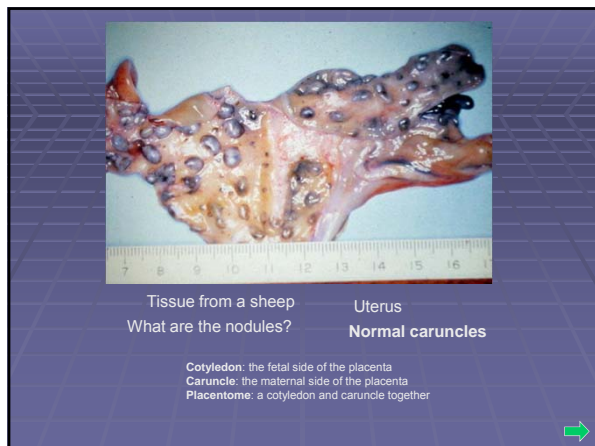
## Ruminants

Bovine  
Ovine  
Caprine

## Reproductive Characteristics Ruminants

- Seasonal Polyestrous
- Gestation lengths (days) ?
  - Sheep 147-150
  - Goats 145-155
  - Cattle 270-292
- Placentation ?

Epitheliochorial, cotyledonary



## Bovine Research Use

- Cardiac Transplantation
- Cardiac prosthetics- stents, valves
- Reproductive research
  - Artificial insemination
  - Embryo transfer
  - Genetic engineering

Cloned Transgenic Calves

## Bovine

Order	Artiodactyla (even-toed ungulates)
Family	Bovidae
Genus species	<i>Bos taurus</i> <i>Bos indicus</i>
Female	Cow/Heifer
Male	Bull/Steer
Young	Calf

Semmental  
(*Bos taurus*)

Brahman  
(*Bos indicus*)

## Bovine Infectious Disease Model

*Trichostrongylus axei*

Bovine trichostrongylosis

Animal model for human *Trichostrongylus axei*

Breed ?    Holstein

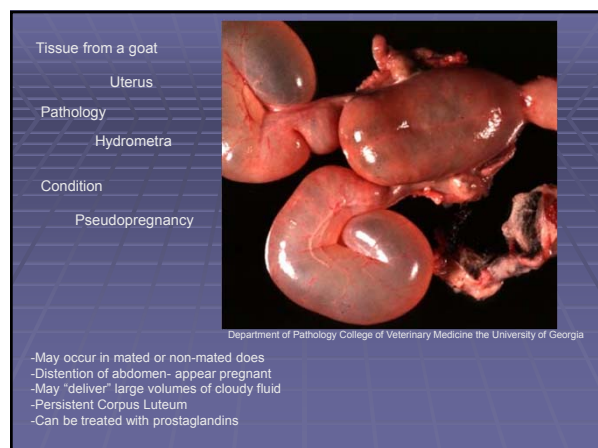
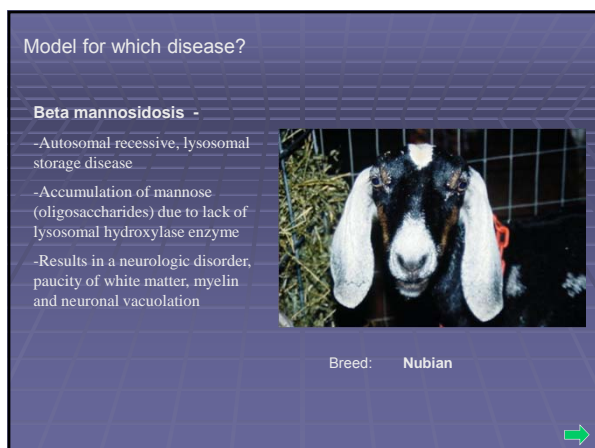
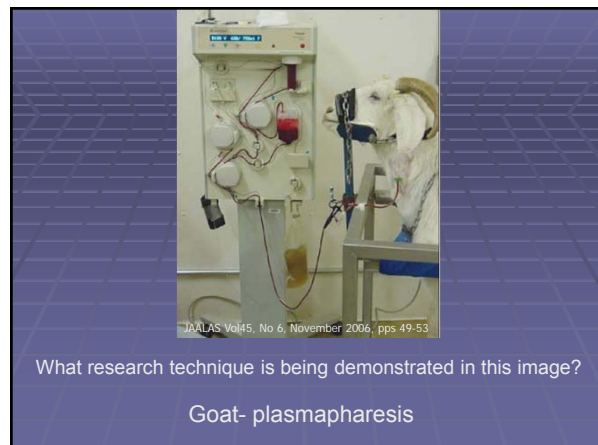
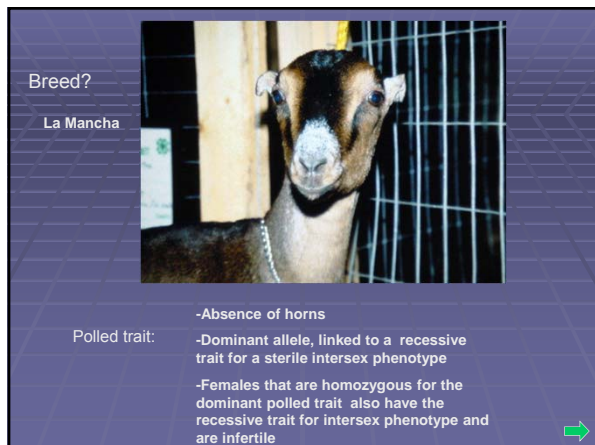
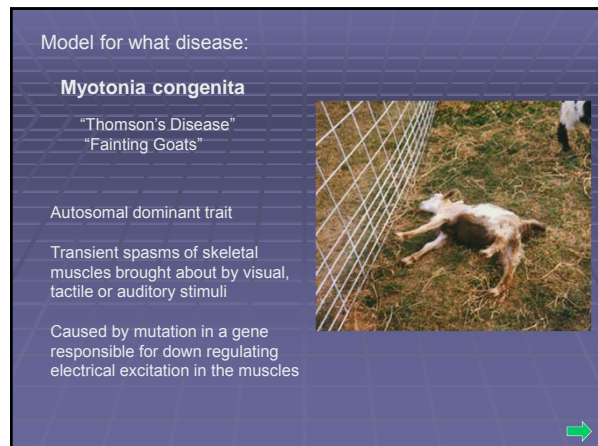
Disease Model?

