Improving Diagnostic Pathology Results: Help us to help you come to a more definitive diagnosis

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University of Missouri, Columbia

Challenges

Clinician

- Getting a helpful diagnosis as soon as possible
- Maintaining health of animal or remaining animals
- Costs

Pathologist

- Helpful interpretation of specimens
- Making appropriate differential lists and rule outs

Dispelling Myths

- “If I provide too much history, it will lead the pathologist.”
- “Oh, they can figure out everything they need to know from the carcass.”
- “If we are going to perform a necropsy, we should FREEZE the carcass immediately!!!”

Necropsy at Clinic vs. Lab

Clinic

- Personal/Business time
- Clean up
- Formalin on hand
- Tools
- Write necropsy report
- Submit samples for additional testing
- Storage of specimens
- Time = $$$

Diagnostic lab

- Fill out history form
  - List tests requested
- Mail package and pay shipping fees
- Wait for result



Surgical Biopsies



- Make sure you fill out history form!!!
  - Detail pertinent information to case
  - If multiple tissues, please indicate
  - Include photos, clinical records, or chemistry values
    - **NOTE:** More is ***MORE!***
- Use appropriate amount of formalin and containers.
    - **NOTE:** We provide shipping containers for average surgical biopsy samples.

Use Appropriate Containers

- Leakproof, double package
- Try to provide some absorbent material
- May have irate mail carriers, FedEx, or the FAA coming to see you.
- Be sure to respect federal regulations concerning shipping (USDA, CDC, and shipping companies)

Veterinary Medical Diagnostic Laboratory, College of Veterinary Medicine

1-800-UMC-VMDL 800-862-8635 Fax 573-882-1411

Courier Address
1600 E. Rollins, Columbia, MO 65211

www.vmdl.missouri.edu

US Mail Address
PO Box 6023, Columbia, MO 65205

Acct No _____ □ **Fax or** □ **Email** _____ **DATE SAMPLE TAKEN** _____ Date Sent _____

Phone _____ Fax _____ Owner _____

Veterinarian _____ Address _____

Clinic _____ City _____ State ___ Zip _____

Address _____ Phone _____

City _____ State _____ Zip _____ Third Party _____ **Bill__ Vet __Owner**

Animal Name/ID_____ **Species** _____ **Breed** _____ **Sex**_____ **Age**_____ **Weight**_____

 # Samples submitted __ □ Biopsy □ Blood □ Feces □ Milk □ Referral culture □ Semen □ Serum □ Swab from _____

□ Tissue from _____ □ Urine: __**Cystocentesis** __**Free Catch** □ **Other** _____

No. in Group _____ Sick ____ Dead ___ Raised on Premises? ____ If purchased, when?_____ New introductions? ____

Date Introduced _____ Date noticed sick _____ Euthanized? ____ Time/ Date of Death _____ Clinical signs, history, vaccinations, postmortem findings: _____

Field Diagnosis _____

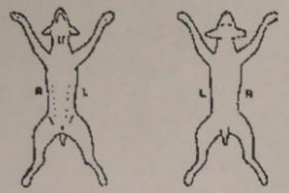
□ **Gross necropsy** □ **Necropsy/histopathology** □ **Necropsy/histopathology/labs** □ **Fixed & fresh tissue exam**

Histopathology If skin biopsy,*(circle all applicable)* erythema, pruritus, macules, papules, pustules, ulceration, crusts, hyperkeratosis, scales, alopecia, lichenification, hyperpigmentation, depigmentation, excoriation, **other** _____

*Duration _____ *Treatment _____

When started/how long? _____ Response to therapy? ___ **yes** ___ **no** ___ **partially**

If tumor, Size _____ Duration _____ Rate of growth ___ **slow** ___ **fast** Recurrence ___**yes** ___**no**



Circle lesions, mark "X" for biopsy locations.



Cytologic Exam—Label slides with animal ID & site

□ Washes □ Aspirates □ Imprints □ Brushes

□ Scrapings □ Other (specify) _____

Fluid Analysis (includes Cytologic Exam)
(CALL for instructions)

□ CSF □ Pleural □ Peritoneal □ Synovial □ Pericardial

Pertinent clinical information (source, lesion description) _____

Bacteriology/Mycology

□Aerobic culture □Anaerobic culture □Blood culture □Enteric screen □Johne's culture □Johne's direct PCR fecal

□Antimicrobial susceptibility □Fungal Culture □Salmonella □Tritrichomonas foetus □Culture □ PCR

□ **Other** _____

Toxicology (Include number of samples by each sample type)

□ Feed____ □ Blood ____ □ Serum____ □ Fresh Tissue____ □ Other_____ □ Consult with Toxicologist on duty

□ Lead □ Copper □ Mycotoxin Screen □ Ergot Alkaloids □ Ergot/Fescue Alkaloids **Other**_____

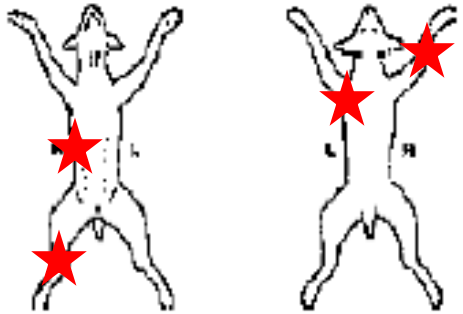
Clients submitting specimens are considered to have agreed to VMDL testing procedures, policies and fees (view at: http://www.cvm.missouri.edu/vmdl/). Specimens become the property of the VMDL.

Histopathology **If skin biopsy,** *(circle all applicable)* erythema, pruritus, macules, papules, pustules, ulceration, crusts, hyperkeratosis, scales, alopecia, lichenification, hyperpigmentation, depigmentation, excoriation, **other** _____

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Handwriting

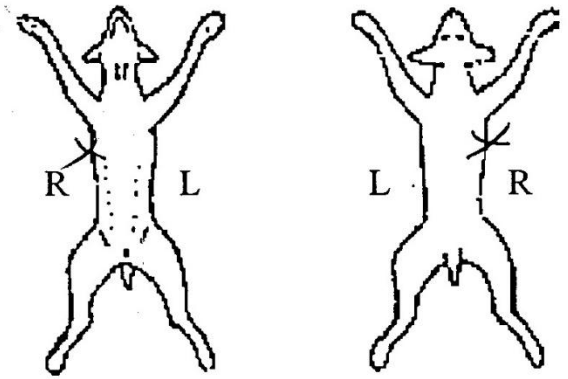
History
Animal ID Eddie Species Feline Breed DSH Sex FE Age 1 day mo. (yr.) Weight 6 lb
Number in Group _____ Number Sick (excluding dead) _____ Number Dead _____ Raised on Premises? **yes / no**
If purchased, when? _____ Recent introductions? **yes / no** Date Introduced _____
Date first noticed sick _____ Euthanized? **yes / no** Method used _____
Time & Date of Death _____ Previous submissions? **yes / no** VMDL accession no. _____
Clinical signs, vaccinations & dates & postmortem findings: Presented w/ leg infection 10/12/10 w/ hx of vertical skin. Examined 11/19 w/ [illegible] lesions [illegible], [illegible], [illegible]. When examined 3/8/11 lesions still present, ranged [illegible] wide w/ 1/2 - 1 cm wart-like granulomas scattered over body. One granuloma was excised from skin over right thorax & formalin fixed for histopath.

Field Diagnosis feline papilloma?

