Ferret Coronavirus Diseases

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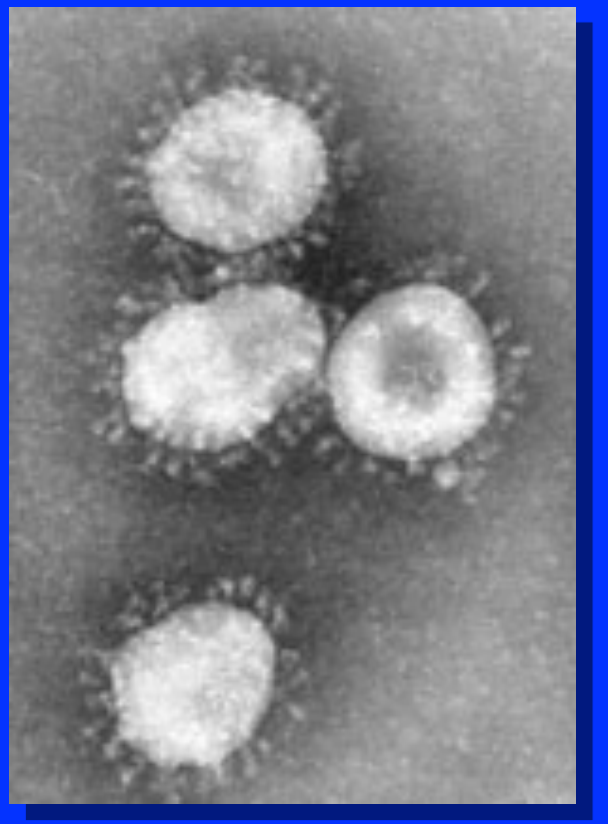
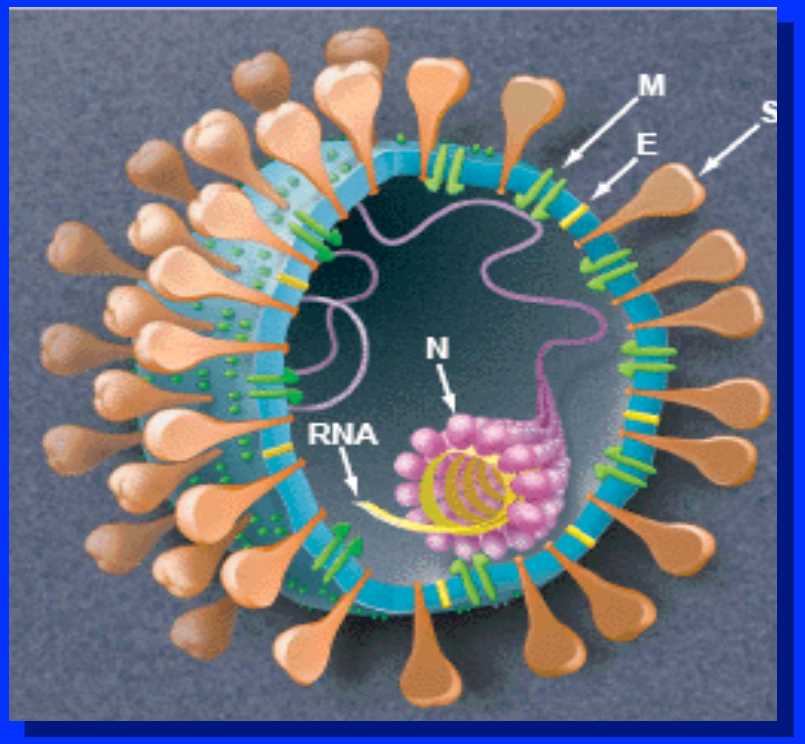
Coronaviruses

- Coronaviridae family
- Irregularly-shaped particles
- 60-220 nm in diameter
- Club-shaped peplomers
- embedded in the envelope
- "Crown/*corona*-like" appearance



Coronavirus Characteristics

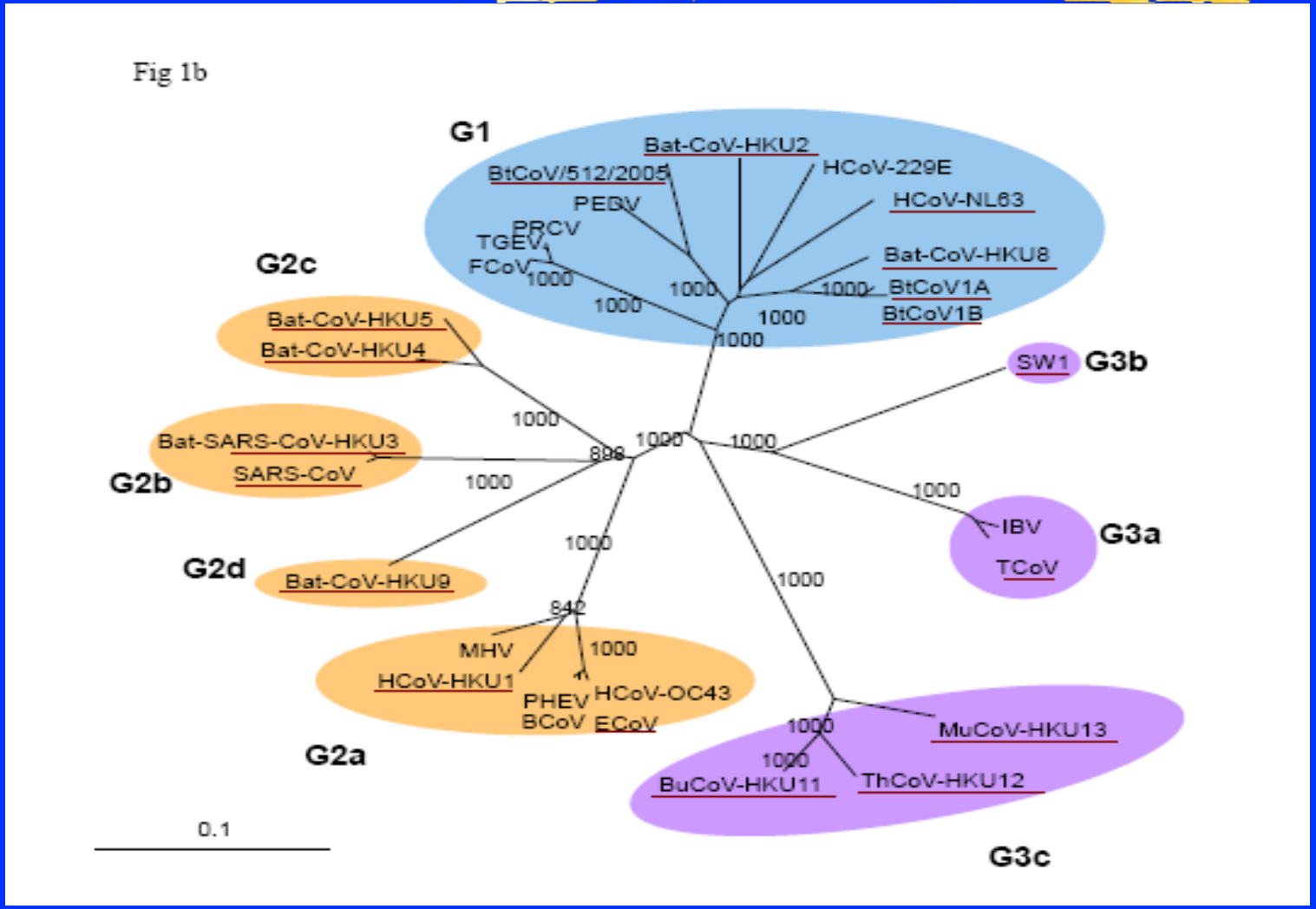
- Glycoproteins of envelope:
  - S – Spike
  - M – Membrane
  - E – Envelope
- Genome: + sense RNA
  - non-segmented
  - single-stranded
  - 27-31 kb
- RNA enclosed in capsid of N protein monomers



Coronavirus Antigenic Groupings

1 (mammalian)	Human coronavirus 229 E
	TGEV of swine
	Feline infectious peritonitis virus
	Canine coronavirus
2 (mammalian)	Human coronavirus OC43
	Mouse hepatitis virus
	Bovine coronavirus
	Porcine hemagglutinating encephalo-myelitis virus
3 (avian)	Infectious bronchitis virus of chickens
	Bluecomb disease virus of turkeys