Leukocyte adhesion deficiency syndrome  
Hereditary orotic aciduria  
Inherited Cardiomyopathies

## Caprine

Genus and species:	<i>Capra hircus</i>
Order:	Artiodactyla
Family:	Bovidae
Male:	Buck (billy)
Castrated Male:	Wether
Female:	Doe (nanny)
Young:	Kid

Breed:    toggenburg



## Sheep

Genus and species: *Ovis aries*

Male: Ram  
Castrated male: Wether  
Female: Ewe  
Young: Lamb

Location of Sebaceous Glands?  
Below eye and between toes



Breed: Suffolk

- Pruritis
- Nervous
- Excitable
- Tremors



Tissue from a sheep

Intestinal adenocarcinoma

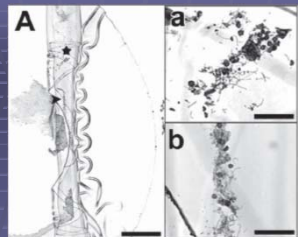
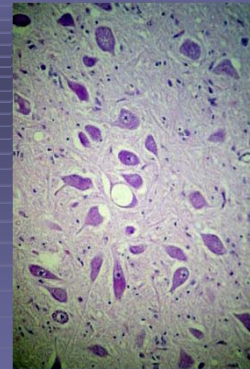


Comp Med, Vol 56, No 2 pps 136-141

- Occur in aged, unthrifty sheep
- 100% in the small intestine
- Proposed as a model for human intestinal adenocarcinoma

Diagnosis: Scrapie

- Neuron - large cytoplasmic vacuoles (spongiform)
- Medulla oblongata, pons and midbrain
- Prion disease
- Transmissible spongiform encephalopathy
- Genetic component
  - Suffolk are susceptible
  - Targees are resistant
- Specific codon/genes identified
  - 171- genes Q, R or H
  - 136- genes A or V
  - R and A confer resistance



JALAA Vol 45 No 4 July 2006

- Research complication in sheep/goats
- Infection associated with chronic catheterization
  - Most often present clinically silent
  - In comparison to the organisms colonizing catheter tips in humans and nonhuman primates, the catheters in these sheep were colonized by gram negative bacteria

What procedure is being performed?