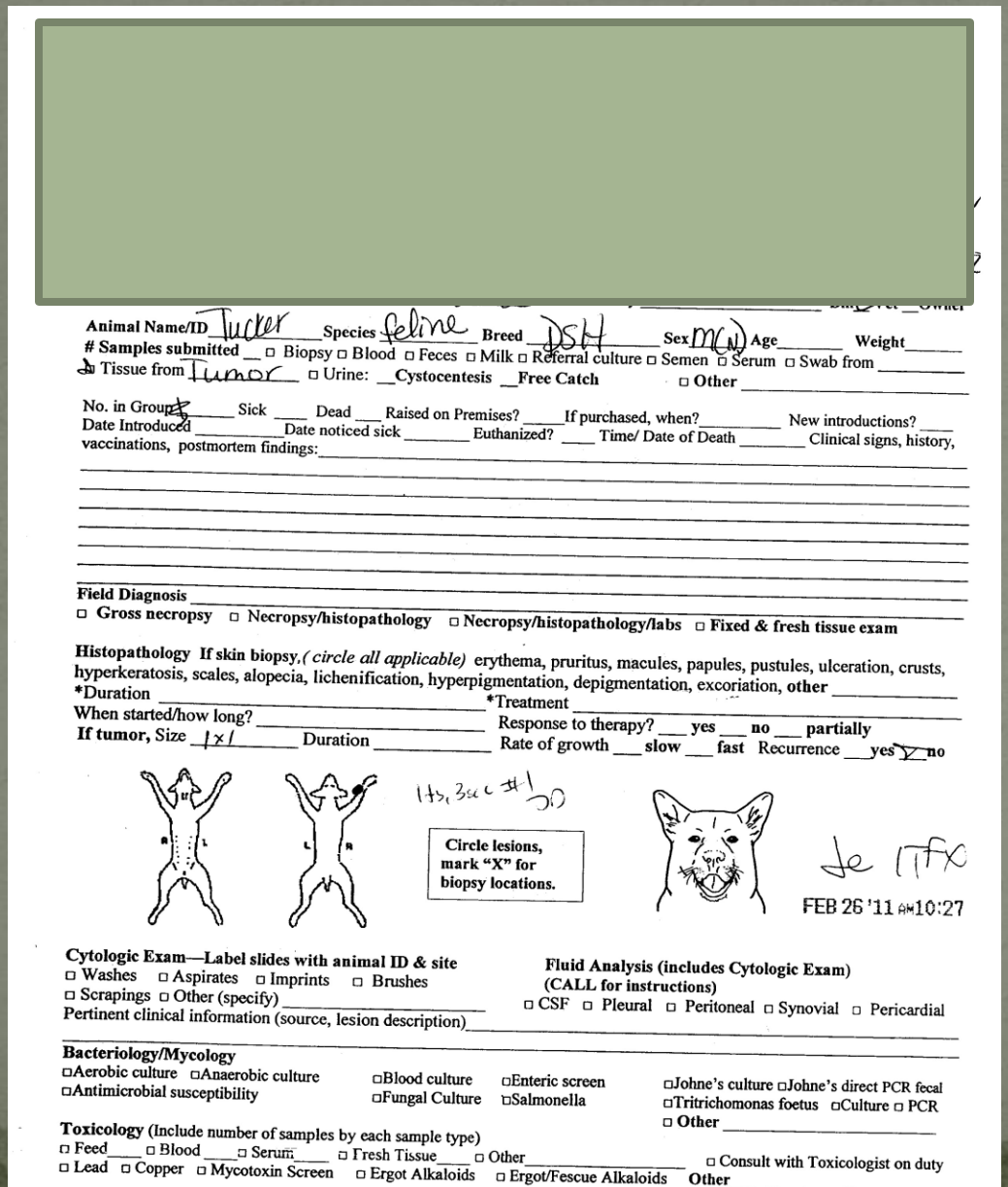
If tumor Size 1 cm Duration 2 mo Rate of growth: **slow/fast** Recurrence **yes / no**
Site of lesion: Internal—Location in organ or tissue _____ External—Location on skin or mucous membrane _____

Specimen Submitted	Qty	Examinations Requested
☐ Live Animal		☐ Gross Necropsy only
☐ Dead Animal		☐ Gross Necropsy with Histopathology
☐ Fetus		☐ Necropsy with other labs
☐ Blood		☒ Histopathology *
☐ Serum		☐ Immunohistochemistry
☒ Fixed Tissue	1	☐ **Clinical Pathology (See reverse side)**
☐ Fresh Tissue		☐ Mycoplasma Isolation**
☐ Milk		Bacteriology
☐ Urine		☐ E. coli Typing
☐ Feces		☐ Isolation/Identification**
☐ Stomach Contents		☐ Aerobic Culture
☐ Referral Plate		☐ Anaerobic Culture
☐ Swab (original)		☐ Antimicrobial Susceptibility
☐ Feed		☐ Parasitology
☐ Water		☐ Mycology

Sample Information



Wall of shame, scant information



Animal Name/ID Tucker Species feline Breed DSH Sex M(N) Age _____ Weight _____
Samples submitted __ ☐ Biopsy ☐ Blood ☐ Feces ☐ Milk ☐ Referral culture ☐ Semen ☐ Serum ☐ Swab from _____
Tissue from Tumor ☐ Urine: __Cystocentesis __Free Catch ☐ Other _____

No. in Group _____ Sick ____ Dead ___ Raised on Premises? _____ If purchased, when? _____ New introductions? ____
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*Duration _____ *Treatment _____
When started/how long? _____ Response to therapy? ___ yes ___ no ___ partially
If tumor, Size 1x1 Duration _____ Rate of growth ___ slow ___ fast Recurrence ___ yes X no

Circle lesions, mark "X" for biopsy locations.

FEB 26 '11 AM10:27

Cytologic Exam—Label slides with animal ID & site
☐ Washes ☐ Aspirates ☐ Imprints ☐ Brushes
☐ Scrapings ☐ Other (specify) _____
Pertinent clinical information (source, lesion description) _____

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☐ Other _____

Toxicology (Include number of samples by each sample type)
☐ Feed____ ☐ Blood ____ ☐ Serum____ ☐ Fresh Tissue____ ☐ Other_____ ☐ Consult with Toxicologist on duty
☐ Lead ☐ Copper ☐ Mycotoxin Screen ☐ Ergot Alkaloids ☐ Ergot/Fescue Alkaloids Other

- How long has the mass been there?
- Is it pruritic?
- Ulcerated?
- Limited to the SQ?
- Inquiring minds want to know!!

Surgical Biopsies (cont'd)

- Wait for tissue to become tacky b/f you ink margins
- Butterfly and/or bread-loaf section tissue
- If multiple samples, LABEL TISSUES!!!!
- Separate potential bacteriology and histopath specimens!

Zoonoses

- PLEASE, INDICATE POTENTIAL ZOONOTIC DISEASE SUSPICION ON FORM
- IF uncertain, call and ask us



Wall of shame

- Too little formalin
- Specimen too big for container
- Specimen not in formalin at all
  - Water
  - Alcohol
- Specimen not sectioned appropriately
- Formalin exposure
  - Fresh tissues for culture
  - Cytology specimens

