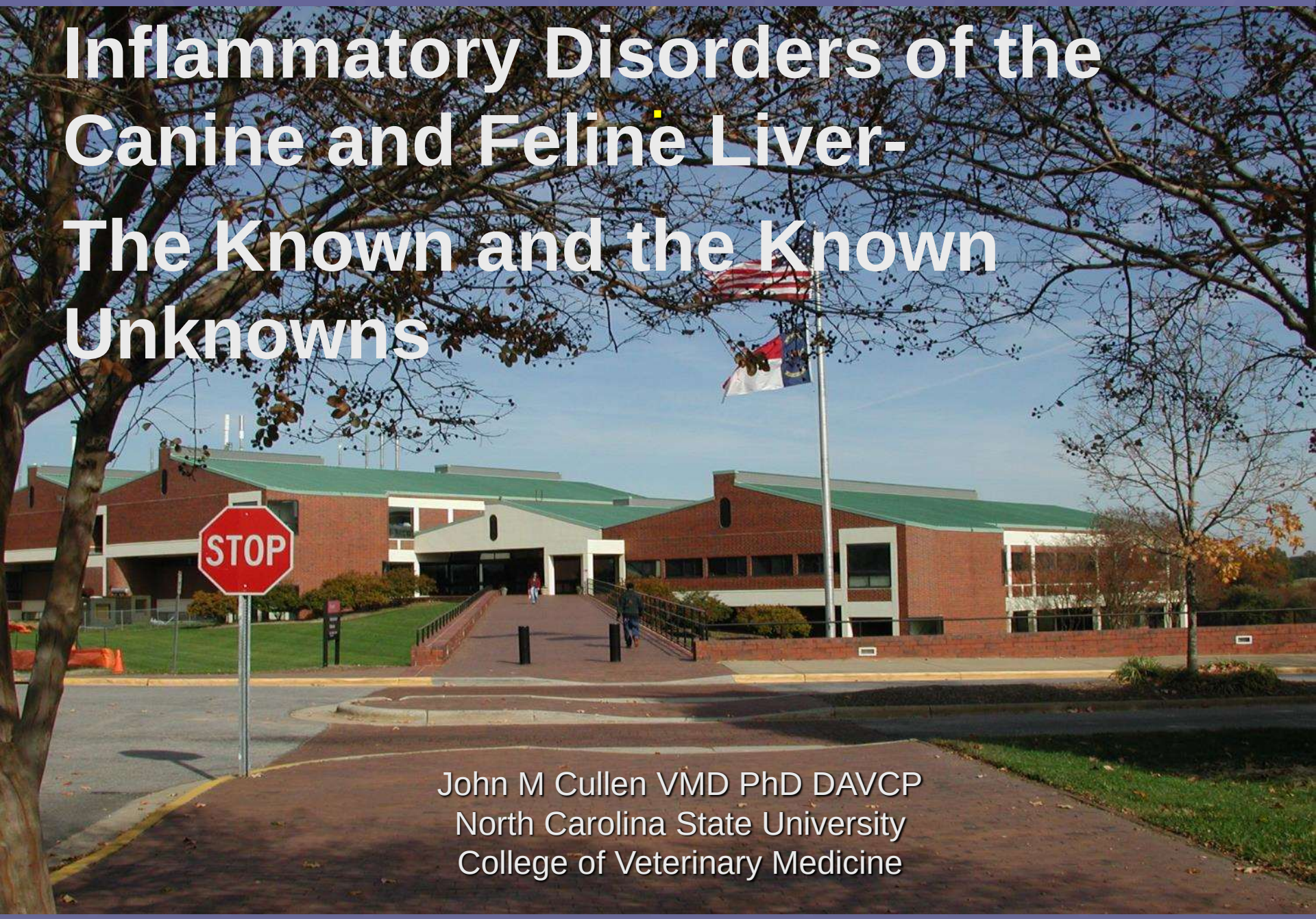
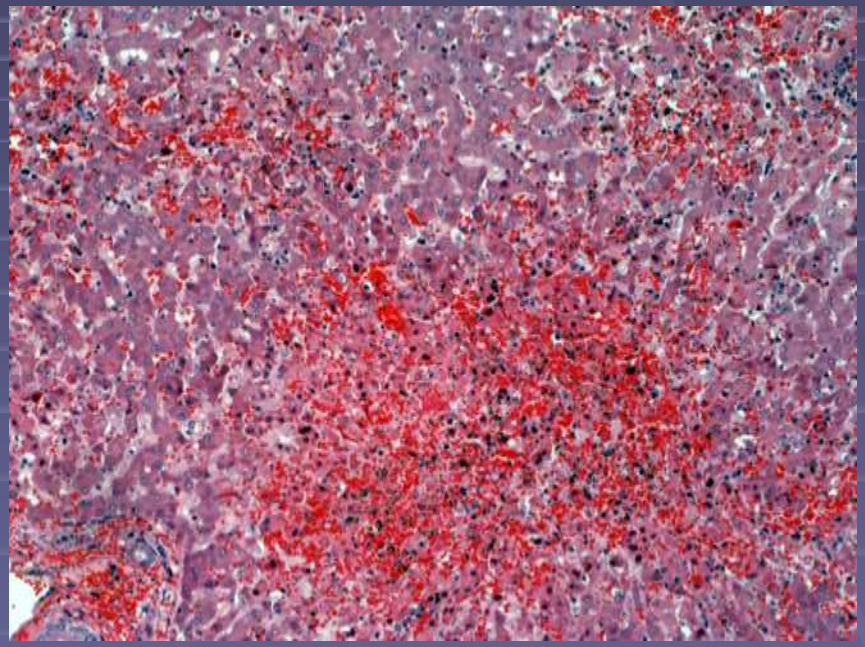
Inflammatory Disorders of the Canine and Feline Liver- The Known and the Known Unknowns



John M Cullen VMD PhD DAVCP
North Carolina State University
College of Veterinary Medicine

Acute Hepatitis

- Inflammation
- Hepatocellular apoptosis
- Hepatocellular necrosis
- +/- Regeneration



Specific Causes of Acute Hepatitis

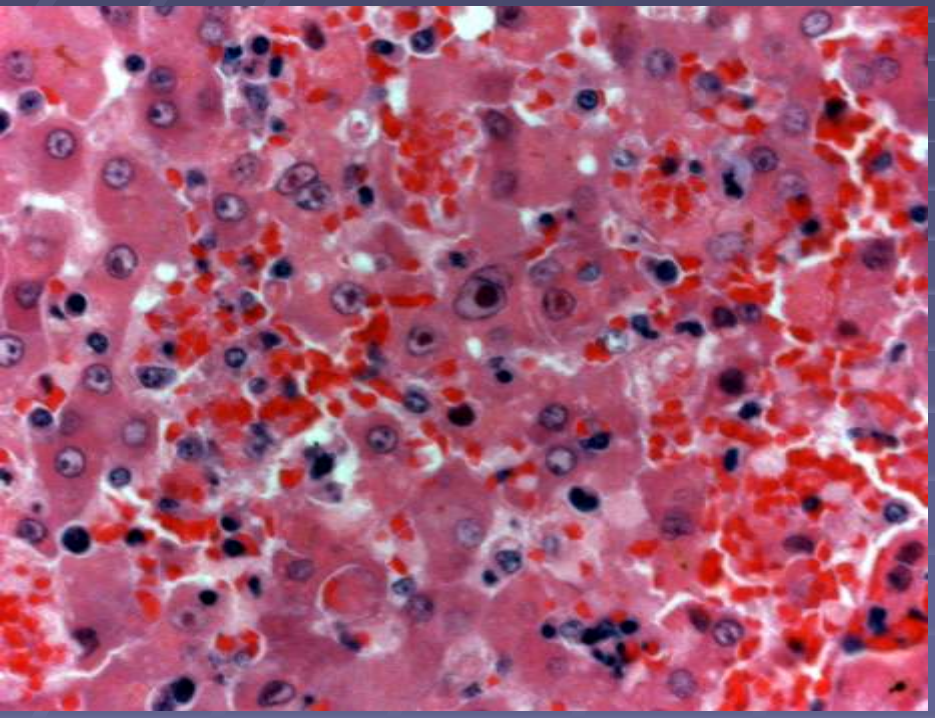
Dog

- Infectious canine hepatitis (adenovirus)
- Herpes virus
- *Clostridium piliformis*
- *Leptospirosis spp.*
- Septicemic bacteria
- *Toxoplasma gondii*

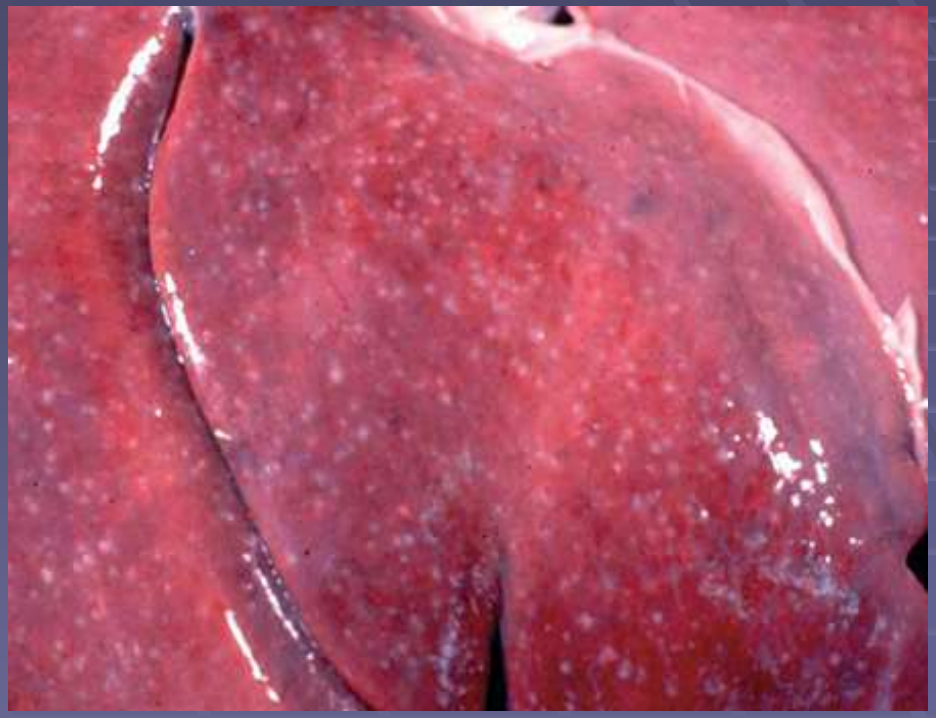
Cat

- Herpes virus
- Feline Infectious Peritonitis (corona virus mutant)
- *Clostridium piliformis*
- Septicemic bacteria
- *Toxoplasma gondii*

