Pathology of Marmoset Diseases

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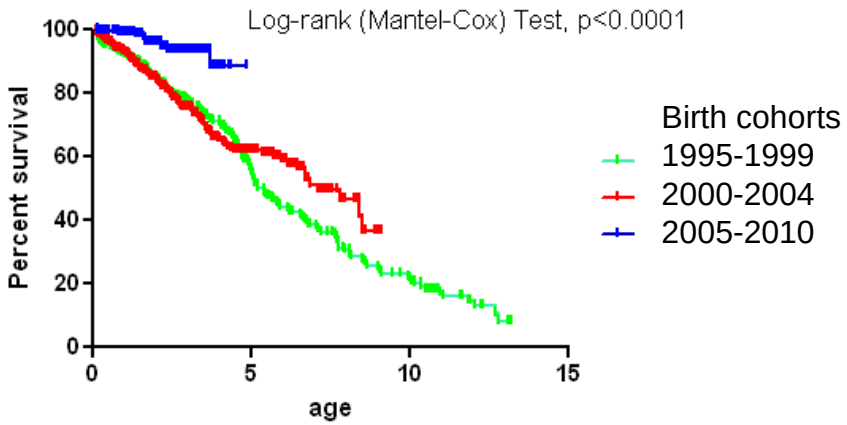
Pathology of Common Marmosets

- Degenerate
- Autoimmune
- Metabolic
- Nutritional
- Neoplastic
- Inflammatory and infectious
- Iatrogenic
- Traumatic

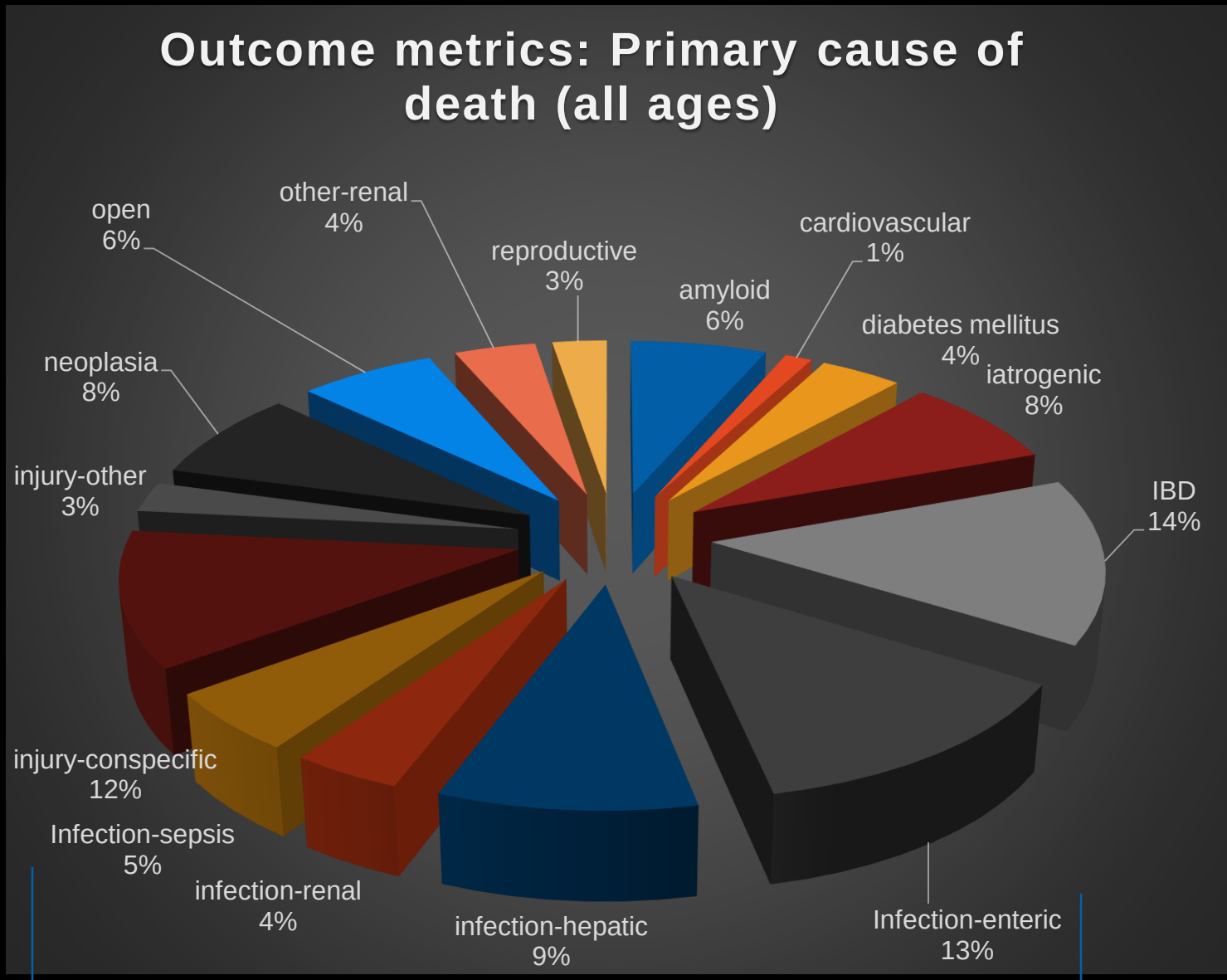
Pathology of marmoset diseases

- Specific causes of morbidity and mortality may differ significantly from colony to colony due to differences in:
  - Husbandry practices
  - Diet
  - Environment/housing
  - Genetic characteristics
  - Infectious diseases



- **Important to define the spectrum of disease entities in individual colonies and understand their impact on animal health and research programs.**

Outcome metrics: Primary cause of death (all ages)



Infectious-31%

Causes of mortality in a common marmoset colony >5.78 years

	<5.78 years	>5.78 years	> 10 years
Amyloid	**0.0%**	**12.1%**	**28.6%**
Cardiovascular	0.0%	3.0%	0.0%
Diabetes mellitus	**2.3%**	**6.1%**	**7.1%**
Iatrogenic	9.1%	6.1%	14.3%
IBD	15.9%	12.1%	0.0%
Infection-enteric	18.2%	6.1%	7.1%
Infection-hepatic	9.1%	9.1%	0.0%
Infection-renal	4.5%	3.0%	0.0%
Infection-sepsis	6.8%	3.0%	0.0%
Injury-conspecific	20.5%	0.0%	0.0%
Injury-other	4.5%	0.0%	0.0%
Neoplasia	**2.3%**	**15.2%**	**21.4%**
Open	4.5%	9.1%	7.1%
Other-renal	**0.0%**	**6.1%**	**14.3%**
Reproductive	2.3%	3.0%	0.0%

Causes of mortality in a common marmoset colony <5.78 years

	<5.78 years	>5.78 years	> 10 years
Amyloid	0.0%	12.1%	28.6%
Cardiovascular	0.0%	3.0%	0.0%
Diabetes mellitus	2.3%	6.1%	7.1%
Iatrogenic	9.1%	6.1%	14.3%
IBD	**15.9%**	**12.1%**	**0.0%**
Infection-enteric	**18.2%**	**6.1%**	**7.1%**
Infection-hepatic	9.1%	9.1%	0.0%
Infection-renal	4.5%	3.0%	0.0%
Infection-sepsis	**6.8%**	**3.0%**	**0.0%**
Injury-conspecific	**20.5%**	**0.0%**	**0.0%**
Injury-other	**4.5%**	**0.0%**	**0.0%**
Neoplasia	2.3%	15.2%	21.4%
Open	4.5%	9.1%	7.1%
Other-renal	0.0%	6.1%	14.3%
Reproductive	2.3%	3.0%	0.0%

Survey of gastrointestinal pathology in common marmoset colonies

Condition	Colony 1	Colony 2	Colony 3
Estimated monthly incidence of diarrhea requiring treatment	1.0%	2.2%	3.8%
Inflammatory			
gastritis	5%	1.9%	1%
Chronic lymphocytic enteritis	5%	20.4%	48%
enteritis other	2%	1.9%	1%
neutrophilic colitis	0%	0%	1%
lymphoplasmacytic colitis	10%	31.1%	16%
necrotizing colitis	0%	6.8%	0%
pancreatitis	1%	2.9%	0%
Degenerative			
Amyloidosis	6%	13.6%	1%
Infectious			
Enteropathogenic E. coli	10%	1%	0%
Campylobacter	0%	0%	0%
Giardia	0%	8.3%	1%
nematodiasis	0%	1.5%	0%
Atypical mycobacterial infection	2%	0%	0%
Neoplastic			
Adenocarcinoma of SI	12%	0%	1%
Adenocarcinoma of LI	0%	0%	0%
Malignant lymphoma	2%	4.9%	12%
Other			
fecal impaction	0%	3.9%	0%

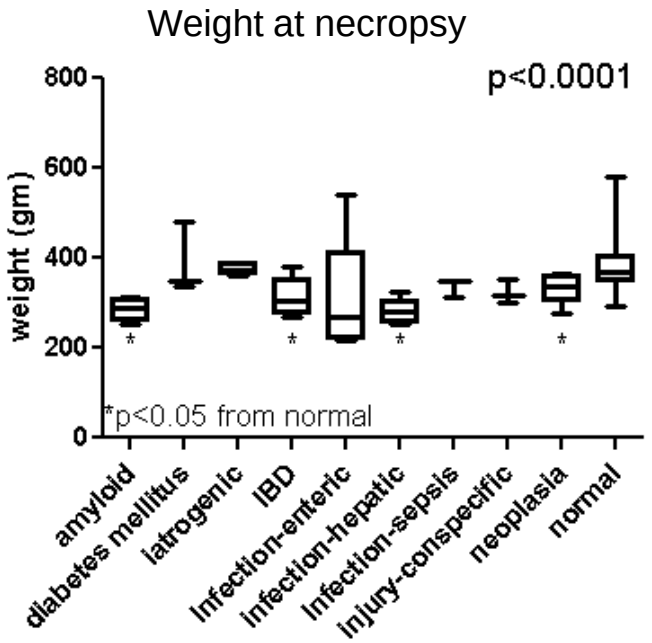
Genetics?
Infectious agents?
Microbiome?
Diet?
Housing?
Stress?