Sample size vs. container





The ill-fated pill bottle




Academic
CM 1
2
3
4
5
6
7
10
11
12


CM
1
2
3
4
5
6
7
8
9
10
11
12
Academic



Bacteriology Samples: Rectal Sleeve



Better choices



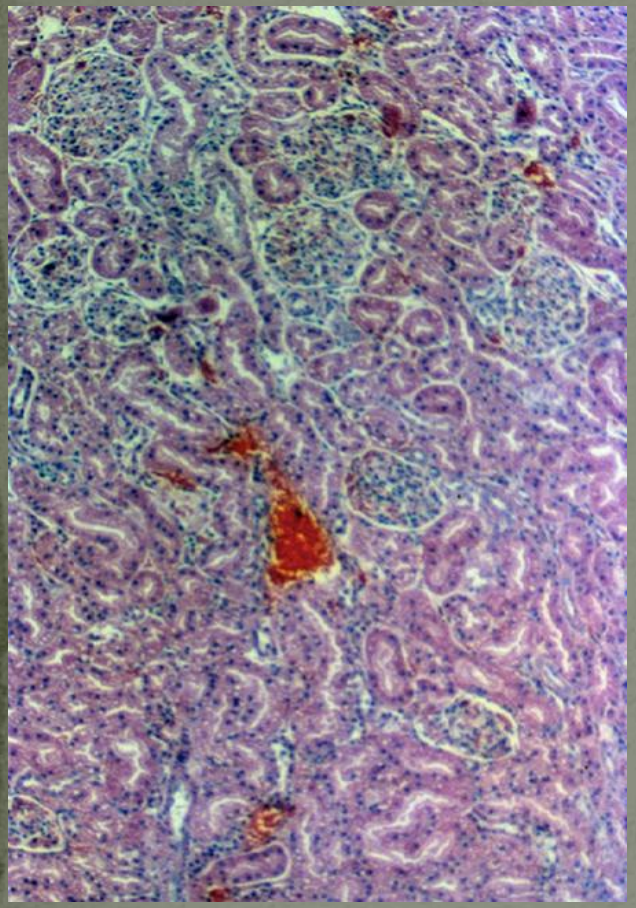
In a pinch…



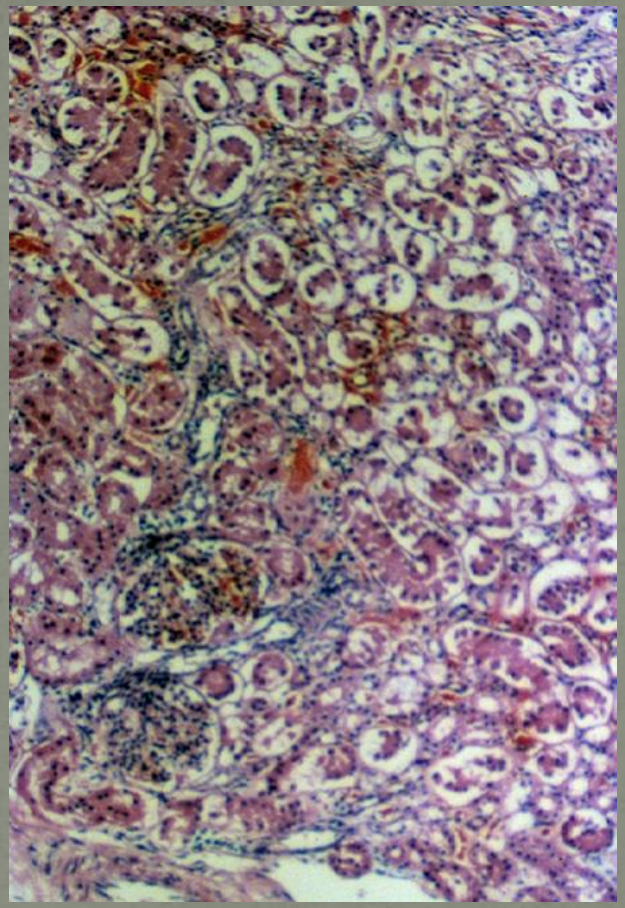
Wall of Pride!