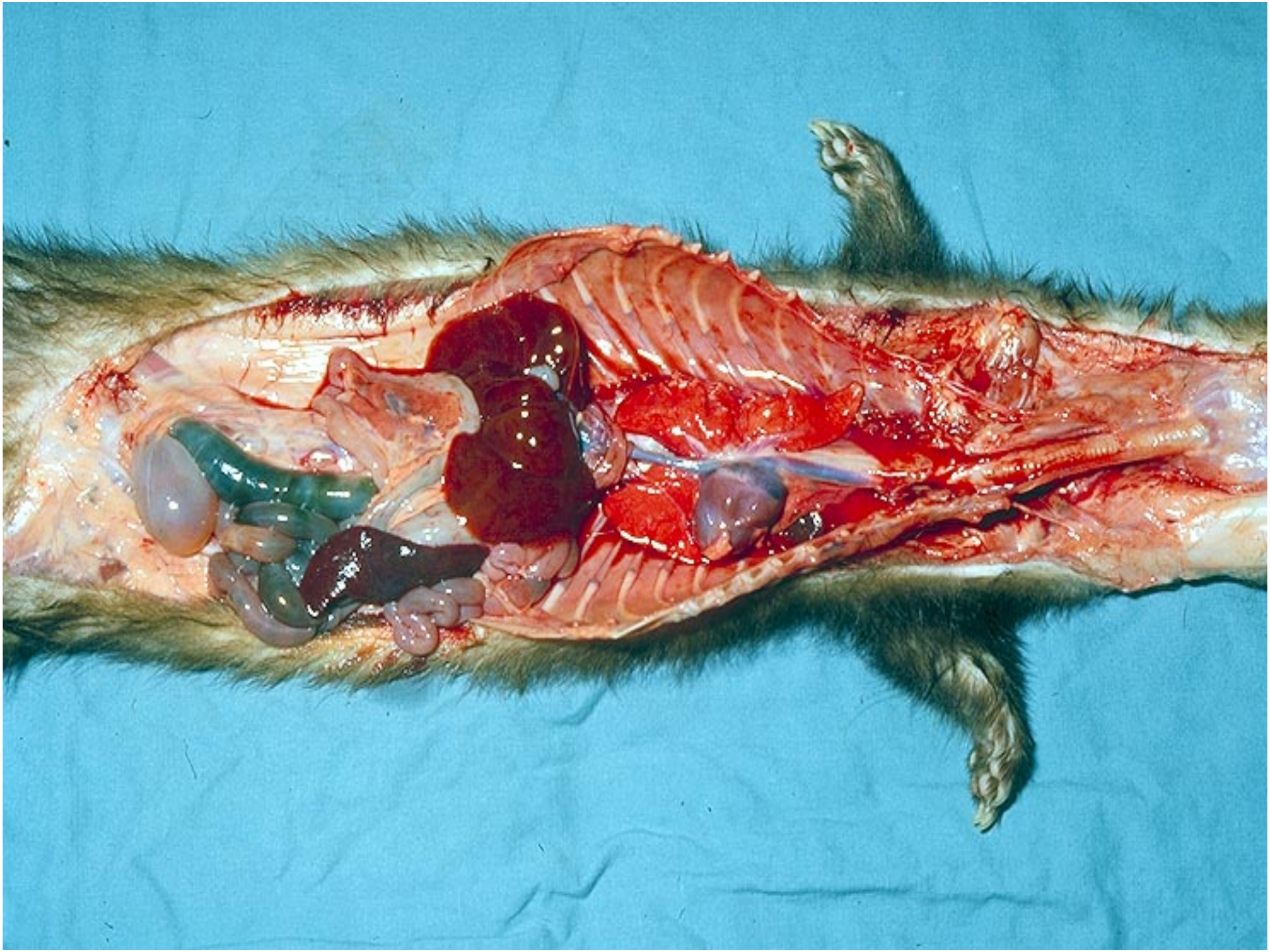
Coronavirus Phylogeny



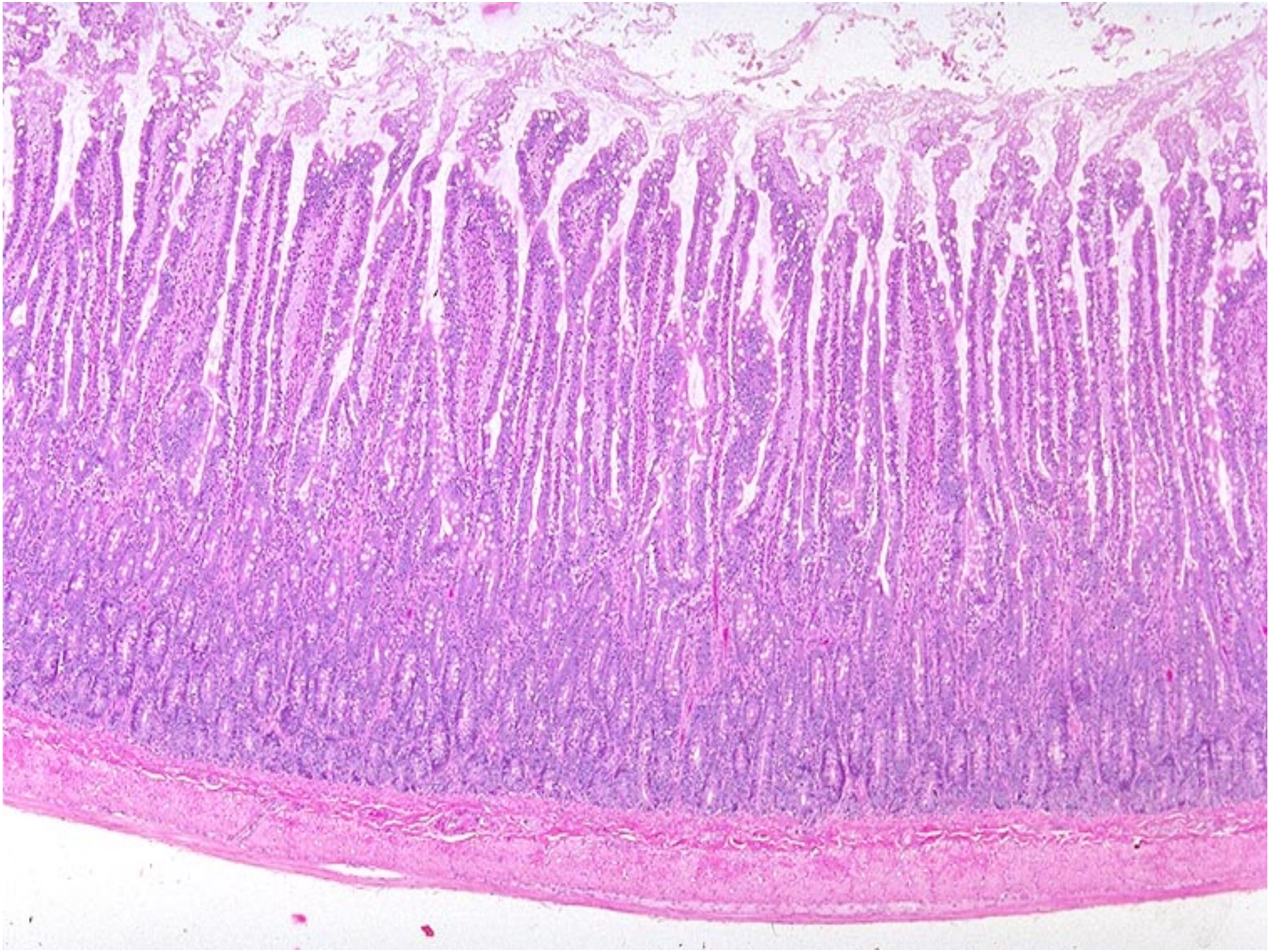
Woo et al. Exp. Biol. and Medicine (2009)

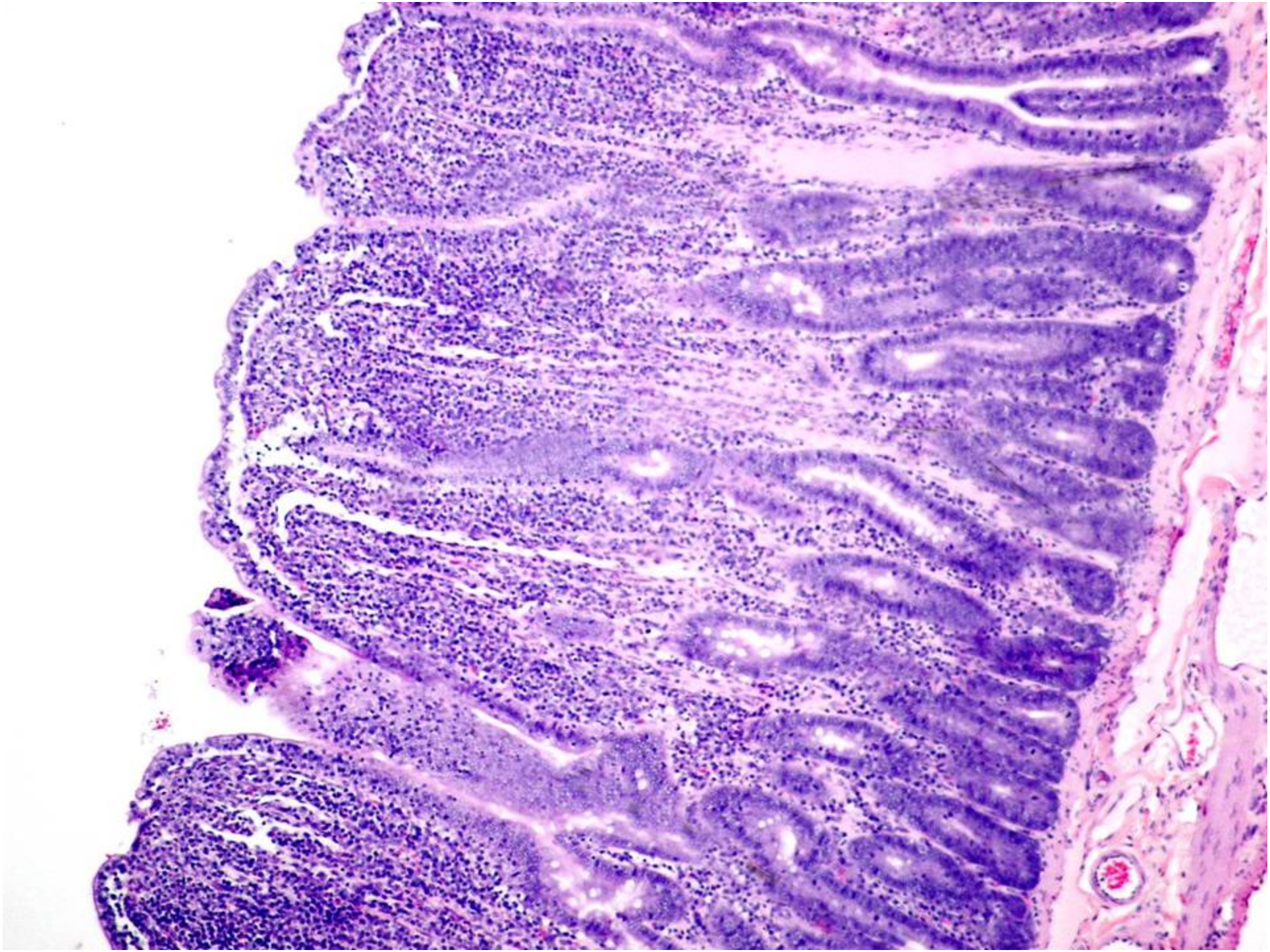
Epizootic Catarrhal Enteritis (ECE)

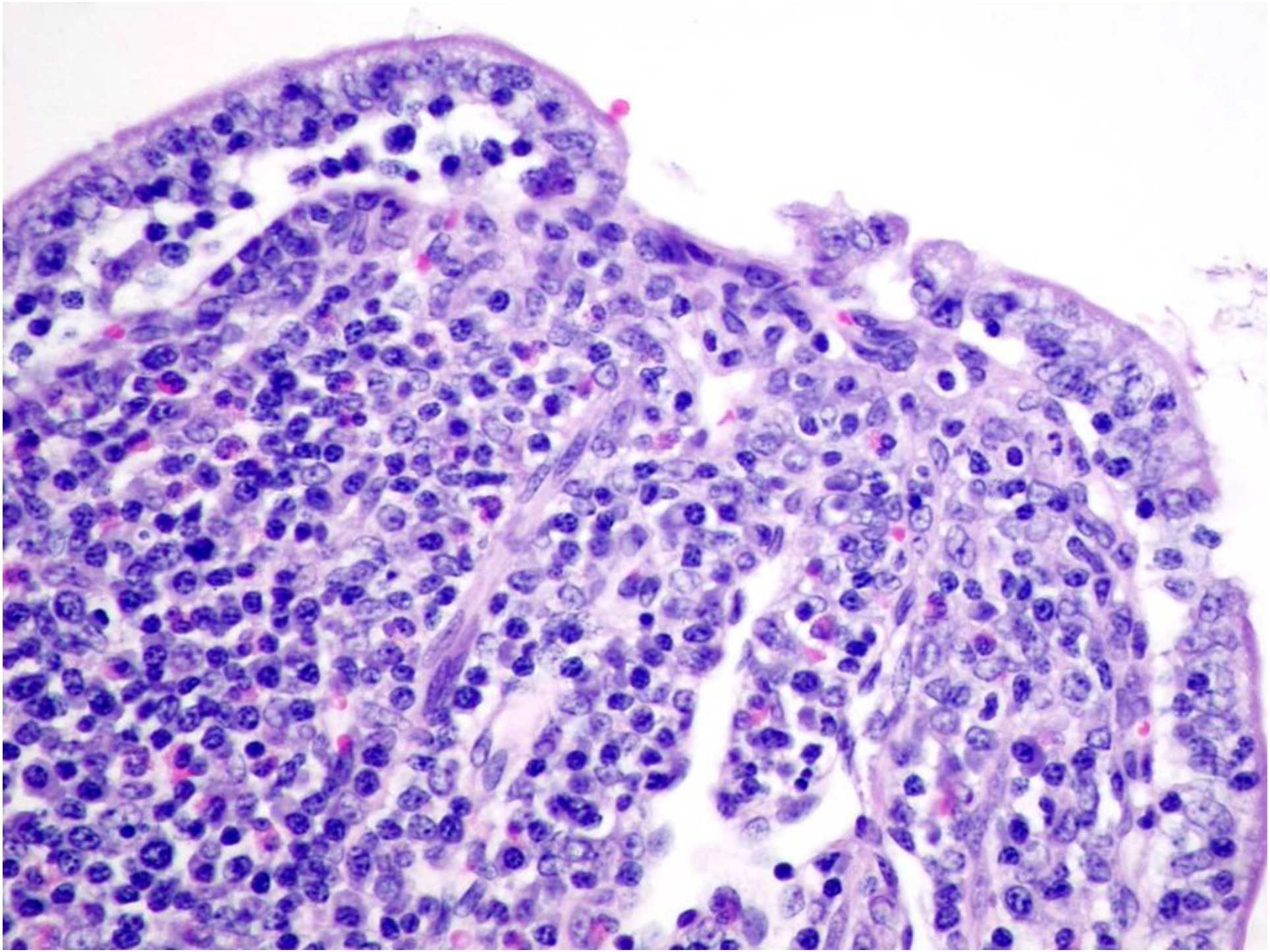
- New diarrheal disease of ferrets (1993)
- First described on the East Coast of the U.S.
- "Green slime disease", "Green diarrhea", or "The Greenies"
- Clinical signs: lethargy, anorexia, vomiting, followed by profuse diarrhea
- Mortality rate 5%, Morbidity rate 100%
- Disease more severe in older ferrets
- Present throughout the U.S. and several other countries