Chronic lymphocytic enteritis
A distinct form of Inflammatory Bowel Disease

- **Clinical disease:** waxing and waning illness; intermittent to chronic diarrhea accompanied by wasting; results in significant maldigestion/malabsorption syndrome.
- **Clinical pathology:** hypoalbuminemia; hyperglobulinemia; microcytic hypochromic anemia; abnormal d-xylose absorption test
- **Pathology:** segmental lymphocytic enteritis accompanied by crypt epithelial cell hyperplasia and villous atrophy; colon largely unaffected.
- **Etiology:** unknown; confirmed in multiple colonies; affected animals may have anti-gliadin IgA antibodies but minimal response to gluten withdrawal.

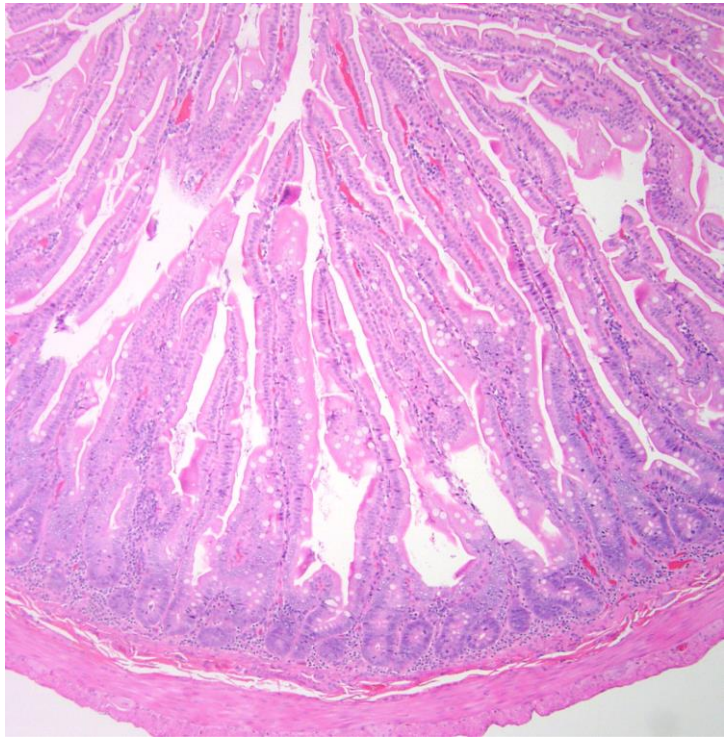
- **Other forms of inflammatory bowel disease associated with weight loss/wasting are recognized in common marmosets.**
- **These may differ in etiology, pathogenesis, clinical manifestation and response to treatment and should be treated as separate entities.**

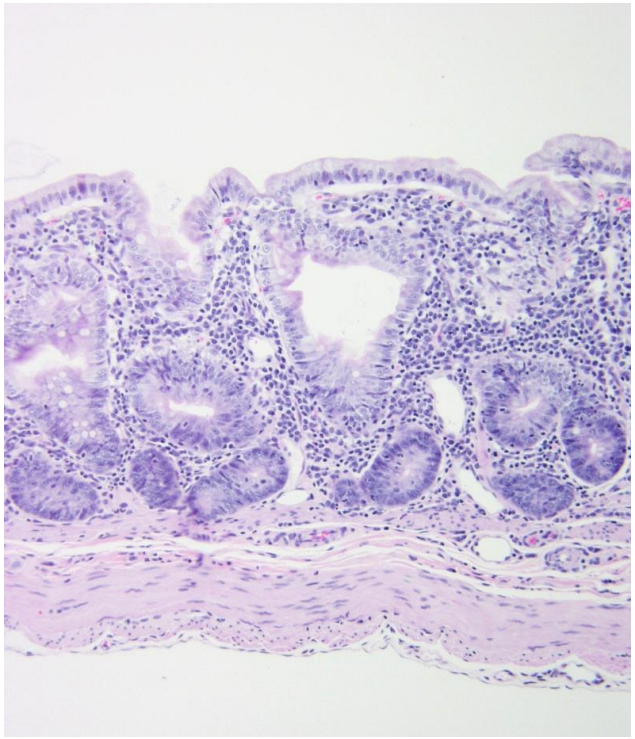
Weight loss is common in a variety of disease processes and *Marmoset Wasting Syndrome* does not define a single entity in the literature



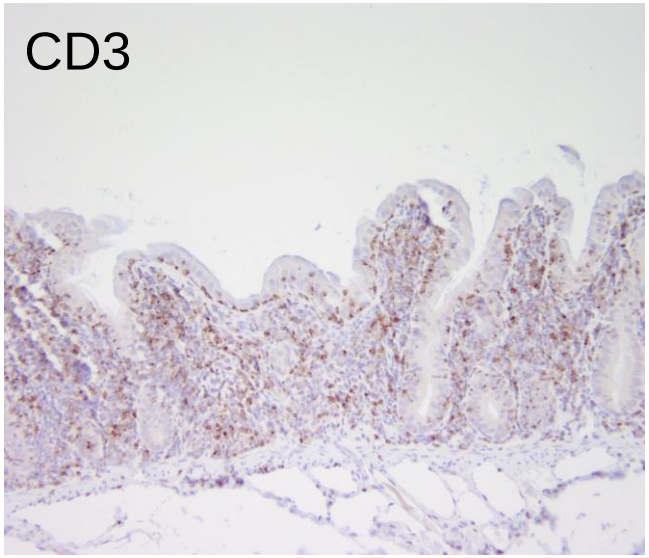
- ***Use of the terms marmoset wasting syndrome or marmoset wasting disease should be discouraged as they lack diagnostic specificity.***
- ***An etiologic or morphologic diagnosis based on a pathological assessment of tissues should be used.***

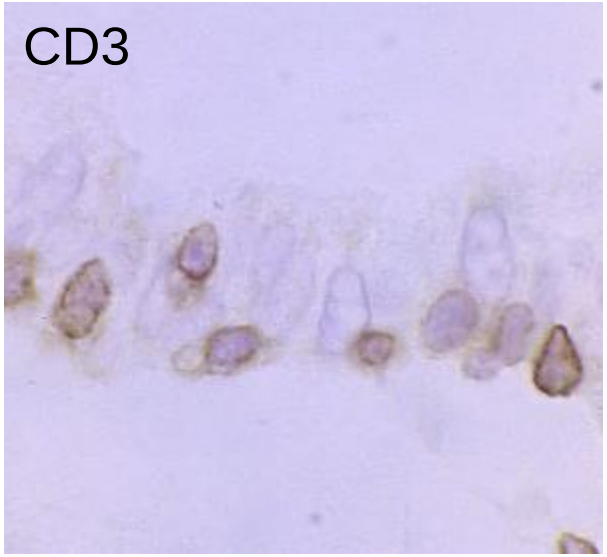
Chronic lymphocytic enteritis
Morphologic alterations