Quiz

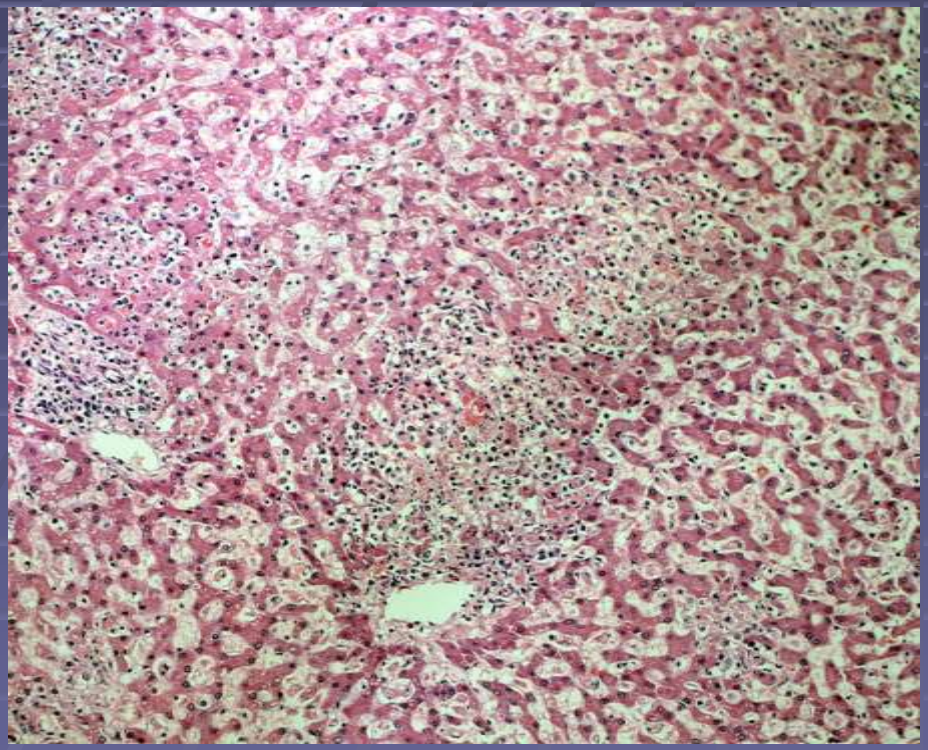


Dog

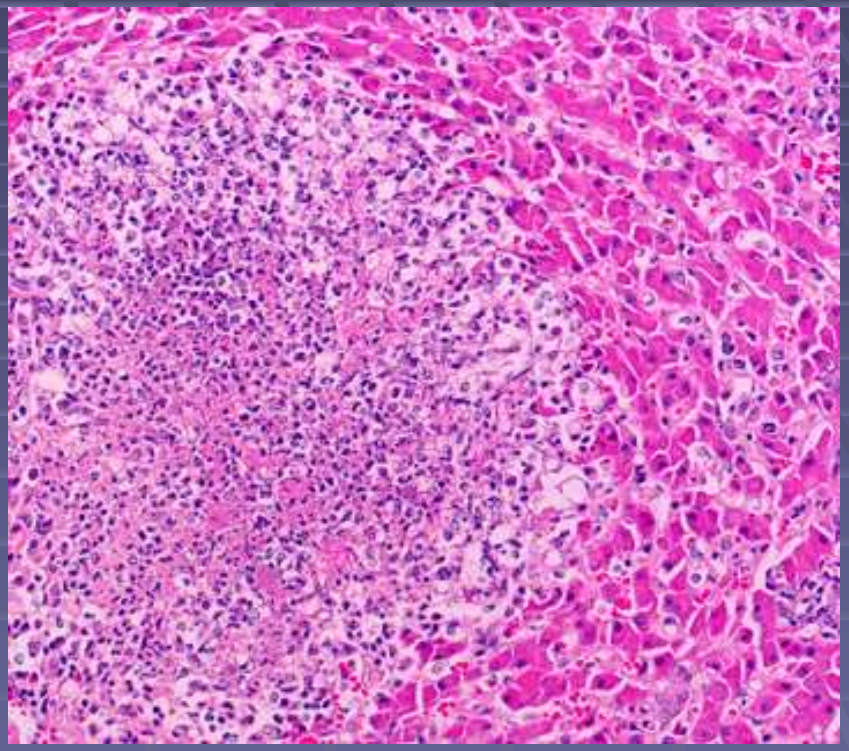


Any species
Neonate

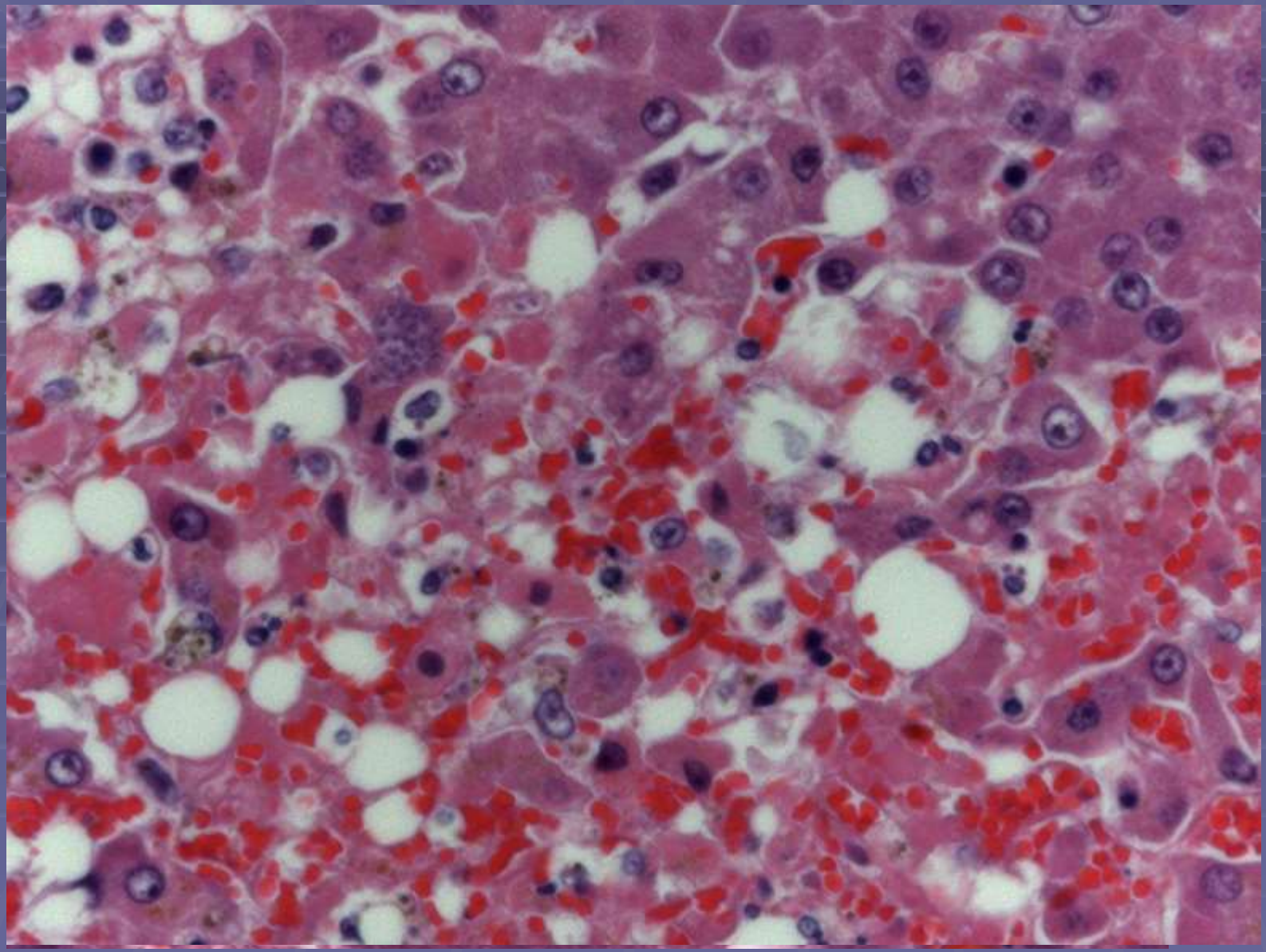
Quiz

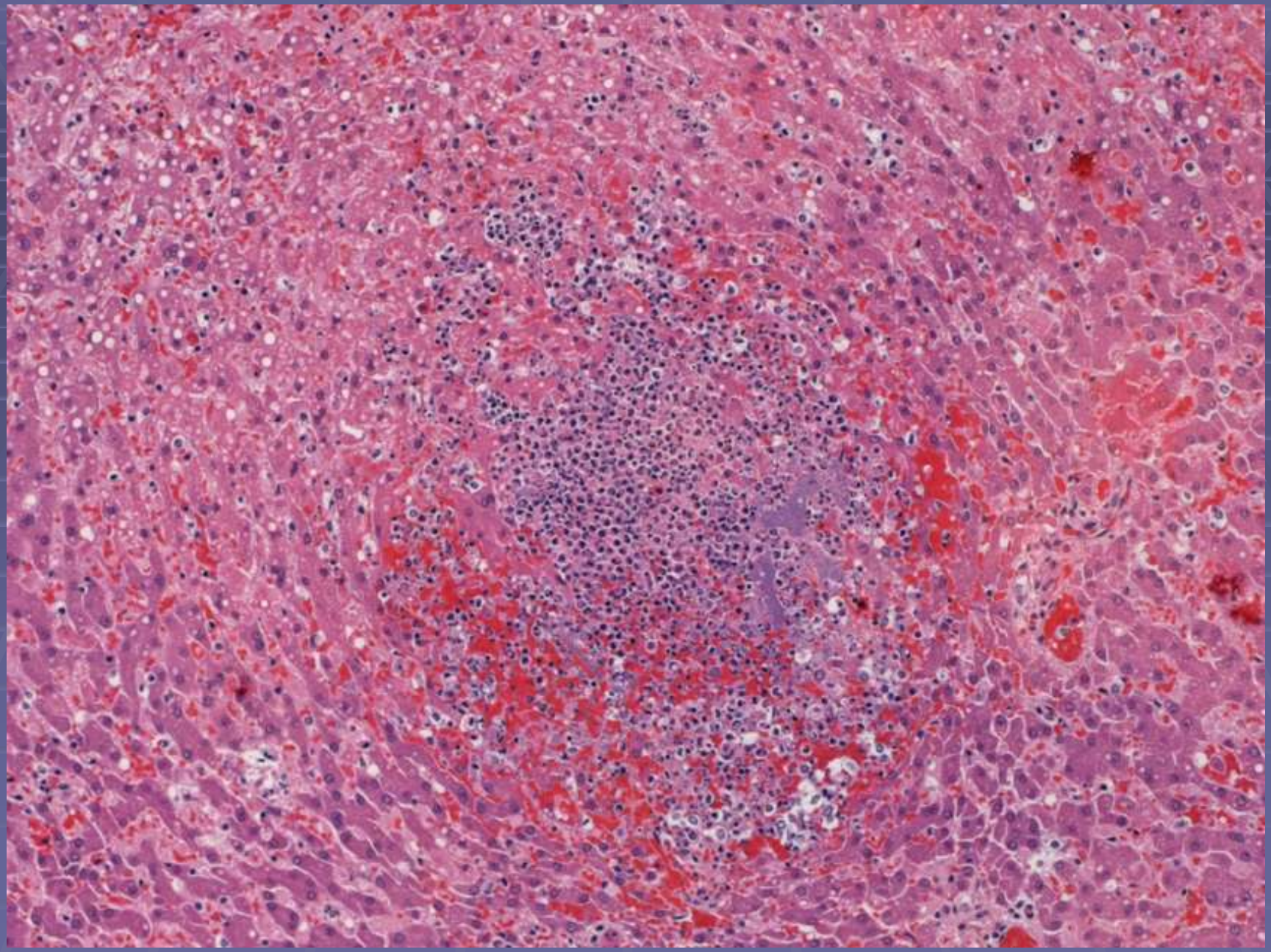


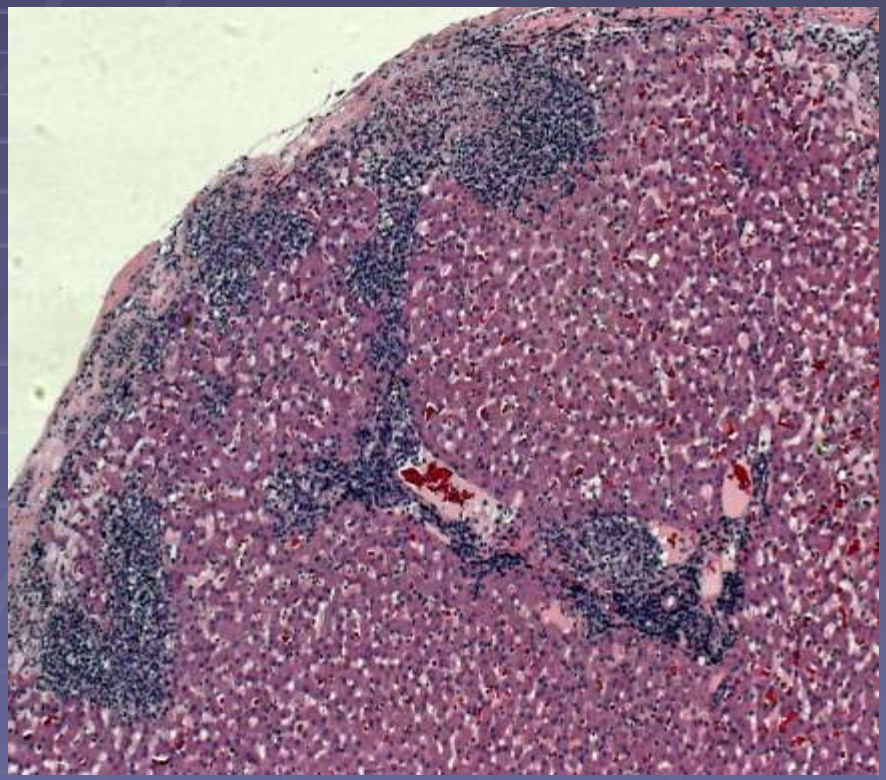
Dog
Neonate



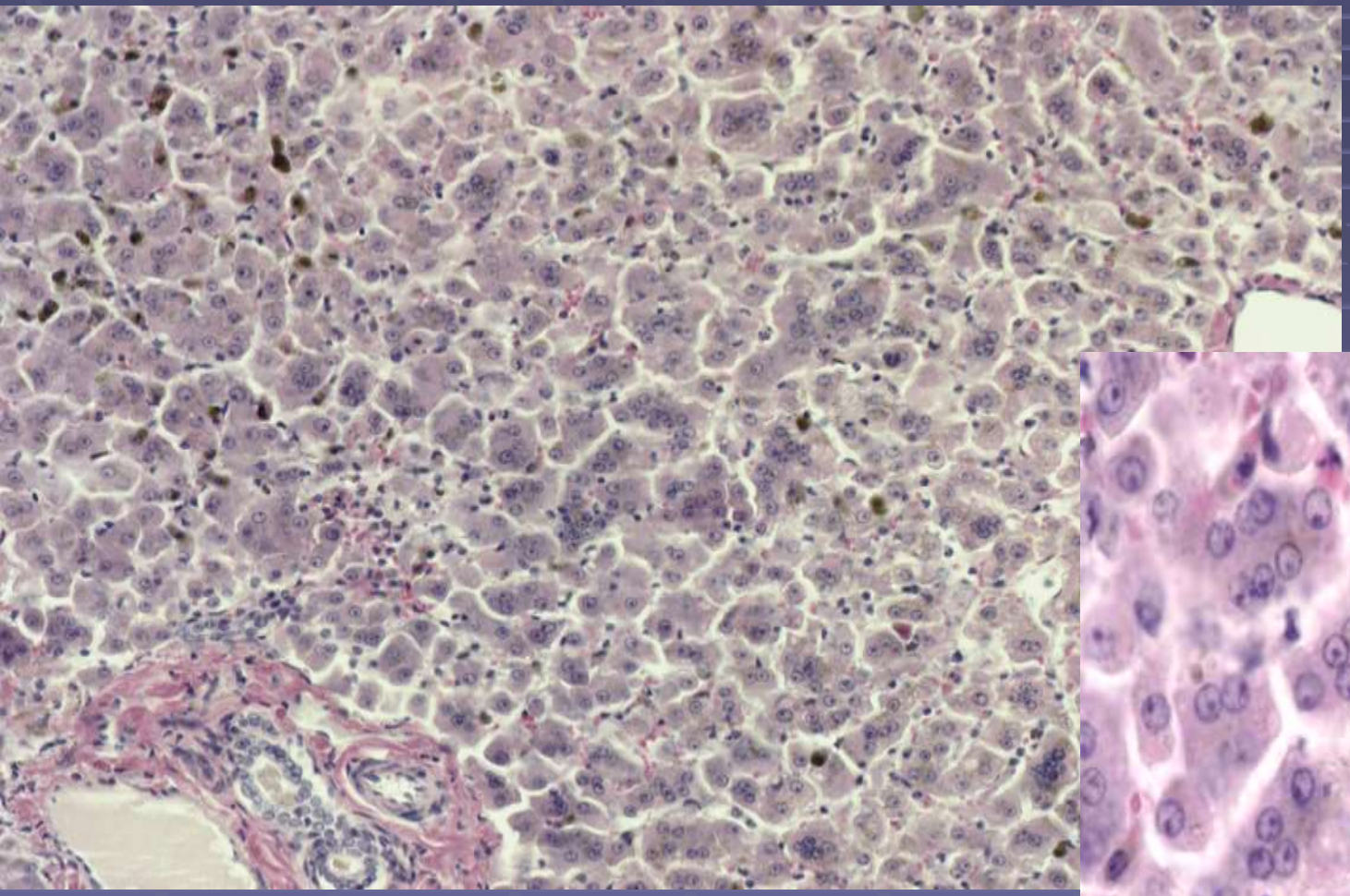
Cat
Lives outdoor
Enlarged multinodular spleen

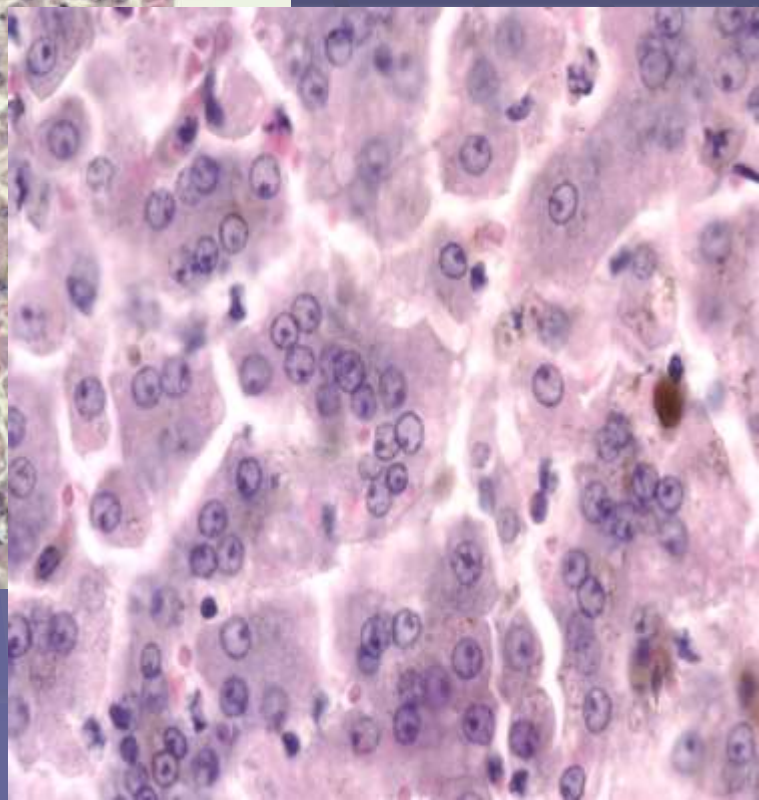






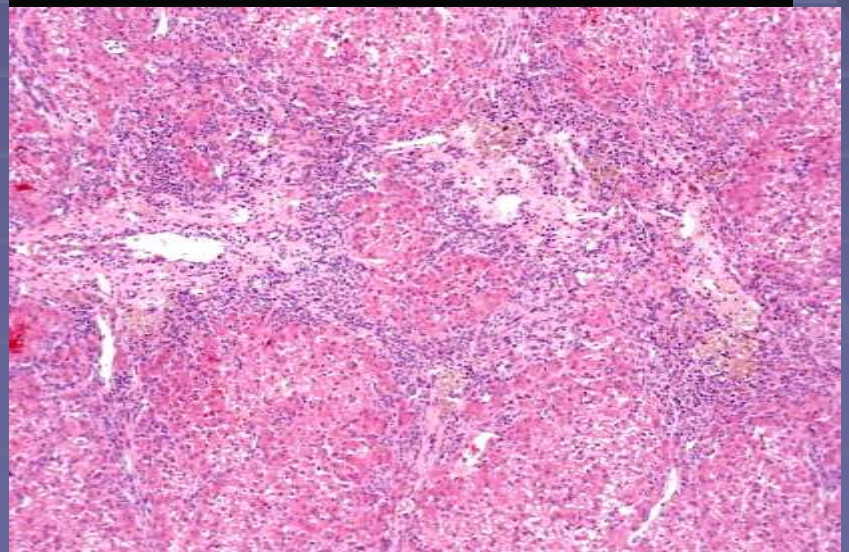
Giant cell Hepatitis





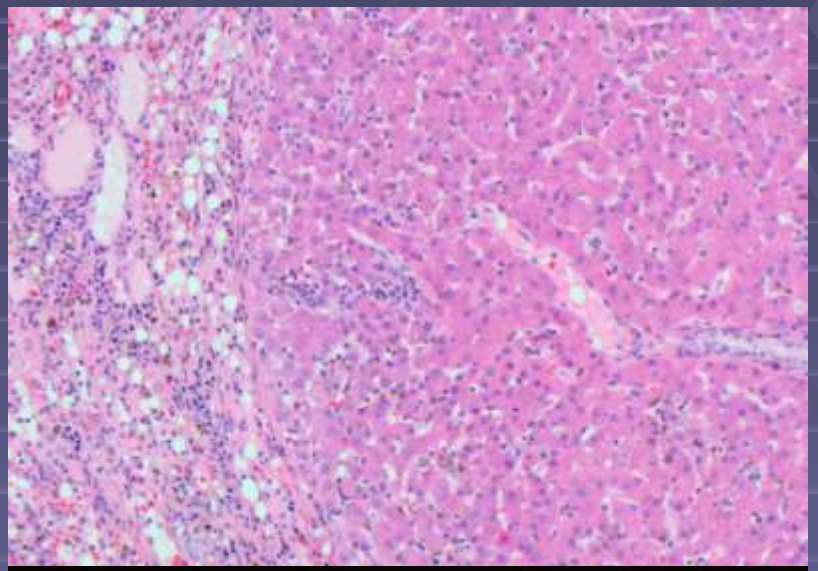
Chronic Hepatitis:

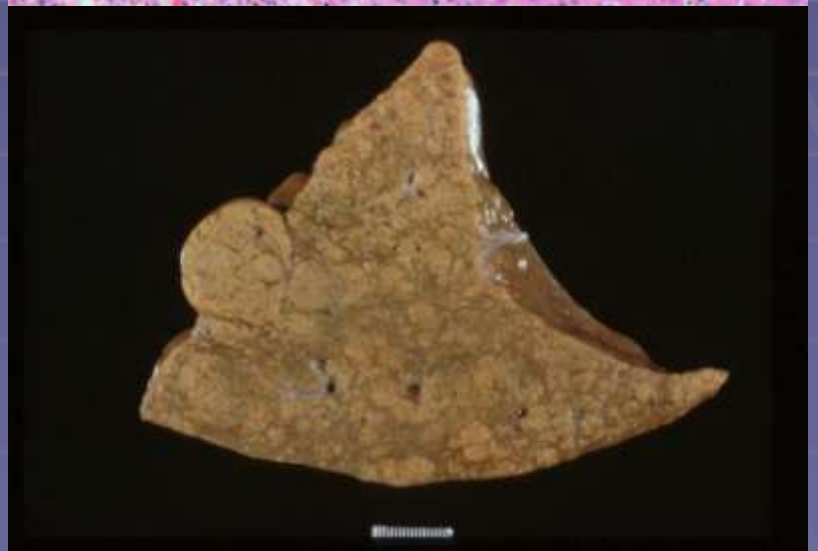
- Hepatocellular apoptosis or necrosis
- Variable inflammatory infiltrate
- Fibrosis
- Regeneration



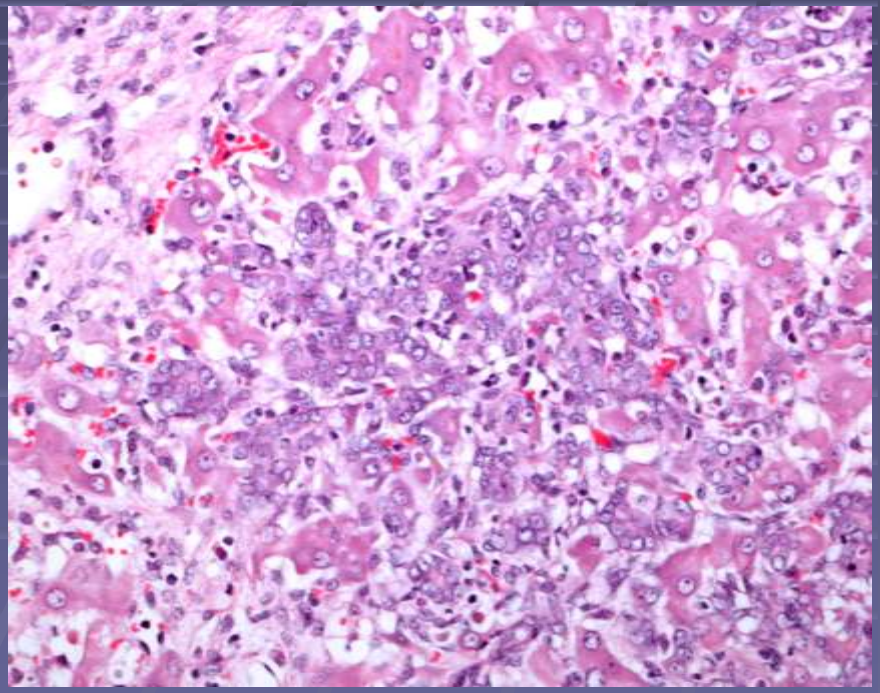
Fibrosis and Nodular Regeneration

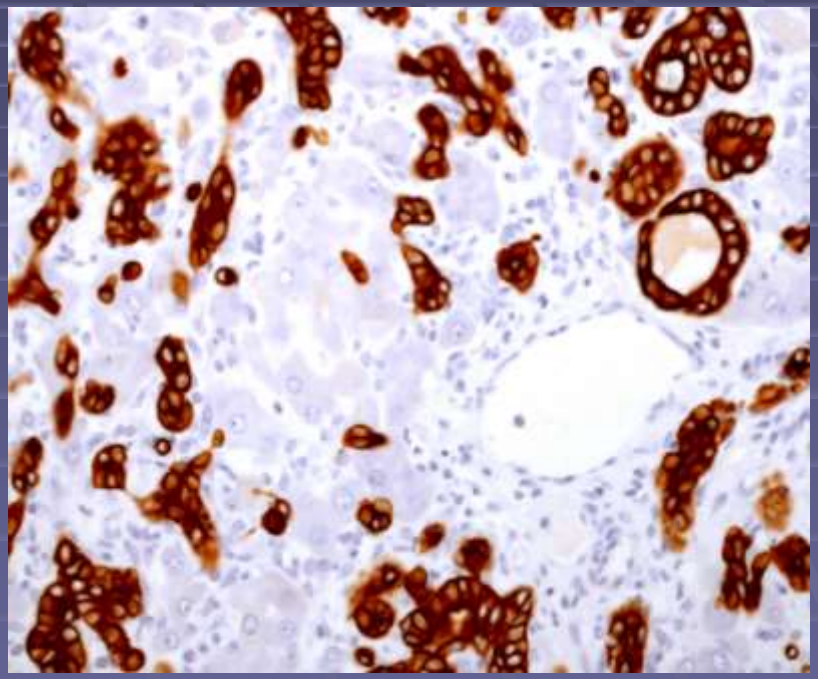
- May disrupt the lobular architecture and be accompanied by:
  - nodules of regeneration in dogs only





Ductular Proliferation





Chronic Hepatitis

- Dogs
- Not common
  - diagnosed in < 0.5% of dogs
- Chronic hepatitis more common than acute hepatitis
  - About half of acute hepatitis → chronic
- Cats
  - Rare