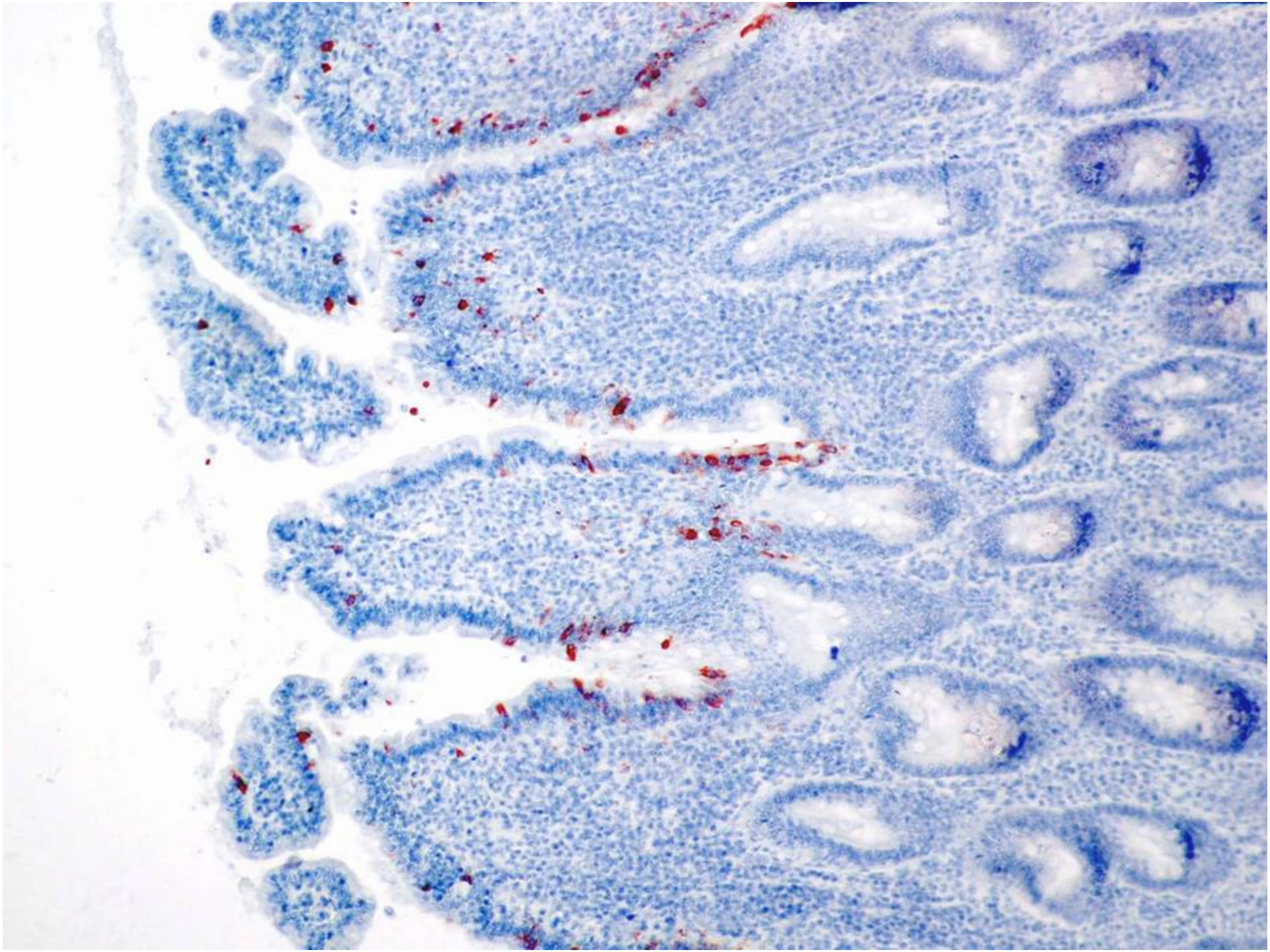


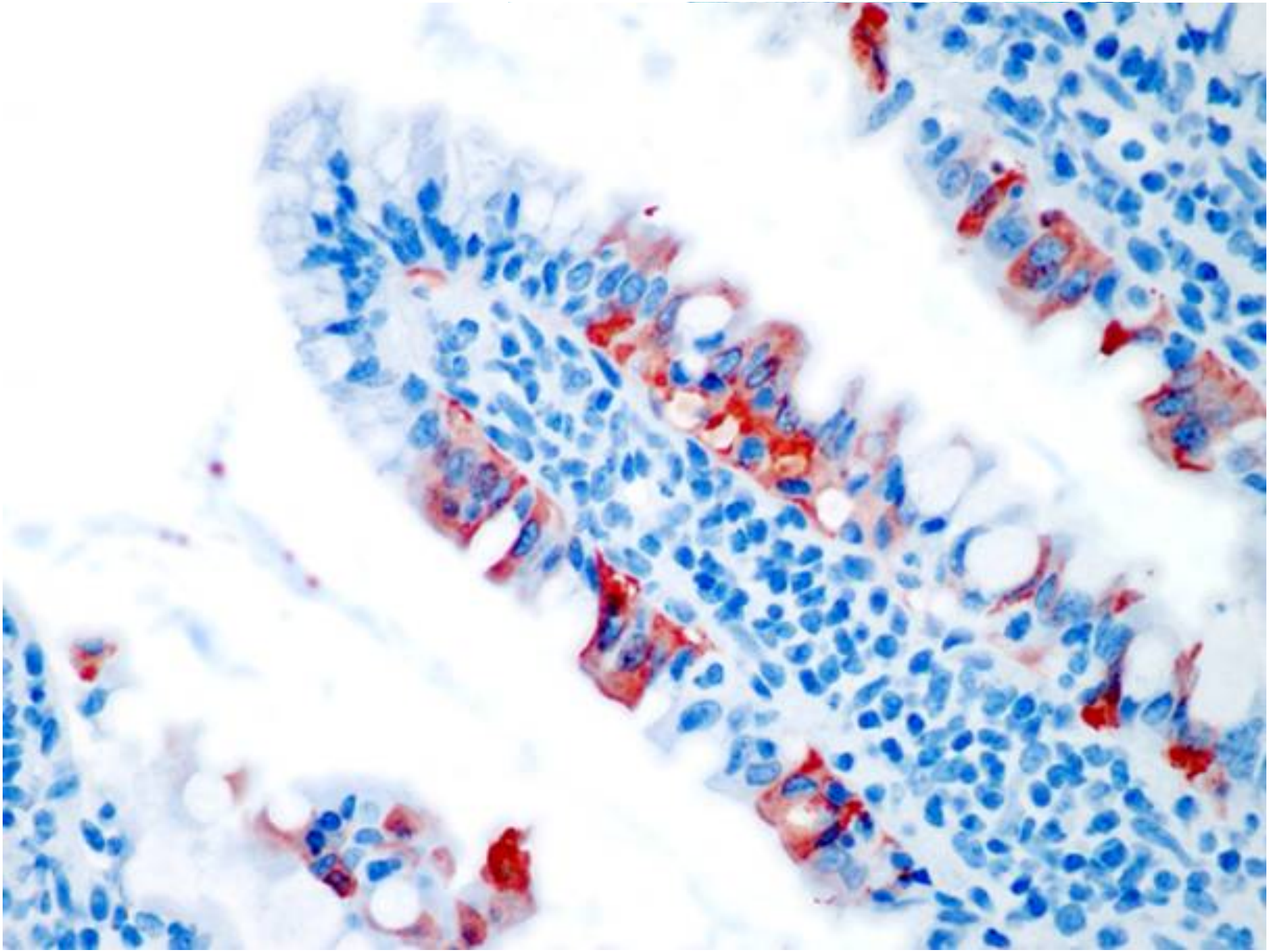

Epizootic Catarrhal Enteritis (ECE)





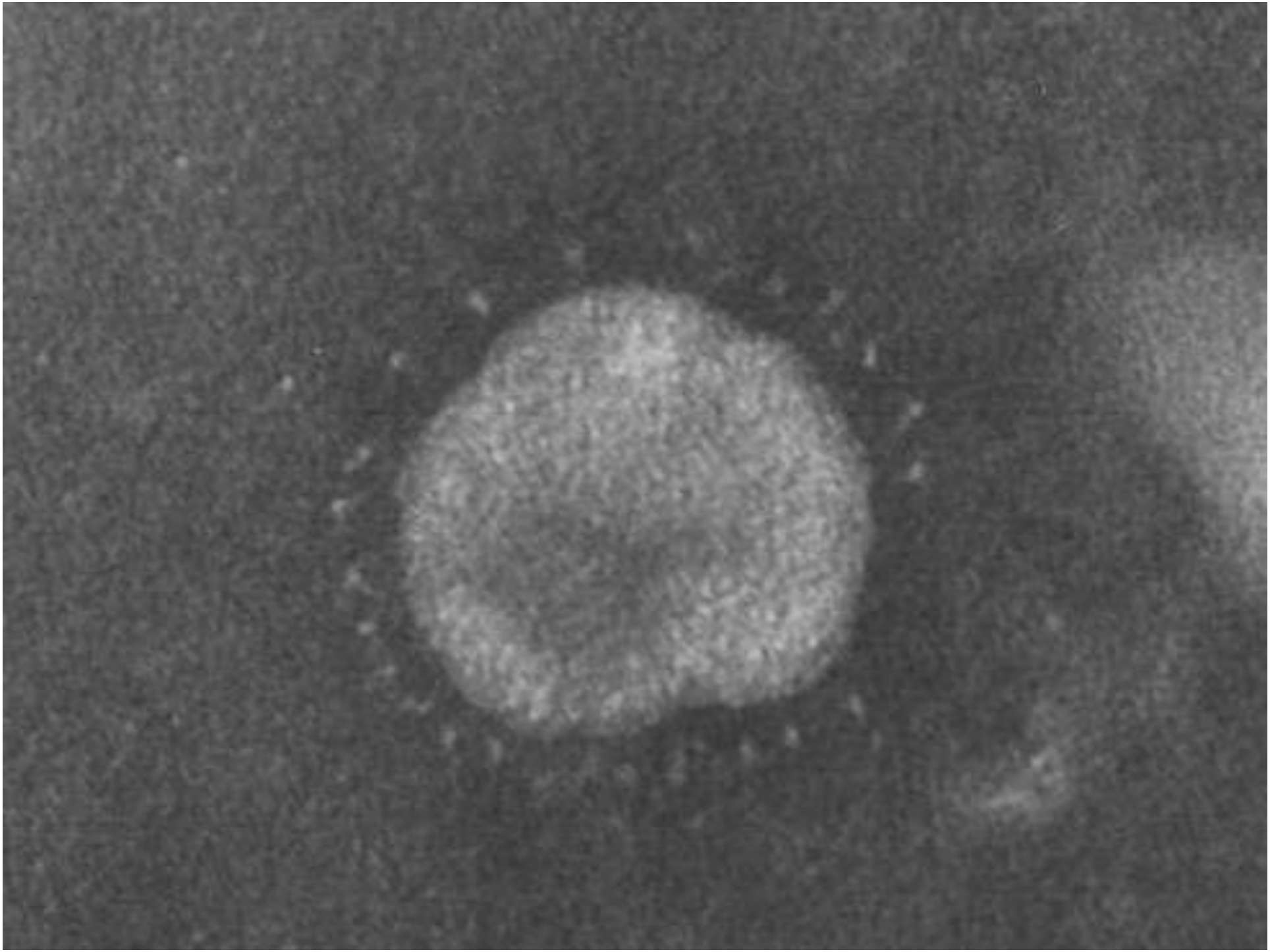


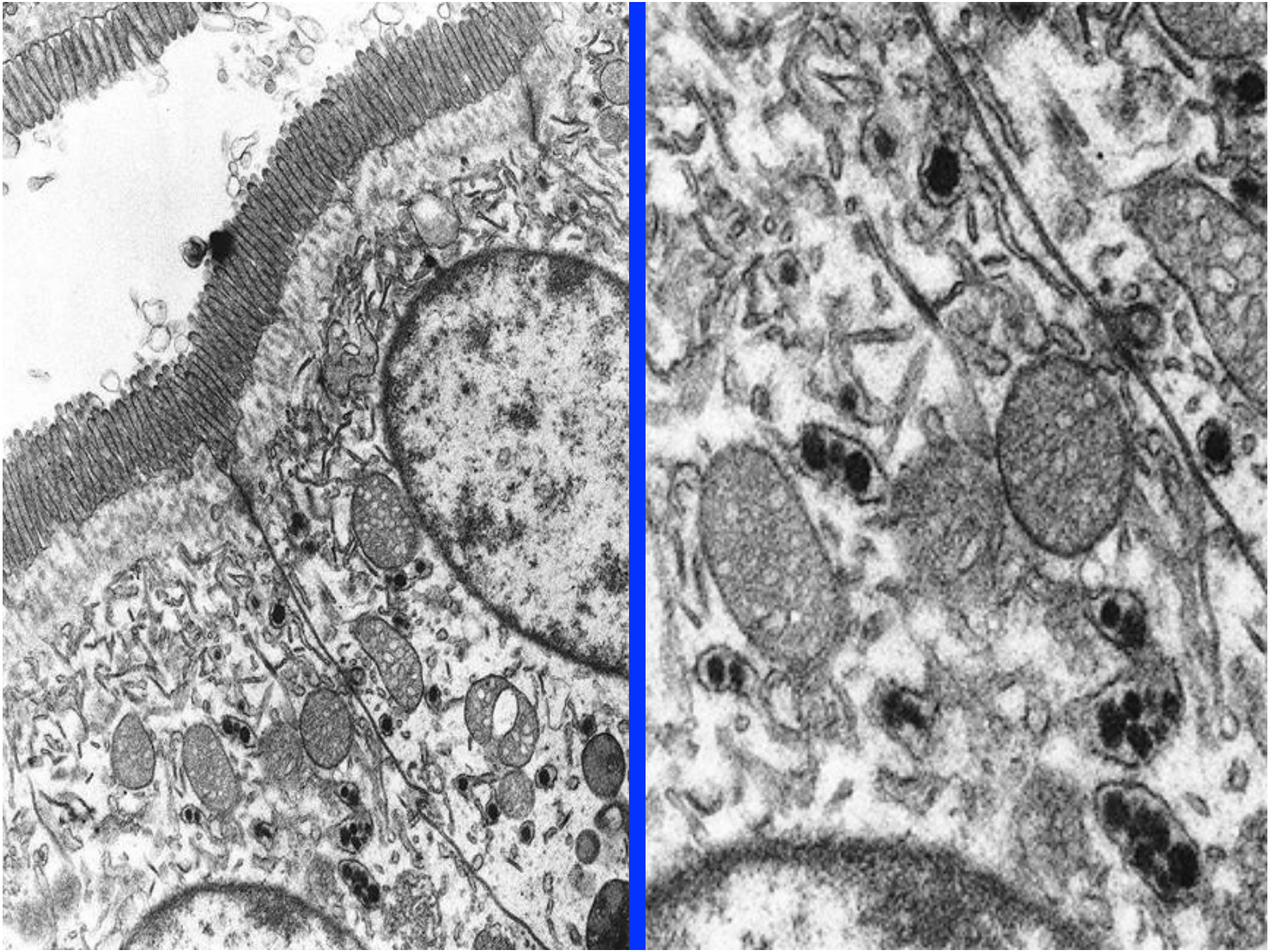




Morphologic Diagnosis

Small Intestine, atrophic and lymphoplasmacytic enteritis with intralesional coronavirus antigen





Coronavirus-associated epizootic catarrhal enteritis in ferrets

Bruce H. Williams, DVM, DACVP; Matti Kiupel, MS, Dr med vet; Keith H. West, DVM, PhD;
James T. Raymond, DVM, DACVP; Christopher K. Grant, DVM, PhD; Lawrence T. Glickman, VMD, DrPH

Objective—To characterize clinical signs and lesions and identify the etiologic agent associated with epizootic catarrhal enteritis in domestic ferrets.