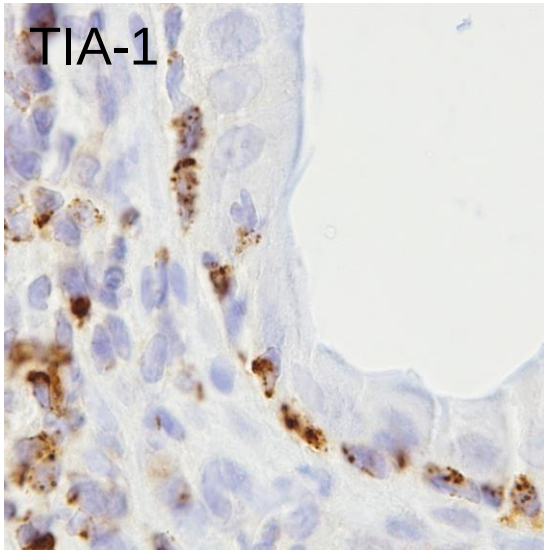




Chronic lymphocytic enteritis
Morphologic alterations

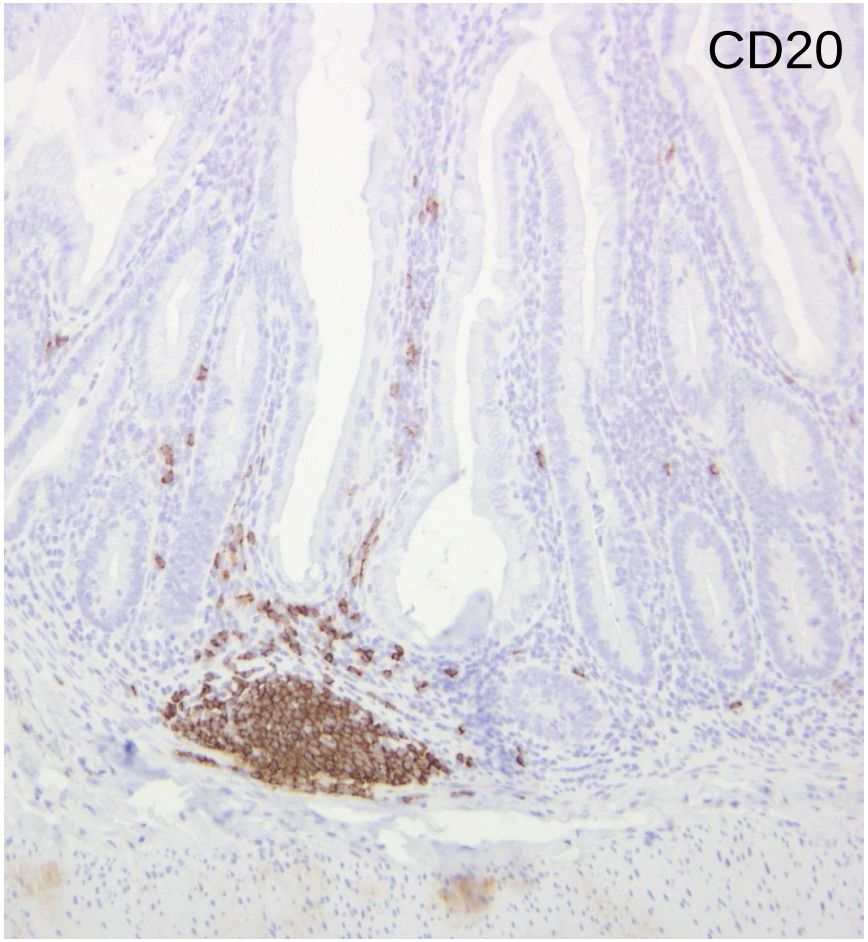


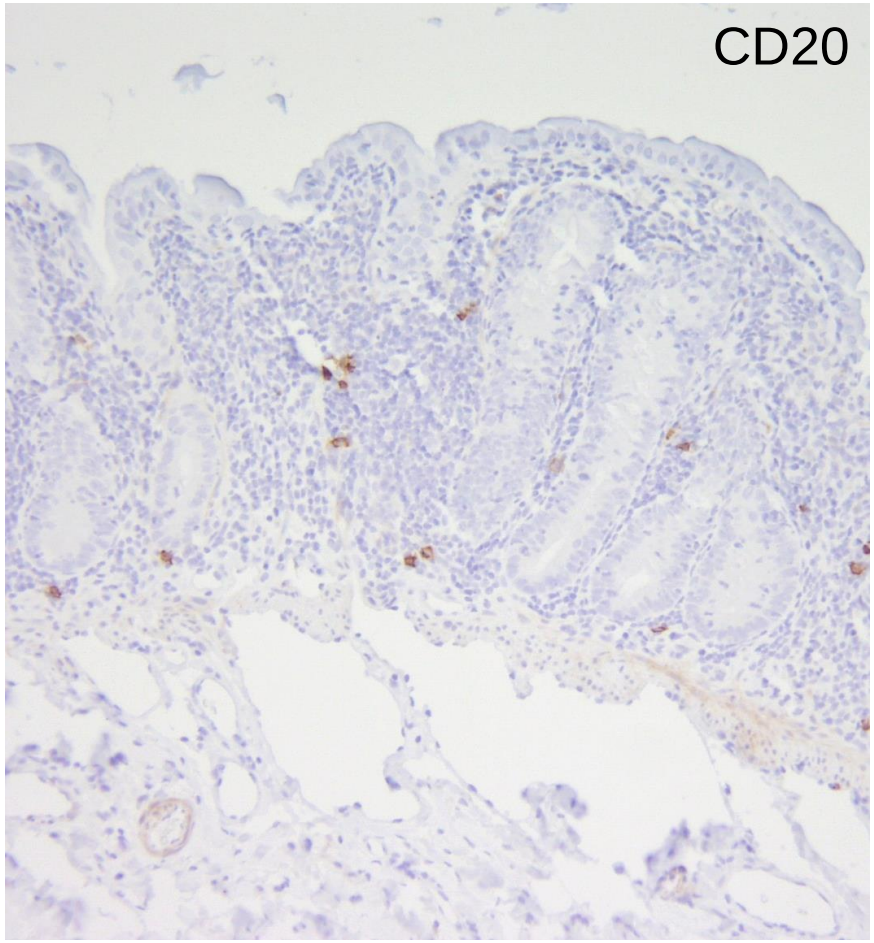




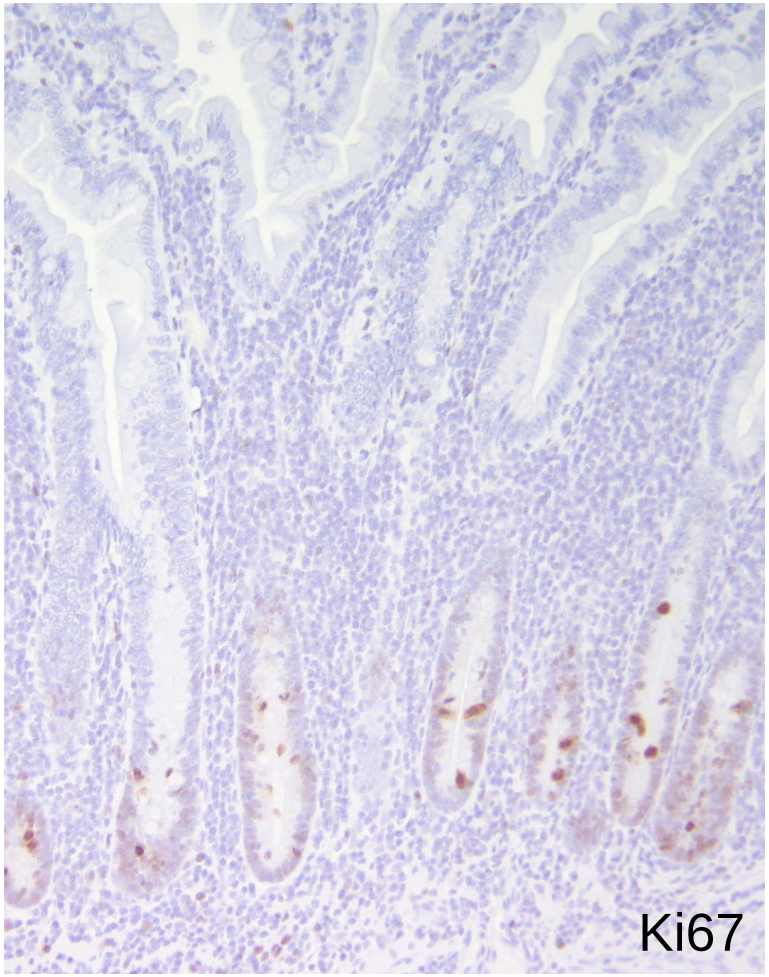
- Inflammatory cell infiltrate consists primarily of cytotoxic CD3 T lymphocytes which infiltrate the lamina propria and epithelium

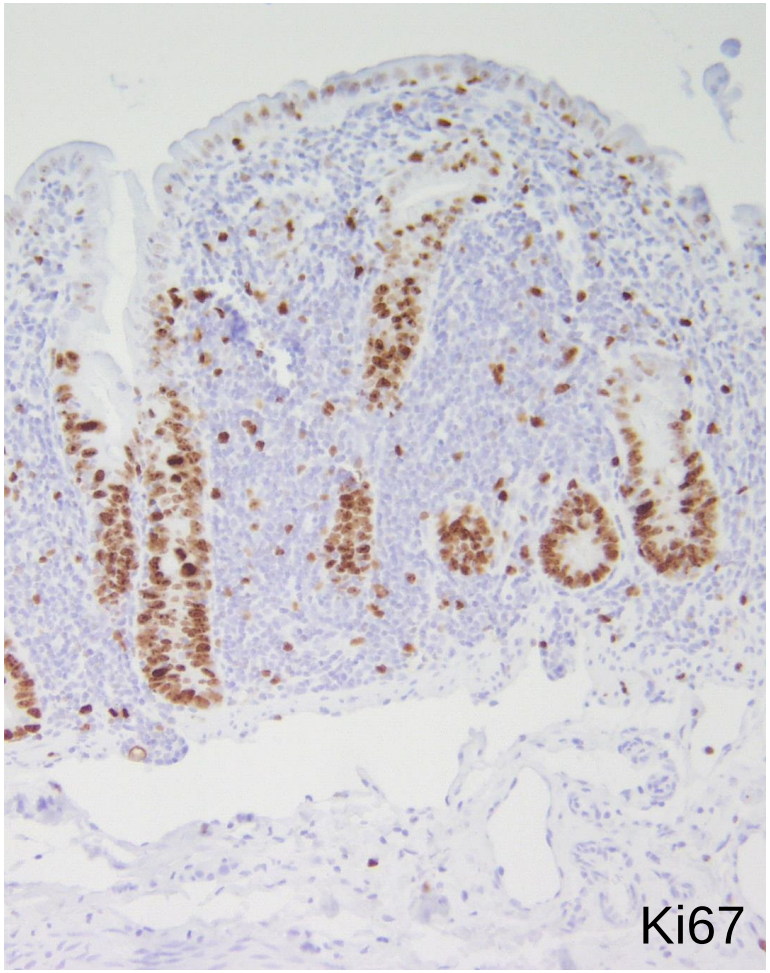
Chronic lymphocytic enteritis
Morphologic alterations





Chronic lymphocytic enteritis
Morphologic alterations





Chronic lymphocytic enteritis
Diagnostic criteria

Infiltrate lamina propria

0- Normal; small number of lymphocytes and plasma cells within deep lamina propria; B cell aggregates;

1- Mild; small numbers of lymphocytes within the lamina propria extend into the villous tips

2- Moderate; moderate numbers of lymphocytes within the lamina propria

3- Severe; large numbers of lymphocytes expand lamina propria and infiltrate the distal villous tips; B cell areas not evident.

IELS

0- Normal; rare IELs evident

1- Mild; >3-4 IELS per hpf

2- Moderate; 5-10 IELS per hpf

3- Severe; large numbers of IELS evident; >10 IELS per villous; multifocally disrupt the basement membrane; aggregates of IELS

Villous atrophy

0- Normal; villous to crypt ratio >6:1; branching of villous tips evident

1- Mild; villous to crypt ratio <6:1; multifocal fusion of villous tips

2- Moderate; villous to crypt ratio <3:1;

3- Severe; multifocally complete loss of villous architecture; villous to crypt ratio <1:1

Crypt hyperplasia

0- Normal; goblet cells evident

1- Mild basophilia

2- Moderate; increase crypt length; few goblet cells; moderate basophilia

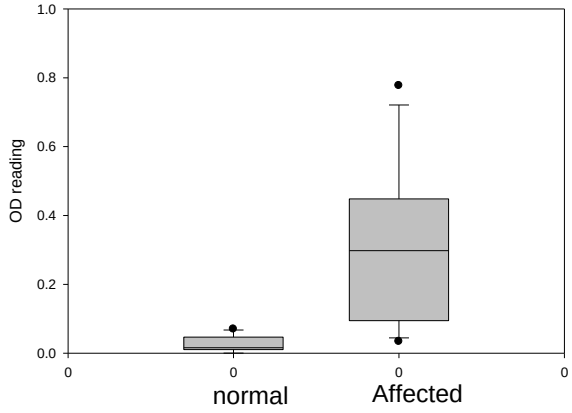
3- Severe; complete loss of goblet cells; cytoplasmic basophilia;

score > 6 meets diagnostic criteria

Chronic lymphocytic enteritis
Etiology

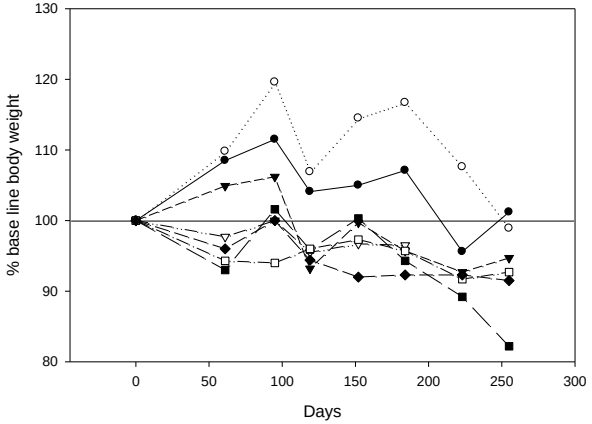
- Morphologic similarities to celiac disease
- Despite the presence of IgA-AGA in a subset of affected animals, minimal response to gluten withdrawal.