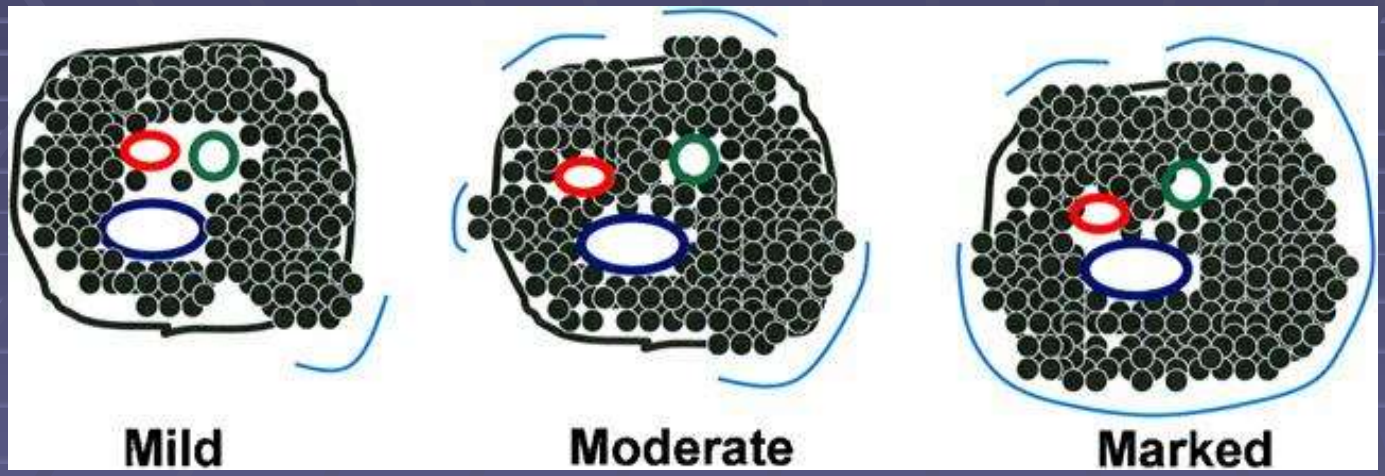
Poldervaart JH et al. JVIM 2009

Chronic Hepatitis

- Activity
  - Inflammation
- Stage
  - Fibrosis

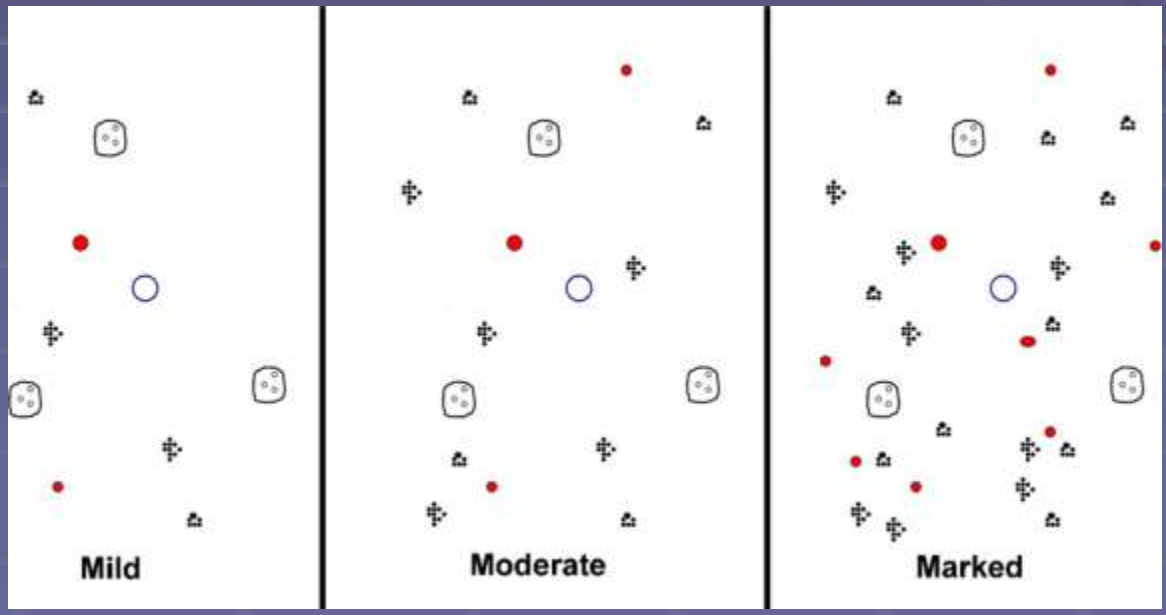
- We need a standardized grading scheme for both

Grading Inflammation: Options



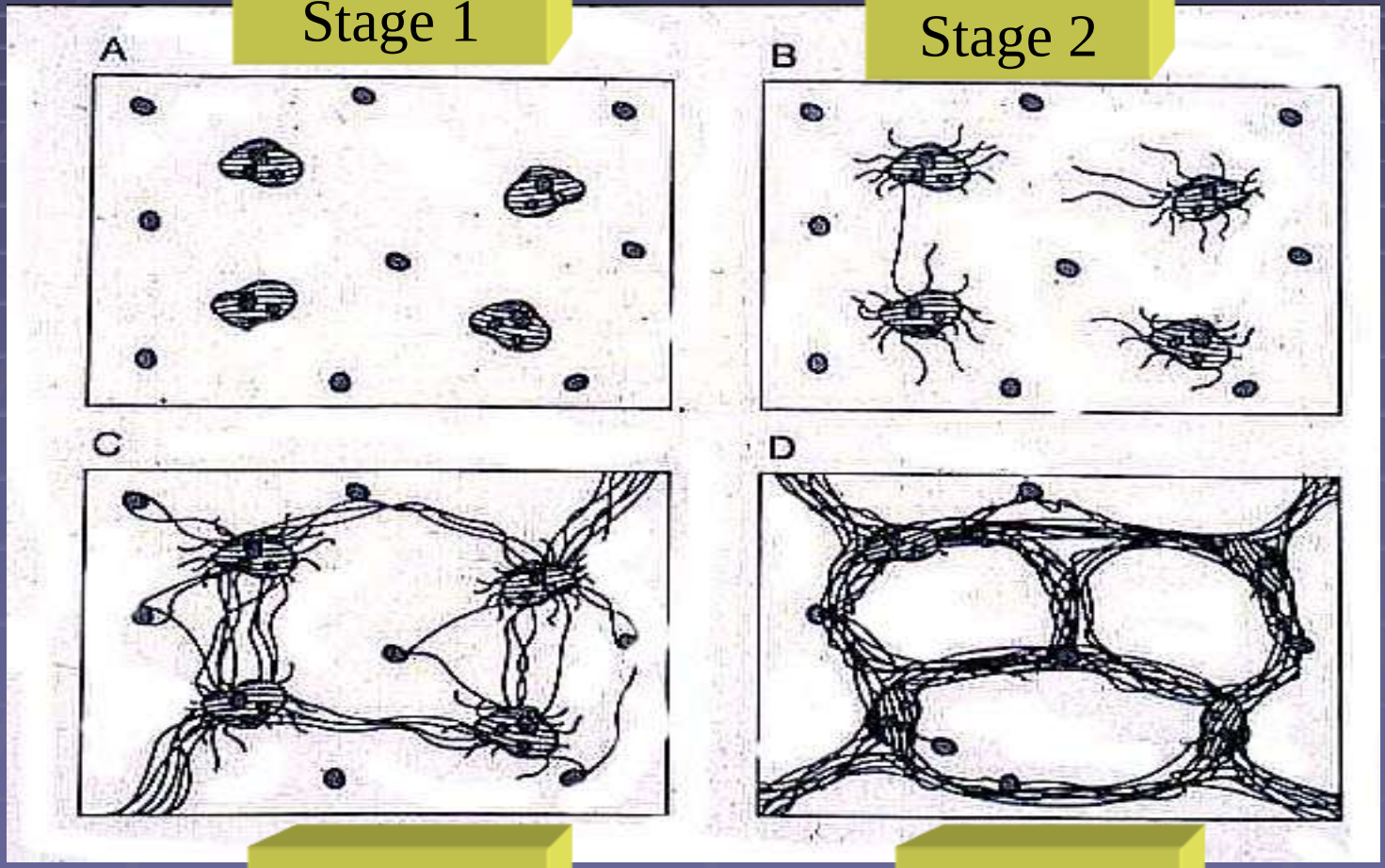
Interface Hepatitis

Parenchymal activity



Dr. Z. Goodwin

Staging Scheme



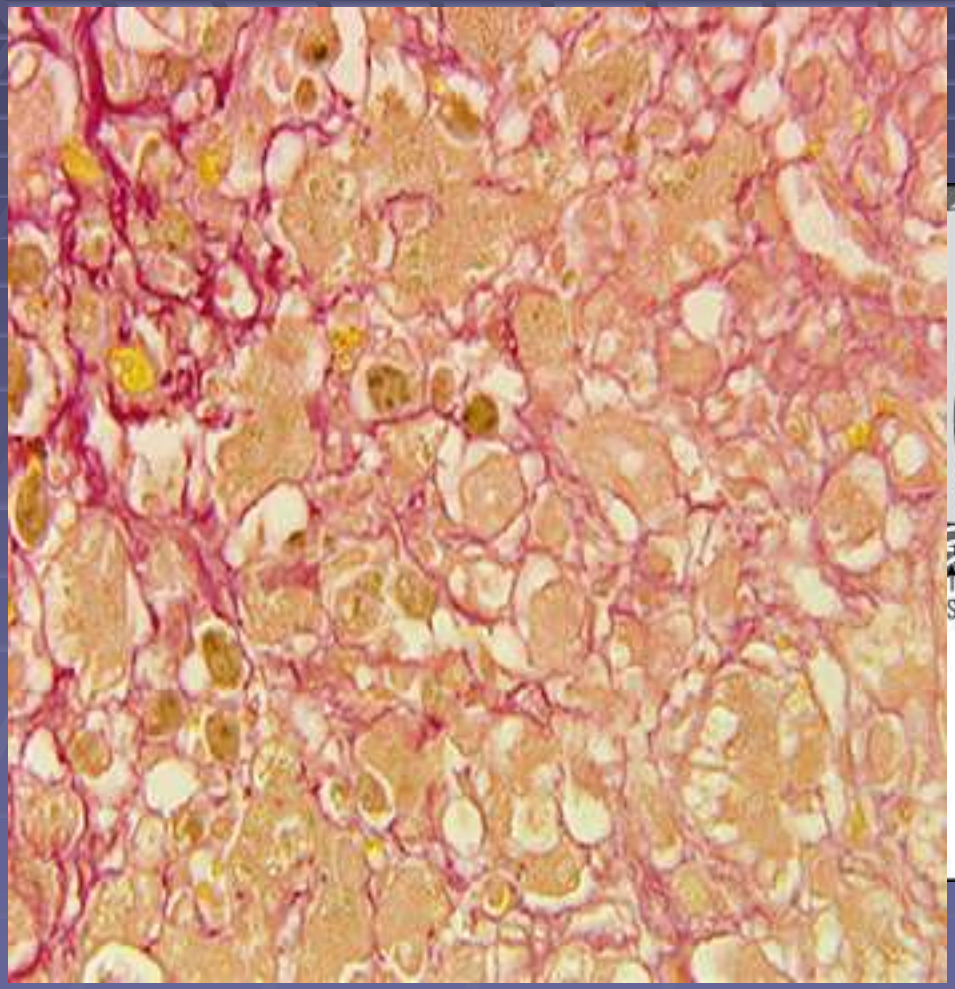
Batt K et al., 1995

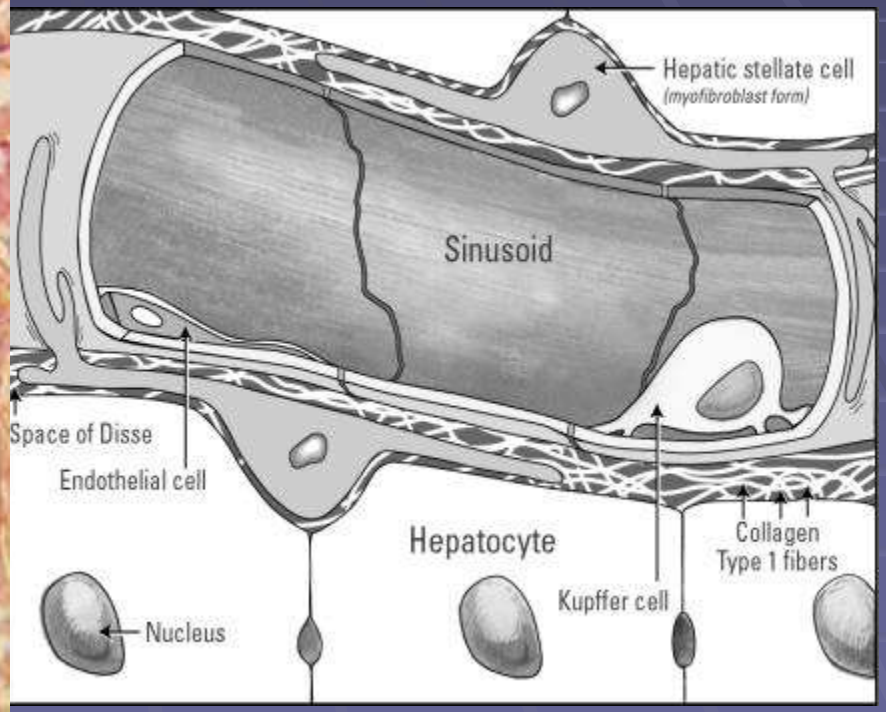
Simple grading and staging systems for chronic viral or autoimmune hepatitis

	IASL	Batts–Ludwig	Metavir
Grade			
Chronic hepatitis with minimal activity		Grade 1	A1
Chronic hepatitis with mild activity		Grade 2	A1
Chronic hepatitis with moderate activity		Grade 3	A2
Chronic hepatitis with marked activity		Grade 4	A3
Chronic hepatitis with marked activity and bridging or multiacinar necrosis		Grade 4	A3
Stage			
No fibrosis		No fibrosis	Stage 0 F0
Fibrous portal expansion		Mild fibrosis	Stage 1 F1
Few bridges or septa		Moderate fibrosis	Stage 2 F2
Numerous bridges or septa		Severe fibrosis	Stage 3 F3
Cirrhosis		Cirrhosis	Stage 4 F4

Corresponding terms in the IASL [17], Batts–Ludwig [18] and Metavir [14] scores.

Assessing fibrosis: Sirius red





Lobular dissecting hepatitis:

Chronic Hepatitis

- Sampling errors can be a serious problem in evaluating livers with chronic hepatitis as there may be considerable variation in severity between and within liver lobes

Causes of Chronic Hepatitis

- Known
  - Copper retention
  - 36% of 47 dogs with CH
    - (Poldervaart, JVIM, 23:2009)
  - Drug toxicity
    - Anticonvulsants
    - NSAIDS

Known Unknowns

Most are idiopathic

- Speculations
- Infectious disease
  - Bacterial
  - Viral
- Immune-Mediated?

Copper-associated hepatitis affects dogs primarily

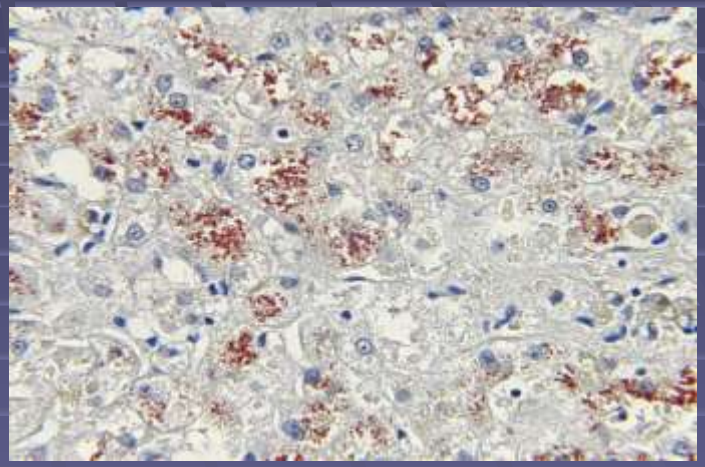
- Hepatitis associated with accumulation of copper in hepatocytes
- Starting in the centrilobular regions
- Progressive accumulation results in hepatocellular necrosis and inflammation with copper-laden macrophage aggregates
- Acute and chronic hepatitis and cirrhosis

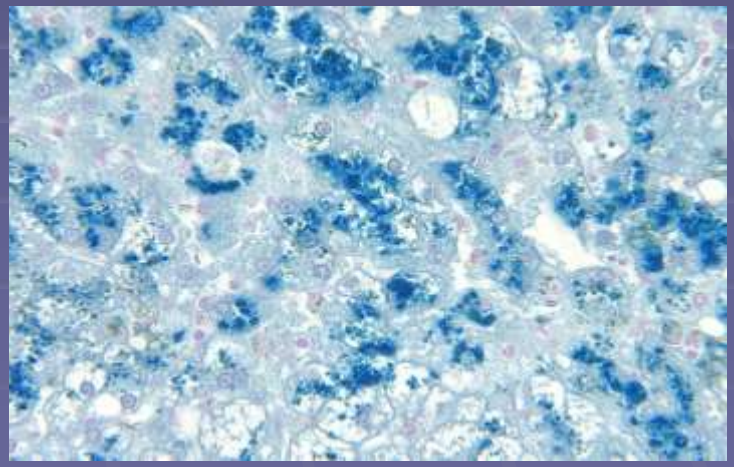
Canine copper metabolism

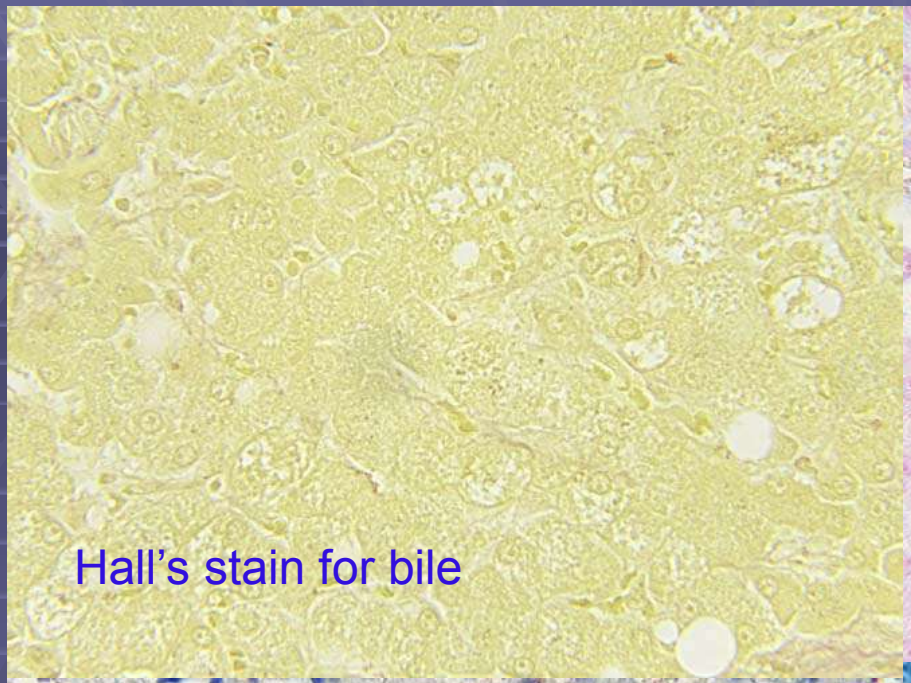
- Absorbed in proximal GI tract
- Intestinal dose cleared in bile
- In 80 min hepatic copper normal or reduced following dose in normal dogs-High in bile
- In 80 min affected Bedlingtons hepatic copper increased ~50%-low in bile
  - In lysosomes
  - Kaneko et al.