Collection of the third eyelid lymphoid tissue to detect Scrapie

- Immunohistochemical detection of PrP which accumulates in the lymphatic tissue of the inner eyelid of sheep
- Test is positive one year before clinical signs develop (Vet Forum; June 1998; April 98, [New Scientist](#))



#### Other Spongiform Encephalopathies

- Scrapie – sheep and goats
- Bovine Spongiform Encephalopathy - bovine
- Transmissible Mink Encephalopathy - mink
- Chronic Wasting Disease of deer and elk
- Feline spongiform encephalopathy
- Kuru - human
- Cruetzfield-Jakob Disease - human
- Gerstmann-Straussler – human



#### Diagnosis:

##### Contagious ecthyma (Orf, sore mouth)

- Parapoxvirus
- Primary lesions on lips and mouth
- Usually seen in animals < 1yr
- High morbidity/Low mortality

#### Rule outs: Other vesicular/ulcerative diseases of sheep and goats

##### Blue tongue

- orbivirus
- cyanosis, ulcers of the dental pad, gingiva and tongue, chorioretinitis, conjunctivitis, coronitis

##### Ulcerative dermatosis

- poxvirus
- ulcers of face, genitals and feet

##### Foot and Mouth Disease

- picornavirus
- vesicles around the mouth, hooves and teats

##### Vesicular Stomatitis

- rhabdovirus
- vesicles on the oral mucus membranes, teats, interdigital spaces



Submandibular edema with abscesses, draining tracts and granulomas

#### Diagnosis:

##### "Wooden tongue"

*Actinobacillus lignieresii*

Gram negative rod

Goats- not affected

Sheep – lip

Cattle – tongue

#### Rule out:

##### "Lumpy Jaw"

*Arcanobacterium pyogenes, A. bovis*

Gram positive rod/coccobacillus

Affects bone

Rare in sheep and goats



## Contagious ecthyma

- Zoonotic disease
- human-to-human transmission can occur
- Handlers should wear PPE
- Disinfect clippers, ear taggers etc between infected animals
- Commercial vaccine available, but should be used with caution, may induce lesions in handlers
- Do not vaccinate herds that are already free of the disease



## Diagnosis:

Haemonchus Haemonchus Haemonchus

*Haemonchus contortus*

"barber pole worm"



Tissue from a sheep



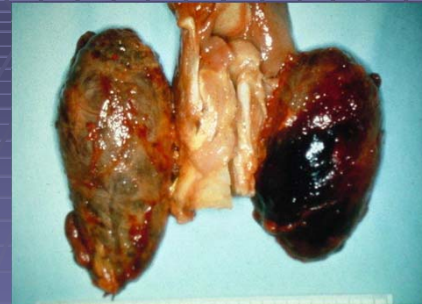
Diagnosis: Nodule worm  
*Oesophagostomum columbianum*  
*Oesophagostomum venulosum*

## Clinical signs-

- Pallor, severe anemia
- Submandibular edema ("bottlejaw")- hypoproteinemia
- Weight loss, diarrhea
- Unthriftiness, decreased milk production, poor wool coat



Tissue from a sheep



## Pathogenesis

- Direct life cycle
  - Ingestion of larvae from eggs passed in feces
- Hypobiotic (arrested) larvae may exist in host
- "spring rise"- large number of larvae passed from periparturient ewes onto pasture
- Blood meals from mucosa of abomasum
- Treatment Control
  - Anthelmintics
    - Severe resistance has developed!
  - Facility sanitation and pasture management and rotation
  - Susceptible to freezing and dry conditions

Diagnosis:

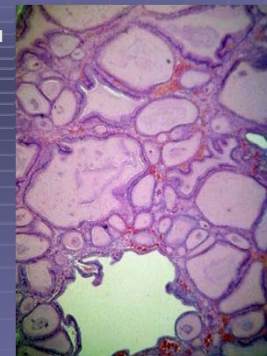
**Goiter- enlarged thyroid gland**

Congenital Goiter

-Merino sheep

Nutritional Goiter

-due to iodine deficiency  
-consumption of goitrogenic plants (soybeans, rape, kale, cabbage and turnips)



### Clinical exam



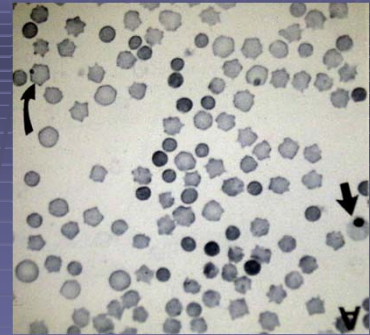
### Blood smear (Wright Geimsa)