IgA-AGA



IgA anti-gliadin antibodies in sera of affected and non-affected marmosets
Assay lacks specificity for gluten enteropathy

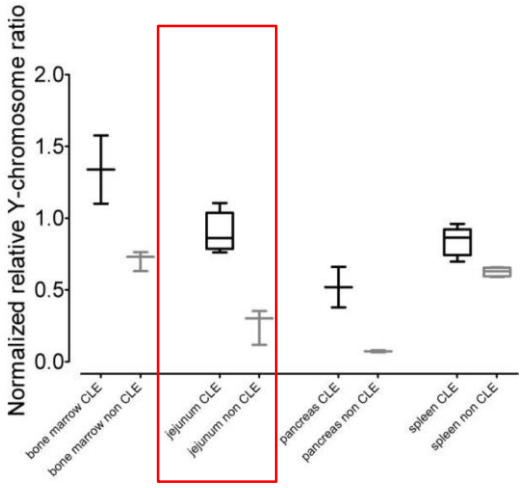
Gluten dietary restriction



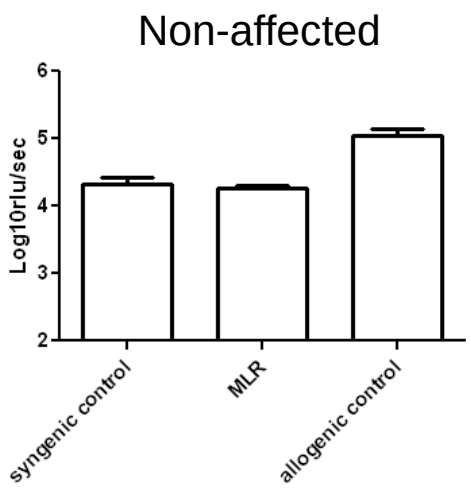
No sustained response in weight or biopsy scores from marmosets following initiation of a gluten free diet

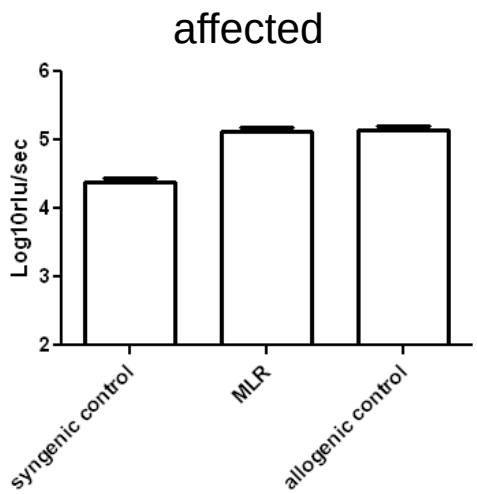
Chronic lymphocytic enteritis
Etiology

- Enrichment of chimeric twin cells in inflammatory lesion.
- Positive mixed lymphocyte reaction detected in a subset of animals suggesting a breakdown in tolerance and GvHD-like condition.

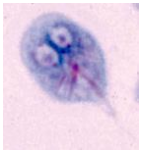


Marmosets with CLE showed significantly greater levels of donor twin chimerism in jejunum ($p < 0.001$).



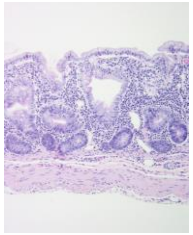


Potential pathogenesis of chronic lymphocytic enteritis



Environmental trigger
Giardia and/or other enteric pathogens

Break in local tolerance and induction of GvHD



GI inflammation, disruption of mucosal barrier and alterations in villous architecture

Exposure to enteric antigens (e.g. gluten)

Maldigestion/malabsorption

Alterations in microbiome

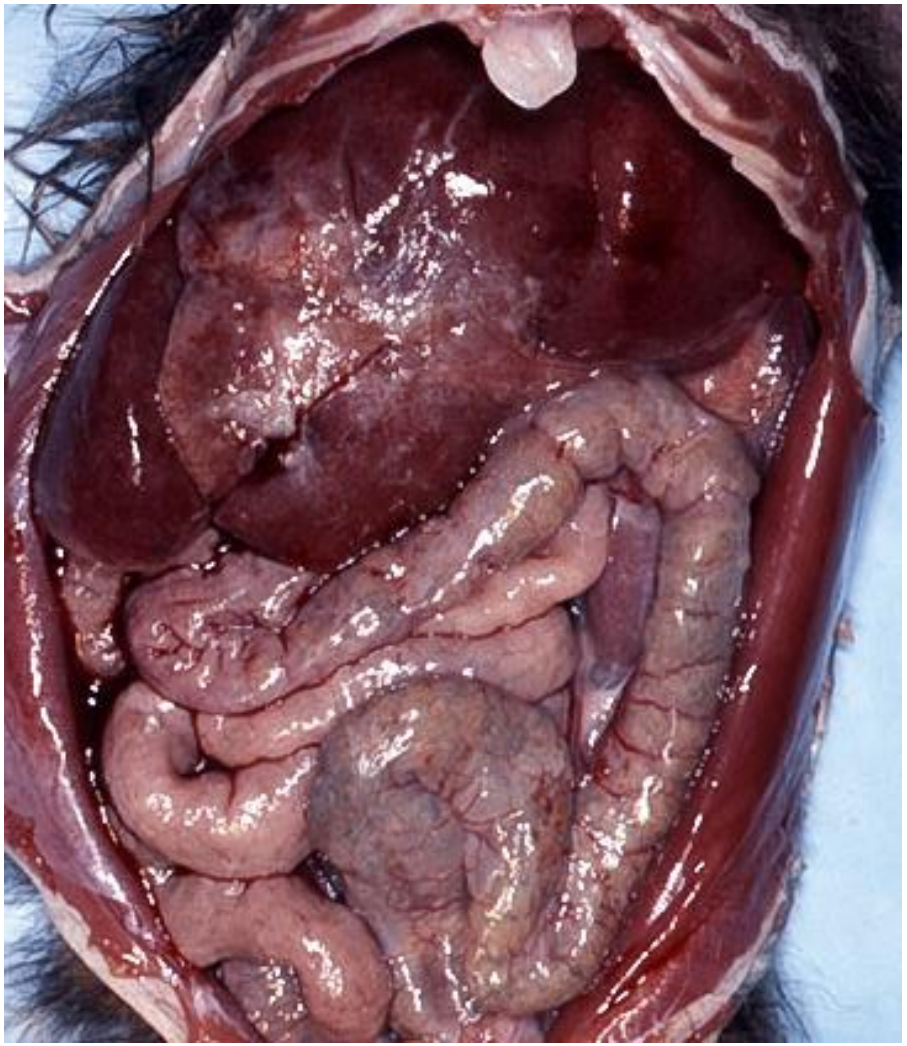
Bone disease, alopecia, anemia, lymphomas, susceptibility to GI pathogens