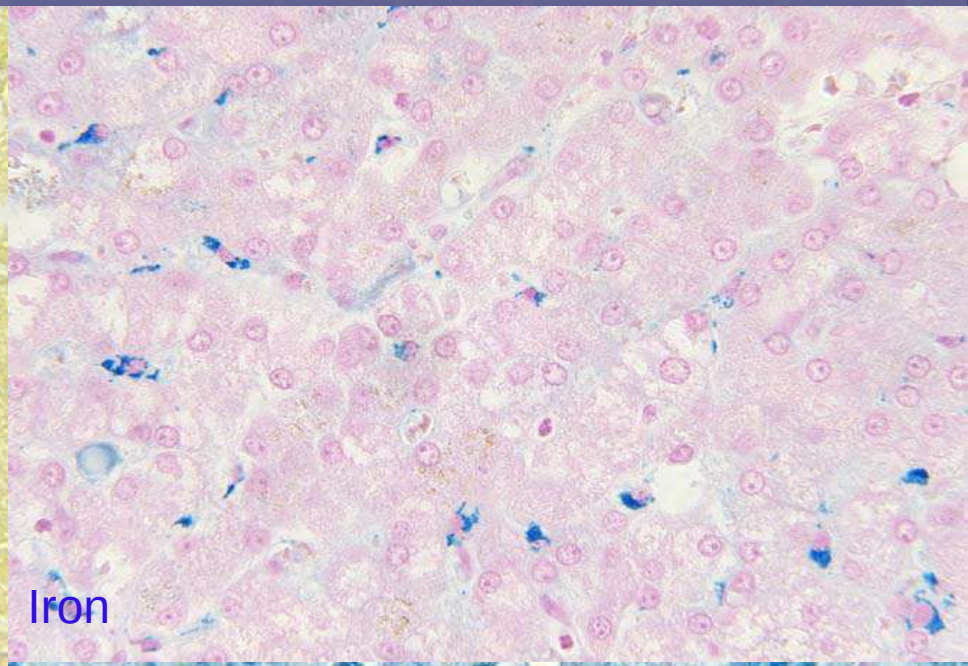
Mechanism of Toxicity

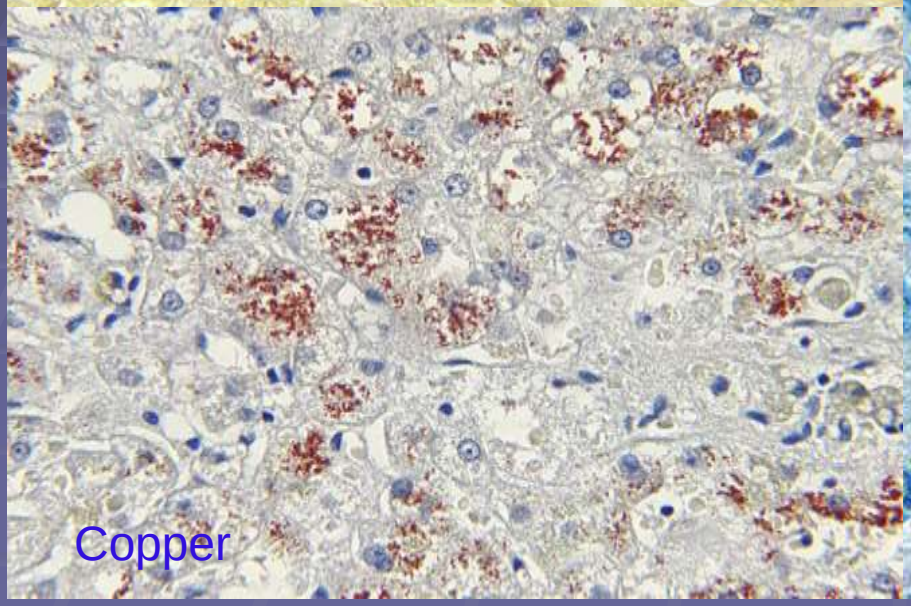
- CU initially stored in MT in cytoplasm, then lysosomes
- CU can participate in the Fenton Reaction (like iron) and produces oxygen radicals
- Oxygen radicals damage cell membranes
- Lipofuscin typically seen with chronic copper toxicosis

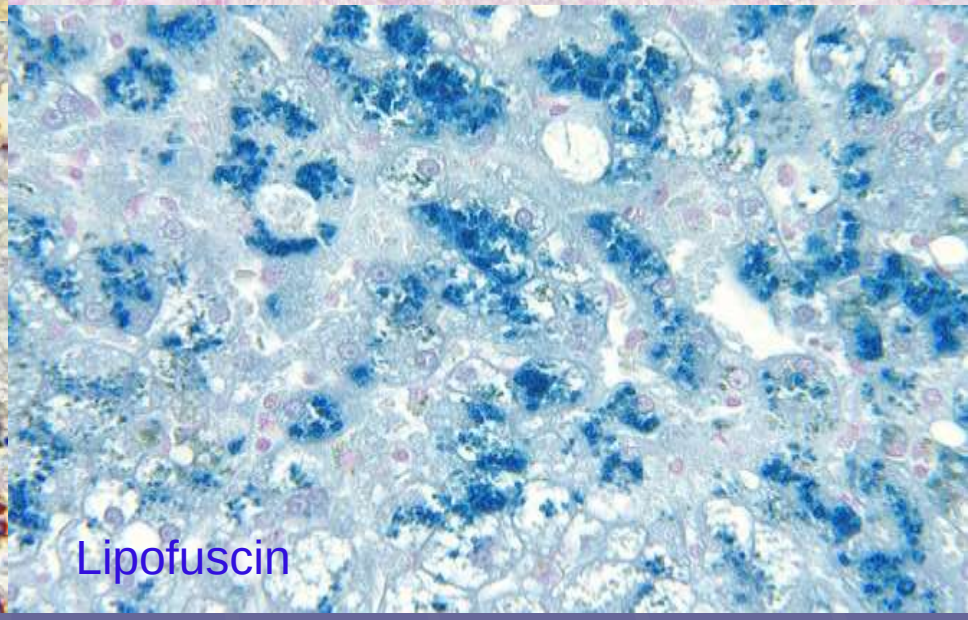





Hall’s stain for bile

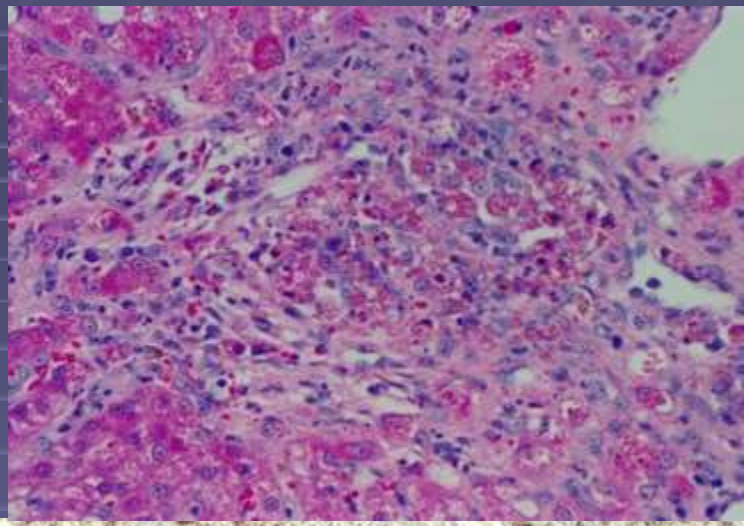

Iron

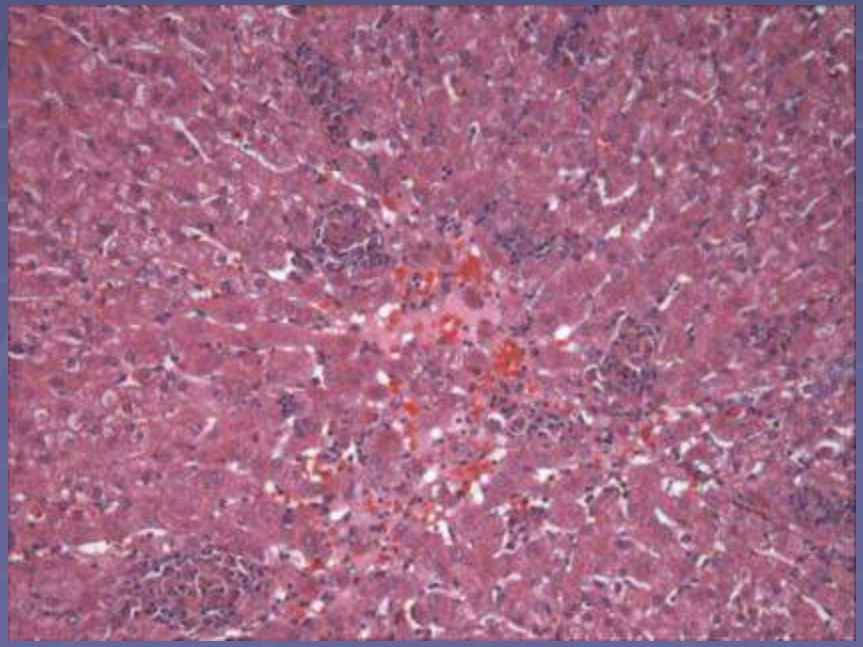

Copper

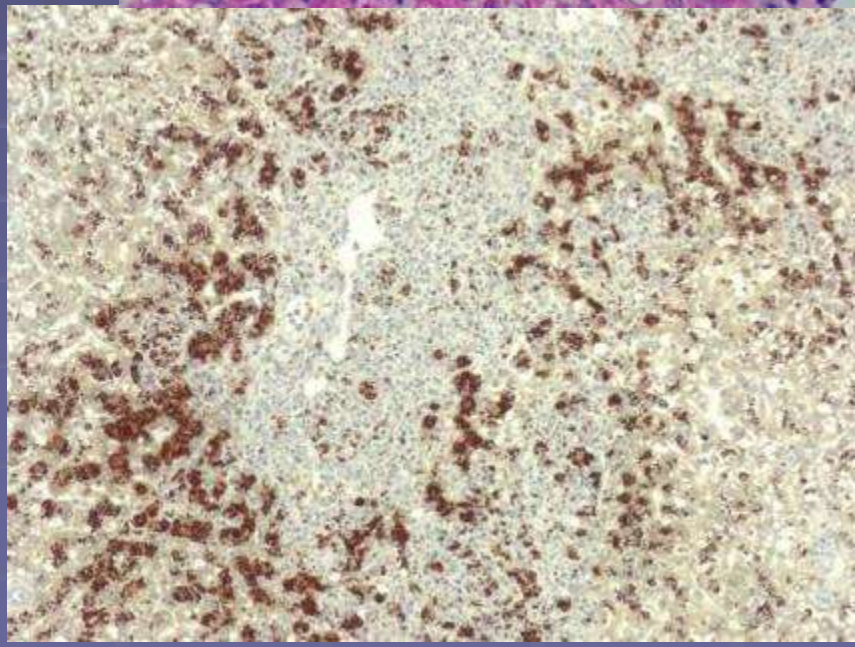

Lipofuscin

Inflamed livers should be stained for copper

- Histology
- Initial centrilobular accumulation of copper
- Primarily mononuclear infiltrate
  - Histiocytic infiltrates to aggregate formation
- Individual cell necrosis







Liver Copper Determination

- Beware
  - Needle biopsies are often inaccurate
- Can use tissue from paraffin block

How Much Copper is Too Much?

- < 400 ppm d.w. in normal dogs
- >1200-1500 ppm d.w. cause for concern
- > 2000 ppm d.w. likely associated with disease
- Effect of antioxidant status?
- Accuracy of measurement?

Causes for Copper Accumulation

- Genetic
  - Bedlington Terriers
- Cholestasis?
  - Cu in bile
- Inflammation?
- Cirrhosis?

Bedlington Terriers

- Murr1 gene-exon 2 deletion (variants)
- Copper increases with age in affected dogs
- Copper accumulates in lysosomes
- Liver disease -> end stage liver likely
- Liver copper and liver disease do not correlate precisely

Copper Excess

- Bedlington Terriers
  - Only breed with age-related Cu increases
  - Homozygous > 10,000 ppm
  - Gene Comm1 (Murr1) possible chaperone
    - Not ATP7B or ATOX1

Doberman Pinchers-Thornburg

- Middle aged female dogs
- Lesion starts in C.L.
  - Copper accumulation in same site as inflammation
  - 30 with increased copper
    - 10>2,000 ppm up to 4,700 ppm
    - 7~ 650-1,900 ppm
    - 5 < 250 ppm

  - Inflammation/disease not driven by copper levels as histology similar with or without copper elevation (Thornburg L. Vet Pathol, 35:1998)

Doberman Hepatitis: Utrecht

- ~30% 3 yo dogs with enzyme elevations have hepatitis
  - 6:1 F:M
- Liver copper increases with time
- Hepatitis increases with time in ~ 30% (Mandigers, JVIM, 2004)
- Copper metabolism gene expression
  - Reduced mRNA for chaperones and membrane pumps-ATP7A
  - Reduced antioxidant levels
- Chelation improves histology of subclinical liver disease
  - Cu vs. anti-inflammatory action

Doberman Hepatitis

- Some form likely related to copper
- Other types of hepatitis in this breed may occur
  - Autoimmune?
- Some dogs have impaired copper metabolism

Labrador Retrievers

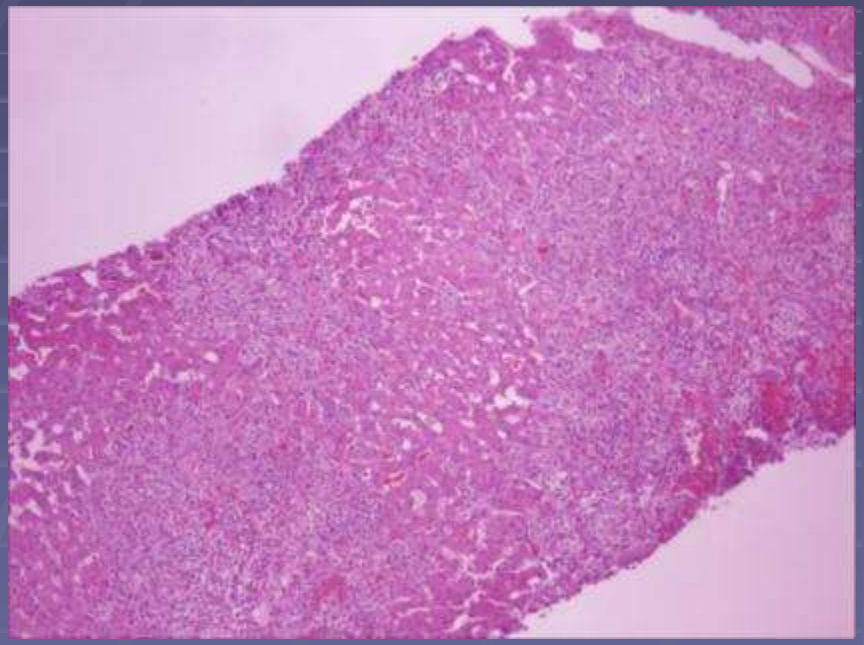
- Clinically affected (N=15)
- 3:1 M:F
- Copper 400-2,600 ppm in affected
  - 100-300 ppm in normals
- Hereditary?
- Typical lesions
- Not all chronic hepatitis in Labs is copper-related

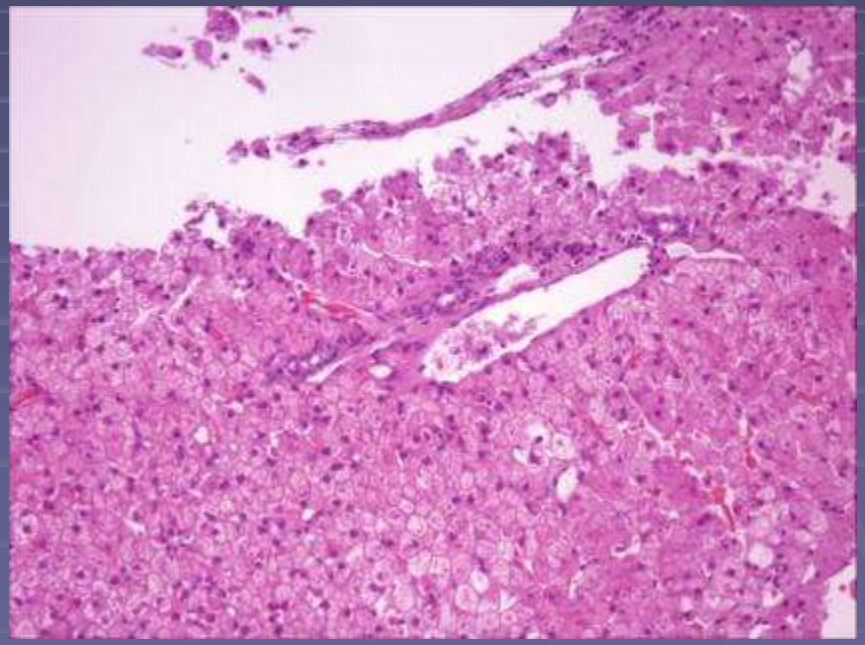
Hoffman et al. JVIM 2006

Other Breeds with Copper Issues

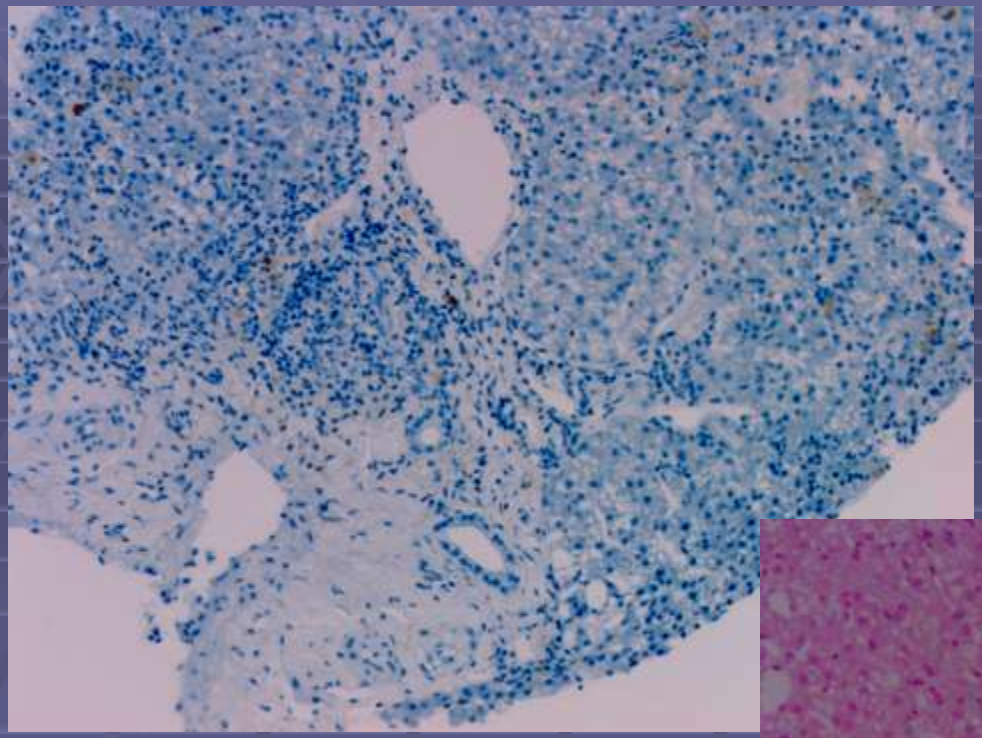
- Skye Terriers
- Dalmatians
- West Highland White

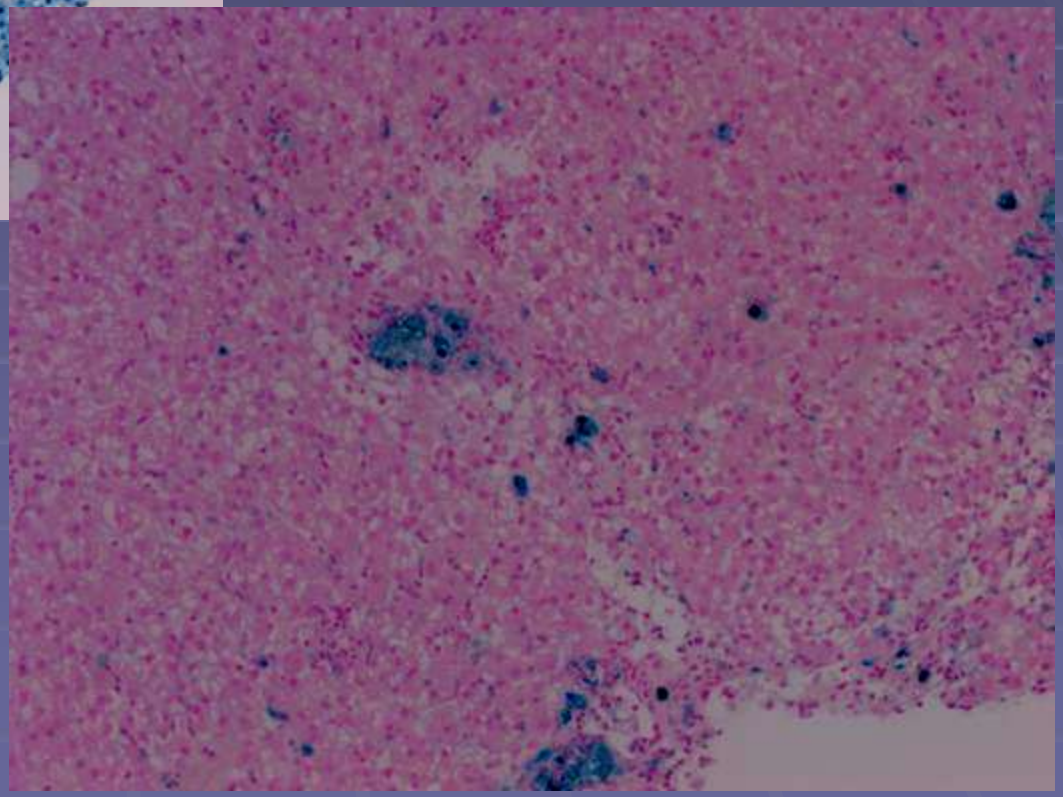
Post chelation
2700 ppm





It's not all Copper





Labrador Retriever
Chronic Hepatitis

Copper/no copper

American and English Cocker Spaniels

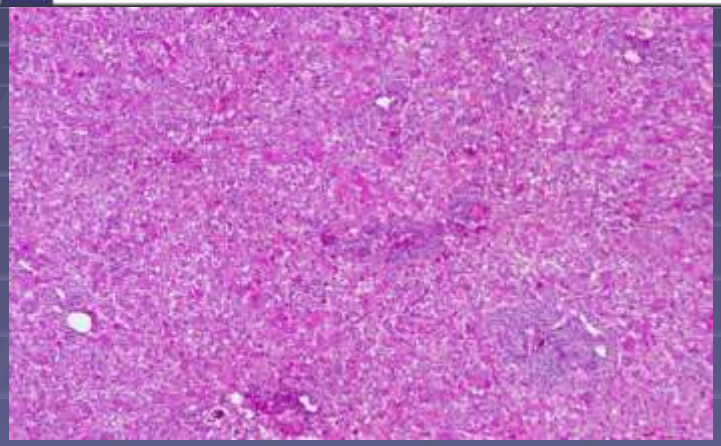
- Chronic hepatitis
- Copper not always an issue