Design—Cross-sectional study.

Animals—119 ferrets with epizootic diarrhea of presumed viral cause and 5 control ferrets.

Procedure—Clinical records and biopsy or necropsy specimens of ferrets with presumed epizootic catarrhal enteritis were reviewed. Immunohistochemical staining for coronavirus antigen was performed on paraffin-embedded tissues from approximately 10% of affected ferrets to identify viral antigen and determine its distribution. Transmission electron microscopy was performed on fecal samples and sections of jejunum. Virus isolation studies as well as immunofluorescent tests for other similar viruses were performed.

Results—Characteristic microscopic lesions consistent with intestinal coronavirus infection (vacuolar degeneration and necrosis of villus enterocytes; villus atrophy, fusion, and blunting; and lymphocytic enteritis) were consistently detected in affected ferrets. Coronavirus particles were identified in feces and jejunal enterocytes by use of transmission electron microscopy. Immunohistochemical staining of jejunal sections revealed coronavirus antigens. Antigen staining was not detected in healthy ferrets or ferrets with other gastrointestinal tract diseases. Virus isolation was unsuccessful, and other similar viruses were not detected.

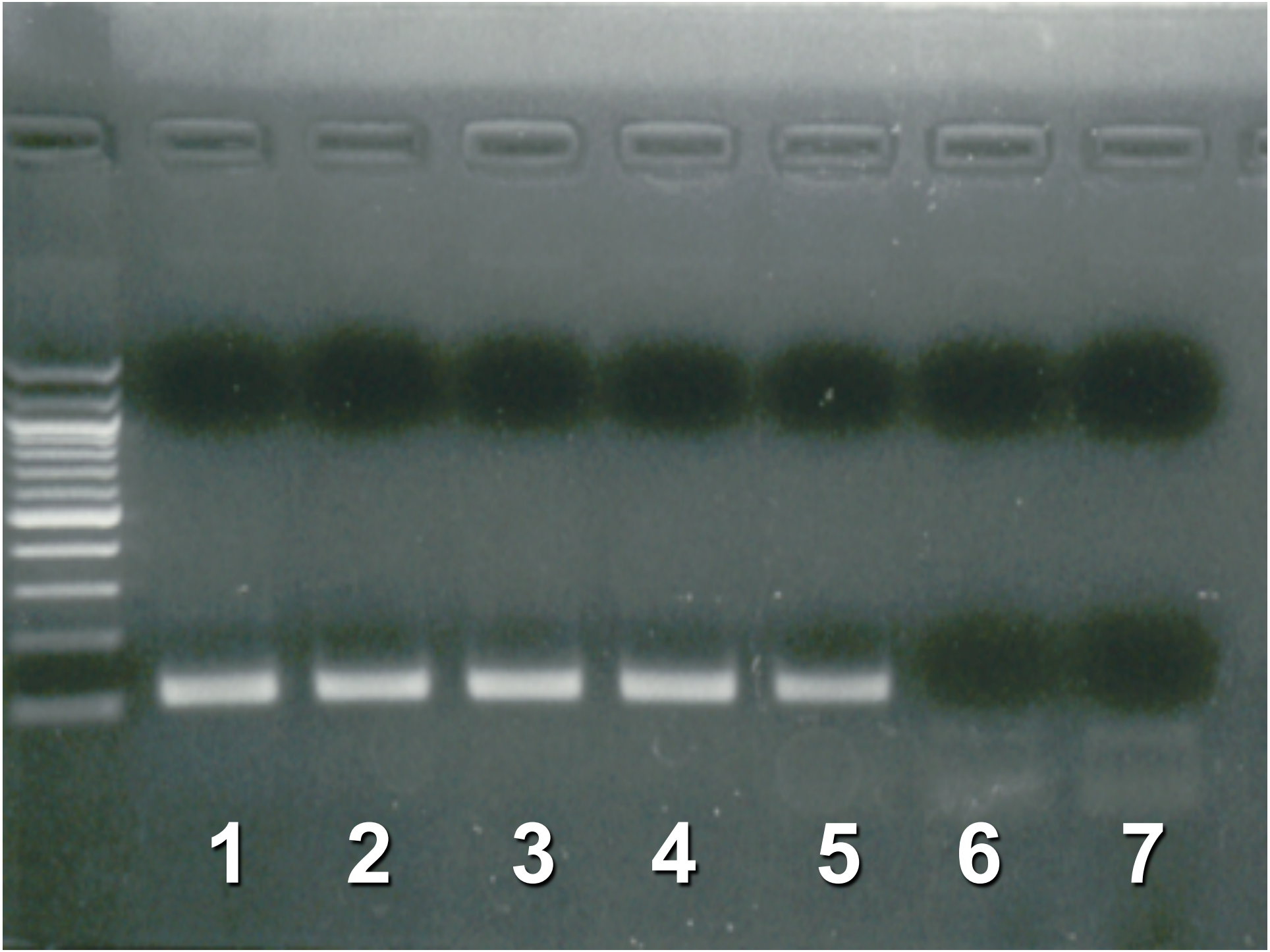
Conclusions and Clinical Relevance—Results strongly implicate a coronavirus as the causative agent of epizootic catarrhal enteritis in ferrets. Diagnosis may be made on the basis of a combination of historical, clinical, and microscopic findings. (*J Am Vet Med Assoc* 2000;217:526–530)

terized as having high morbidity and low mortality with initial signs of lethargy, inappetence, and vomiting. Subsequently, profuse bright green diarrhea with high mucus content developed, often affecting 100% of ferrets in each facility. Disease severity in individuals was highly variable; older ferrets had more severe signs, whereas young ferrets had mild or subclinical infection. Since that time, this disease has been diagnosed throughout the United States and in several other countries, affecting ferrets in breeding and rescue facilities as well as pet ferrets, resulting in substantial economic loss and emotional distress. The disease was named **epizootic catarrhal enteritis** (ECE) after study at the Armed Forces Institute of Pathology on the basis of similarities to a diarrheal disease of mink (epizootic catarrhal gastroenteritis).[1]

In ECE of ferrets, as in epizootic catarrhal gastroenteritis of mink, coronavirus-like particles were seen in feces, and a combination of atrophic and inflammatory enteric lesions characteristic of chronic coronavirus infection was commonly detected in ferrets with long-standing disease. The purpose of the study reported here was to characterize clinical signs and lesions and identify the etiologic agent associated with ECE in domestic ferrets. We hypothesized that a coronavirus was the causative agent.

Materials and Methods

Study population—Medical records of ferrets with nonspecific enteritis diagnosed between March 1993 and July 1999 on the basis of histologic examination of biopsy or necropsy specimens were reviewed at the Armed Forces Institute of Pathology and AccuPath Inc. Criteria for inclusion in the study included a clinical history of diarrhea, lack of a definitive cause of the disease, and one or more of the following microscopic lesions: vacuolar degeneration and necrosis of villus enterocytes; villus atrophy, fusion, and blunting; and lymphoplasmacytic enteritis characterized by a subjective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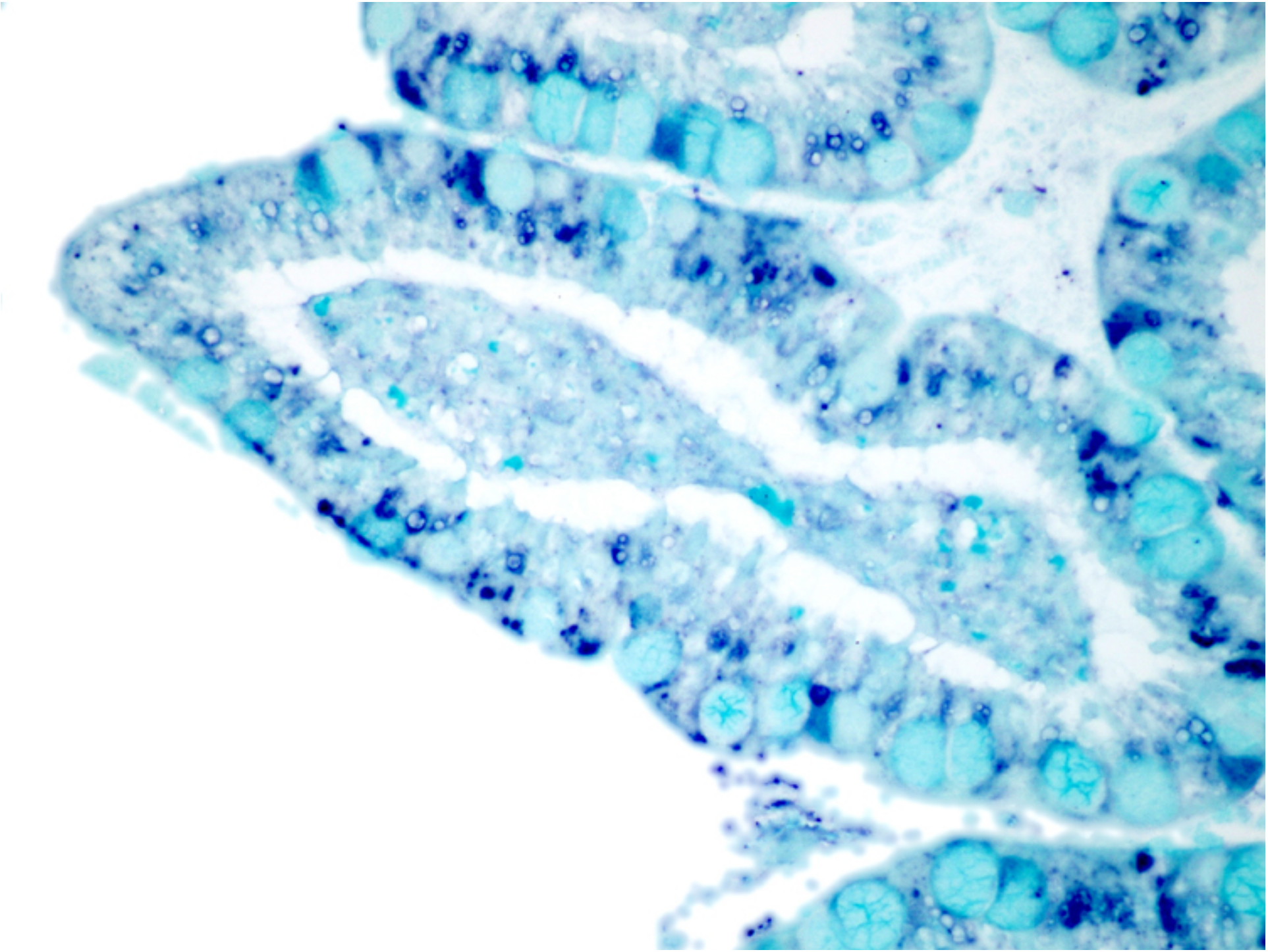
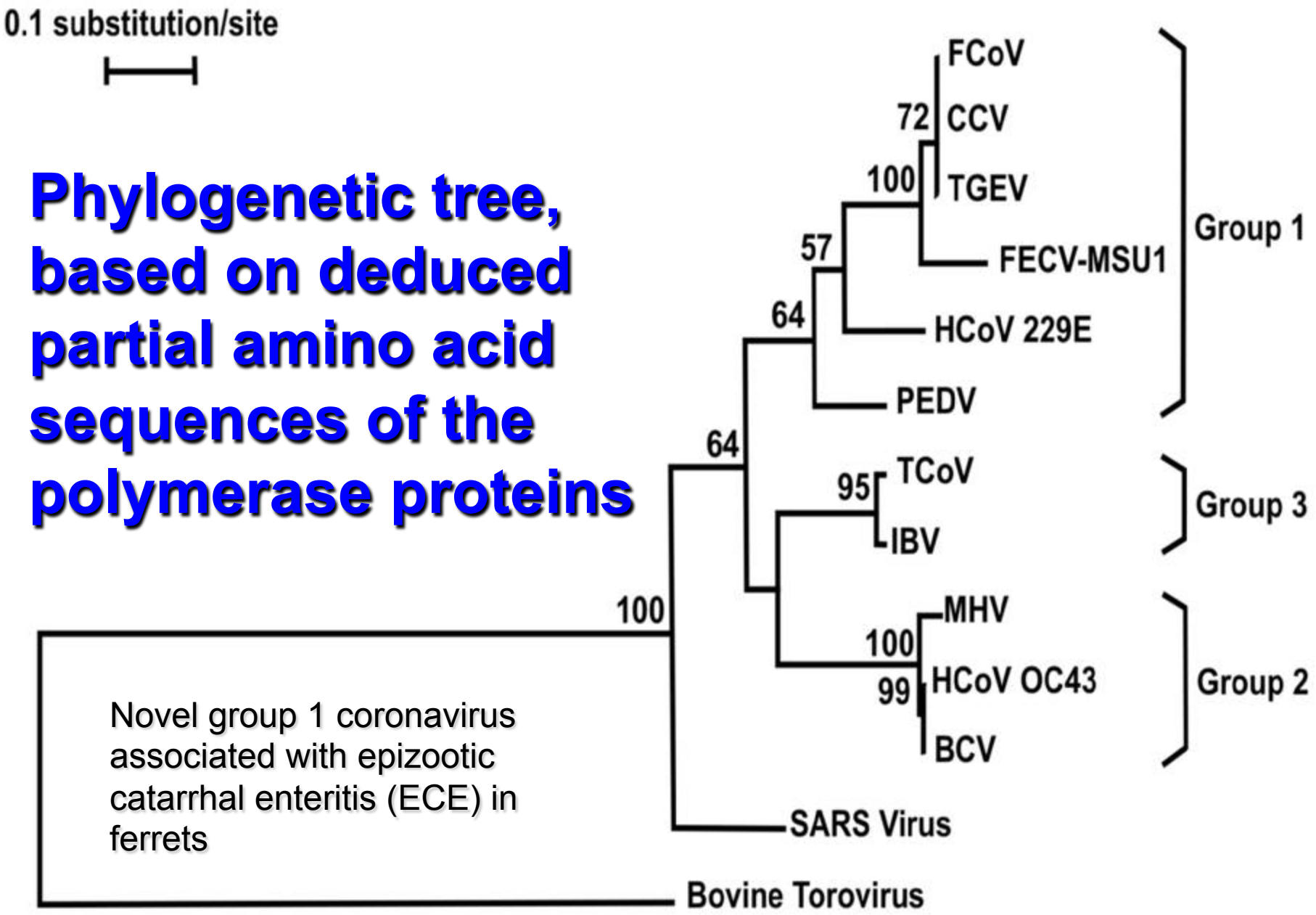