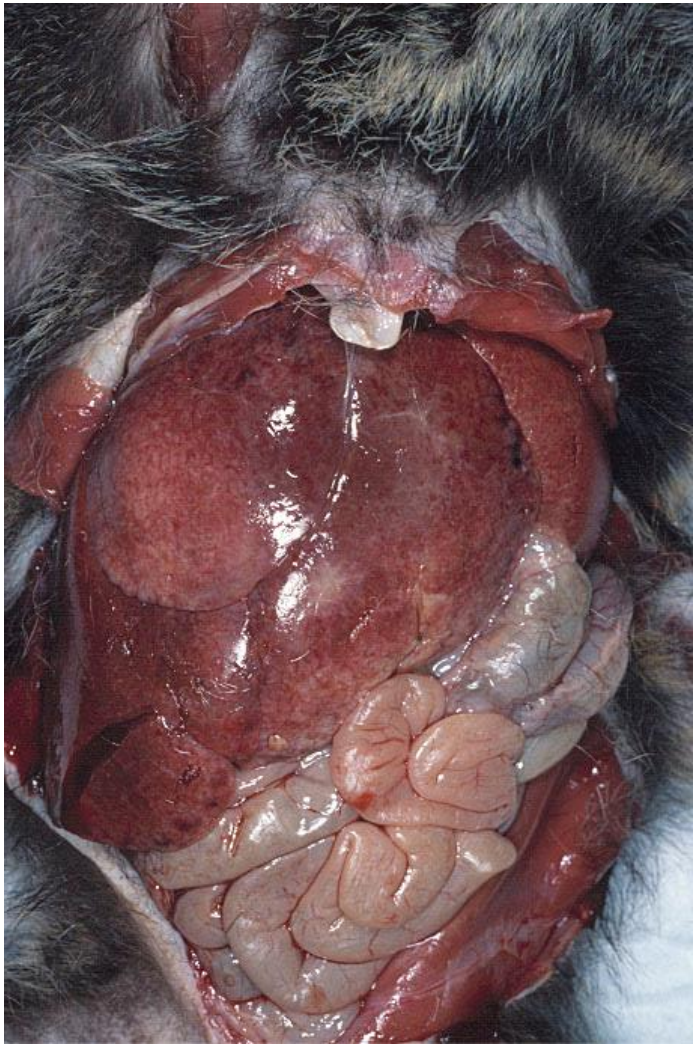
Diarrhea
Weight loss

Systemic AA amyloidosis

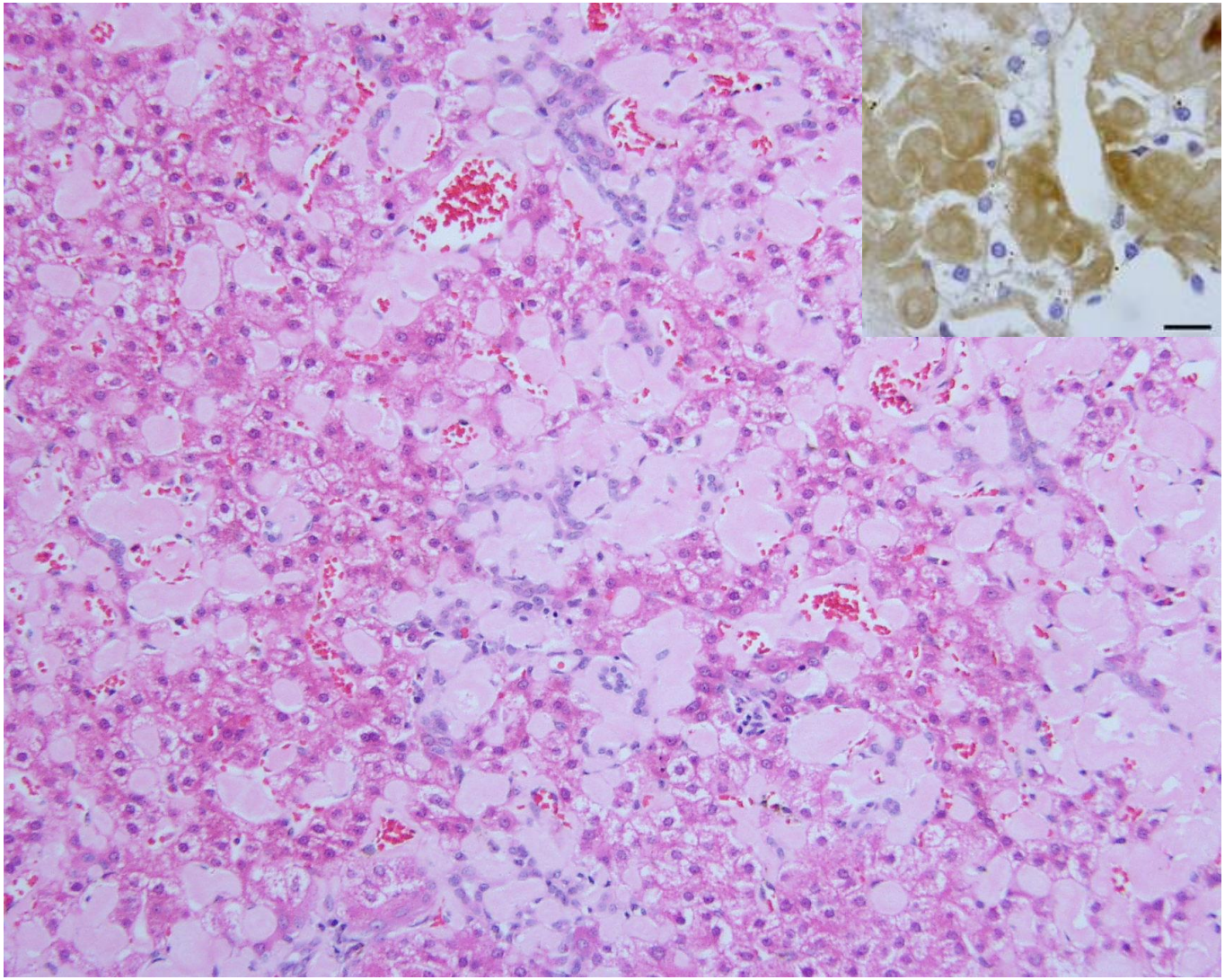
- **Etiology:** chronic inflammation increases SAA which is processed and misfolded to insoluble AA fibrils; Amyloid Enhancing Factor (AEF) has been shown to transmit systemic amyloidosis in other species.
- **Clinical disease:** chronic diarrhea and wasting; hepatomegaly.
- **Clinical pathology:** hypoalbuminemia; **elevated alkaline phosphatase; mild elevation in other liver enzymes.**
- **Pathology:** amyloid deposition in glomeruli, liver, and small intestine >> colon, adrenal glands and renal interstitium; **exposure to AEF from other affected animals in the context of a proinflammatory environment may initiate and accelerate disease.**

Systemic AA amyloidosis

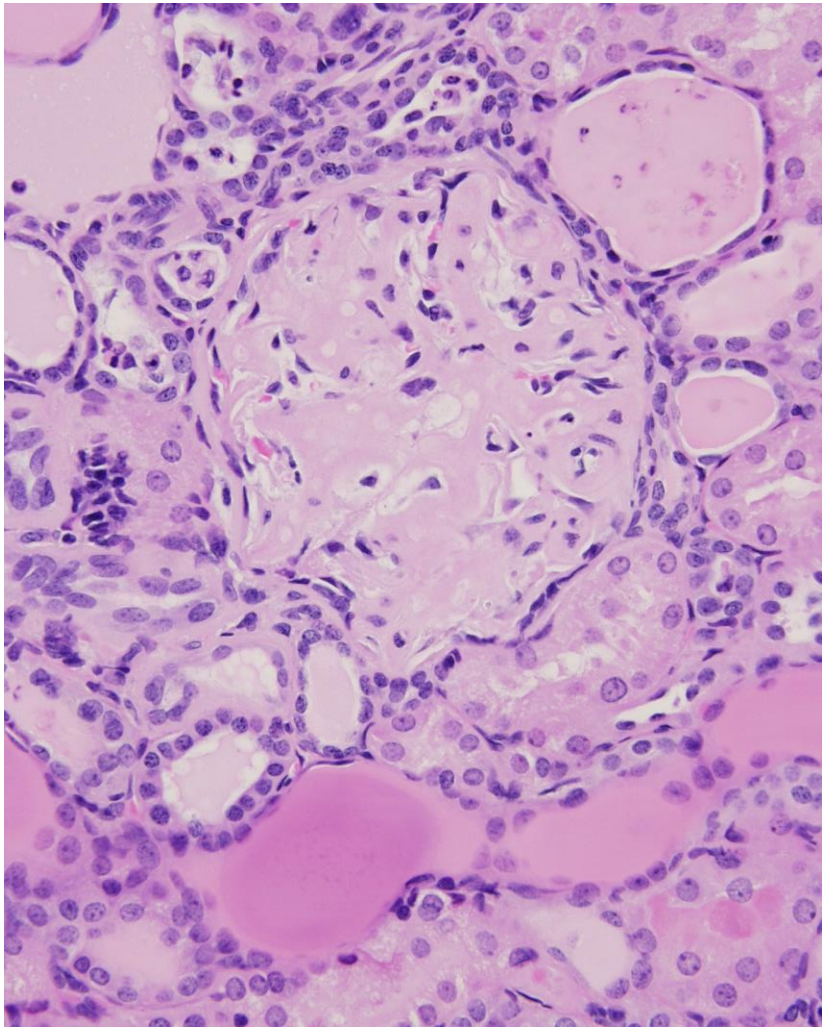


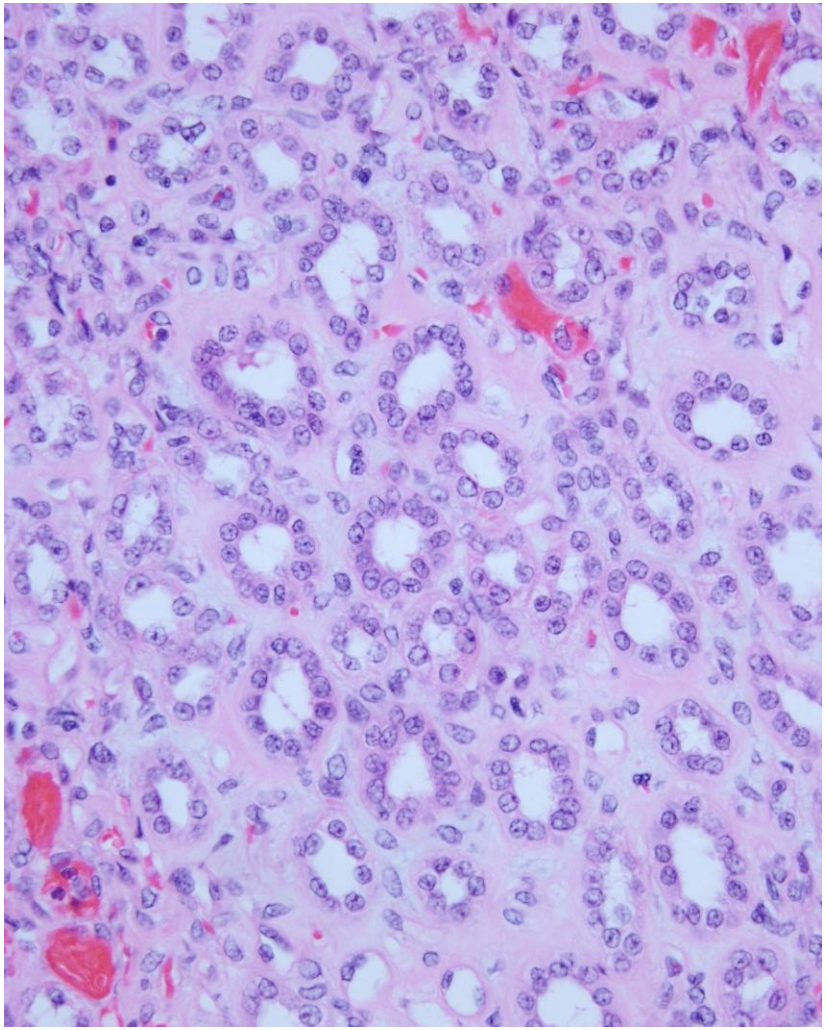


Systemic AA amyloidosis

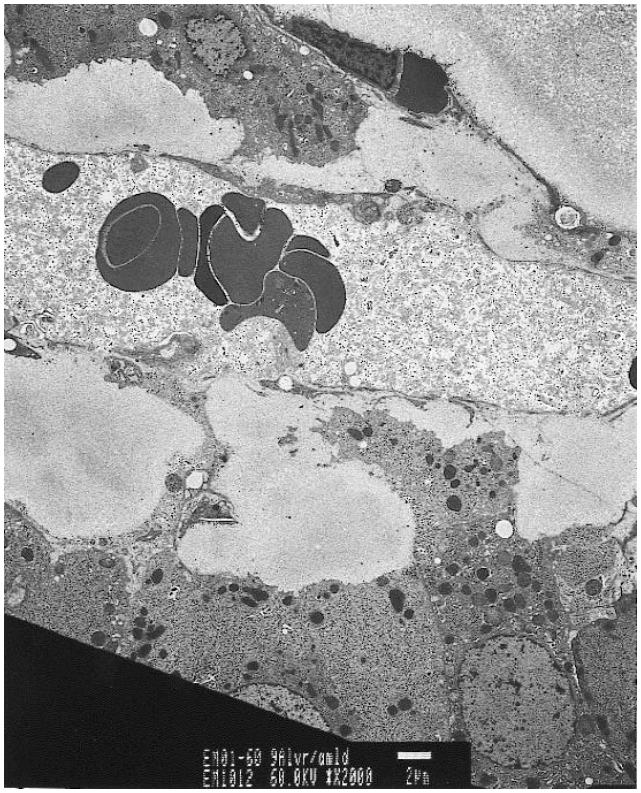


Systemic AA amyloidosis





Systemic AA amyloidosis



- AEF serves as a nidus to accelerate β-pleated sheet formation and is shed into the environment.
- Affected animals may pose a risk to contacts and should be removed from the colony.