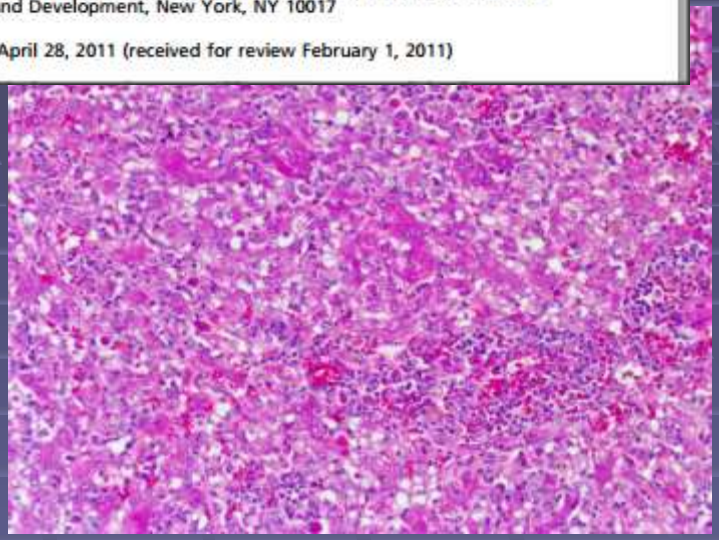
Characterization of a canine homolog of hepatitis C virus

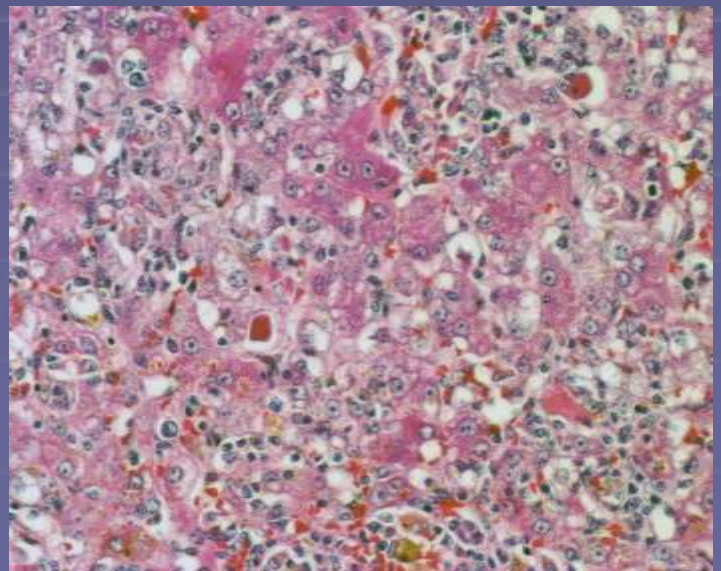
Amit Kapoor[a,1], Peter Simmonds[b], Gisa Gerold[c], Natasha Qaisar[a], Komal Jain[a], Jose A. Henriquez[a], Cadhla Firth[a], David L. Hirschberg[a], Charles M. Rice[c], Shelly Shields[d], and W. Ian Lipkin[a]

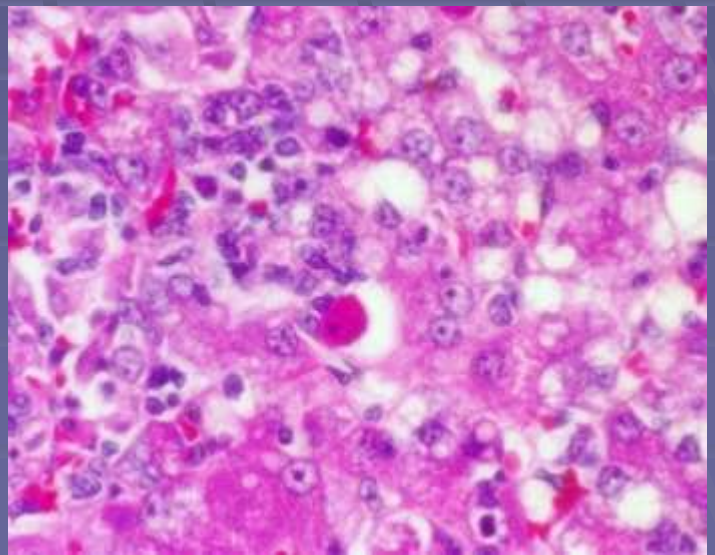
[a]Center for Infection and Immunity, Columbia University, New York, NY 10032; [b]Centre for Immunology, Infection and Evolution, Ashworth Laboratories, University of Edinburgh, Edinburgh EH9 3JT, United Kingdom; [c]Center for the Study of Hepatitis C, Laboratory of Virology and Infectious Disease, The Rockefeller University, New York, NY 10065; and [d]Pfizer Veterinary Medicine Research and Development, New York, NY 10017

Edited by Robert H. Purcell, National Institutes of Health, Bethesda, MD, and approved April 28, 2011 (received for review February 1, 2011)

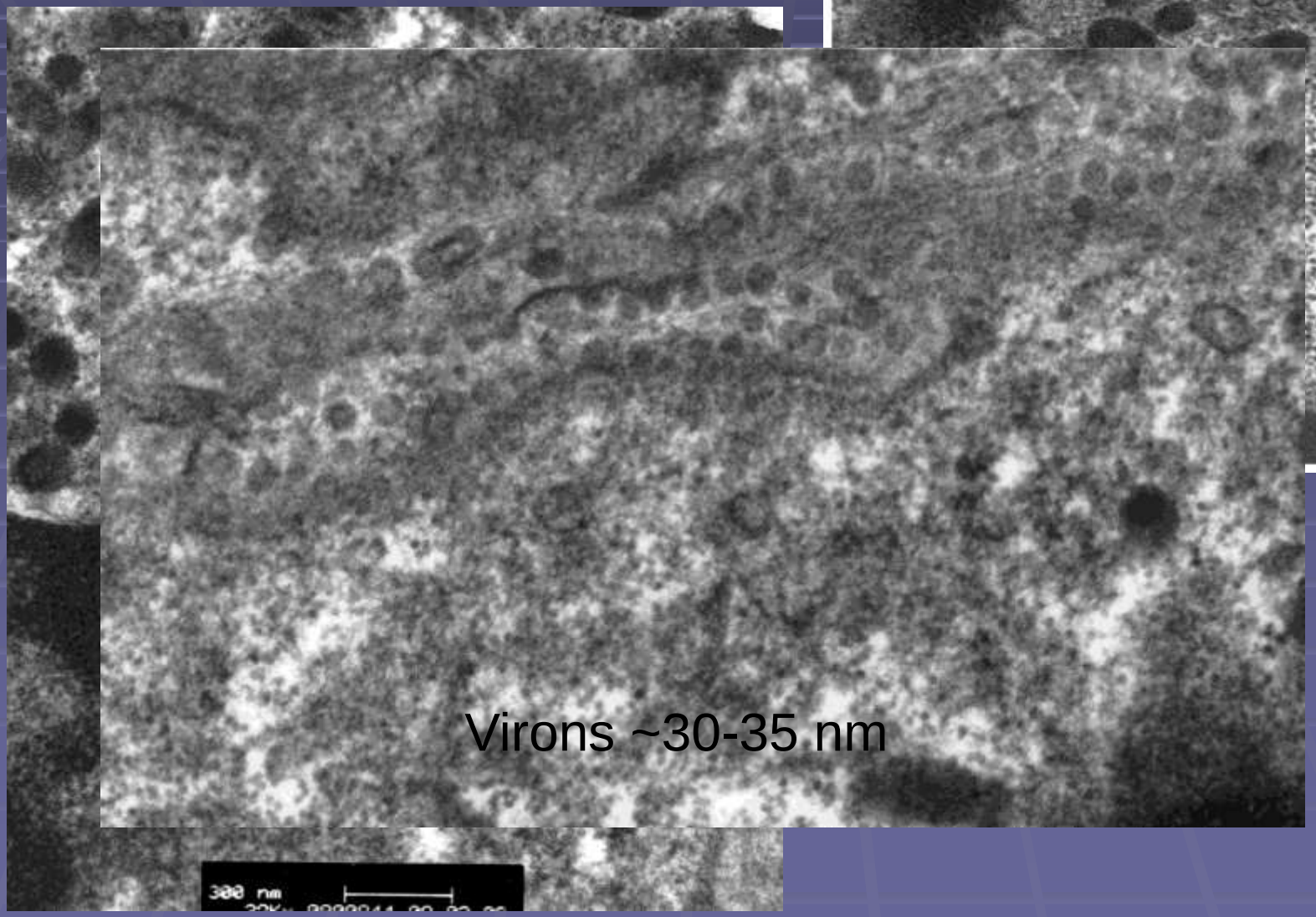








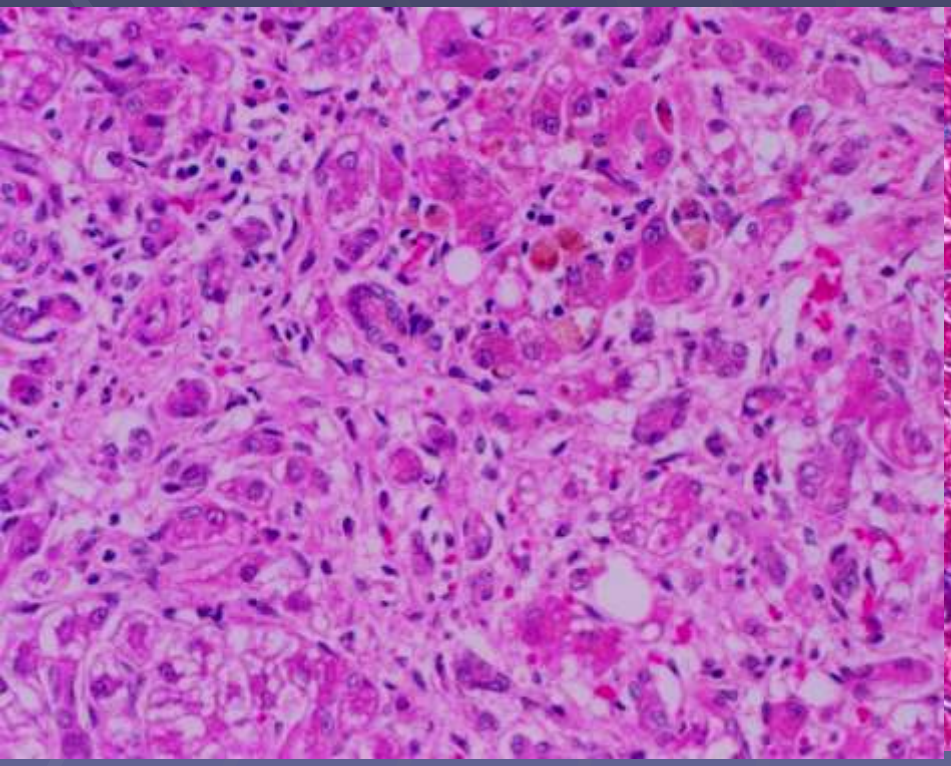
Virus?

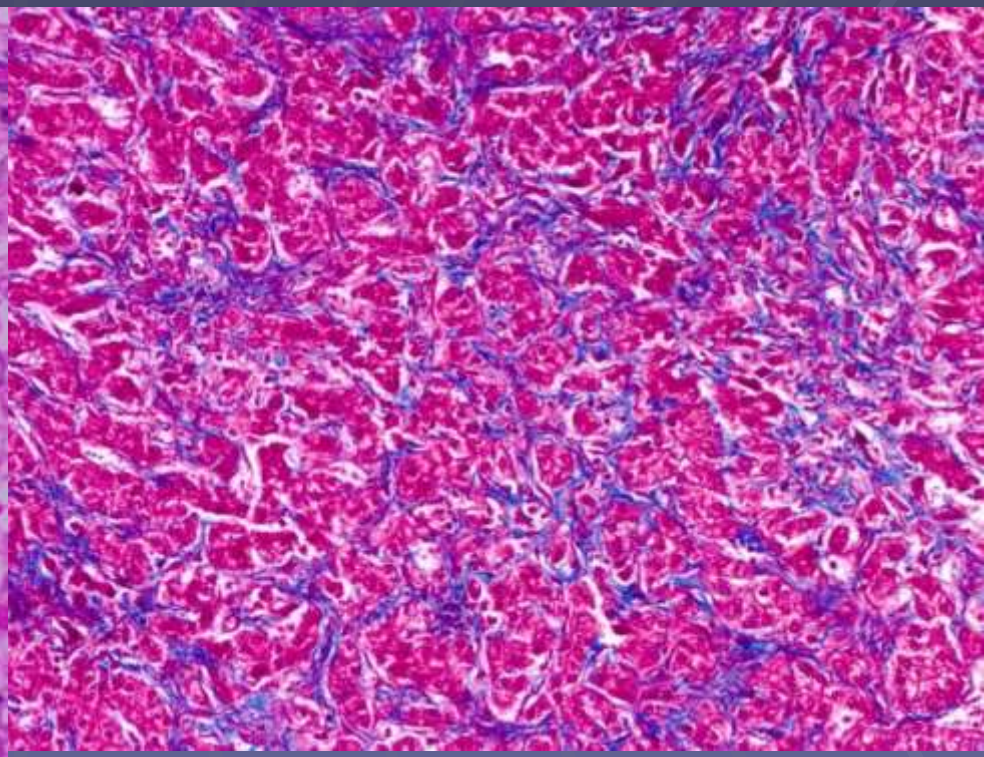


Virons ~30-35 nm

Most Chronic Hepatitis in Dogs is Idiopathic

Lobular Dissecting Hepatitis





Affects young dogs

High mortality rate

Portal hypertension

Ascites

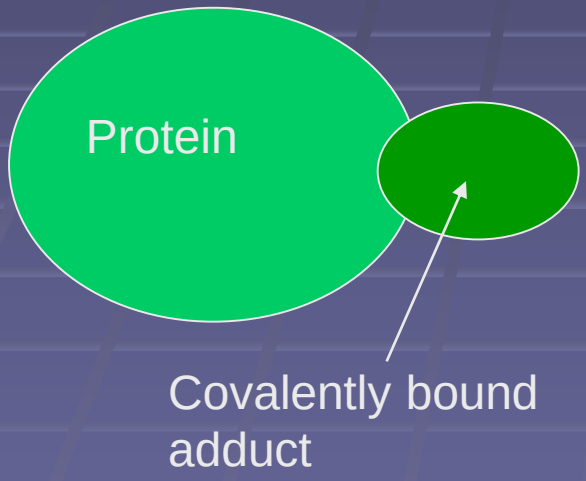
Idiopathic Hepatitis: Possible Pathogenesis

- Possible Autoimmune hepatitis
  - Lymphocyte stimulation in response to hepatocyte membrane preps (primary or secondary)
  - CD3+ lymphs most common in chronic hepatitis
  - MHC II upregulation in chronic hepatitis

Chronic Hepatitis: Immune Issues

- Breed Specific –various breeds
  - Labrador Retrievers, Dobermans, Cocker Spaniels, Terriers and others
  - Some copper and some are not
- CD 4/CD 8 ratio high during chronic hepatitis (2.96 vs ref 0.33 +/- 0.12)
  - Sakai M et al. J Vet Med Sci 68, 2006
- Dobermans have upregulated MCH II antigens on hepatocytes (Speeti M et al. Vet Immunol Immunopatol 103, 2003

Drug-induced Immune-mediated hepatitis



Immune recognition of neoantigen or native protein

Humoral response:Ab

Cell-mediated Response:CTL

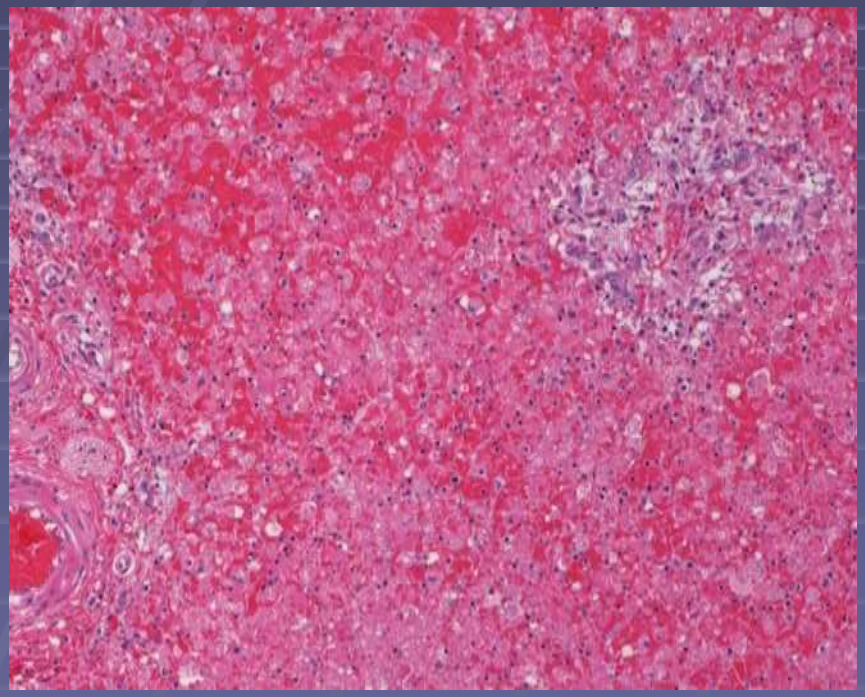
B. Fromenty

Human (and Veterinary) drugs associated with autoimmune hepatitis

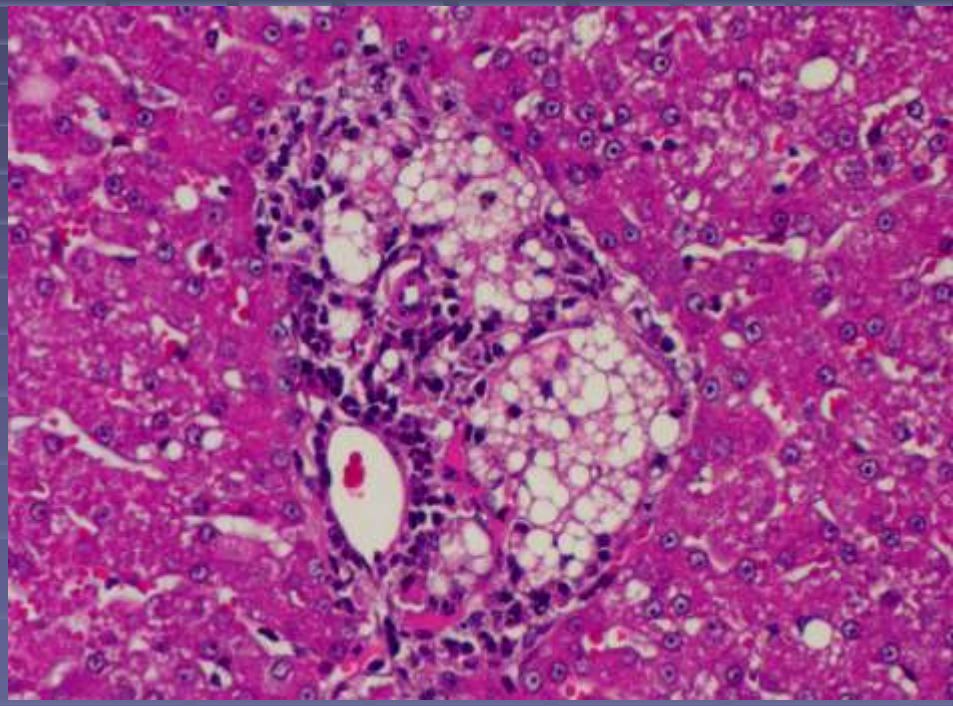
- Diclofenac/carprofen
- Anti-convulsants