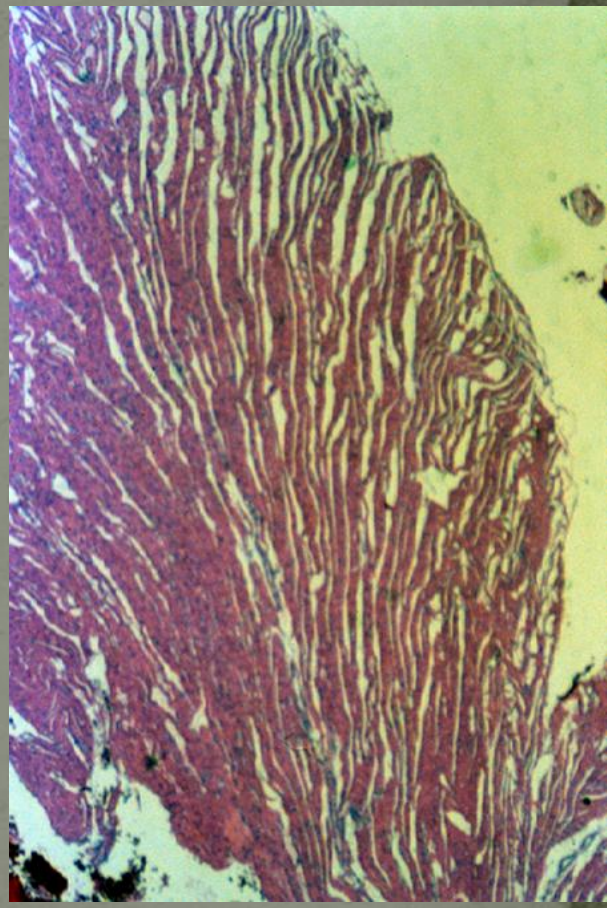
Lack of fixation



formalin

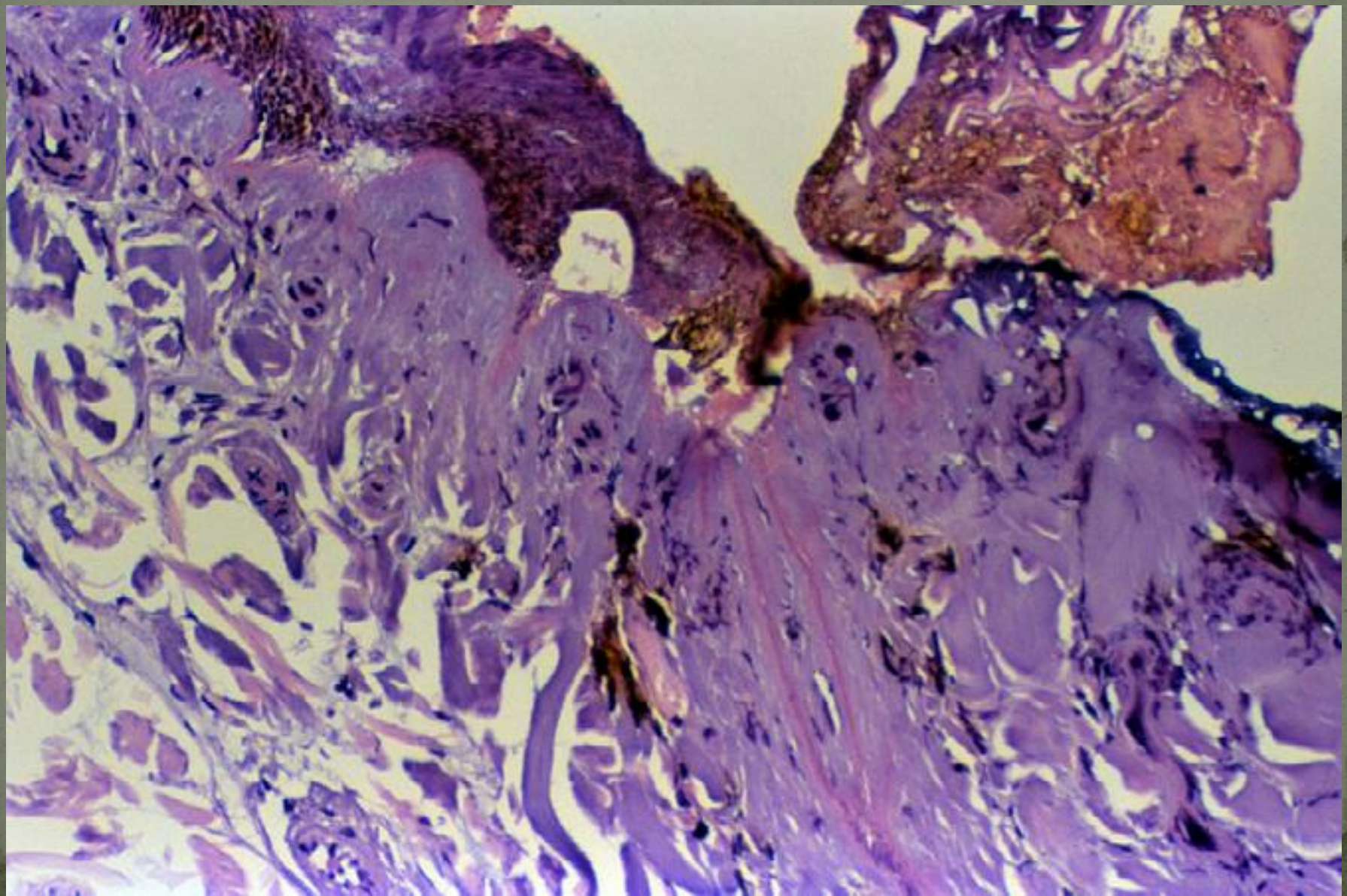


water

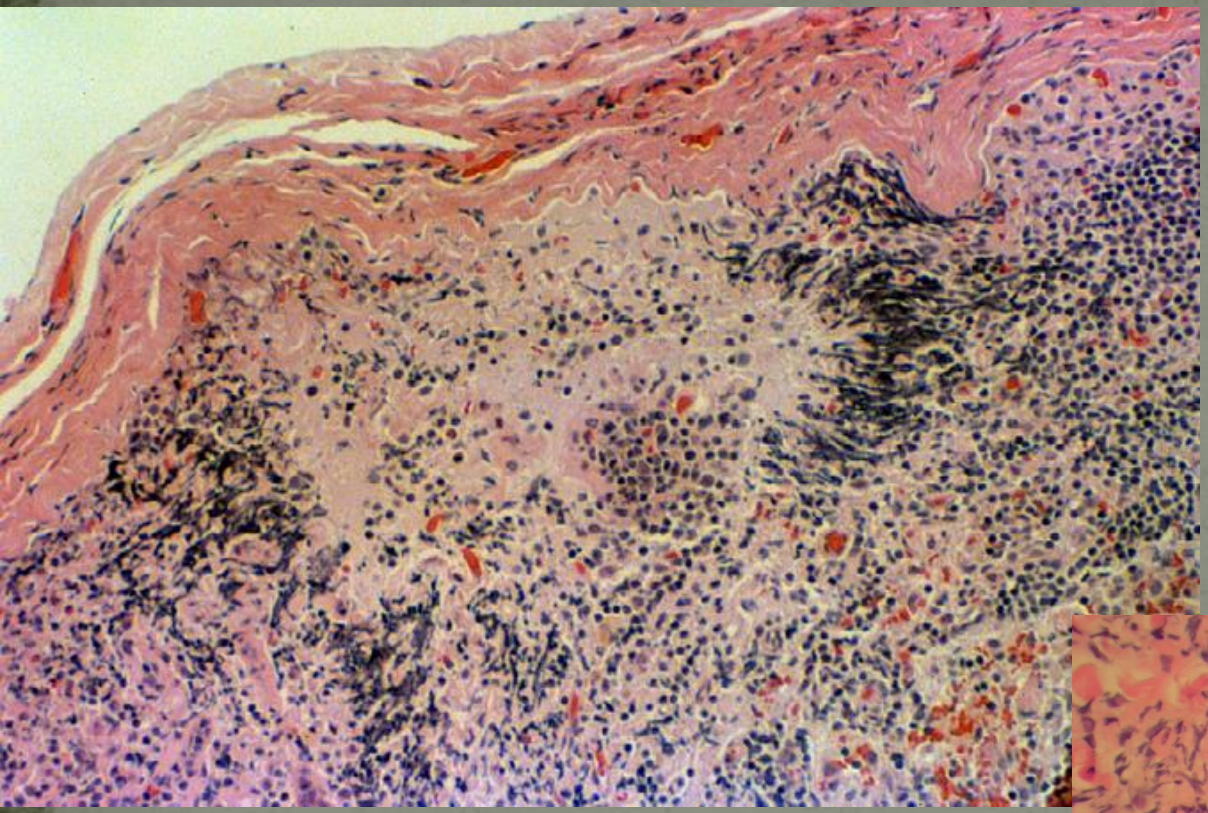


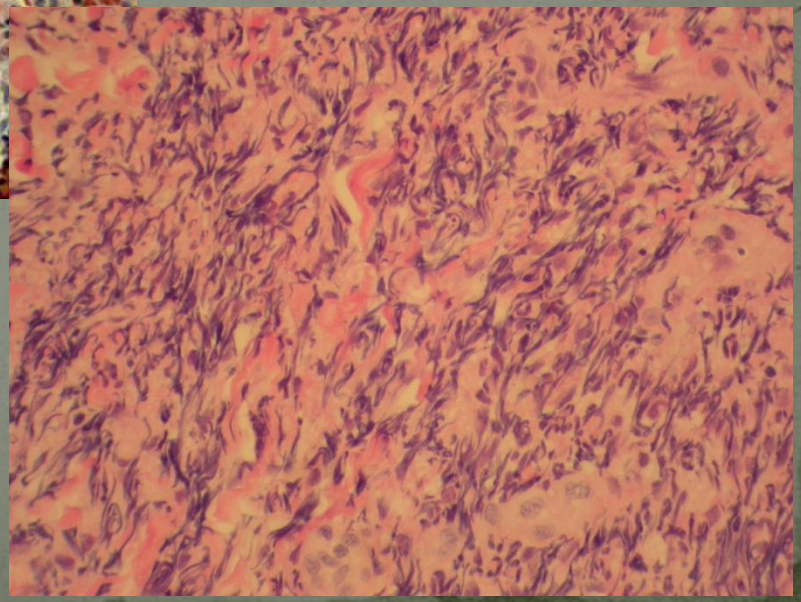
Frozen -> fixed

Beware overuse of the laser



Artifacts of tissue handling





Excessive traction with forceps during surgery

Tools for performing a necropsy

- What do you need to do a necropsy? Or better yet, what fits on your truck?
- Sharp knife
  - Make that TWO sharp knives and a knife sharpener
- Hand ax/hatchet with chisels
- Safety items
  - Goggles/safety glasses
  - GLOVES!!!!!!
  - Plastic apron
- Formalin containers
- Plastic baggies (zip-loc will do)




CORONA
Westlake

“For every mistake we make for not knowing, we make a hundred for not SEEING!!”
~Dr. Padmore

Do a physical!

- Go over history!!!
- Check for pallor.
  - Dead pale vs. anemia pallor
- Determine degree of autolysis
  - Differ b/t lesions and autolytic change
- Guide your search based on external clues
- Think about disease process you are curious about.
  - Relevant anatomy and physiology
- Know when to cease and desist!!!
- Take notes!

Notes on necropsy report

- **Just the facts!**
- Organize report from head to tail
- Use consistent pattern
- **Avoid** using specific ages
  - Fetus, neonate, juvenile, adult, aged adult, geriatric
- **Avoid** using specific breeds
  - Holstein => black and white spotted bovine
- **Avoid** using hearsay . Legal document!
- **Avoid** redundancy
  - Red in color
  - Round in shape
  - Smooth in texture








Two ways to become famous

- Be the person to catch the epidemic

Or

- Be the person who missed it!



Importance of history

- Avoid direct questions.
- Have a list of routine things to ask about.
- Take notes.
- Make a differentials list before you start
  - What lesions do you expect for each differential?

What to look for?

- Differentiate between autolysis, non-lesions, and true lesions
- Consider the common post mortem artifacts
  - Frozen corneas
  - Dependant congestion
  - Melanosis
  - Predation

Freezing artifact: Calf Blue Eye Syndrome!!!



Hypostatic congestion



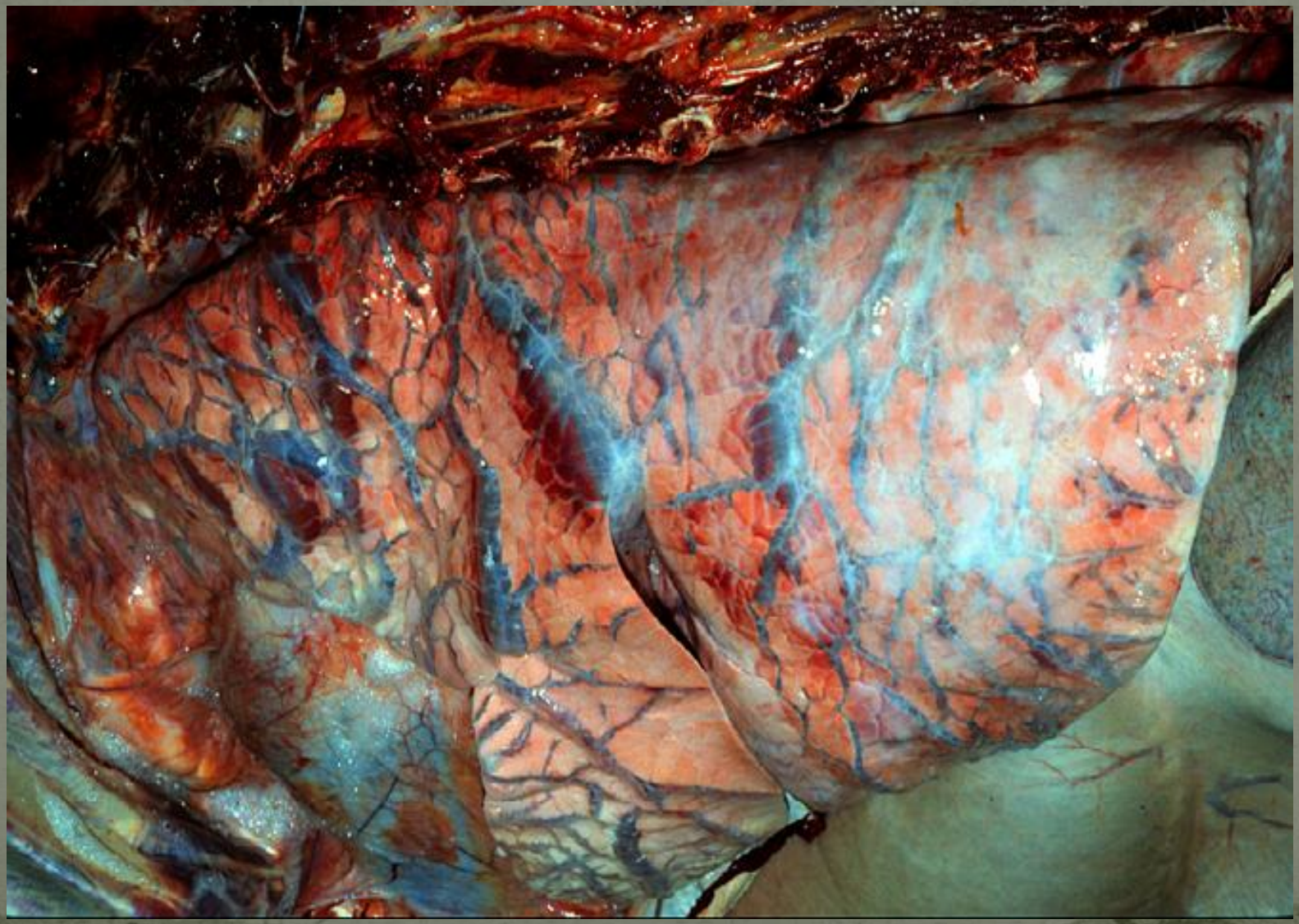
Note red skin on “down side” of carcass

Postmortem peristalsis effects

- Ridges formed by peristalsis trap blood, producing a striped appearance



Emphysematous



Lesion or postmortem change?