Infectious diseases
Impact on morbidity and mortality in the common marmoset

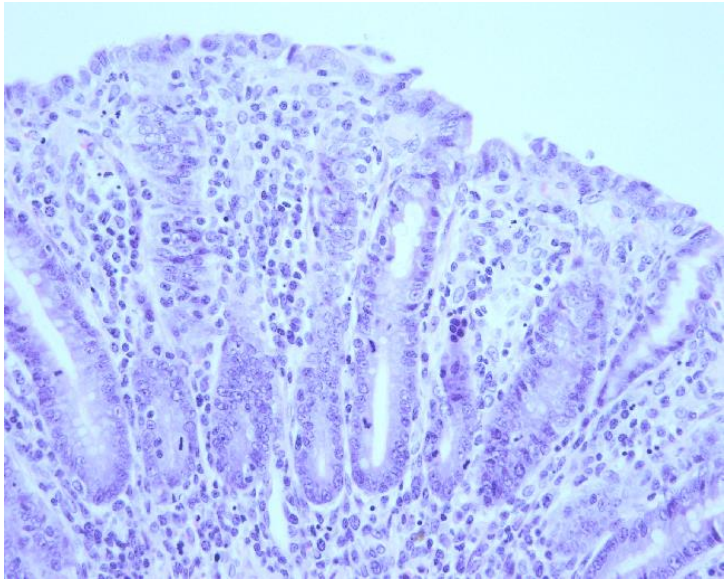
- Marmosets are highly susceptible to a number of severe bacterial and viral infections.
- Enteric pathogens likely effect early survival and elimination of Giardia and Campylobacter appear to have had a positive impact on overall survival.
- Further work is needed to define the enteric virome and understand the impact of chronic viral infections such as GB virus A and CHV3 on animal health.
- **Caution should be used in introducing new animals to a closed colony due to the potential introduction of infectious agents to a naive population.**

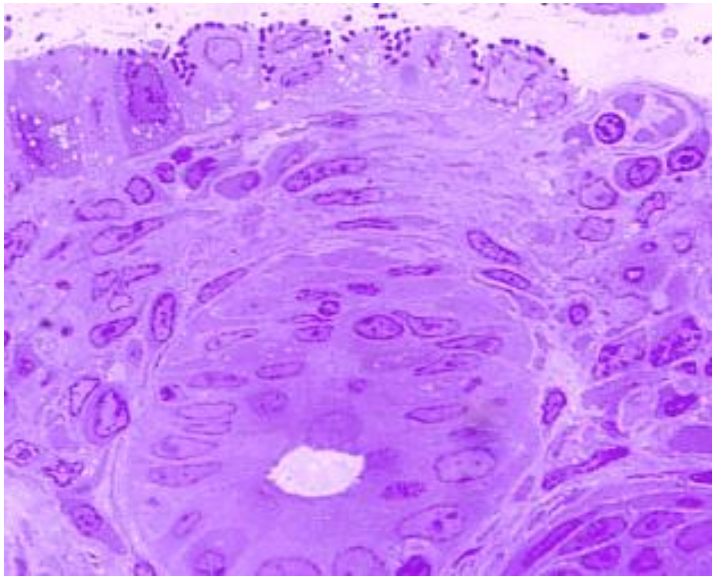
Enteropathogenic *E. coli*

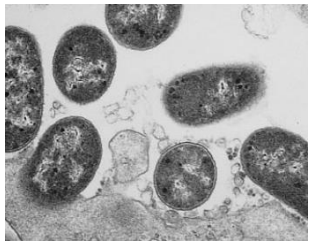
- **Affected species:** NWPs of any age; enzootic and unrecognized pathogen in many colonies.
- **Etiology:** gram negative bacteria
- **Clinical disease:** acute hemorrhagic diarrhea in common marmosets associated with hypovolemia and cardiovascular shock.
- **Pathology:** mild neutrophilic colitis with marked crypt epithelial cell hyperplasia; attenuation of surface epithelium; adherent bacteria forming an attaching and effacing lesion
- **Diagnosis:** morphologic findings (**attaching and effacing lesion**) on biopsy is pathognomonic; bacterial isolation and adhesion assay; PCR detection of intimin gene
- **Treatment:** enrofloxacin, supportive therapy
- **Zoonotic potential:** high?

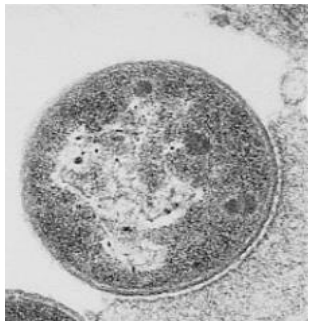
Enteropathogenic *E. coli*

Enteropathogenic *E. coli*
Pathology





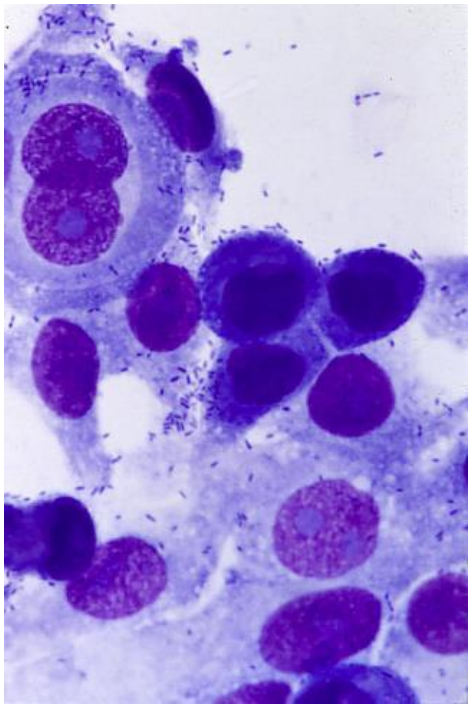




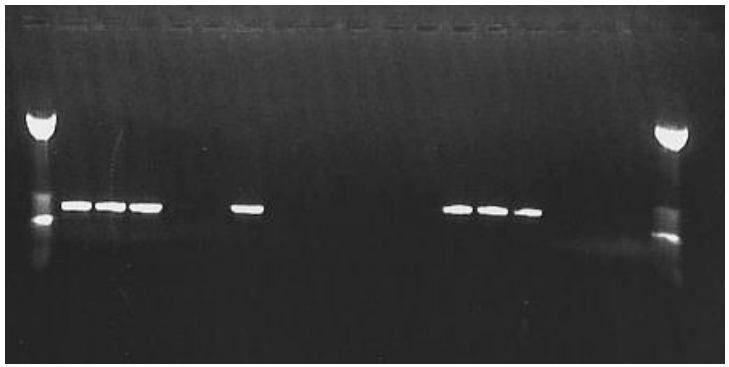
- Attaching and effacing lesion is pathognomonic for EPEC infection.
- Colonic biopsy is rapid and effective method to diagnose clinical disease.

Enteropathogenic *E. coli*
Diagnosis

Bacterial isolation and characterization

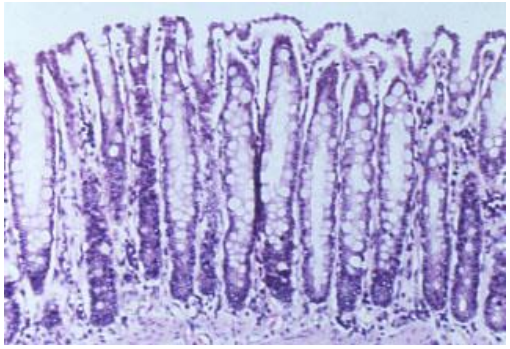


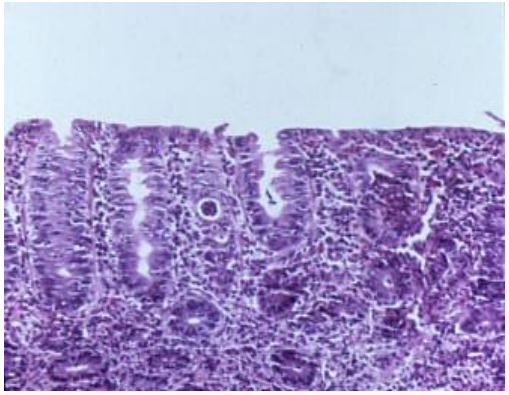
PCR detection of virulence factor genes



Campylobacter

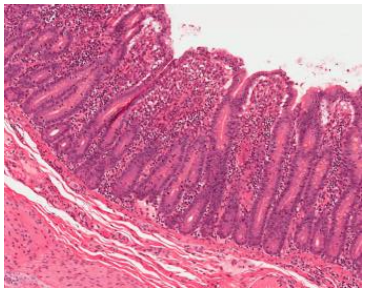
- **Etiology:** gram negative bacteria with slender curved morphology; C. jejuni and C. coli identified in NHP; isolation requires microaerophilic environment; molecular basis of virulence not understood.
- **Clinical disease:** isolation common from OWP and is difficult to associate with disease; should not be present in NWP and often associated with diarrhea; should be eliminated from common marmoset colonies.
- **Pathology:** neutrophilic colitis with crypt abscesses is nonspecific.
- **Zoonotic potential:** species specificity is unknown; primates susceptible to human isolates.