- Halothane hepatitis
- Sulfonamide hepatitis

Trimethoprim Sulfa drugs



Massive Hepatic Necrosis

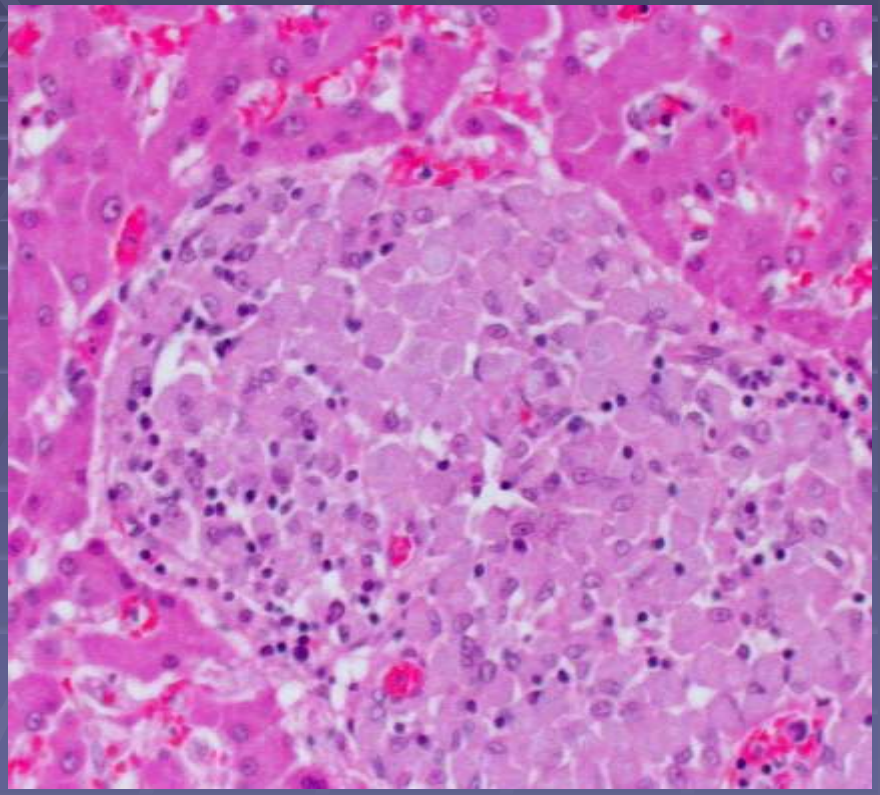


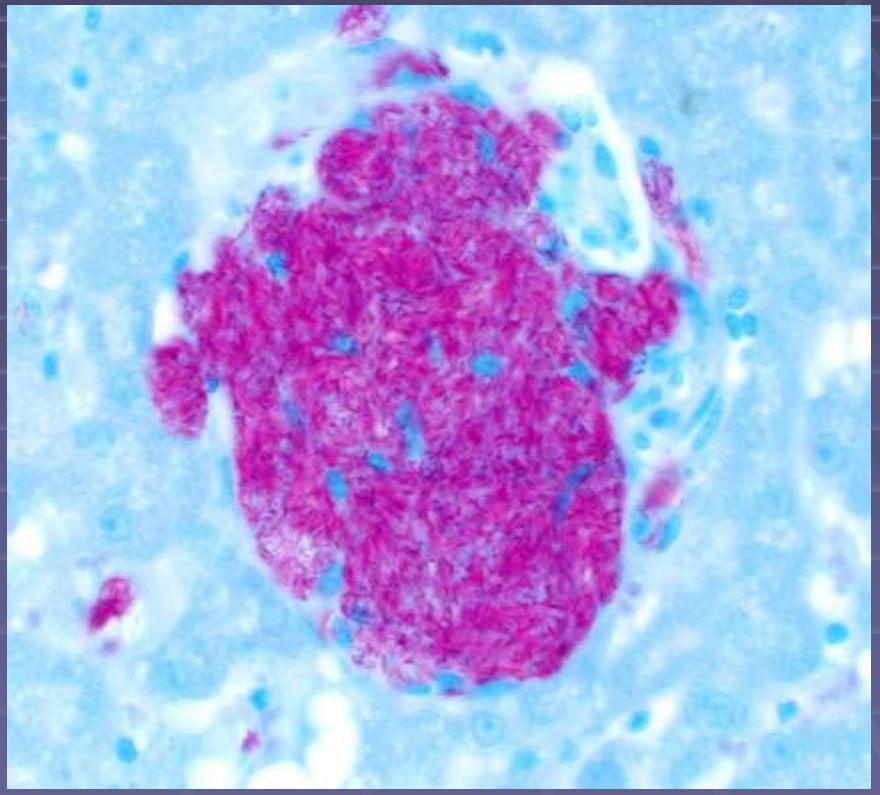
Destructive Cholangitis

Granulomatous/pyogranulomatous hepatitis

- Histochemical
  - Silver stains
  - Acid-fast
    - Fite's
  - PAS
  - Giemsa
  - Tissue Gram
- Bacterial Culture
- PCR
  - *Bartonella canis*
  - *B. henslae*
  - *B. vinsoniae*
  - *Ehrlichia canis*
  - Rock Mountain Spotted Fever
- FISH
  - Bacterial probes

Granulomatous hepatitis

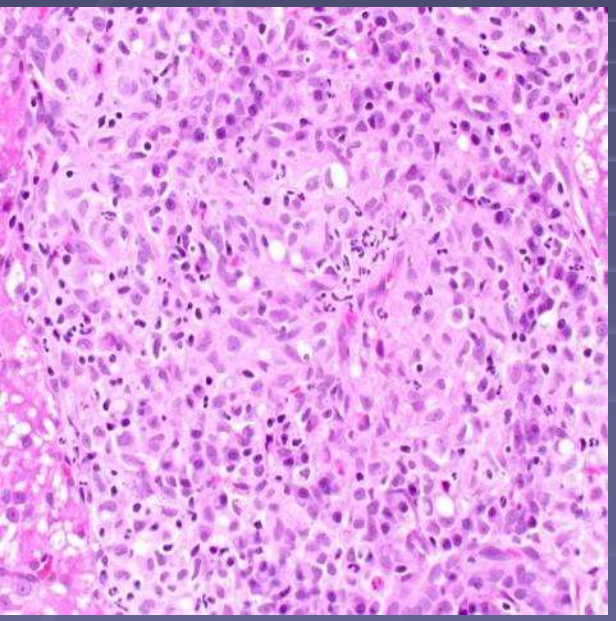


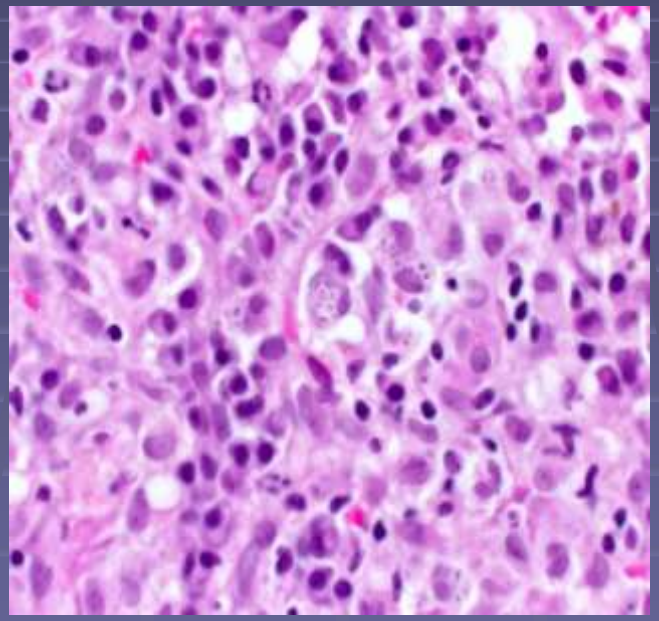


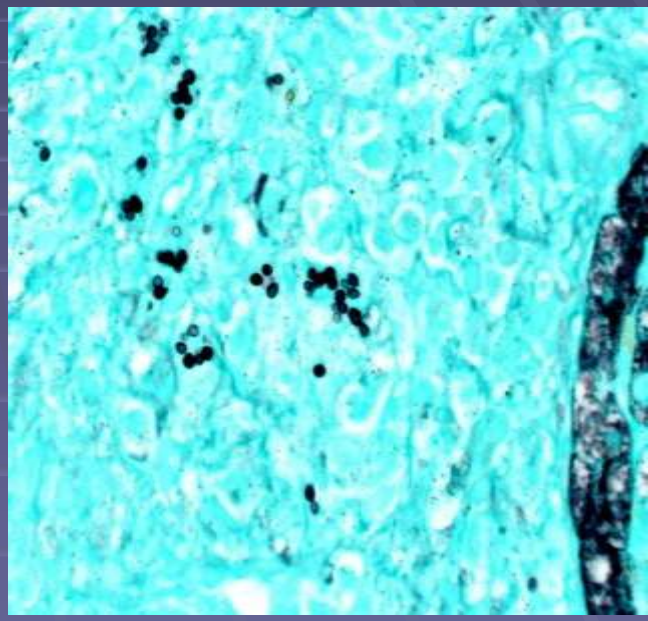
HE Acid-fast

Mycobacterium avium

Granulomatous Hepatitis *Histoplasma capsulatum*

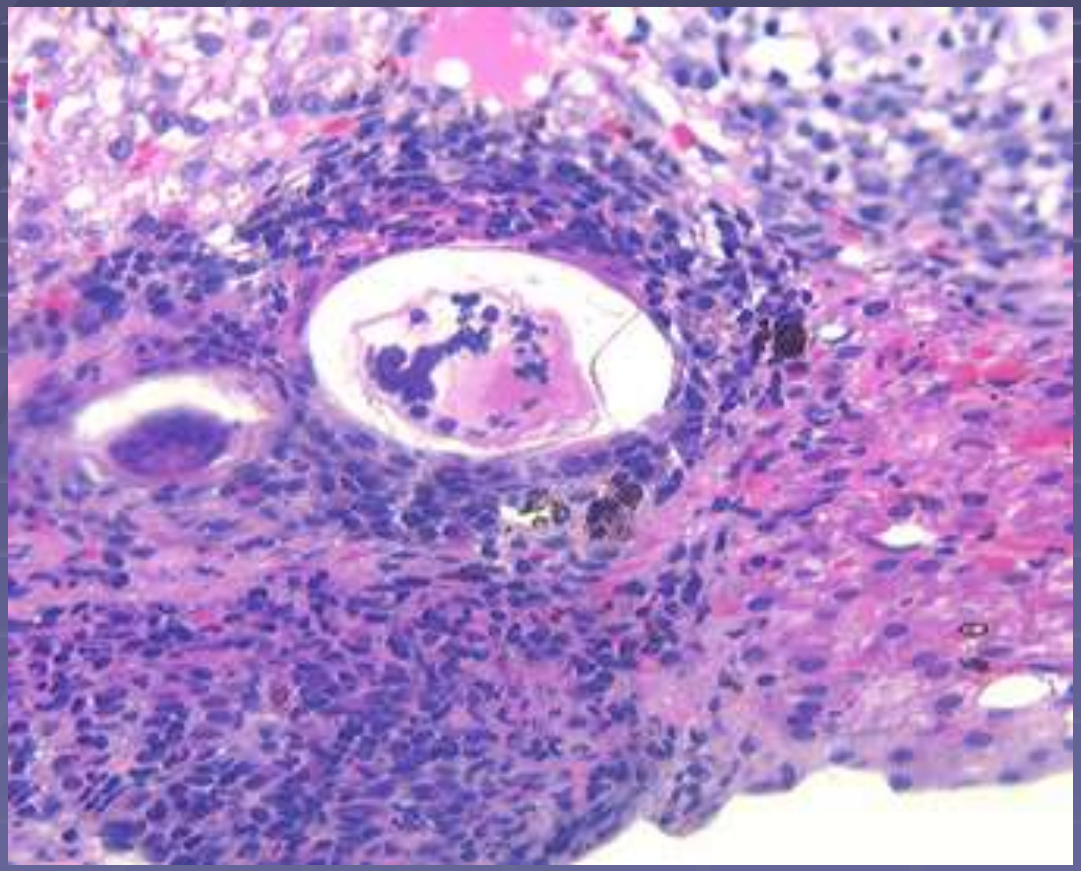






Phlebitis and portal inflammation

Schistosomiasis (*Heterobilharzia americana)*



Pyogranulomas around ova in portal veins and portal tracts

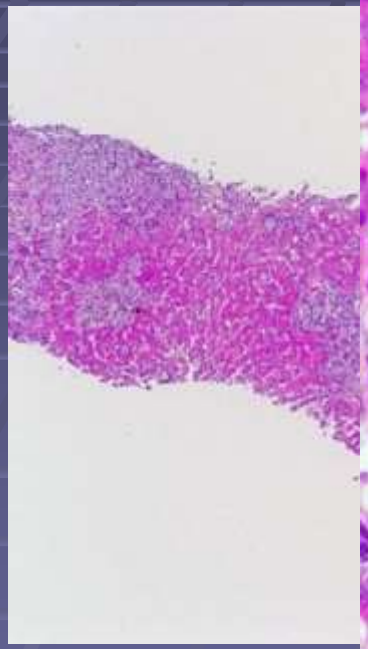
Ova lack spine, hook or knob, may have miracidium

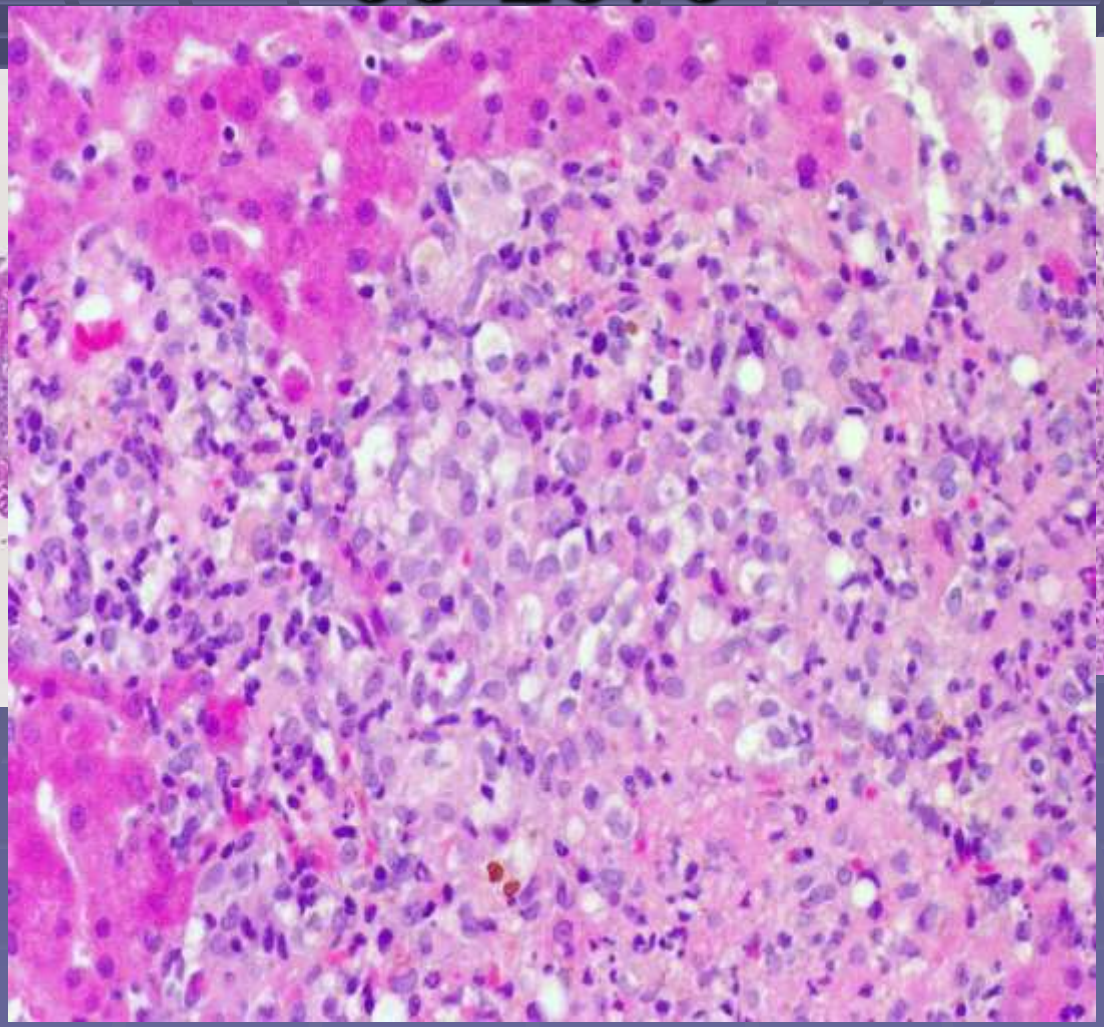
Typical life cycle: Snails to raccoons via cercariae

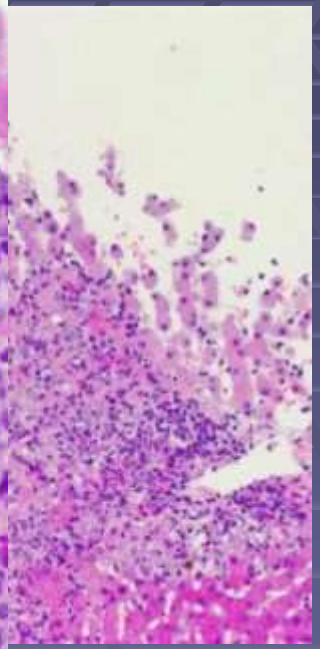
Granulomatous or Histiocytic Disease

- Infectious agents difficult to detect
- Neoplastic (histiocytic, Langerhans cells) variants?