Sample submission

- Big 5, if unsure submit
  - Lung, liver, kidney, spleen, heart
- Fill tubes no more than ¾ full
- Septicemia tissues?

  Clue: Think lots of blood flow
  - **Spleen**
  - **Lung**
  - **Liver**
  - **Kidney**

Toxicology specimens

- The big 6 common specimens
  - Liver
  - Kidney
  - Stomach contents
  - Urine
  - Fat
  - Brain (caudal nucleus)
- Others, based on suspicion

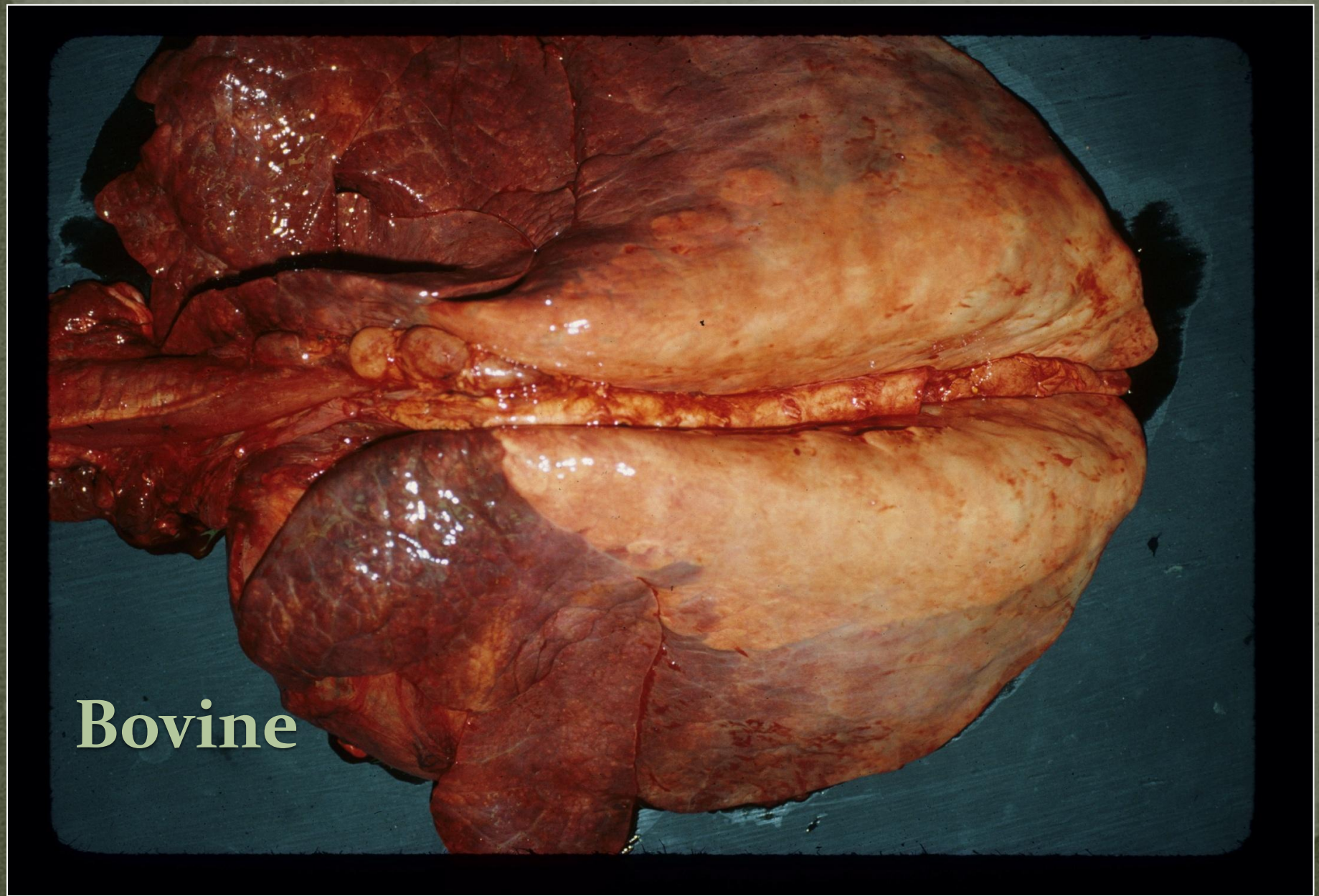
- THERE IS ***NO TOXICOLOGY SCREEN!!!!***
- OK to freeze tissues
- Remember to include feed and water specimens
- Must have an idea of what we are looking for.

Sampling: Three easy steps!

- Thin for the formalin bucket,
  - no more than 0.5 cm
  - (1/4" for the metrically challenged)
- Small for the PCR/virology whirl-pac
  - Looking for DNA, so very small chunks OK!
- BIG for bacteriology
  - Need lots of tissue to culture


SPEC.
DATE

Dependant congestion or lesion?

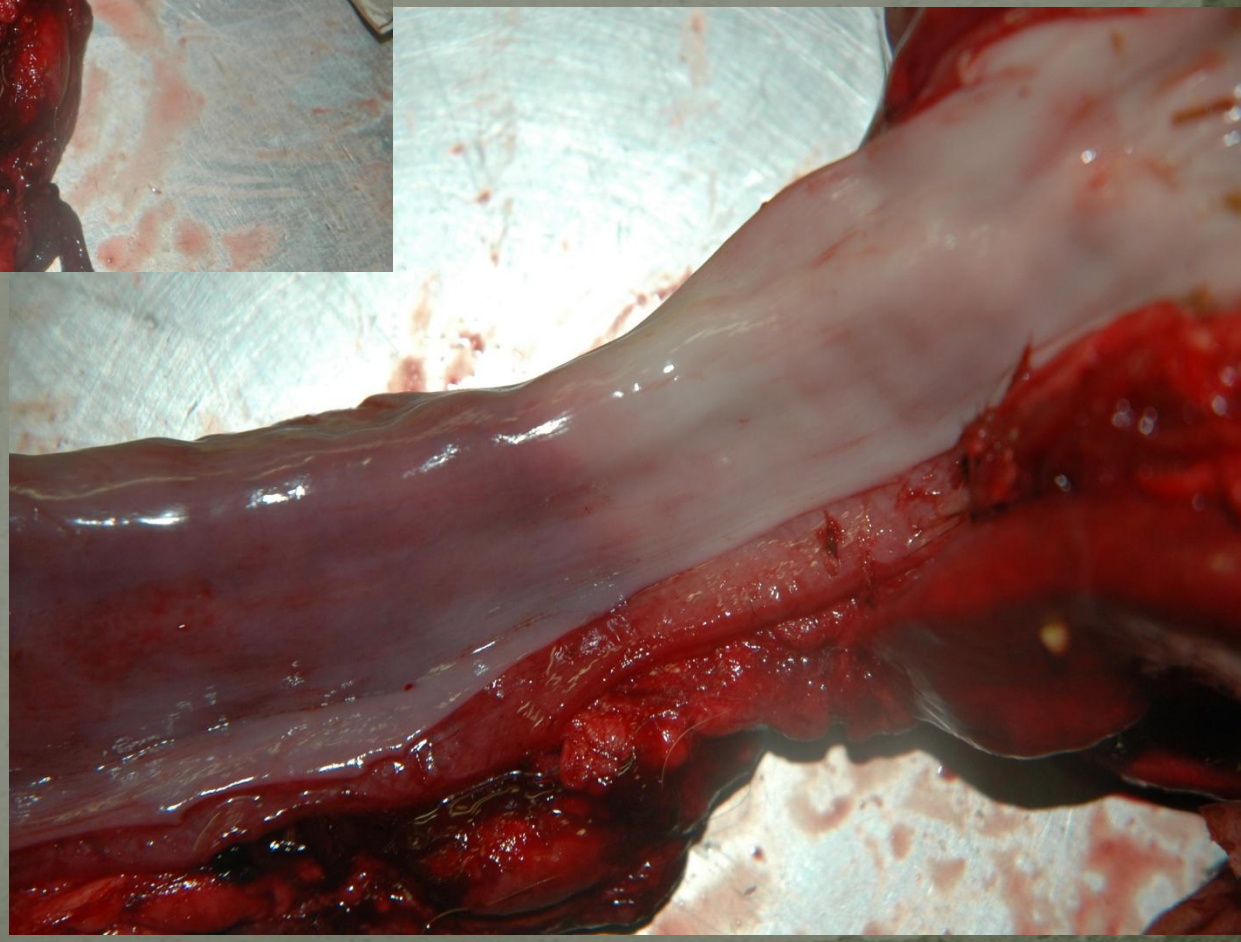


Bloat. Postmortem or Antemortem?