Describe the RBC morphologic changes:

- Polychromasia (Hb)
- Poikilocytosis (shape)
- Anisocytosis (size)

Describe arrows:

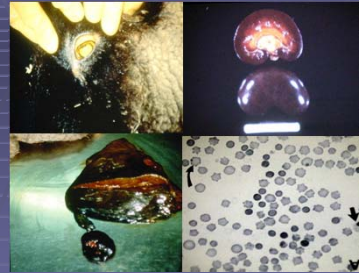
- Heinz body (curved arrow)
- Howell Jolly body (straight arrow)



### Necropsy



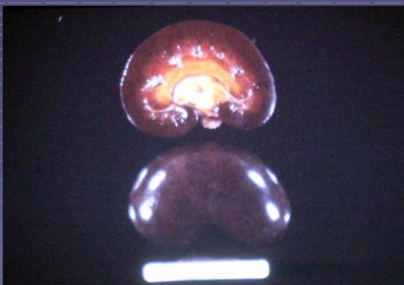
### Presumptive Diagnosis: Copper Toxicosis



- Icterus/hemolysis
- Enlarged black/brown liver/spleen
- "Gun-barrel" black kidneys
- Hematuria/hemoglobinuria



### Necropsy



### Copper Toxicity

#### Pathogenesis:

- Sheep store Cu readily
- Single toxic dose range = 20-100 mg/kg (vs 220-880 mg/kg in cattle)
- Cu released from liver is directly toxic to RBC membranes

#### Cause:

- Sheep fed improperly balanced rations or cattle diets
- Feed low in molybdenum, zinc or calcium
- Phytoegenous sources- subterranean clover
- Merino sheep may be more susceptible to this cause than other breeds

#### Treatment:

- D-penicillamine, Mb, thiosulfate, tetrathiomolybdate



## Model for Wilson's disease

- Human genetic defect in copper transporting p-type ATPase
- Northern Ronaldsay Sheep



Other well known animal model (rodent) for Wilson's disease?

Long Evans Cinnamon (LEC) Rat



Tissue from a Bovine



Tissue from a Bovine – subcutaneous surface of the skin

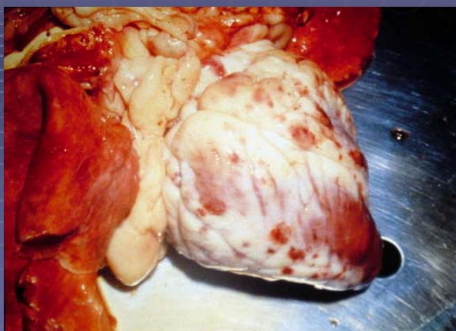


## Diagnosis

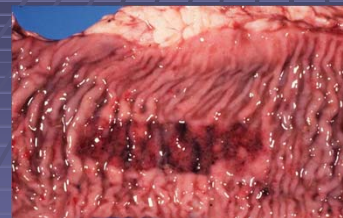
### Lymphosarcoma – Bovine Leukemia Virus

- B lymphocyte associated retro-virus
  - Common Sites
    - Retroorbital
    - Abomasum
    - Uterus
  - Goats seroconvert but do not develop clinical disease
- Sheep can be infected both naturally and experimentally

Tissue from a Bovine



Tissues from a Bovine



© Cornell Veterinary Medicine  
Last Revised: Program - 9/21/2010, Database - 4/20/2011

Peyer's Patch Necrosis



Stomatitis

## Diagnosis

### ■ Bovine Viral Diarrhea Virus

- Flaviviridae, pestivirus
- Disease primarily of cattle
- Very similar virus and disease in sheep called "Border" disease
- Clinical signs: subclinical, fever, anorexia, oral erosions, diarrhea, congenital abnormalities, reduced fertility, abortions, contributing factor to pneumonia
- Persistent infection
  - Animals infected in utero, become immuno tolerant to the virus
  - Usually do not survive to maturity, show signs of mucosal disease
  - Important source for infection of other animals, as they shed large amount of virus, even through the skin
- Vaccination should be integrated into herd health programs

### Clinical signs:

- Dyspnea, paresis, stiffness, inability to stand
- Other lambs found dead
- Creatinine Kinase (CK) and aspartate aminotransferase (AST) elevated

Tissue from a lamb



### Presumptive diagnosis?

#### White Muscle Disease

(aka: stiff lamb disease, nutritional myodegeneration, nutritional muscular dystrophy)