Complete Capsid Gene Sequencing

- Entire capsid (N) gene sequence derived by 3' RACE (1,125 bp-ORF)
- Nucleotide sequence similarities:
  - 51.6 % (CCV)
  - 48.7% (TGEV)
  - 48.2% (FCoV)
  - 34.5% (HCoV 229E)

Phylogenetic tree, based on deduced partial amino acid sequences of the polymerase proteins



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Molecular characterization of a novel coronavirus associated with epizootic catarrhal enteritis (ECE) in ferrets

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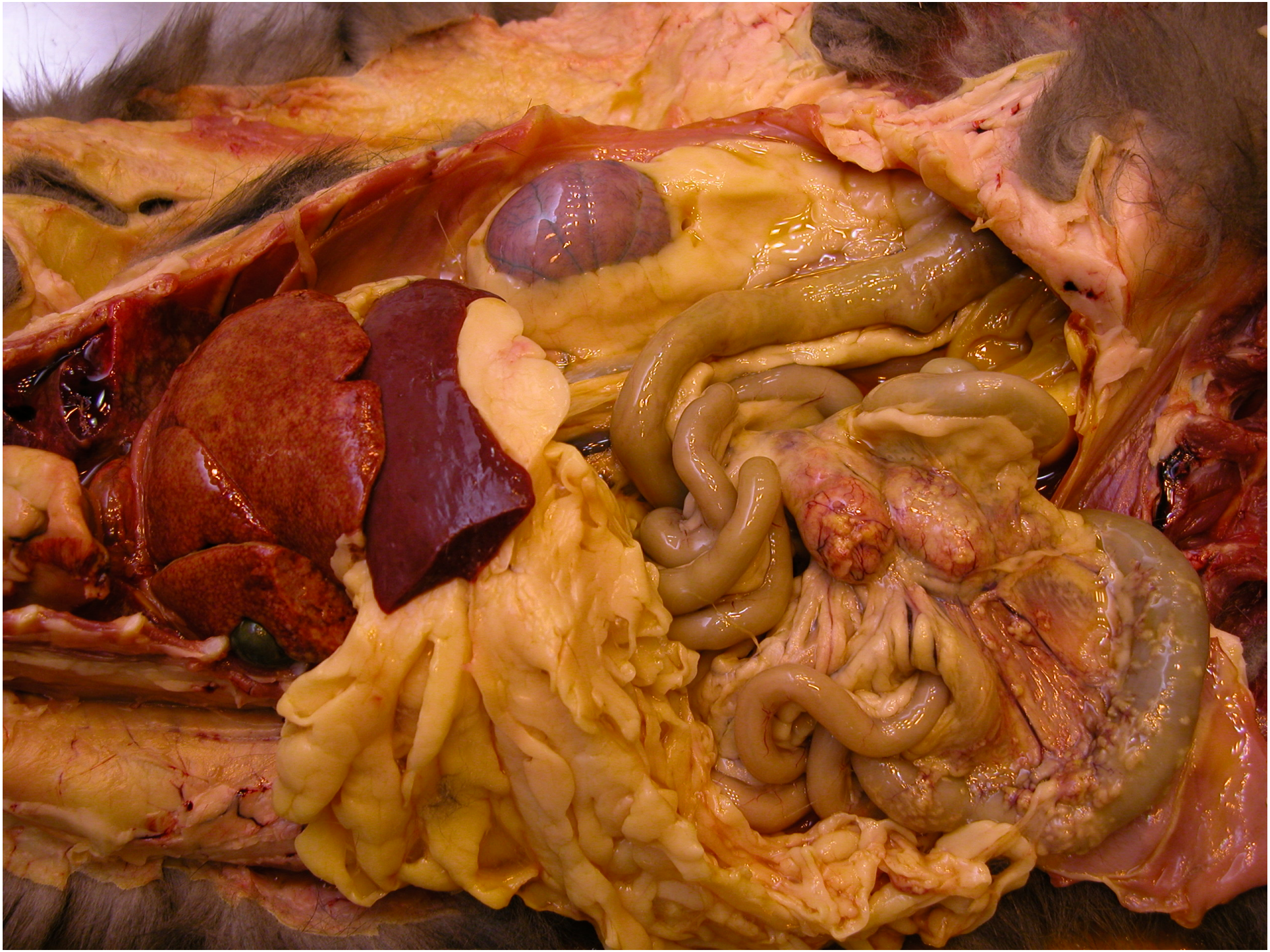
Abstract

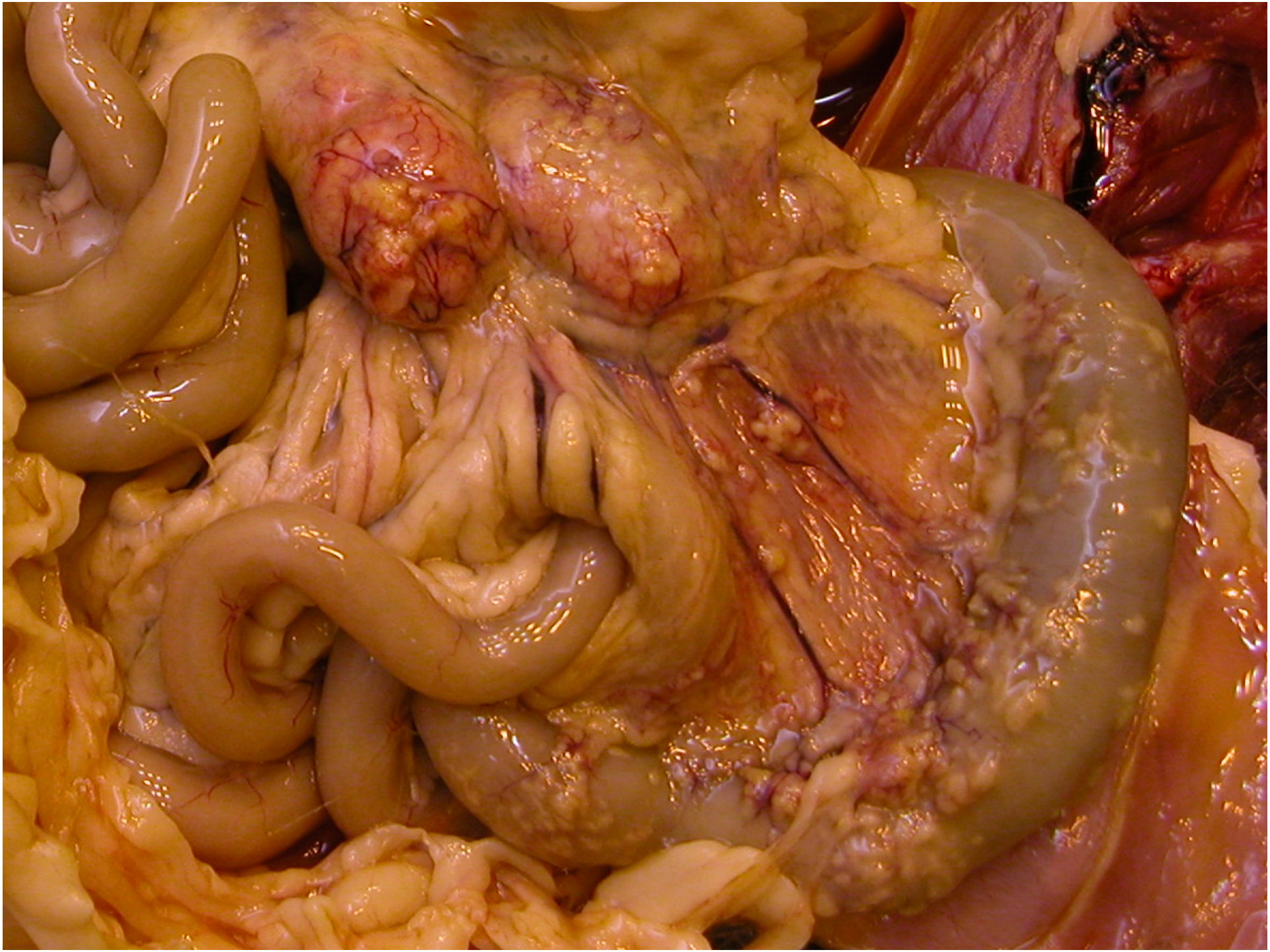
A novel coronavirus, designated as ferret enteric coronavirus (FECV), was identified in feces of domestic ferrets clinically diagnosed with epizootic catarrhal enteritis (ECE). Initially, partial sequences of the polymerase, spike, membrane protein, and nucleocapsid genes were generated using coronavirus consensus PCR assays. Subsequently, the complete sequences of the nucleocapsid gene and the last two open reading frames at the 3′ terminus of the FECV genome were obtained. Phylogenetic analyses based on predicted partial amino acid sequences of the polymerase, spike, and membrane proteins, and full sequence of the nucleocapsid protein showed that FECV is genetically most closely related to group 1 coronaviruses. FECV is more similar to feline coronavirus, porcine transmissible gastroenteritis virus, and canine coronavirus than to porcine epidemic diarrhea virus and human coronavirus 229E. Molecular data presented in this study provide the first genetic evidence for a new coronavirus associated with clinical cases of ECE.

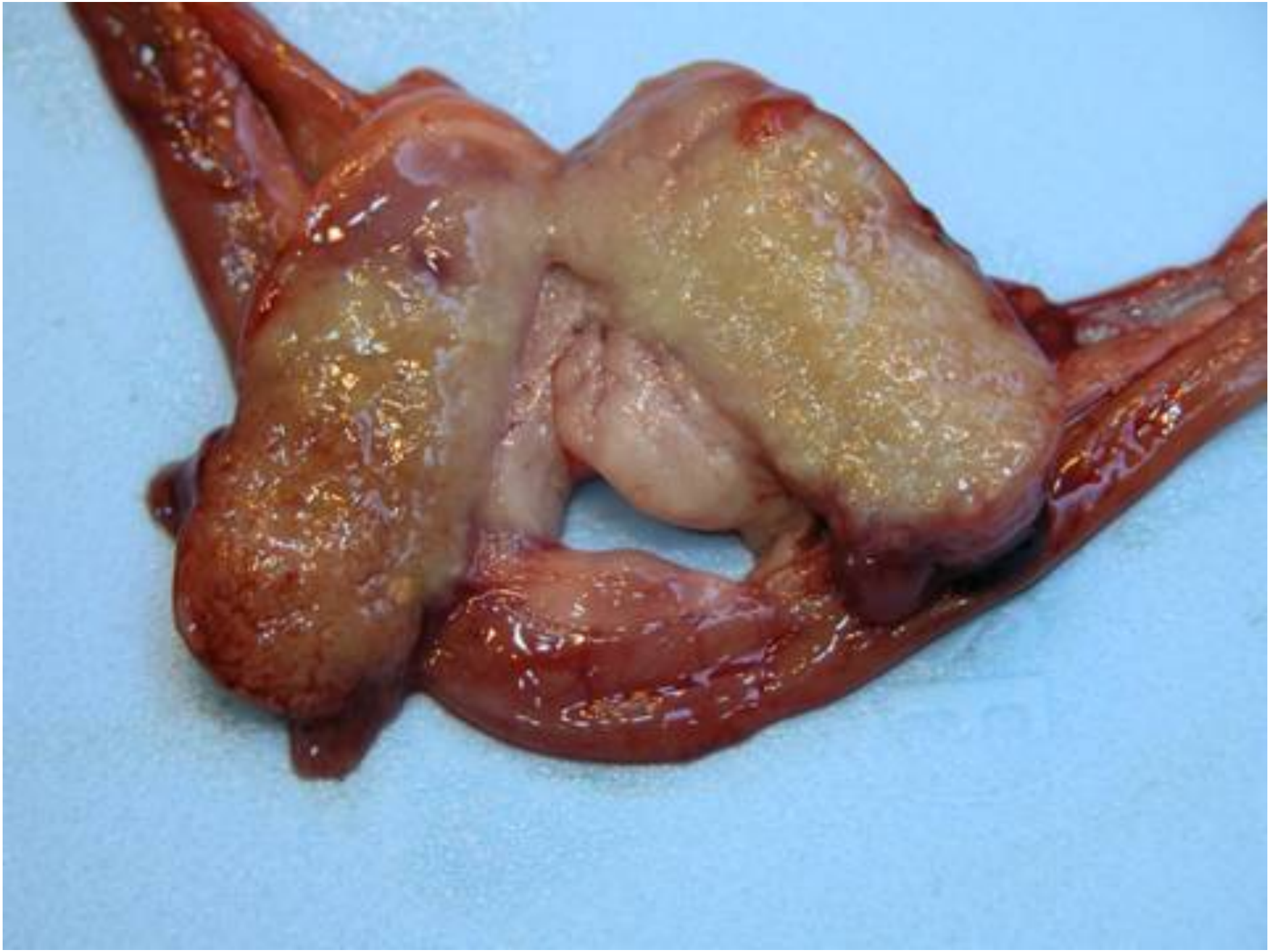
Keywords: Coronavirus; Epizootic catarrhal enteritis; Ferret; *Mustelo putorius furo*; Consensus PCR for coronaviruses