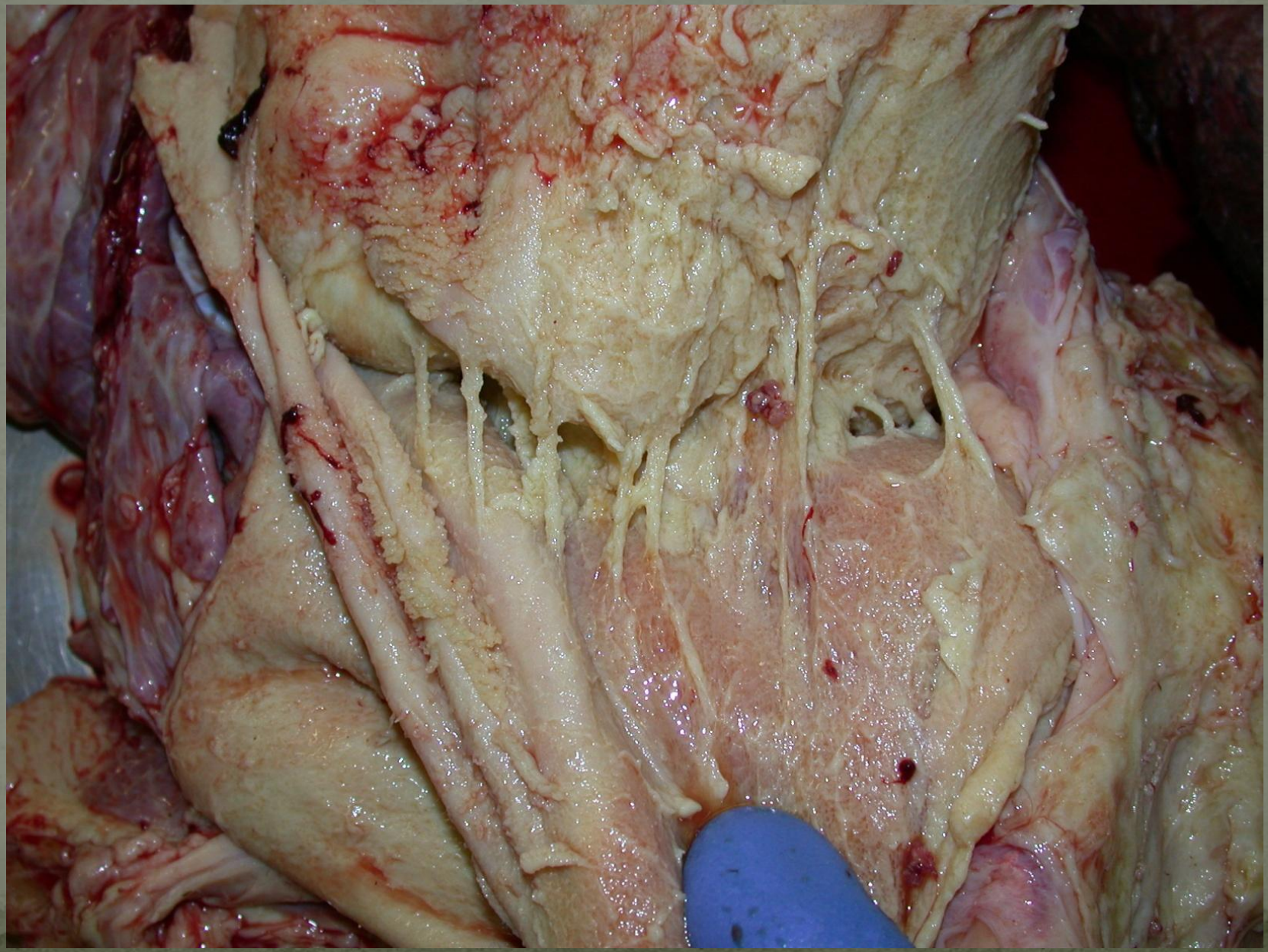


Bloat line

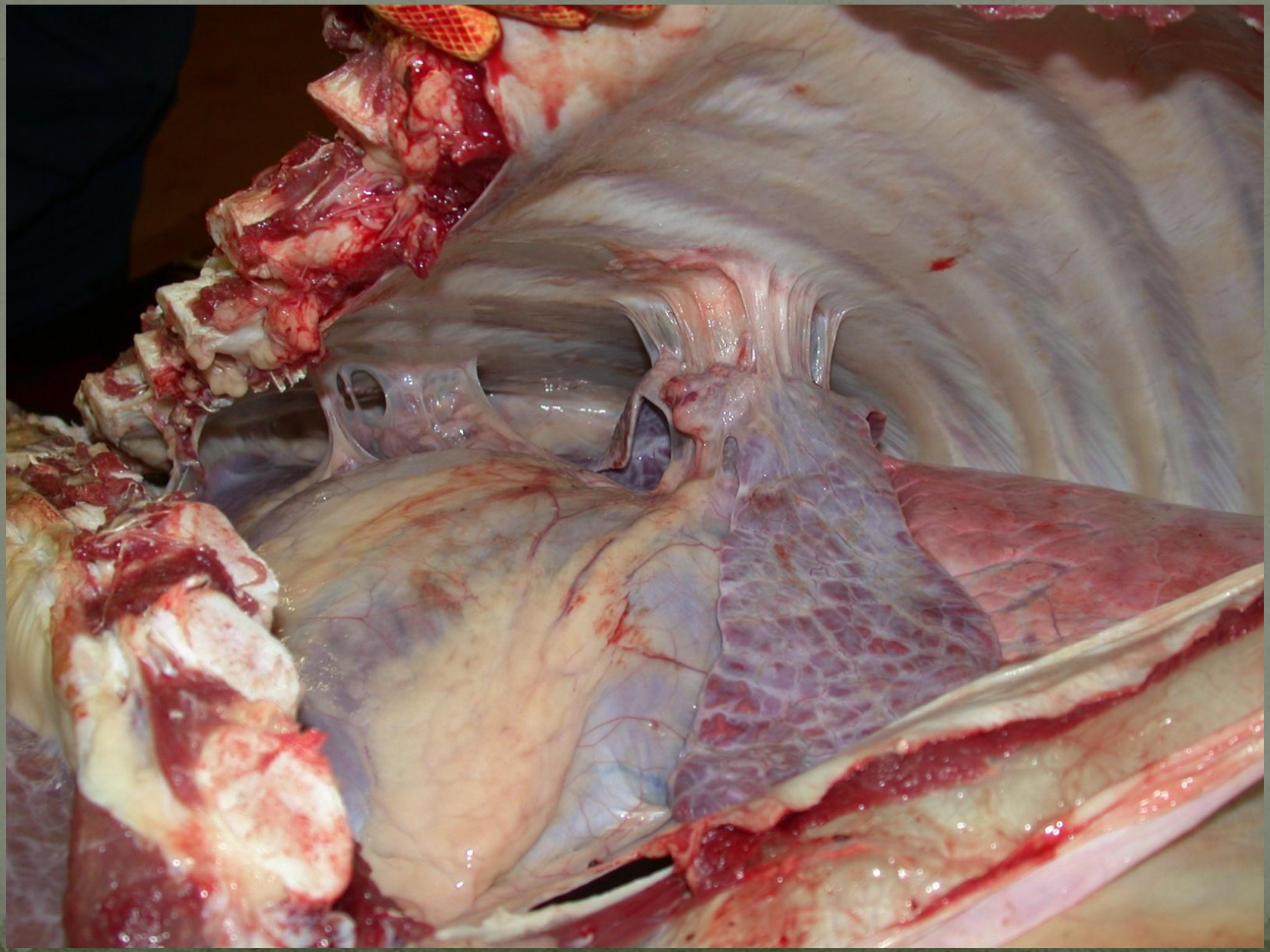


Bovine

Fibrin "cheesy goodness!!"