### Pathogenesis

- Vitamin E and/or Selenium deficiency
- Lack of one or both results in oxidative stress and loss of membrane integrity
- Cardiac form - neonates
- Skeletal form - young



Tissue from a Bovine



## White Muscle Disease

### Diagnosis

- clinical signs
- whole blood levels of vitamin E
- plasma levels of selenium
- glutathione peroxidase levels in red blood cells



### Treatment and Prevention

- Evaluation or awareness of regional soil content
- Supplementation of affected animals or late stage gestational ewes with Vitamin E or Selenium injections
- Properly balanced dietary rations

\*be cautious of selenium "toxicity"

## Diagnosis- Anthrax

### ■ *Bacillus anthracis*

- Large aerobic, spore-forming, gram-positive bacillus;
- Spores reside in alkaline soil, contaminated feeds and water
- Cattle and sheep affected more commonly than goats due to grazing behavior
- Older animals more susceptible than young and bulls more commonly affected than cows
- Clinical signs
  - Septicemia, hyperthermia, anorexia, depression and acute death with bloody discharges from the nostrils, mouth, anus, and vulva
- Necropsy
  - Incomplete rigormortis, dark uncoagulated blood protruding from all body orifices, splenomegaly
- Reportable
  - Report to state officials
- Bioterrorism threat
- Zoonotic

Tissue from a sheep

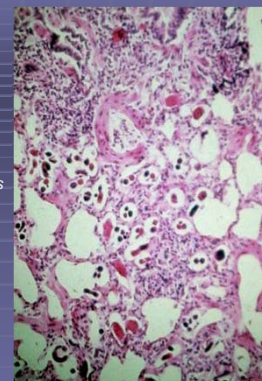
Lung

### Presumptive Diagnosis?

#### Lungworms

*Dictyocaulus filaria*  
*Protostrongylus rufescens*  
*Muellarius capillaris*

Less common in goats



Tissue from a goat

Brain

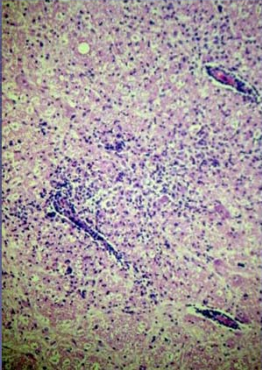
Clinical Signs

- Abortion
- CNS signs

Cold enrichment (20°C) beneficial in culturing the organism

Diagnosis?

*Listeria monocytogenes*



→

Brain from a ruminant with CNS disease

Diagnosis?

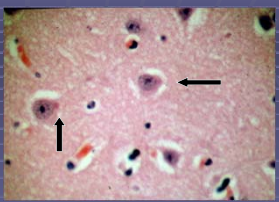
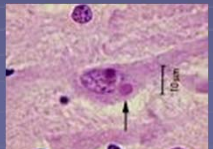
Rabies  
(Lyssavirus-genus)  
(Rhabdovirus-family)

Histology

- Negri bodies in the cytoplasm of the neuron
- Confirmation made by fluorescent antibody stain of the brain

Zoonotic!

Reportable!

→

Most likely diagnosis?

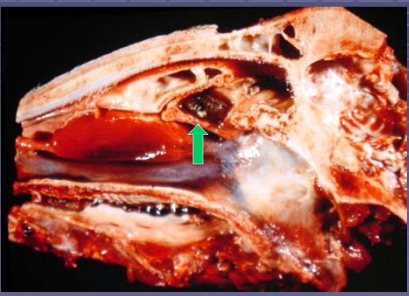


*Corynebacterium pseudotuberculosis*

- Disseminated superficial abscesses of lymph nodes
- Very common
- Gram + coccobacillus
- Thick caseous exudate
- ELISA available

→

Frontal sinus of a sheep





→

Goat small intestine

Young animal with hemorrhagic diarrhea

Presumptive diagnosis?

Coccidiosis  
(*Eimeria ninakohlyakimovae*,  
*E. arloingi*, *E. christenseni*)

Necropsy Findings:  
GI may appear congested, hemorrhagic, or ulcerated and have scattered pale, yellow to white mucosal plaques

- common in young animals
- often associated with stress or intensive housing conditions, or weaning
- 11 *Eimeria* species in sheep, 9 in goats

→

Clinical Presentation




Frontal Sinus of a sheep

→

Name the Parasite



*Oestrus ovis*  
Nasal bot fly - larva

Rule outs?