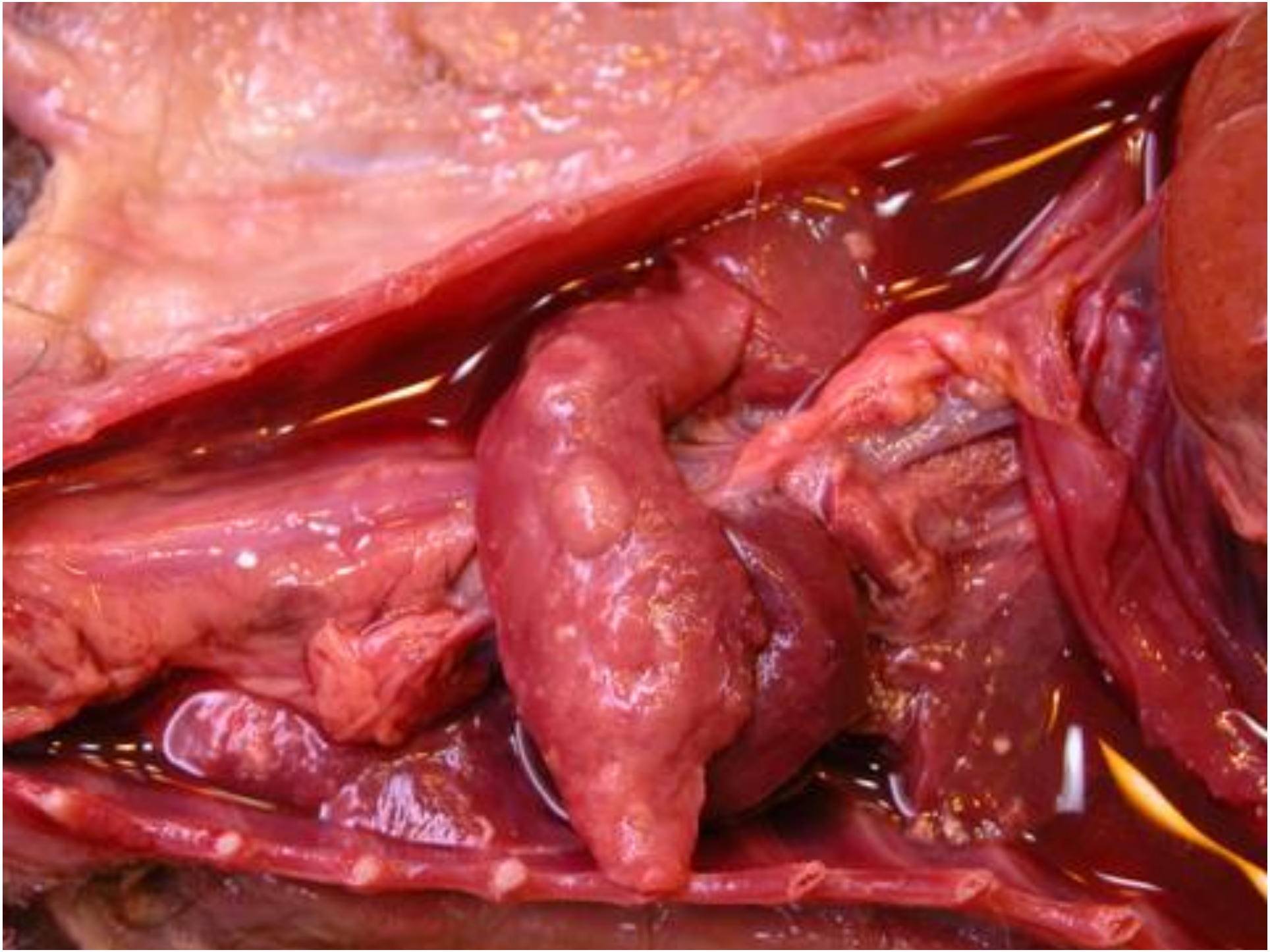
FIP-like Disease

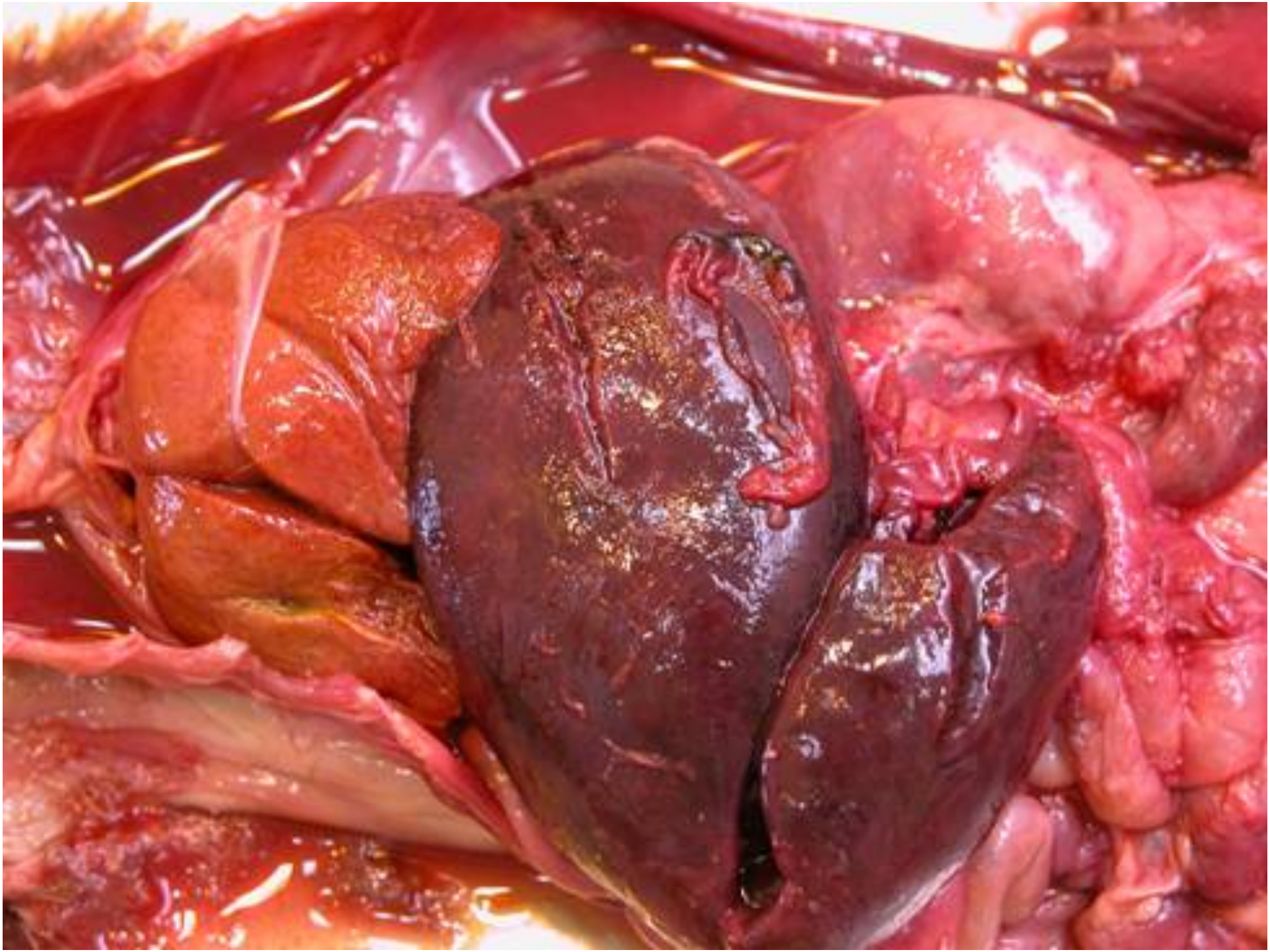
- First confirmed reports in 2002
- Pet ferrets in Spain and US
- 2-36 months old (m = 11 mo.)
- Common clinical findings:
  anorexia, weight loss, diarrhea
  large palpable intra-abdominal masses
- less frequent findings:
  hind limb paresis, CNS signs, vomiting, and dyspnea
- Frequent hematological findings:
  mild anemia, thrombocytopenia, hypergammaglobulinemia