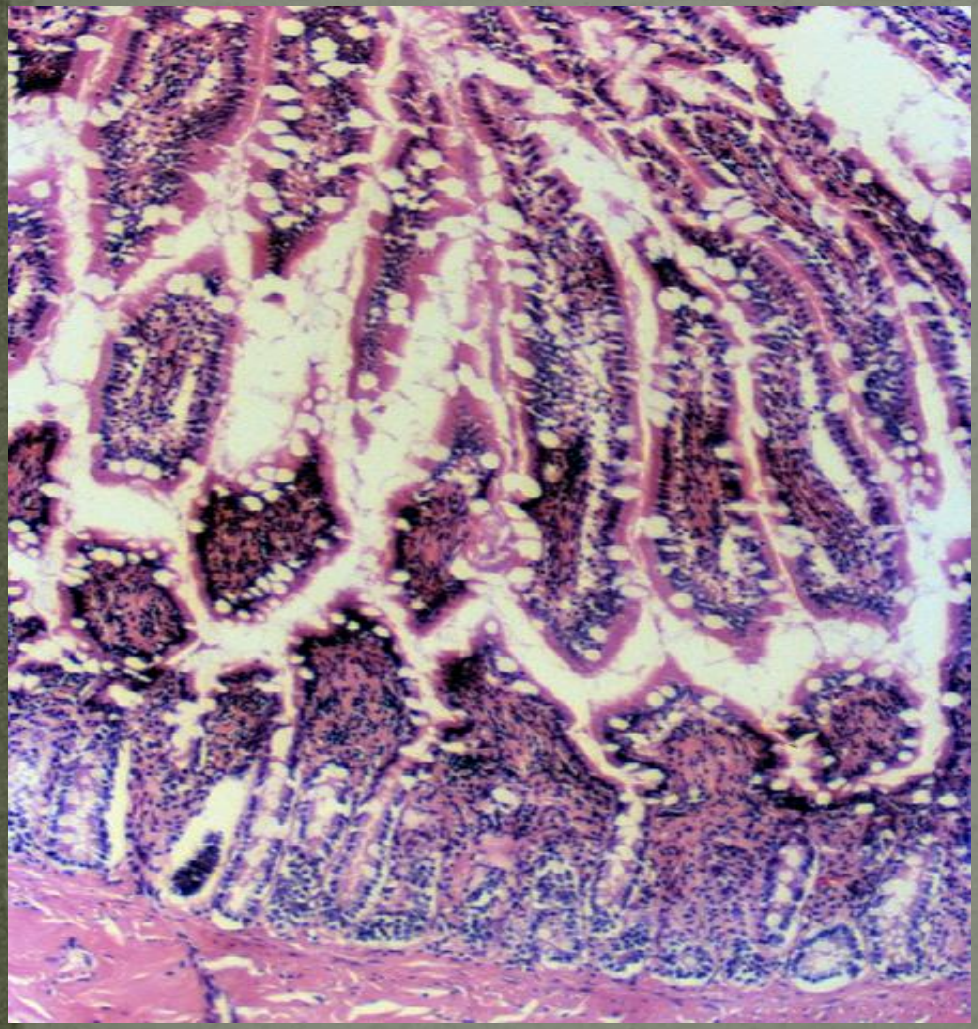
Fibrous

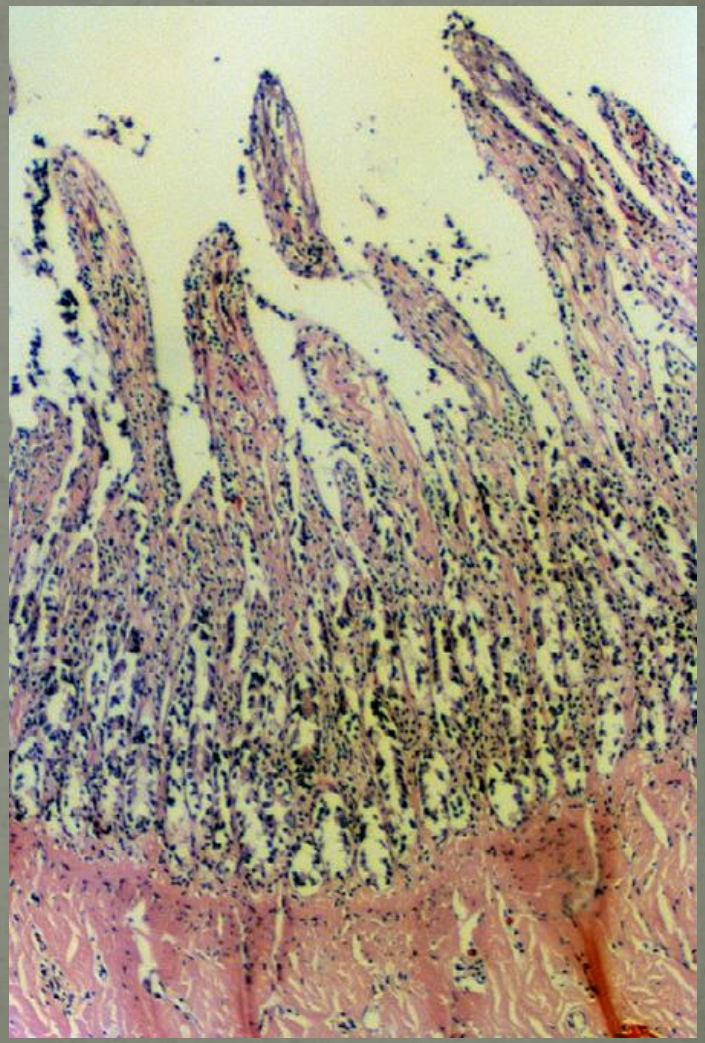


Necropsy protocol (cont'd)

- "Do we have to run the entire gut?"
- Spot check vs. run the entire enteric tract
  - Are there normal feces in the rectum?
  - Is there history of GI disease/symptoms?
  - Spot check
    - Duodenum with pancreas, jejunum, ileum, cecum, colon
    - Take a fecal
- DO NOT SCRAPE MUCOSAL SURFACE!!!

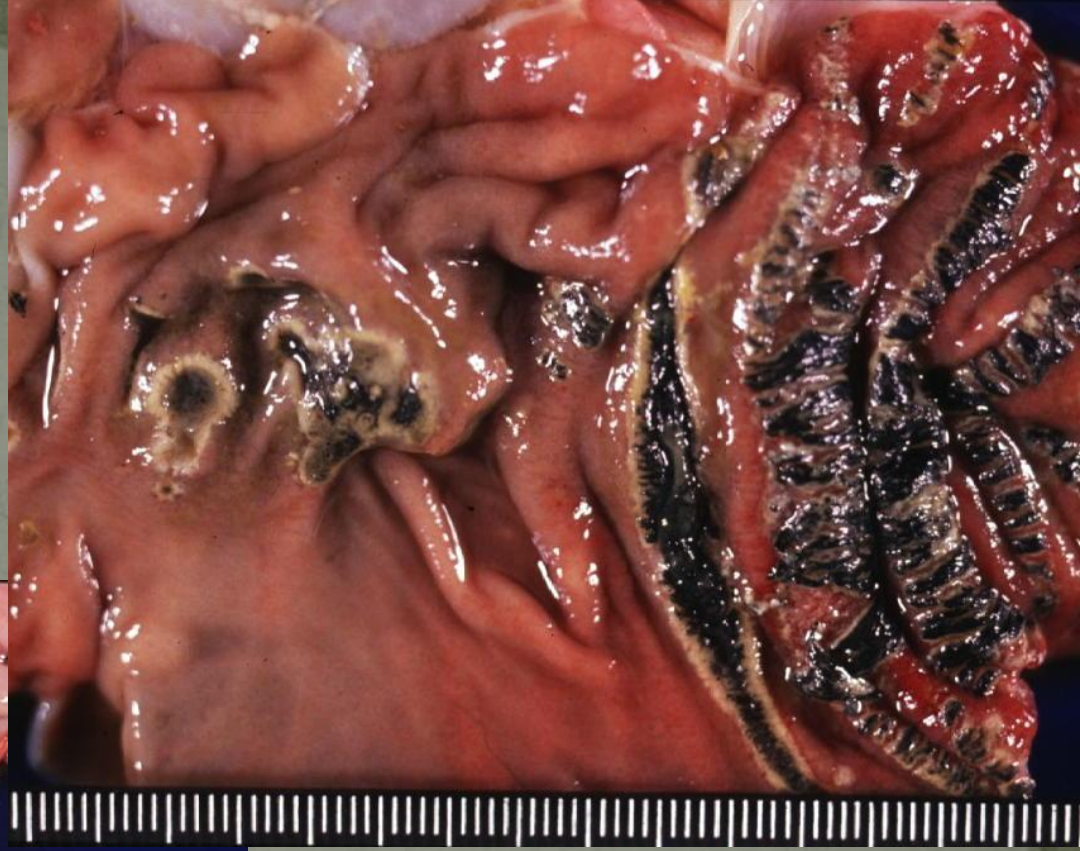
Artifacts introduced during postmortem examination