Middle Aged Dog
08-2879





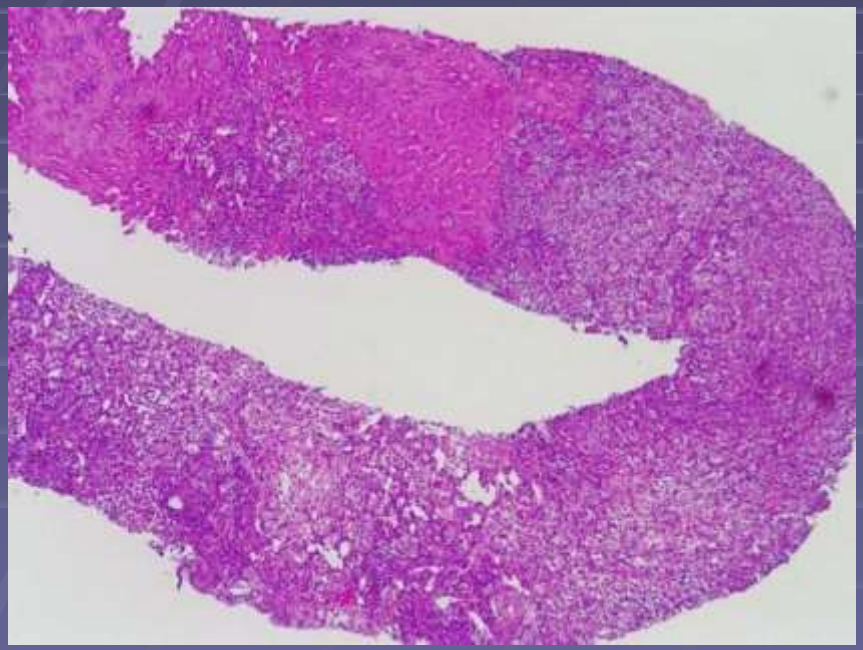


Dramatic recovery following

Doxycyline therapy

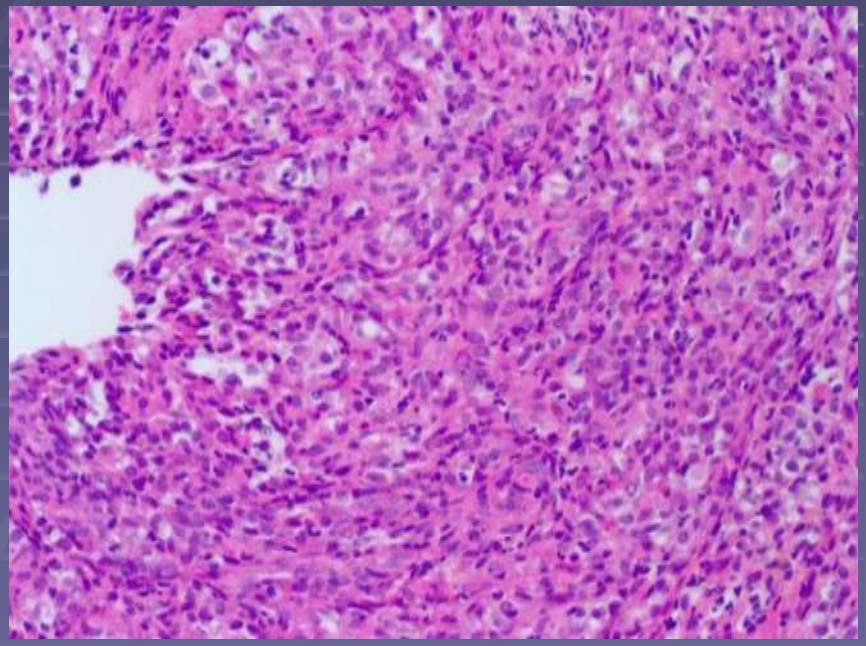
Negative for all serologic and histochemical evaluations

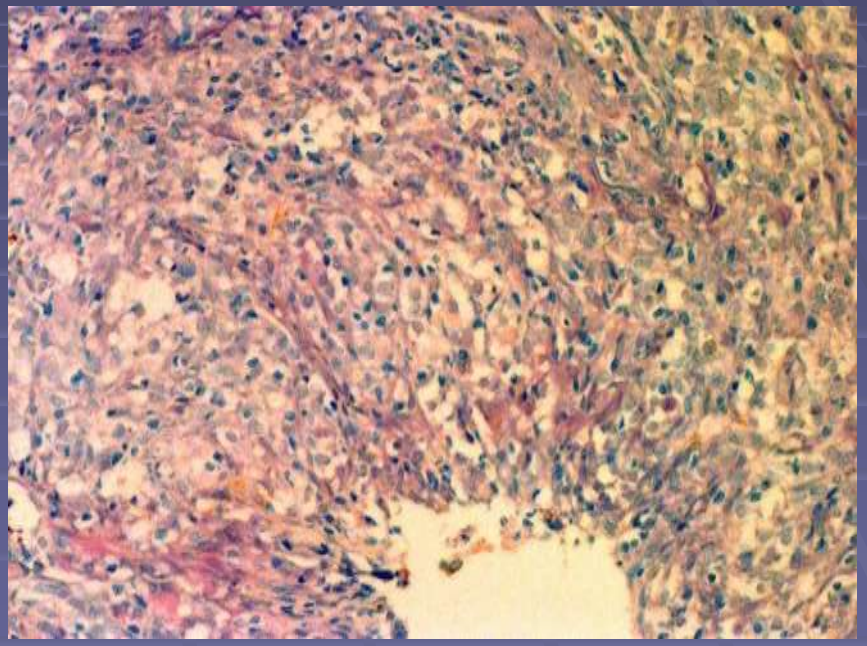
Middle-aged Mixed Breed Dog



- Ehrlichia canis +
- >1:64
- Transaminases, Alk Phos elevated
- Bilirubin mildly elevated
- Clinically in poor condition
- No evidence of other organ involvement

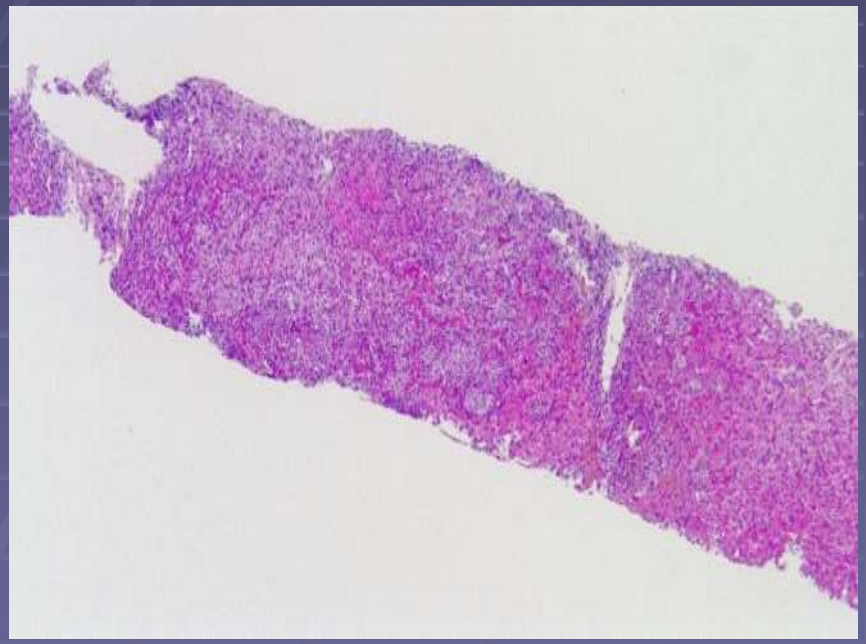
Middle-aged Mixed Breed Dog 09-508

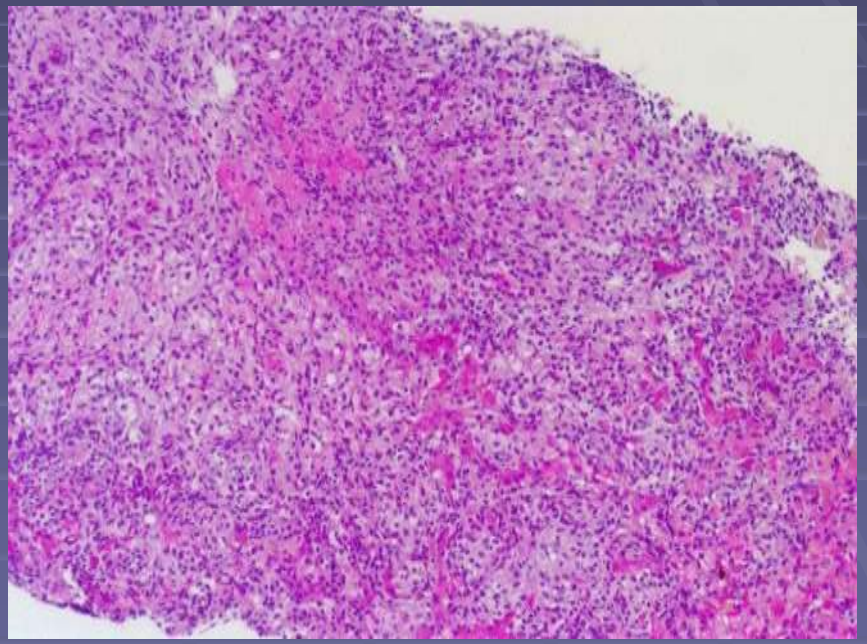




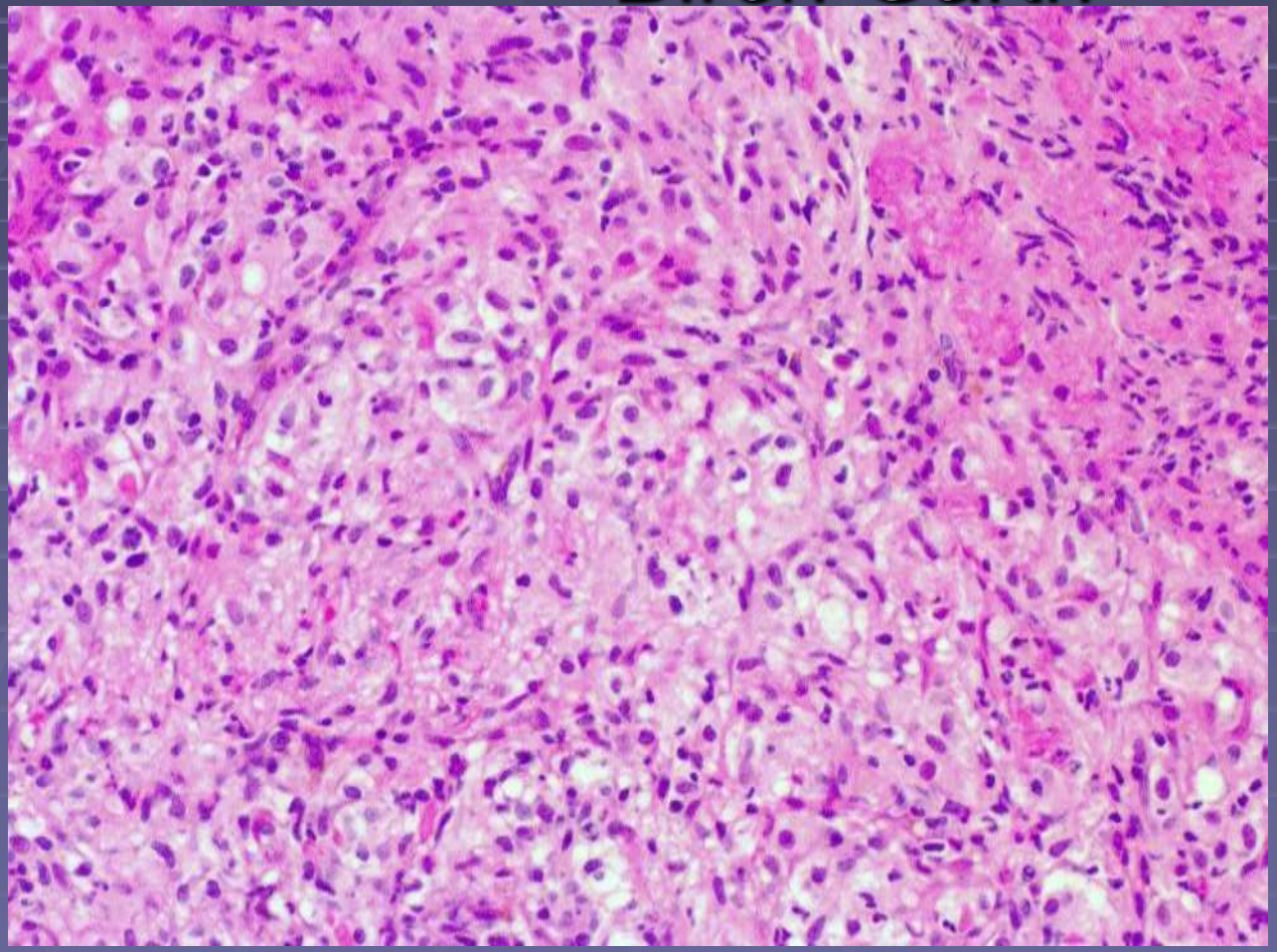
Euthanized 1 week post-biopsy

Middle Aged Dog
08-2102





Middle Aged Dog
08-2102
Birch Garth



Elevated transaminases, ALK Phos, bilirubin

Recurrent fevers

Negative for all serology and histochemical staining

Dramatic recovery on corticosteroids

Nonspecific Reactive Hepatitis

- A hepatic response to systemic or gastrointestinal disease
- Resolution of primary hepatic disease
- Mild enzyme elevations
- Modest inflammatory infiltrate without hepatocellular necrosis
- NOT-cholangiohepatitis, minimal to mild

Eosinophilic hepatitis

- Unusual variant
- Hypersensitivity?
- Parasitic?

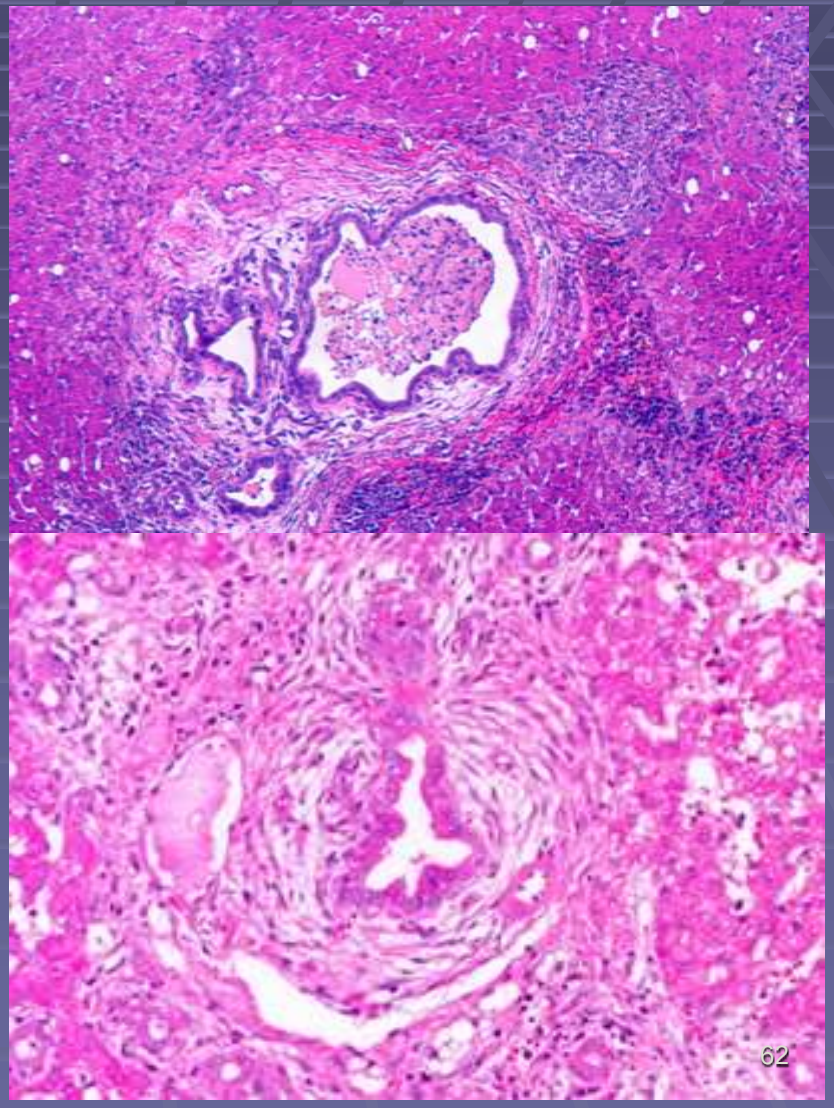
Conclusion

- Chronic Hepatitis remains an enigma in veterinary pathology
- High proportion of idiopathic cases
- Possible etiologies
  - Viruses
  - Other infectious agents
  - Chemical/Drug toxicity
- Alpha1-antitrypsin?
  - Primary or secondary

Biliary Inflammatory Disorders

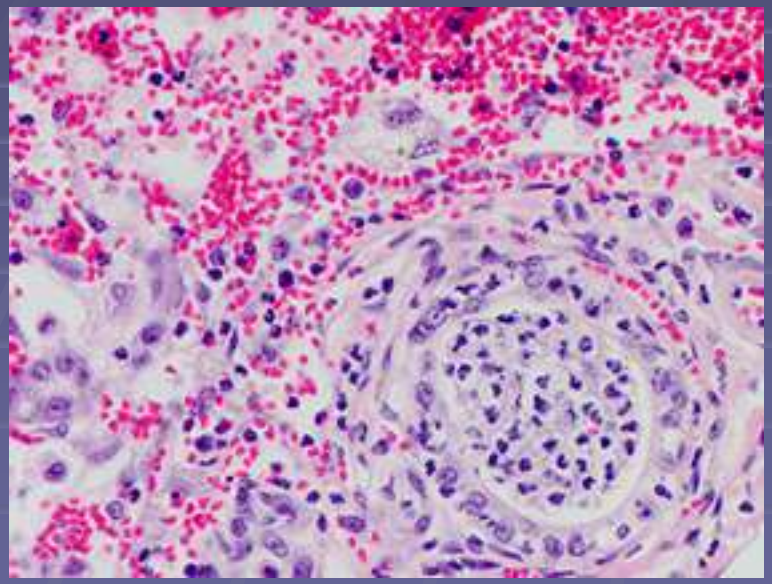
Neutrophilic Cholangitis

- Risk Factors
  - Acute Pancreatitis
  - Chronic Pancreatitis
  - Trauma to sphincter of Oddi
  - Malfunction of sphincter of Oddi
  - Septacemia?

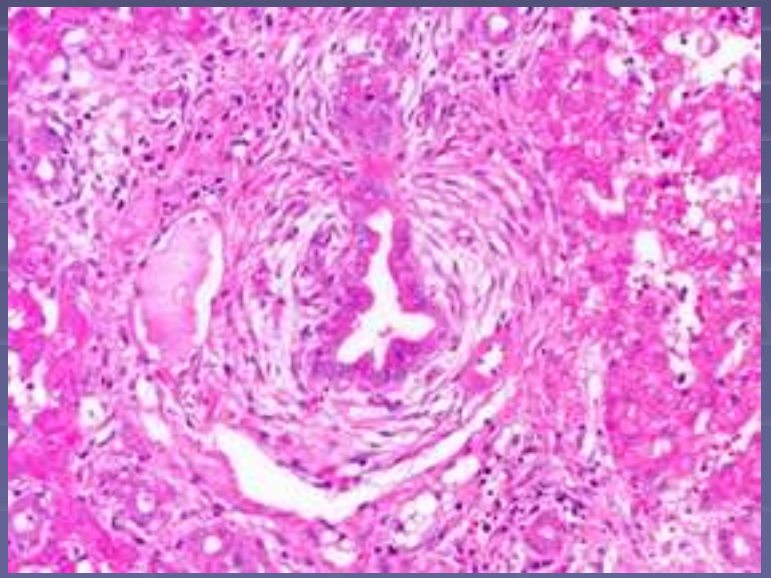


Neutrophilic Cholangitis

Acute

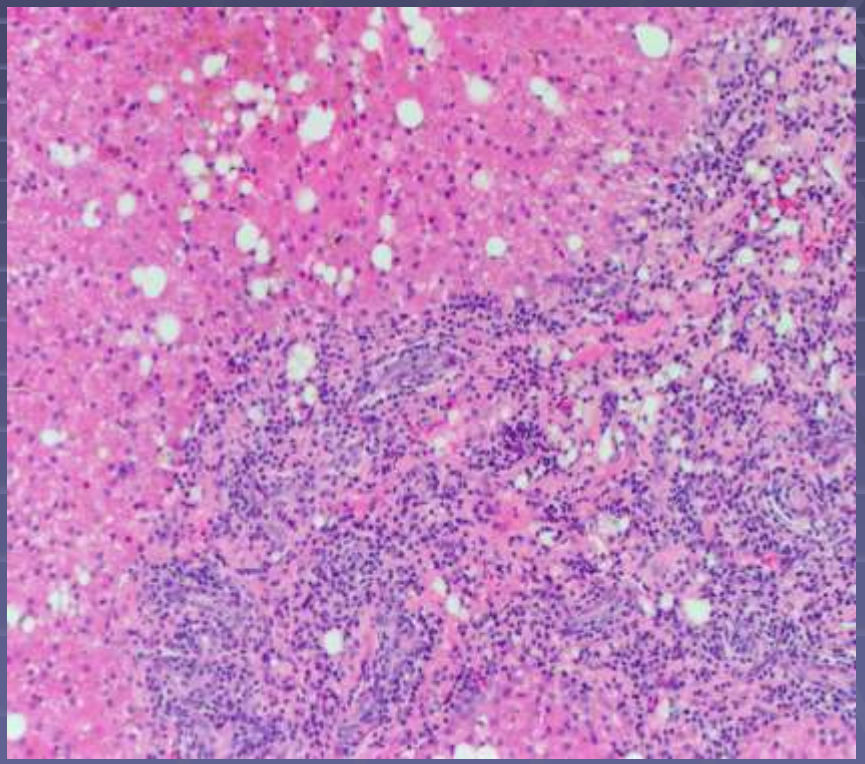


Chronic



Lymphocytic Cholangitis:Cats

- Diagnostic Features
  - Small lymphocytes
  - Variable plasma cells
  - Centered around portal tracts and bile ducts
  - Biliary response
    - Hyperplasia



Lymphocytic Cholangitis

Variants

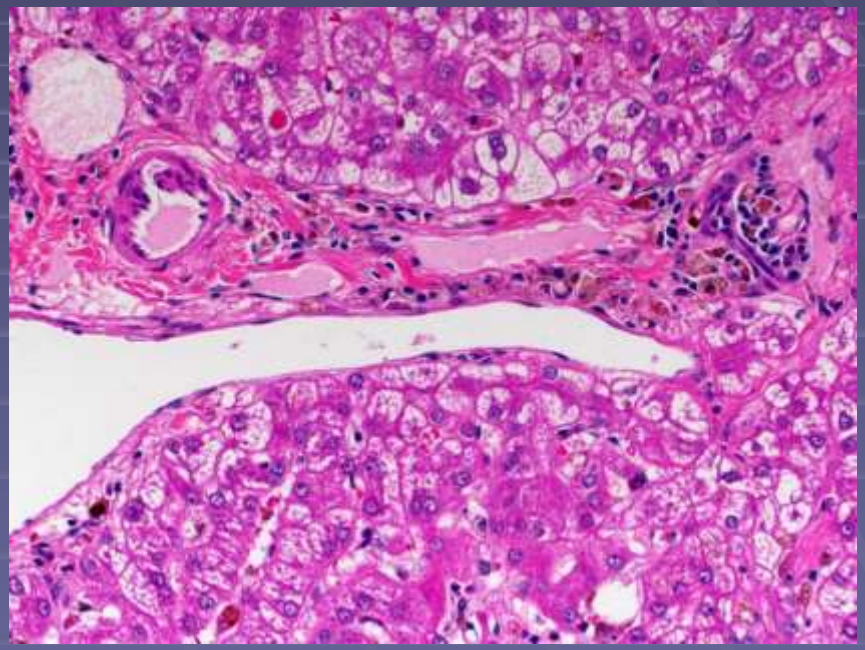
- Destructive forms
- Ductopenia
- Prognosis?