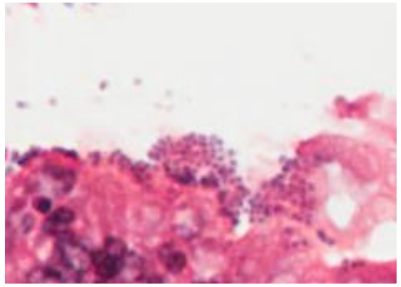


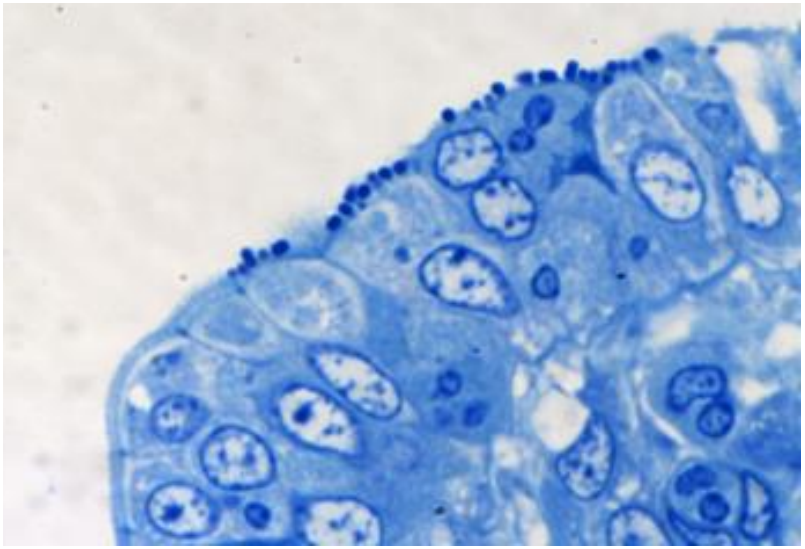


Molecular tools for detection of infectious agents in nonhuman primates

Multiplex PCR detection of enteric pathogens







Organism	Gene
EHEC	Stx1
EHEC	Stx2
EHEC/EPEC	Eae
Salmonella	invA
Shigella/EIEC	ipaH
Shigelle/EIEC	invE
ETEC	Est
ETEC	Elt
EAEC	aggR
Campylobacter jejuni	rim
Campylobacter coli	gyrB
Y enterocolitica/pseudotuberculosis	virF

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A Quantitative Polymerase Chain Reaction Assay for Rapid Detection of 9 Pathogens Directly From Stools of Travelers With Diarrhea

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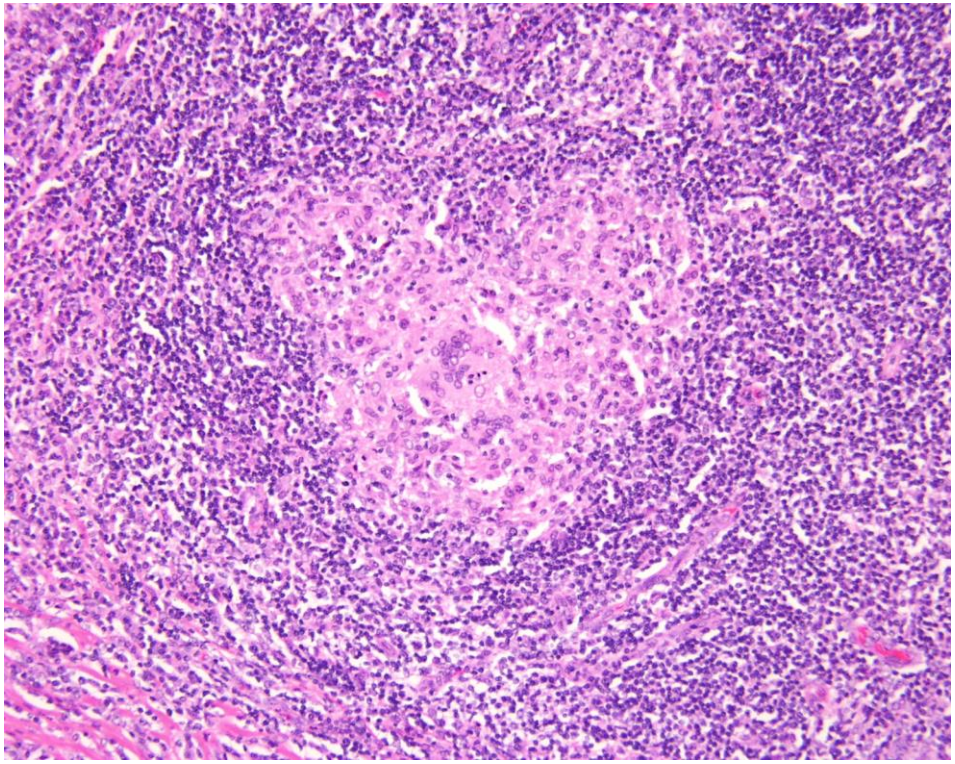
Atypical mycobacteriosis
New World Primates

- **Affected species:** common marmosets, squirrel monkeys
- **Etiology:** *Mycobacterium avium; M. intracellulare; M. Kansasii;* others; believed to be largely non-communicable.
- **Clinical disease:** exposure to atypical mycobacteria is universal but disease is infrequent; most often subclinical and recognized with a **positive intradermal skin test;** less frequently may be associated with weight loss and anorexia; DDx *M. tuberculosis*.
- **Pathology:** Granulomatous lymphadenitis and pneumonia; caseous necrosis with multinucleated giant cells; rare acid fast bacilli; identical to *M. tuberculosis.*
- **Source:** environmental waterborne organisms; water distribution systems in commercial buildings support biofilms allowing survival of environmental mycobacteria.
- **Zoonotic potential:** low

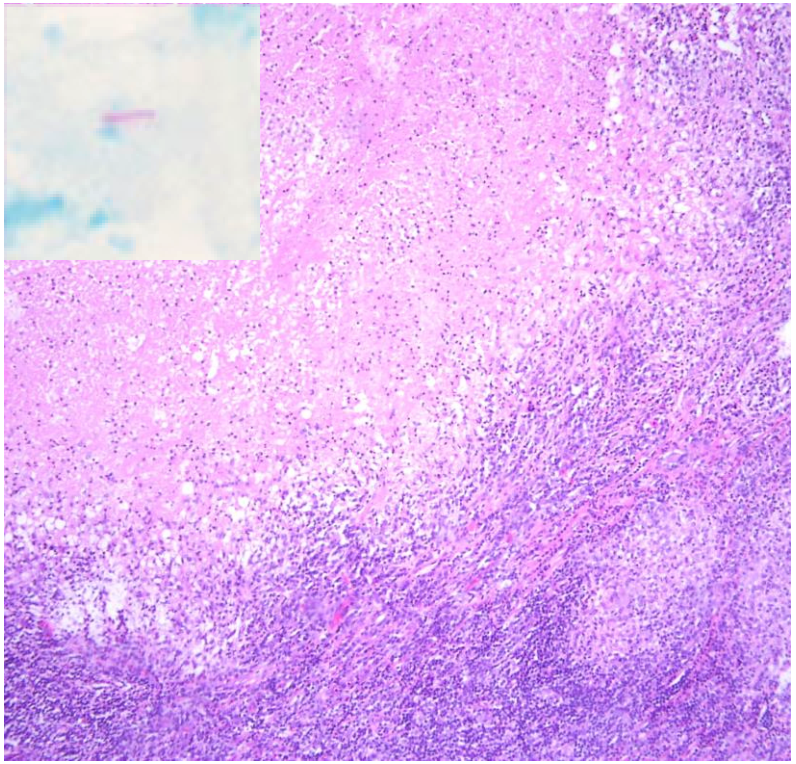
Atypical mycobacteriosis
Common marmoset

Tuberculosis skin test

Atypical mycobacteriosis
New World Primates



Multinucleated giant cells



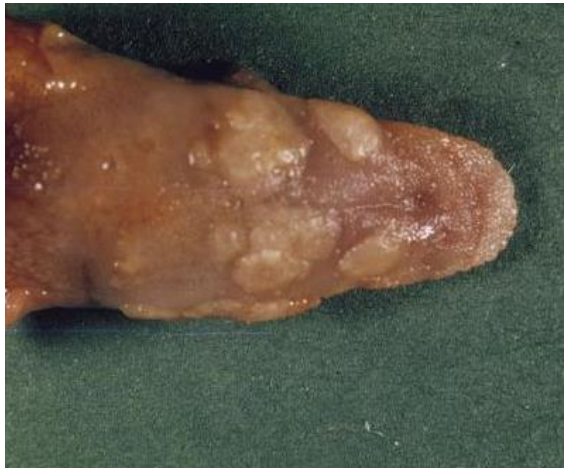
Caseous necrosis in mesenteric lymph node

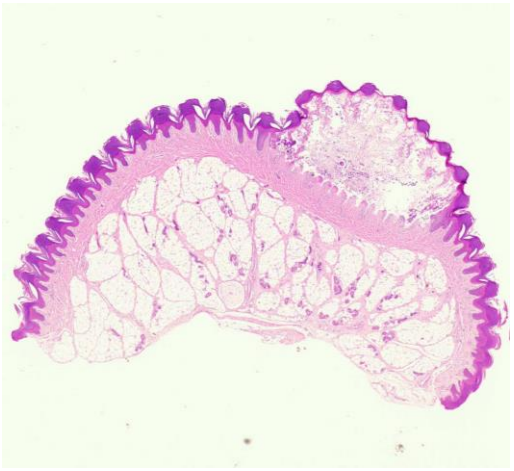
Differential diagnosis of atypical vs typical mycobacterial infections

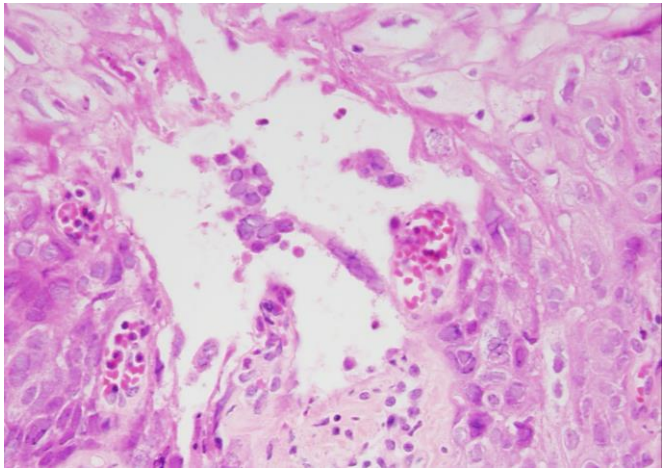
- Comparative skin test
- Radiographs
- Histopathology
- *In vitro* gamma-IFN assays
- Culture-remains the gold standard
- PCR/sequencing