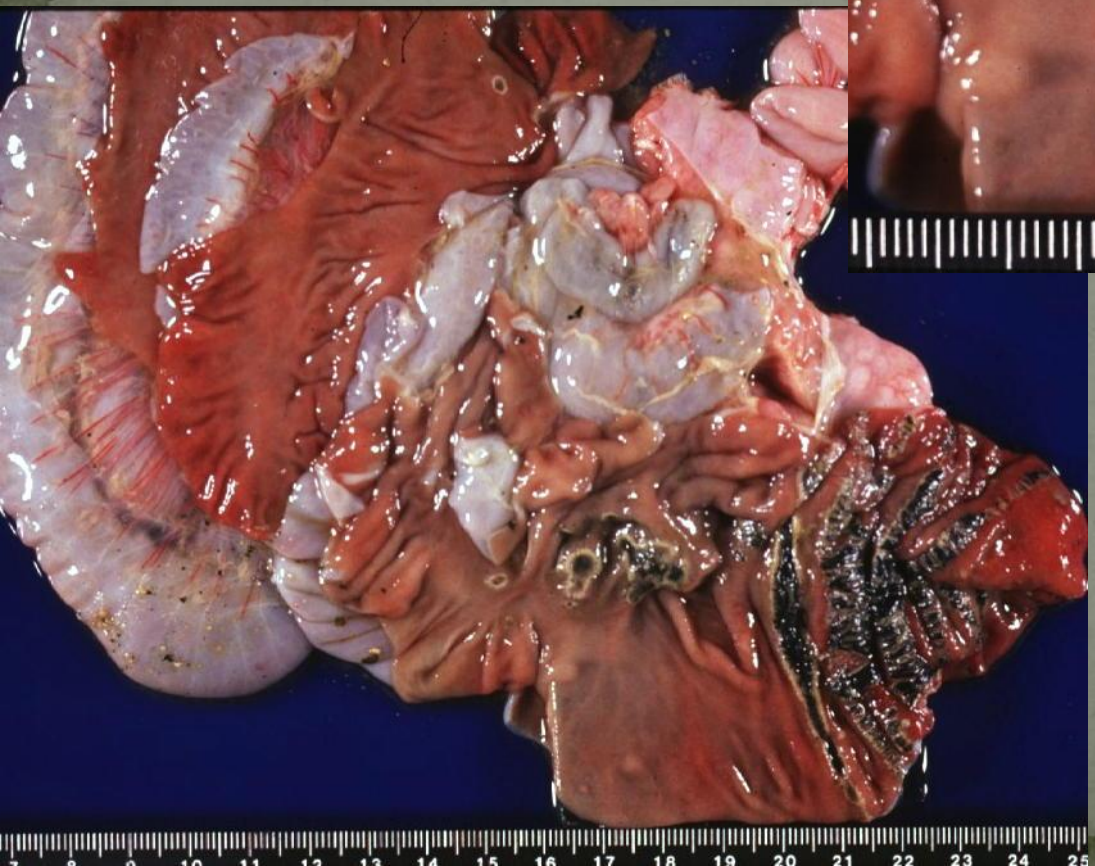




Scraping compared to normal (left)

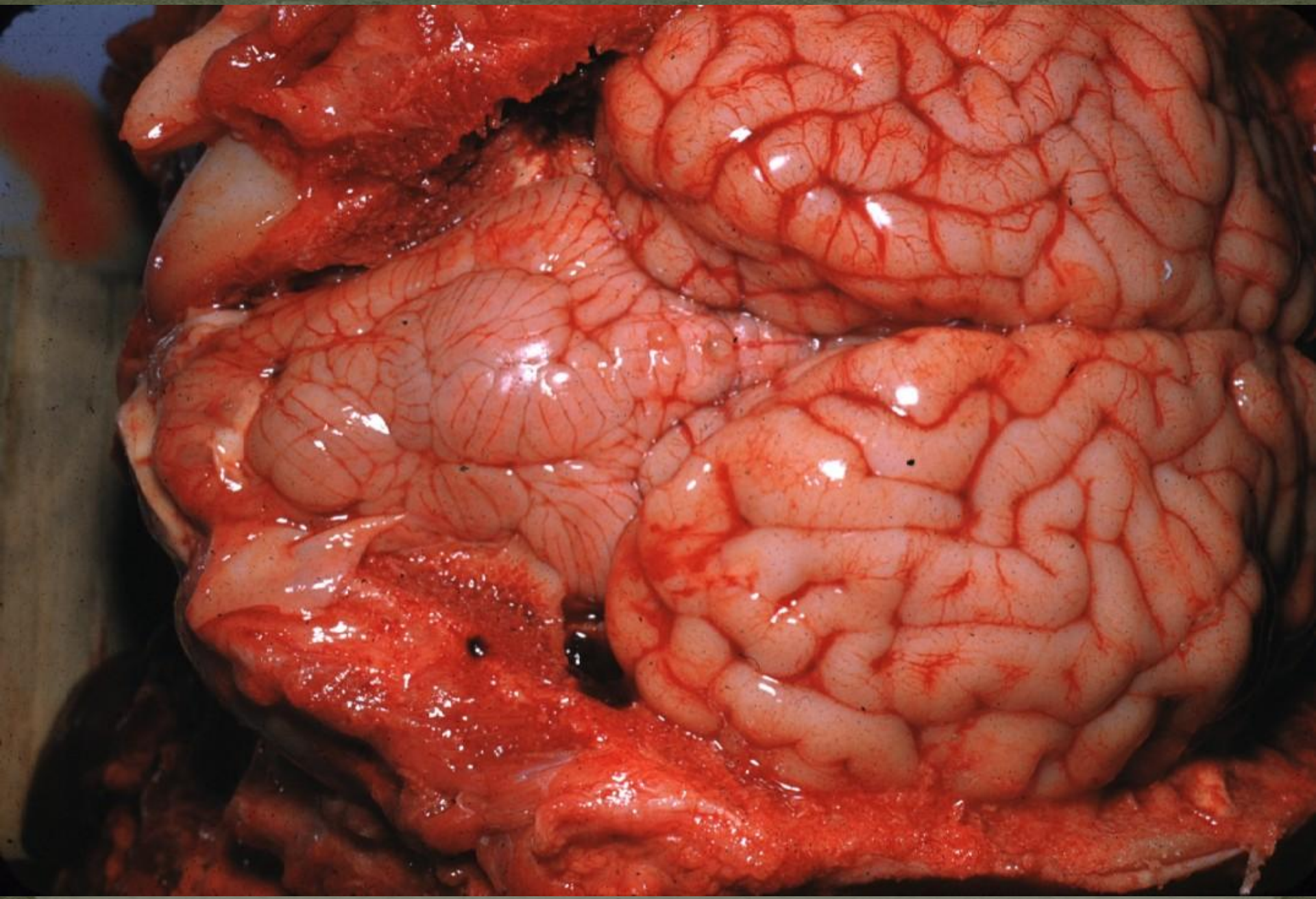
Salmonellosis





Necropsy Protocol (Brain)

- Recommend removal
- Do not section or bread-loaf!
  - You may split it in half
- Use sufficient formalin
- Evaluate for
  - Hemorrhage
  - Edema?



Equine.

Four Easy Steps

- Step 1: Identify strange thing!
- Step 2: Note its characteristics!
  - Color, size, shape, extension
- Step 3: Cut into the lesion!
- Step 4: Take representative sample
  - Step 4a: Remember to save fresh and fixed versions

Conclusion

- Be clear with your history
- Take the appropriate samples
- Prepare samples appropriately
- Recognize typical post mortem artifacts

- Thank you for your attention!
- Questions?