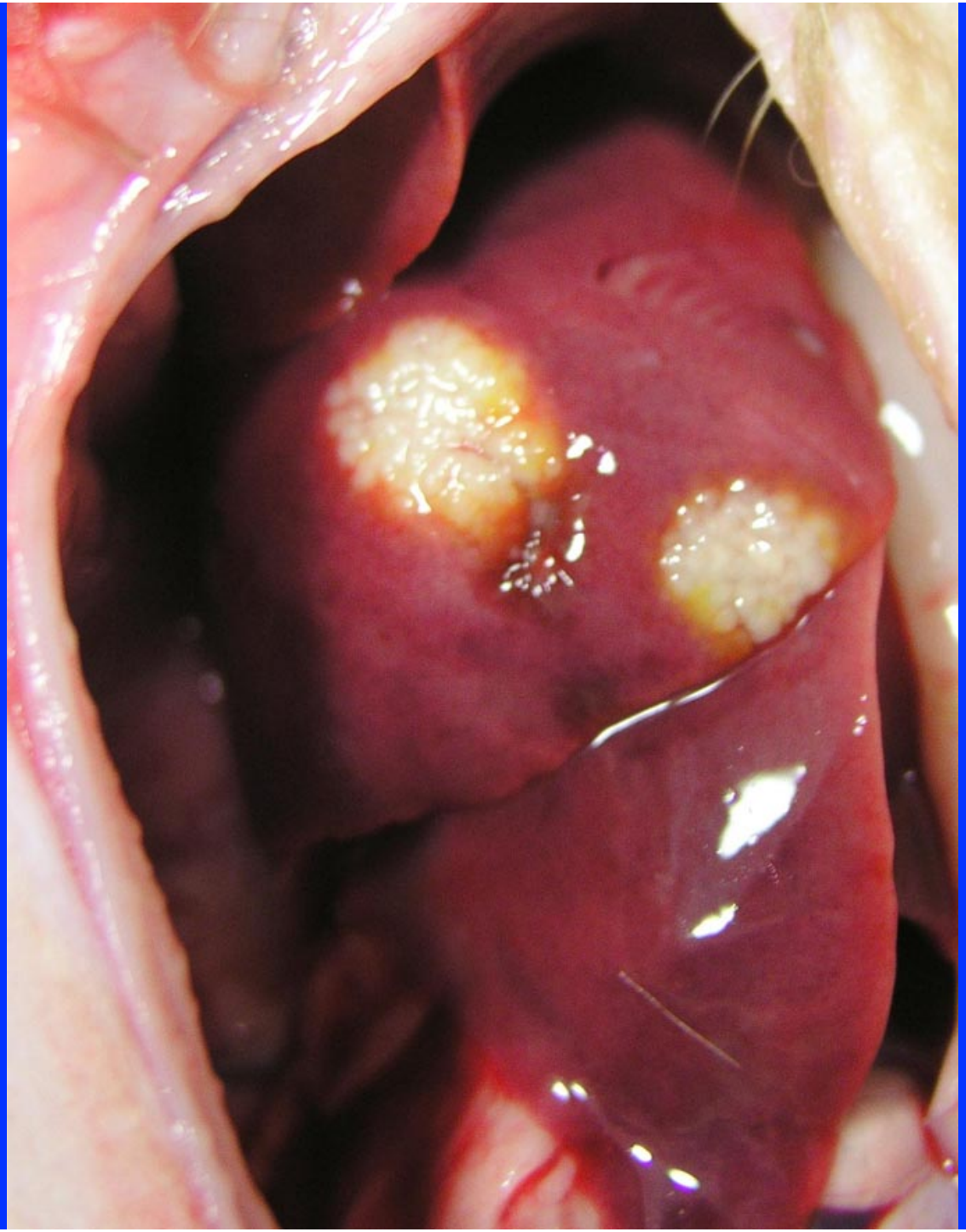


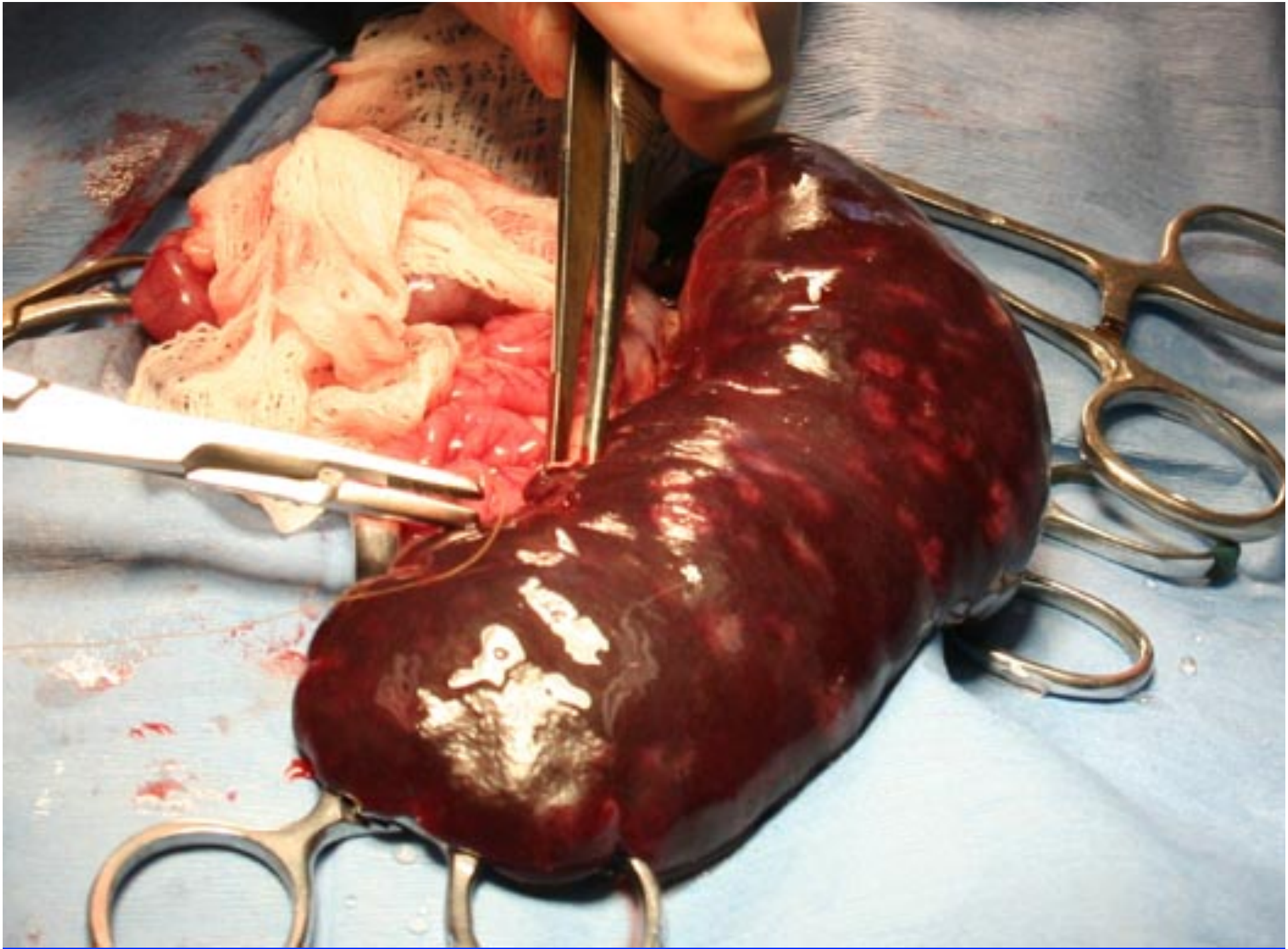


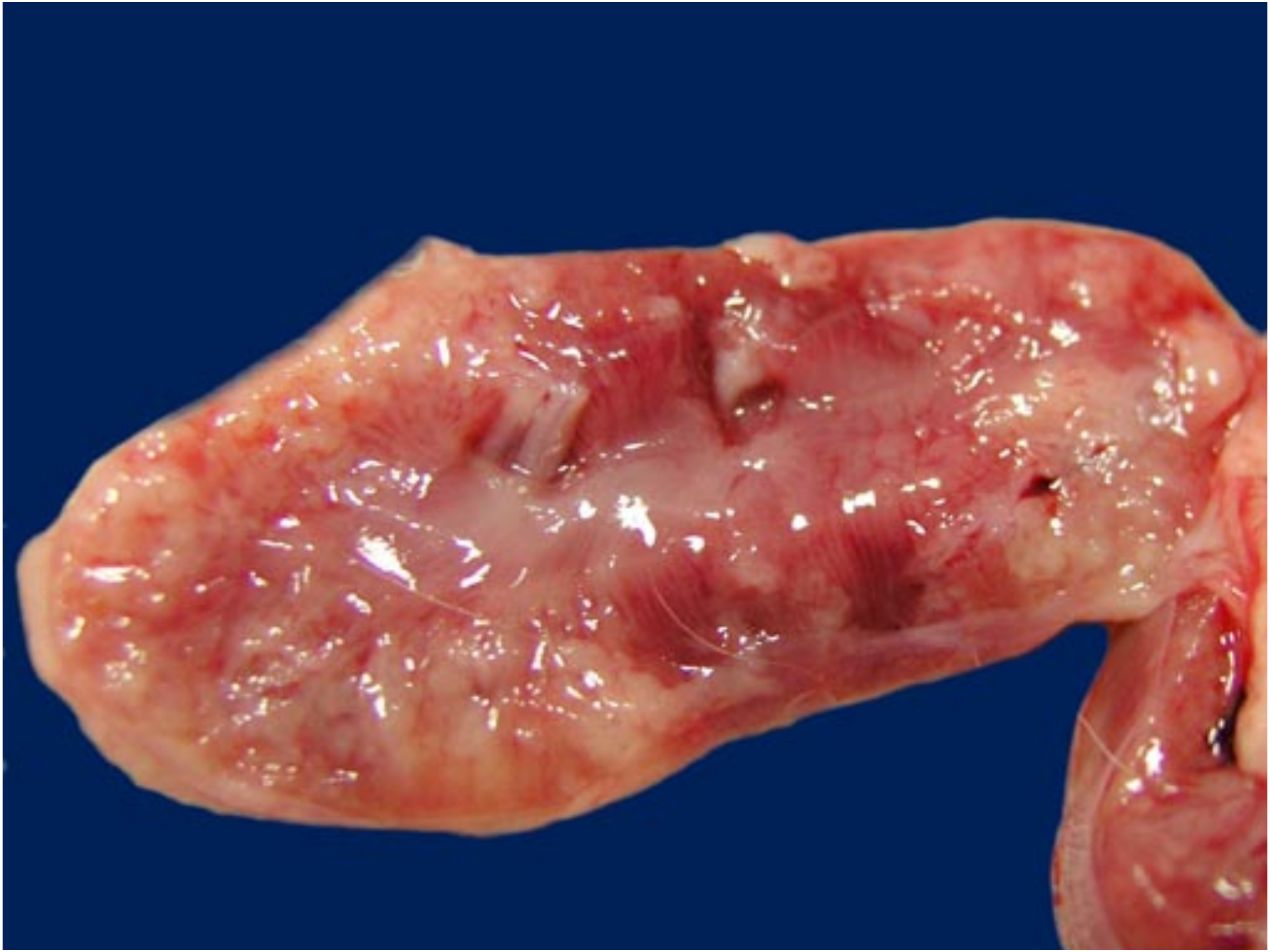


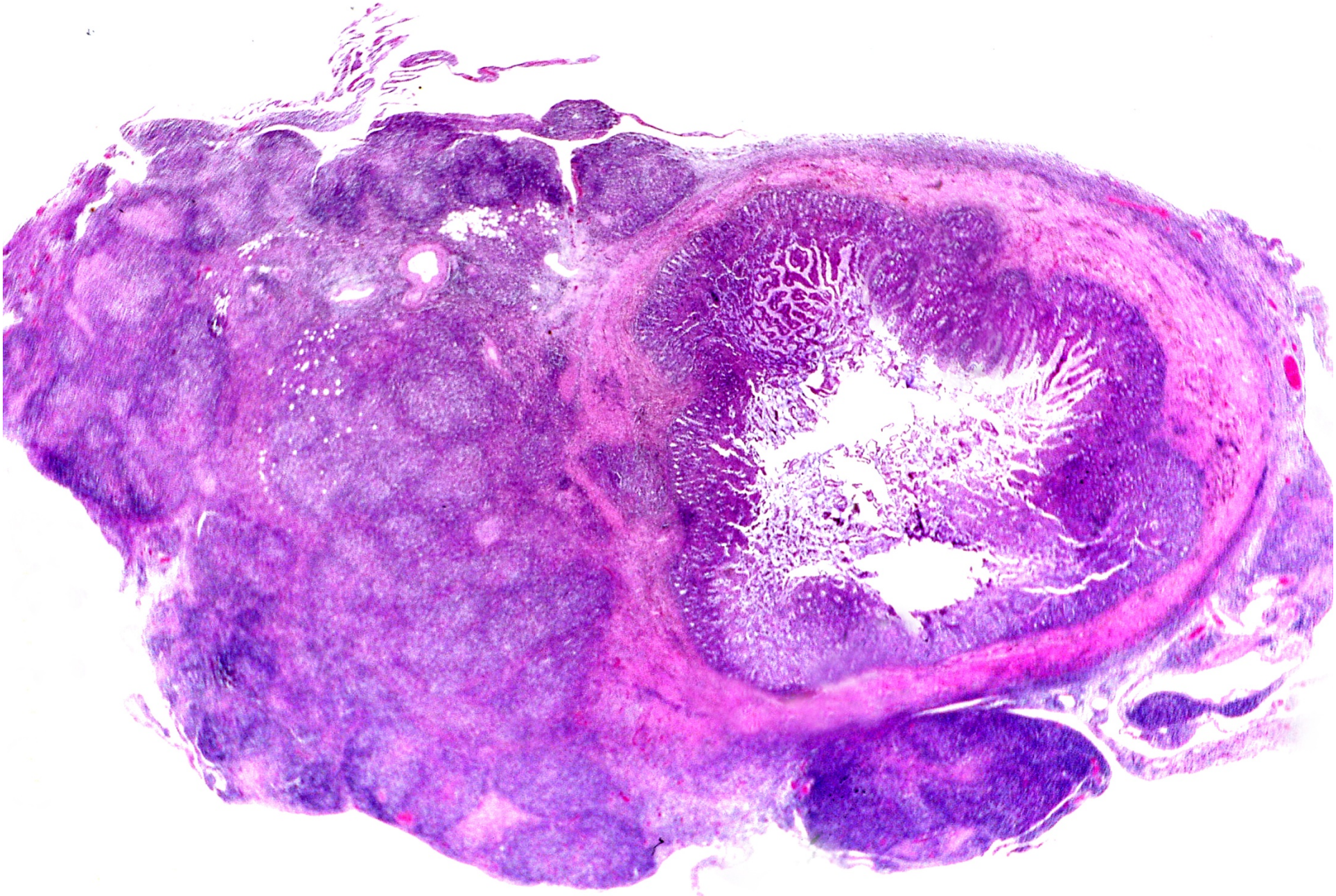