*Psoroptes ovis* (common scabies) -  
Wooled areas

*Psoroptes cuniculi* (ears - rare)

*Sarcoptes scabiei* (head scabies)



Clinical Exam - fetlock of a goat

Pruritus, scales, crusts and hyperkeratosis



Parasite found on sheep with pruritis,  
and chronic dermatitis of the neck,  
sides, abdomen and rump

Diagnosis?

**Sheep keds**

(*Melophagus ovinus*)

wingless, flat, brown, bloodsucking fly

Can transmit which virus?

**Bluetongue**



Diagnosis?

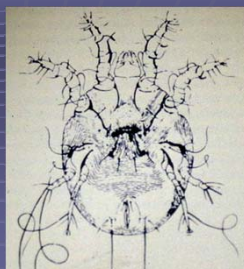
***Chorioptes bovis***

-Affects lower legs and scrotum

-Usually occurs in cooler months

-Ruminant mites have been eradicated  
or are very rare in the US

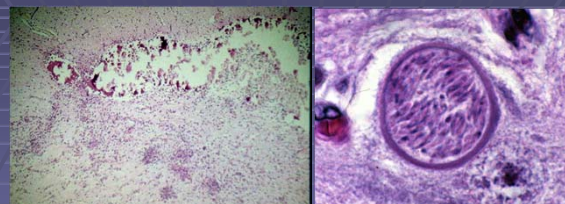
-*Sarcoptes* and *Psorergates* infections  
are reportable!



Scabies in sheep:

*Psoroptes ovis*, *Sarcoptes scabiei*, *Psorergates ovis*, *Chorioptes ovis*

Brain from aborted goat fetus



Moderate gliosis, non-suppurative  
encephalitis, perivascular mononuclear  
infiltrates

### Diagnosis?

#### *Neospora caninum*

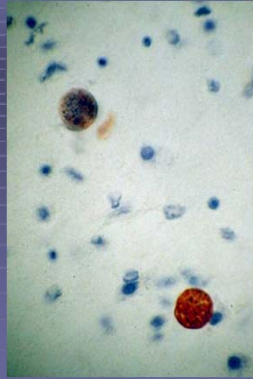
- Widespread worldwide
- Abortion is the only clinical sign in adults
- Young may show weakness or CNS signs
- More common in bovine, but may be seen in sheep and goats
- Immunohistochemical staining specific for the organism (immunoperoxidase)

#### Definitive host?

Dog

#### Rule outs?

*Toxoplasma* sp. (smaller)



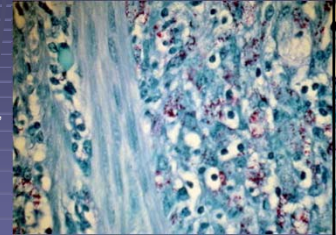
### Diagnosis?

#### *Mycobacterium paratuberculosis* "Johne's Disease"

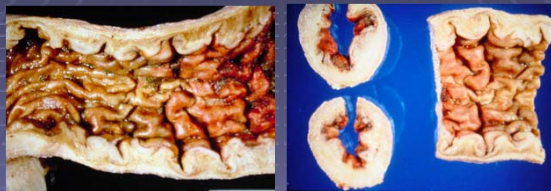
- Non-spore forming, fastidious, acid-fast, gram-positive rod

#### Diagnostic tests?

- Fecal culture: 8-12 weeks
- Serology: ELISA (most reliable), AGID or CF
- Acid-fast organisms on rectal biopsy smears
- Chronic carriers exist
- Most likely route of infection via ingestion
- Vertical transmission reported
- Organisms inhabit macrophages of host



### Tissue from a sheep



unthriftiness, weight loss and intermittent diarrhea



Name three potentially zoonotic organisms associated with abortion and/or lambing in sheep:

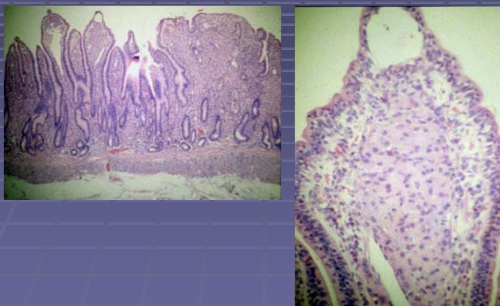
*Coxiella burnetii*- Q fever

*Brucella* \_\_\_\_? \_\_\_\_ *melitensis*

*Campylobacter fetus* subspecies *intestinalis*



### Histopathology



### Q fever

- *Coxiella burnetii*
- Gram-negative coccobacillus-like bacteria, similar to rickettsial organisms
- Found in milk, urine and feces of infected animals
- **Placenta and fetus are particularly dangerous source of infection for people**
- Transmission via inhalation of aerosolized particles
- Likely to be asymptomatic in sheep
- Causes flu-like symptoms in people
- Can be treated with appropriate antibiotics

Goat with stiffness, lameness and swelling of the carpal joints

Diagnosis:

Caprine Arthritis Encephalitis Virus (CAEV)

Genus and family of the virus?

Lentivirus, Retroviridae

- Progressive arthritis or interstitial pneumonia in animals > 6 months
- Encephalitis- in younger animals
- Prevalent in the US
- No treatment exists
- Diagnostic tests- AGID, ELISA
- Test and Cull



\*Same viral genus/family as Ovine Progressive Pneumonia (Maedi/Visna)

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Vol 60, No 4  
December 2010  
Pages 409-425

## Catheterization of Intestinal Loops in Ruminants Does Not Adversely Affect Loop Function

G Douglas Inglis,<sup>1\*</sup> John P Kastelic,<sup>2</sup> and Richard R E Uviera<sup>3</sup>

This recent publication using sheep evaluated the feasibility of using catheterized intestinal loops for studying host response to various treatments with the small intestine. The study concluded that:

- a. The catheters were patent for more than 40 days
- b. Treatments (bacteria) administered into the loops remained localized with the loops
- c. Neither the surgical procedure to place the catheters or the catheters themselves had any long term behavioral or health effects on the sheep
- d. All of the above
- e. None of the above

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Vol 49, No 4  
November 2010  
Pages 806-813

## Evaluation of an Inhouse Rapid ELISA Test for Detection of *Giardia* in Domestic Sheep (*Ovis aries*)

Joline M Wilson<sup>1\*</sup> and F Claire Hankenson<sup>1,2</sup>

This recent publication evaluated whether a commercially available rapid ELISA test used for *Giardia* detection in dogs and cats could be used in sheep. The study concluded that the rapid ELISA test exhibited what percentage of sensitivity for sheep giardiasis?

- a. 0%
- b. 68%
- c. 88%
- d. 100%

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Vol 49, No 4  
November 2010  
Pages 814-824

## Surgical Approaches to Vascular Access for Large-Caliber Devices in Preclinical Research Models

Noah Barak,<sup>1\*</sup> Nancy Rakono,<sup>1</sup> Linnea Lentz,<sup>1</sup> Michael Kropak,<sup>1</sup> Kent Wilks,<sup>1</sup> Ana Menk,<sup>2</sup> and Mike Goss<sup>3</sup>



Figure 2

The objective of this recent publication was to develop, refine and describe access techniques for large caliber devices that model approaches to lower and upper limb vasculature in humans. Figure 2 from the paper details the brachiocephalic access site and demonstrates what suture technique?

- a. Chinese finger trap
- b. Double-diamond purse string
- c. Four quadrant tack
- d. Walking mattress

## Morphometric Comparison of the Lumbar Cancellous Bone of Sheep, Deer, and Humans

Yang Wang,<sup>1\*</sup> Guomin Liu,<sup>2</sup> Ting Li,<sup>1</sup> Yanlong Xue,<sup>1</sup> Qing Han,<sup>1</sup> Rongdong Xu,<sup>1</sup> and Yongjiang Li<sup>1\*</sup>

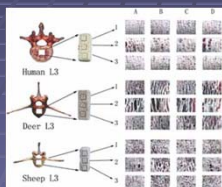


Figure 1

In this recent publication the feasibility of using deer and sheep as animal models for the human spine was studied. This image depicts:

- a. Sampling methods to be used to collect binary images from same sized areas of interest for each species
- b. Morphologic differences in the vertebral bodies of each species
- c. CT scan images of each vertebrae



Electric Dehorners



Barnes-Type Dehorners



## THANK YOU!

- Slides and accompanying information provided by Diane Forsythe and Mary Grant of NIEHS/NIH, RTP, North Carolina and Sue Spray of the Scripps Research Institute
- We wish to extend appreciation to those who contributed to this collection. The contributors are many and without their assistance this collection would not be possible