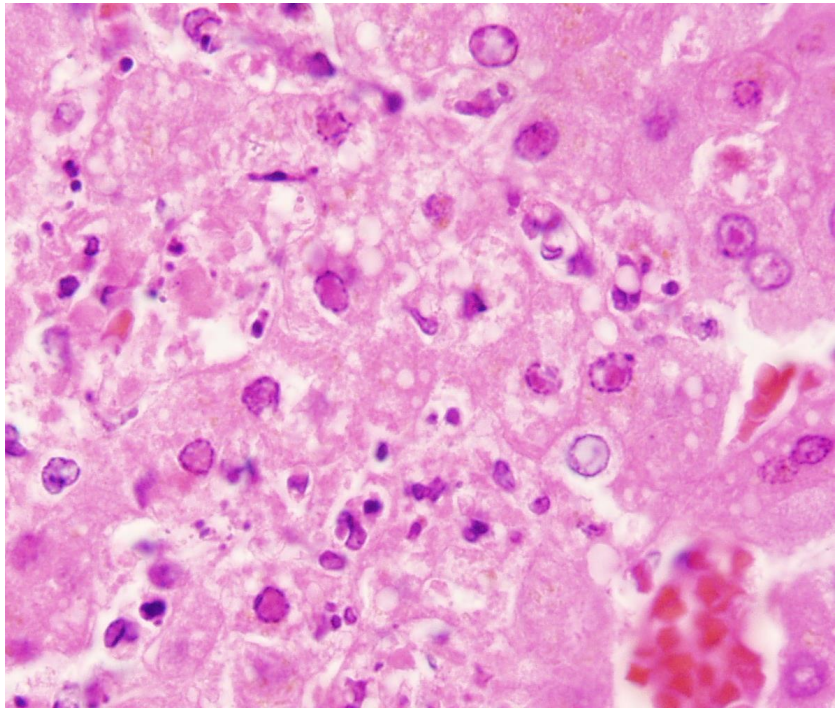
Alphaherpesvirus infections: NWP

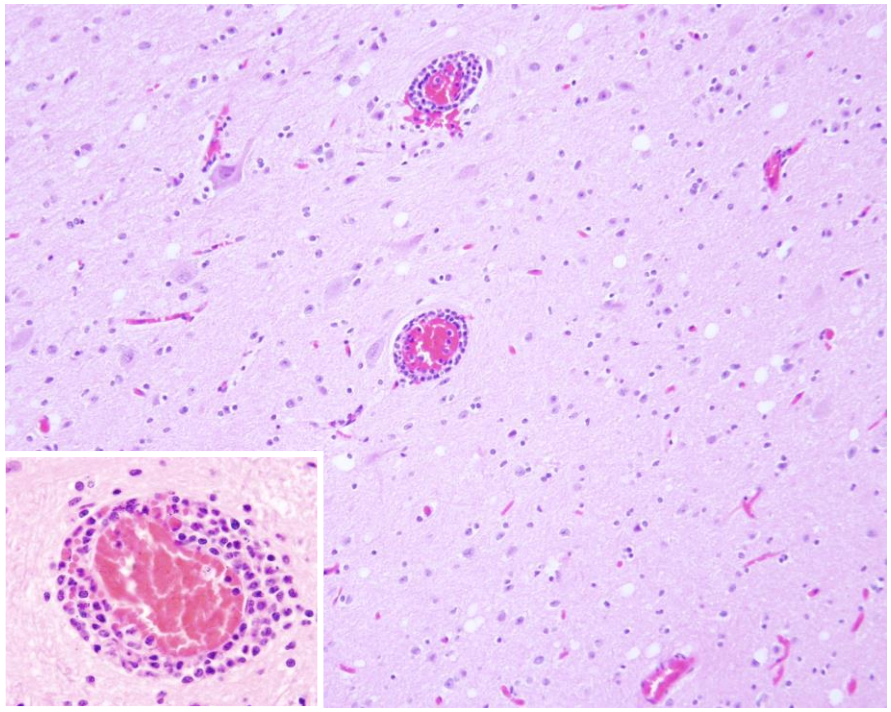
- **Affected species:** Callitrichinae and some cebinae highly susceptible
- **Etiology:** Herpes simplex 1(HSV-1); Herpesvirus tamarinus (HVT)
- **Clinical disease:** Minimal in natural host; rapidly progressive and fatal disseminated viral disease in inadvertent host; results in stomatitis and conjunctivitis>>dermatitis; followed by multiple organ failure (CNS, liver, lung, GI, spleen)
- **Epidemiology:** initial transmission from natural host; once established in colony rapidly transmitted causing a disease of high morbidity and mortality.
- **Pathology:** Multifocal to coalescing necrosis in skin, brain, liver and lymphoid tissue; intranuclear inclusions
- **Prevention:** Strict separation of NHP species; PPE.











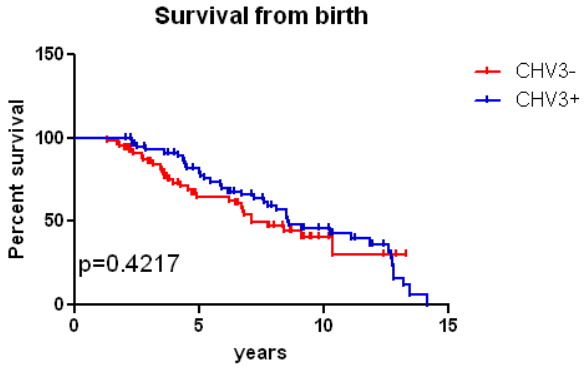
Callitrichid herpesvirus 3

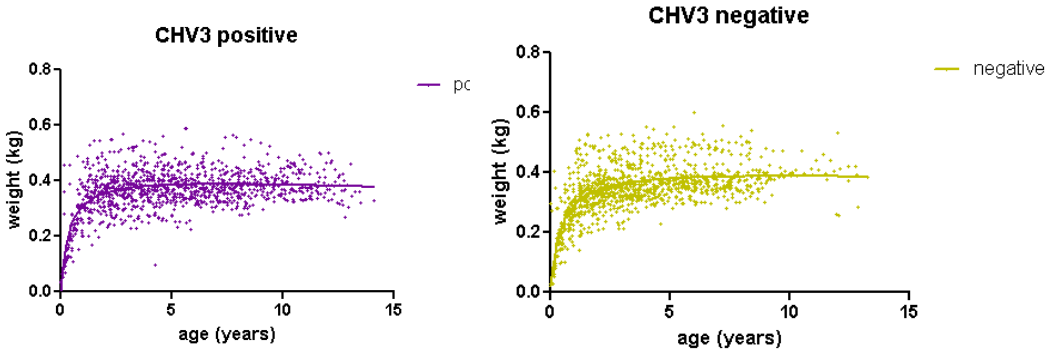
- **Etiology:** gammaherpesvirus (lymphocryptovirus) related to human EBV (HHV4); other γ1-herpesvirus species specific variants recently recognized in NWP.
- **Epidemiology:** Common asymptomatic infection of normal marmosets; serology indicates greater than 60% of animals infected by 3 years of age.
- **Clinical disease:** Unclear; initial association with cluster of GI lymphomas at WiNPRC; common infection in marmoset colonies in NA.
- **Zoonotic potential:** none

- **Additional research is need to understand potential disease associations and genetic variability in CHV3 strains.**

Callitrichid herpesvirus 3

No statistically significant difference in survival from birth based on CHV3 sero-status





No statistically significant difference in weight history based on CHV3 sero-status

Human lymphocryptovirus infection

Outcome variability due to timing, genetics and co-morbidities

Human disease outcome following HHV4/EBV infection

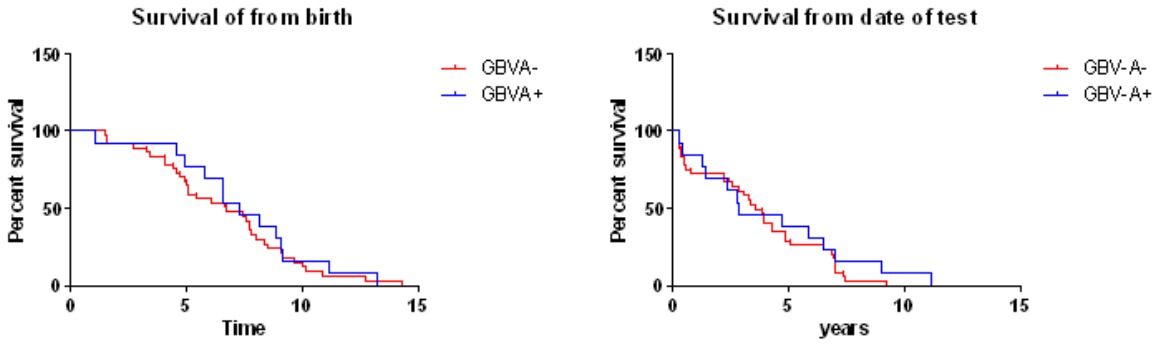
Young age-subclinical or mild disease
Adolescent/Adult-infectious mononucleosis
Perforin gene mutation-chronic active infectious mononucleosis
HIV infection-malignant B lymphoma
Chronic malarial coinfection-endemic Burkitt's lymphoma
HLA susceptibility-nasopharyngeal carcinoma
SLAM associated protein mutation-fatal X-linked lymphoproliferative syndrome

Could differences in genetics and environmental cofactors drive differential disease outcomes for CHV3?

Should we test colonies for CHV3 status?

GB virus A

- **Etiology:** flaviviridae; ssRNA virus; first identified in 1995 in passage 11 GB serum; a number of distinct species specific variants recognized
- **Epidemiology:** readily transmitted in domestic and native colonies
- **Clinical disease:** Common asymptomatic infection of normal marmosets; unknown disease associations.
- **Impact of experimental work:** animals become persistently viremic for up to 7 years; effect of GBV-A on experimental work unknown
- **Human:** GB virus C infects lymphocytes and modulates HIV infection.



Summary

- Common marmoset colonies develop a variety of diseases entities which may present with pathologic features distinct from other primate species.
- Many chronic processes produce a disease phenotype characterized by wasting and must be distinguished.
- A full necropsy with histological examination should be performed on all deceased colony and research animals to better understand the spectrum of diseases processes which may be present in a facility.
- Comparative analysis of colony morbidity and mortality data may inform disease etiology and improve colony health and research utility.
- Publication of disease entities and outbreaks should be encouraged and further research is needed to understand the impact of diet and infectious diseases on animal health.