Differentials

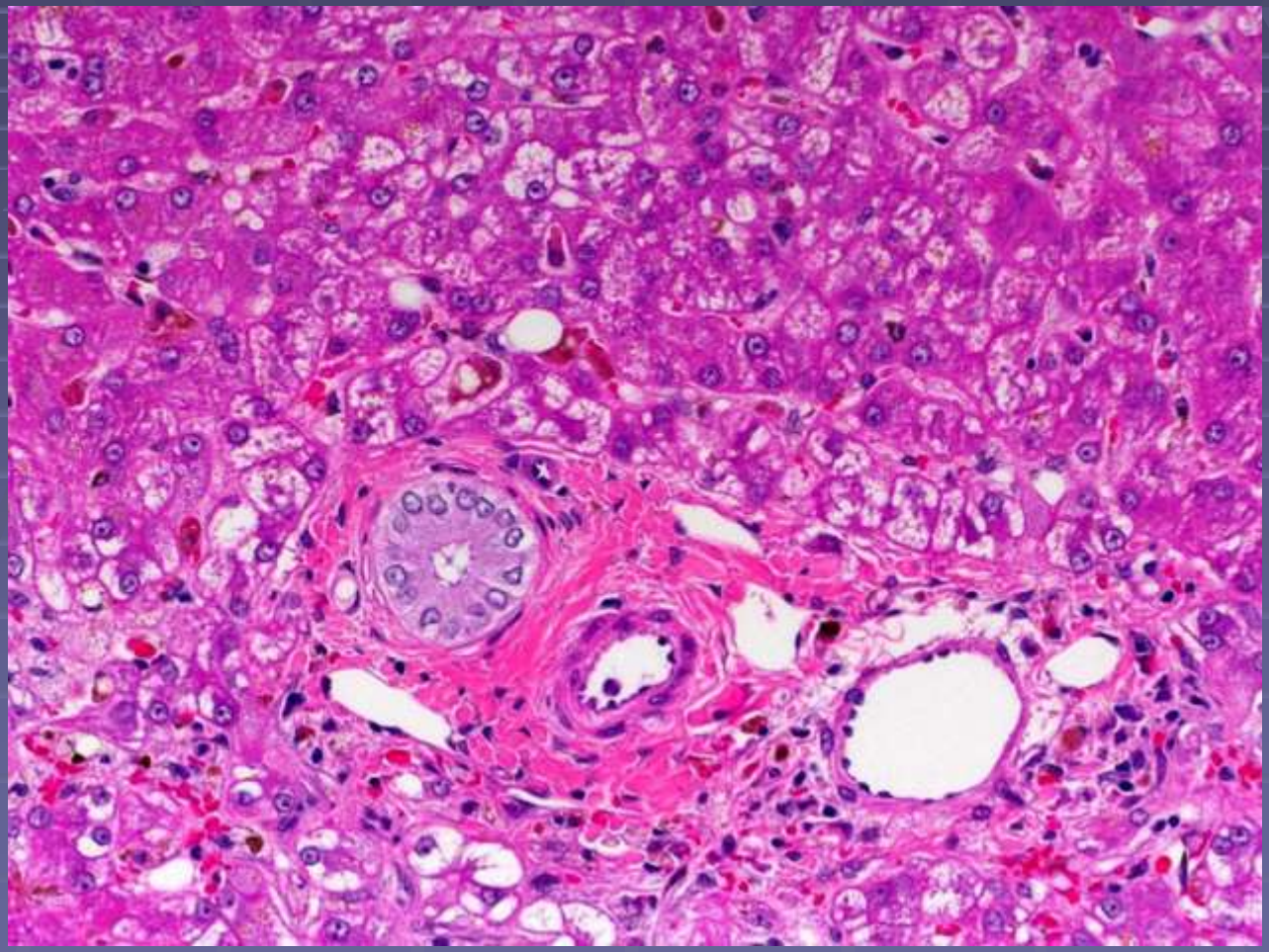
- Lymphoma
  - PAAR
  - Immunophenotype
    - Most lymphs CD3+
    - Not really helpful
- Nonspecific reactive hepatitis

Destructive cholangitis: Drug Hypersensitivity

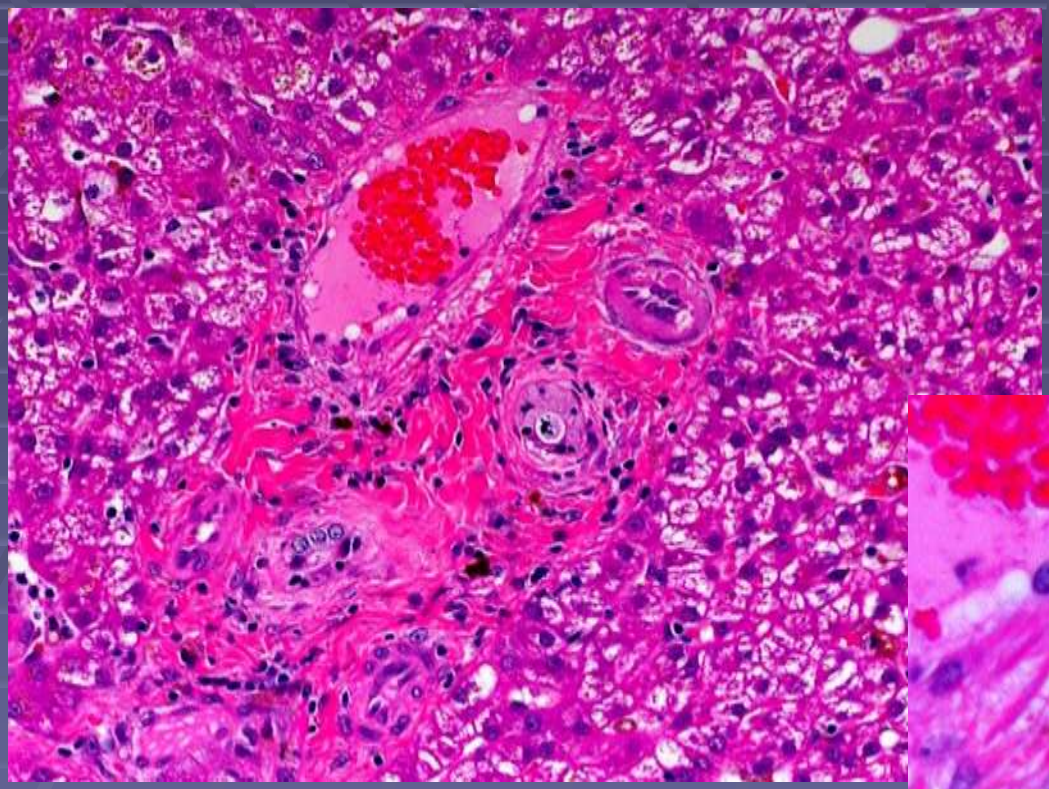
- Hypersensitivity likely
  - Trimethoprim sulfa
- Different sized ducts affected



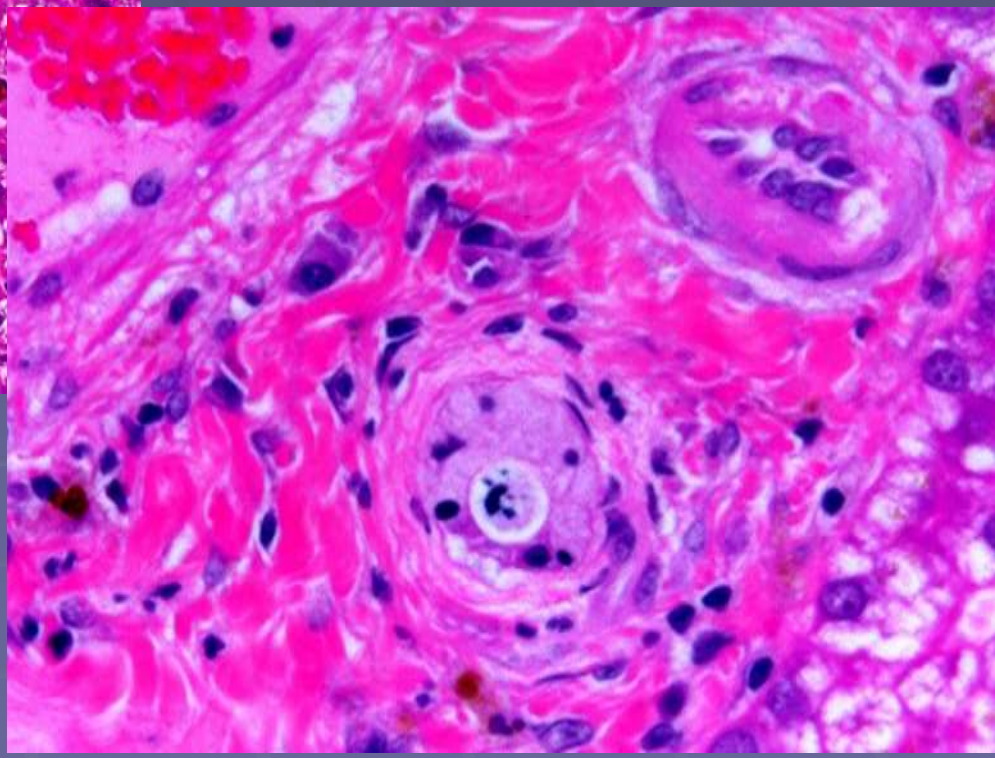
Large ducts spared



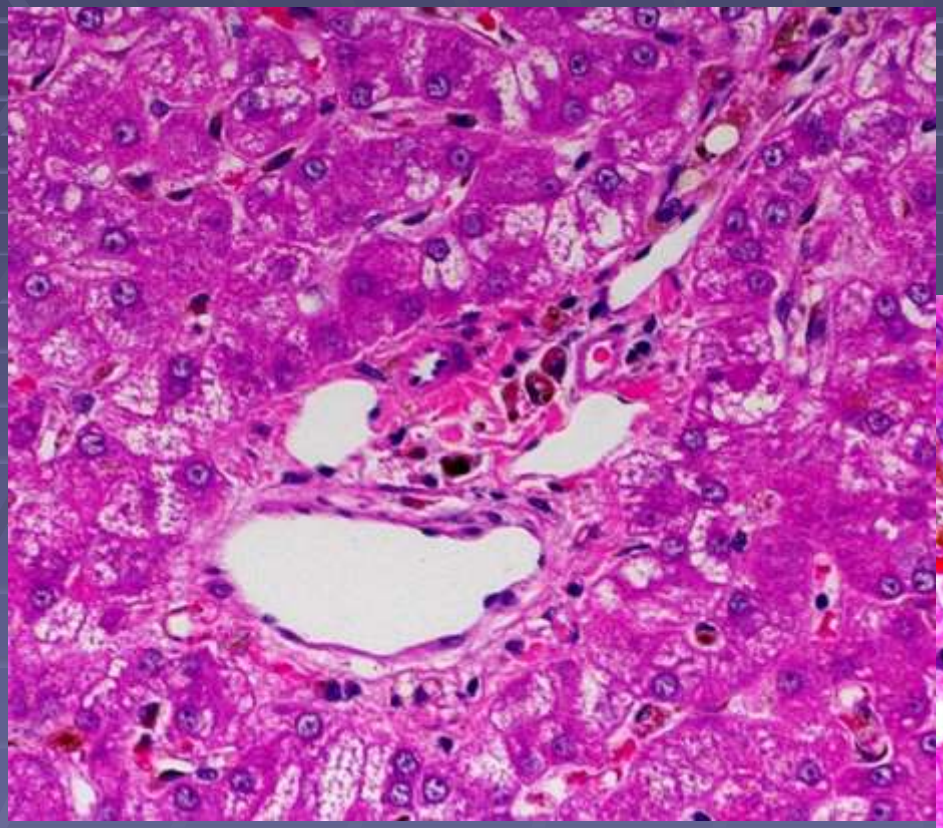
Destructive cholangitis

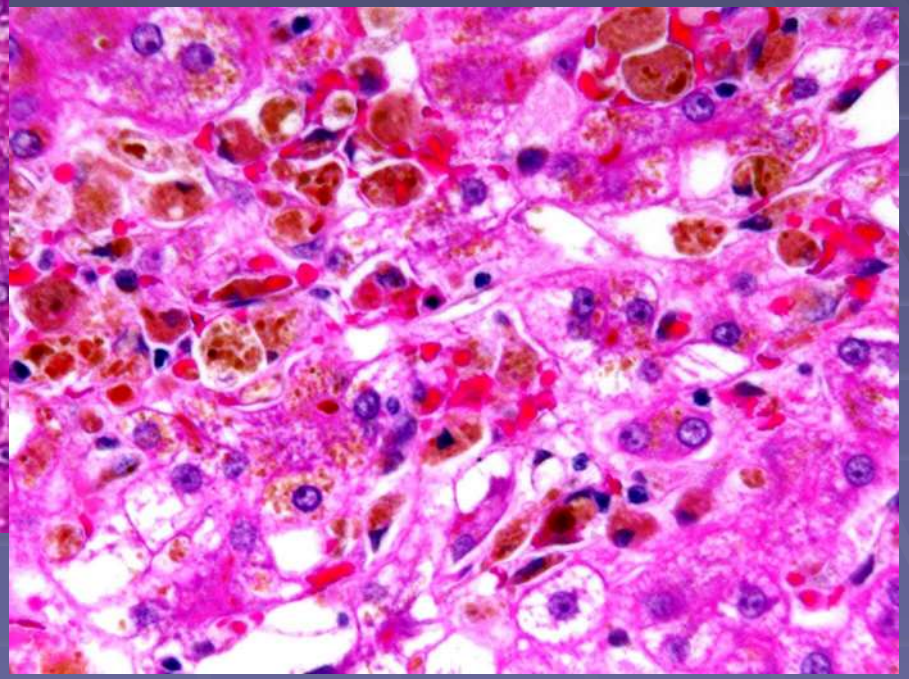


Biopsy

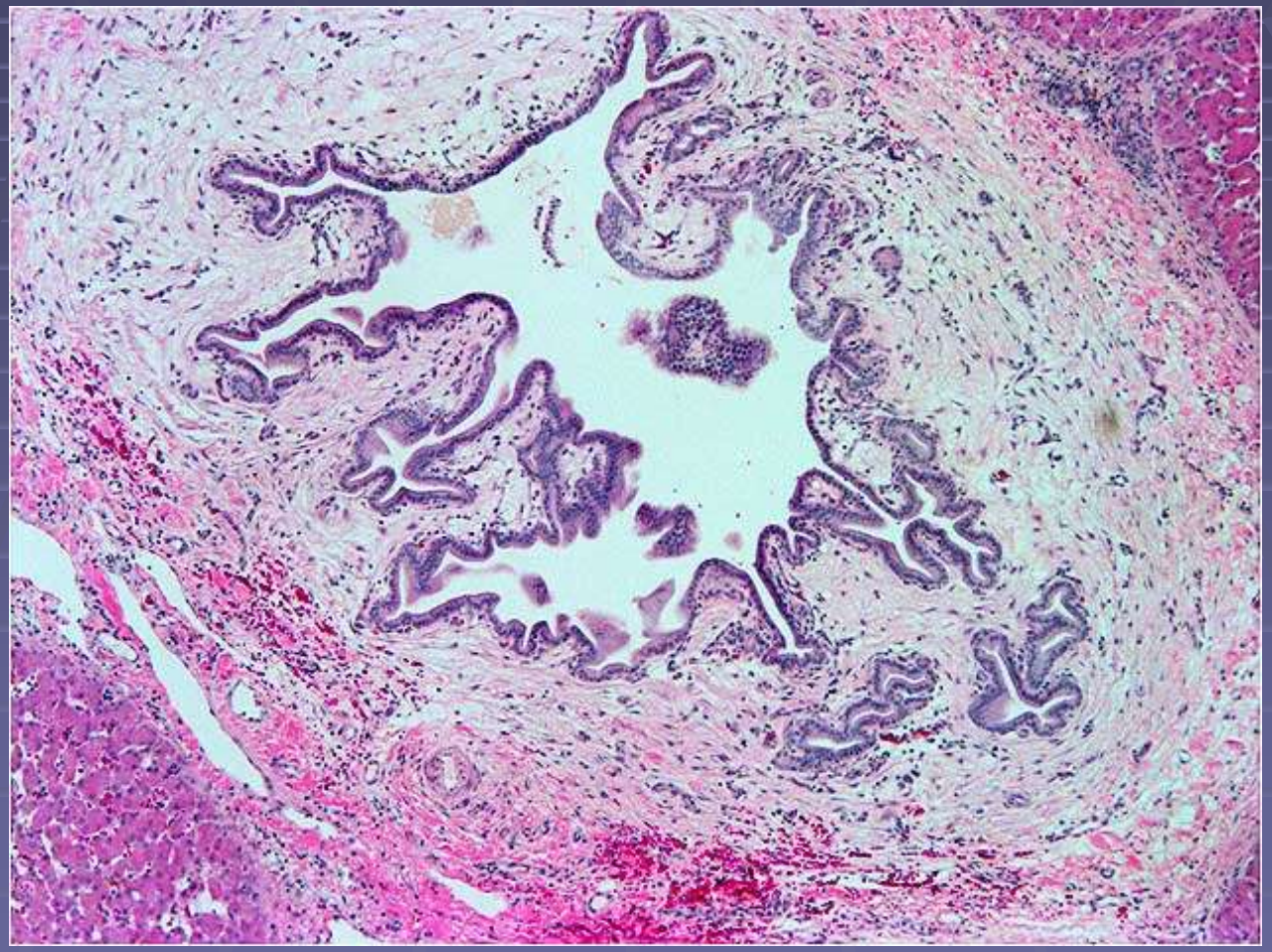


Postmortem





Biliary Flukes



Conclusions

- Many forms of acute hepatitis have a cause that can be diagnosed
- Chronic hepatitis has one main etiology- copper, but this accounts for less than half of the cases.
- Most chronic hepatitis remains idiopathic
- Lymphocytic Cholangitis in cats is idiopathic