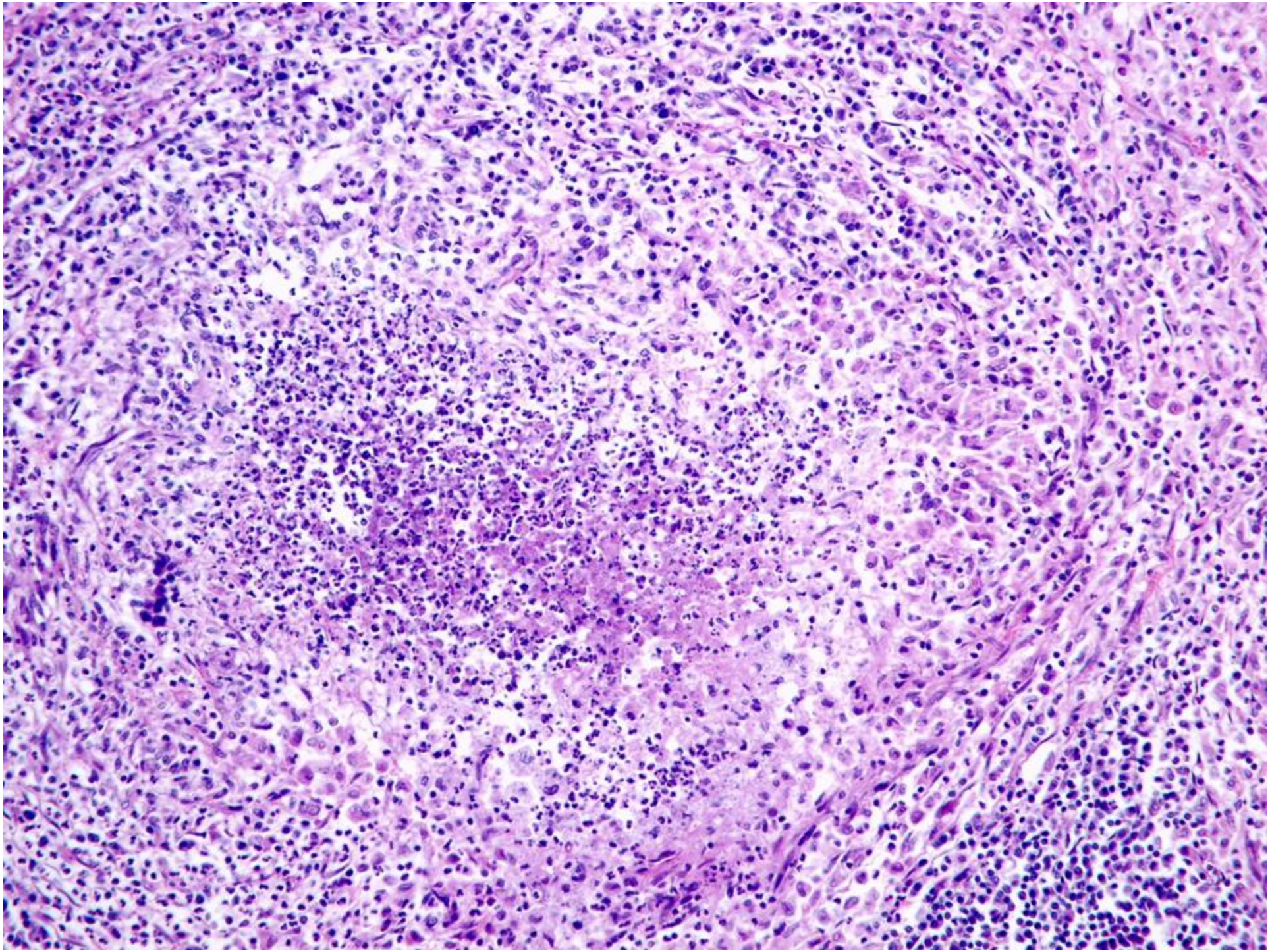


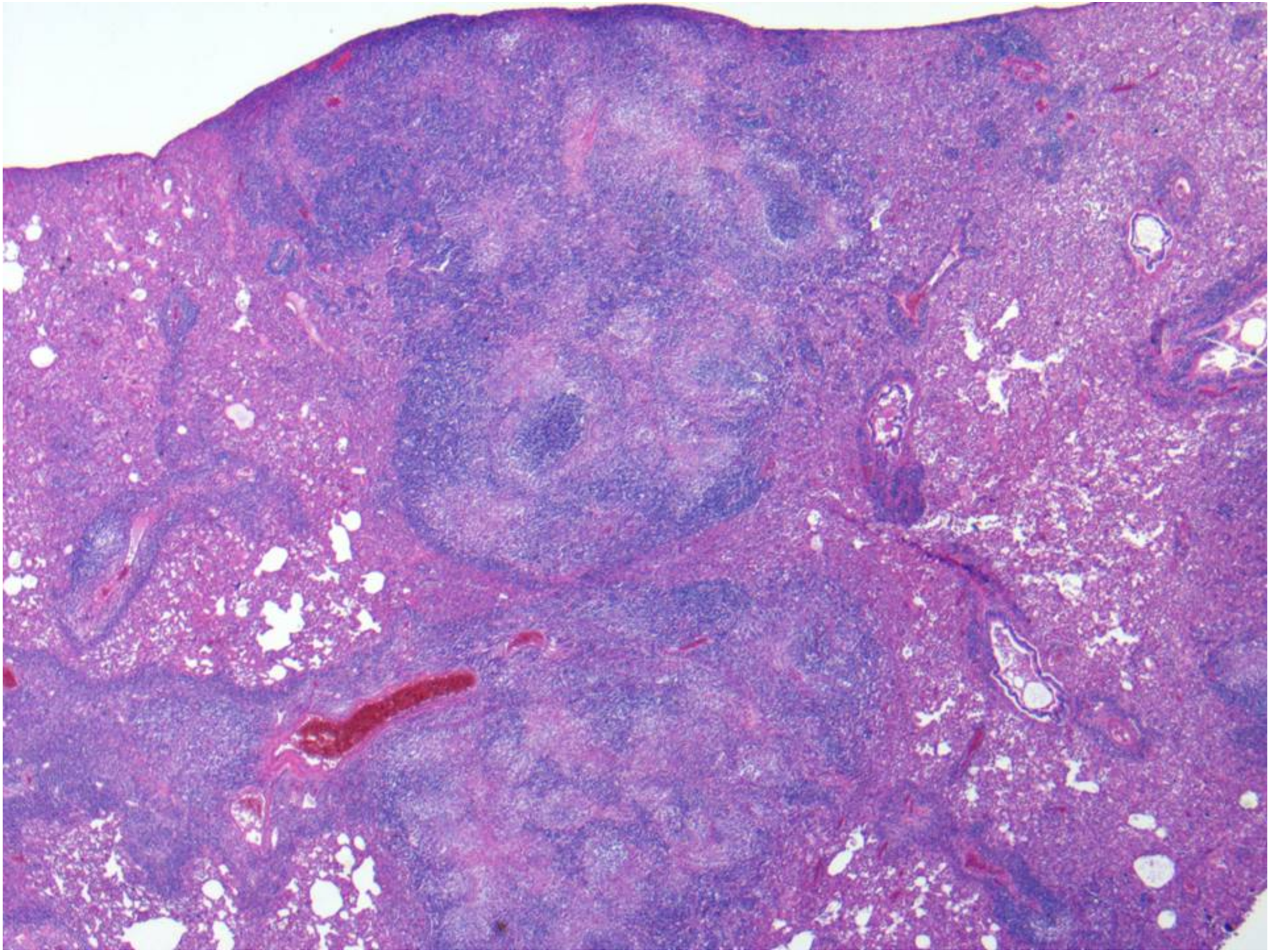


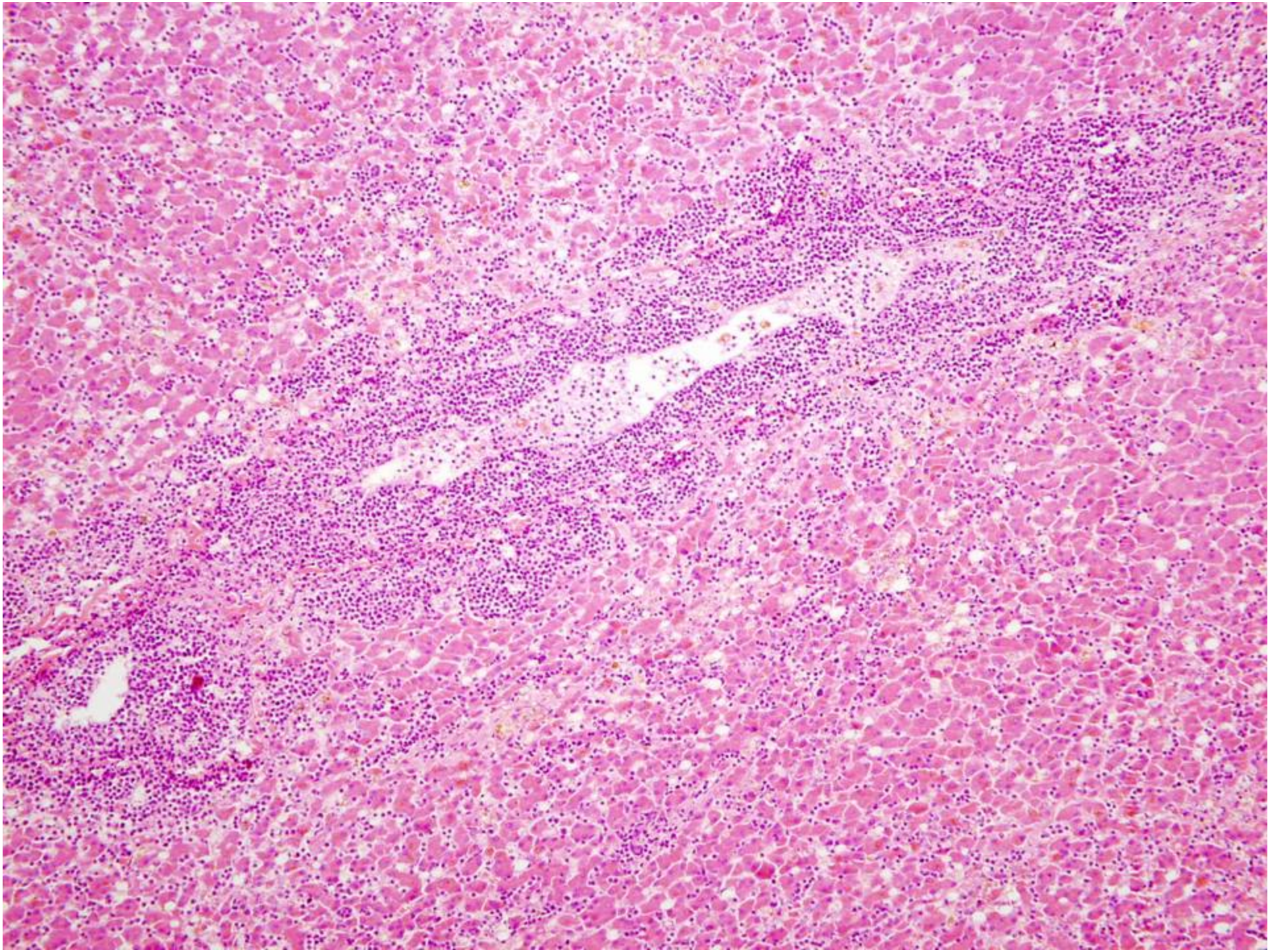


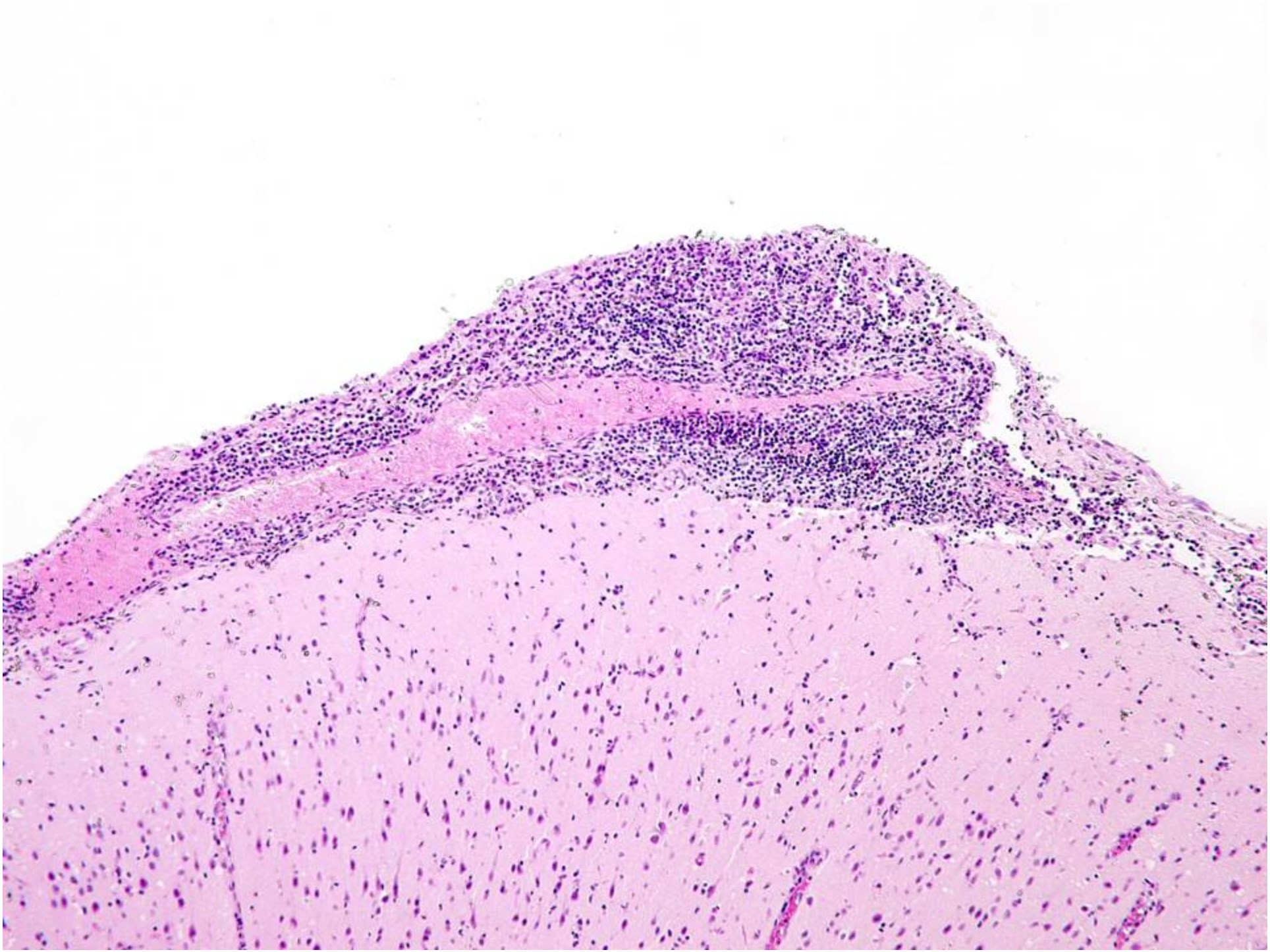




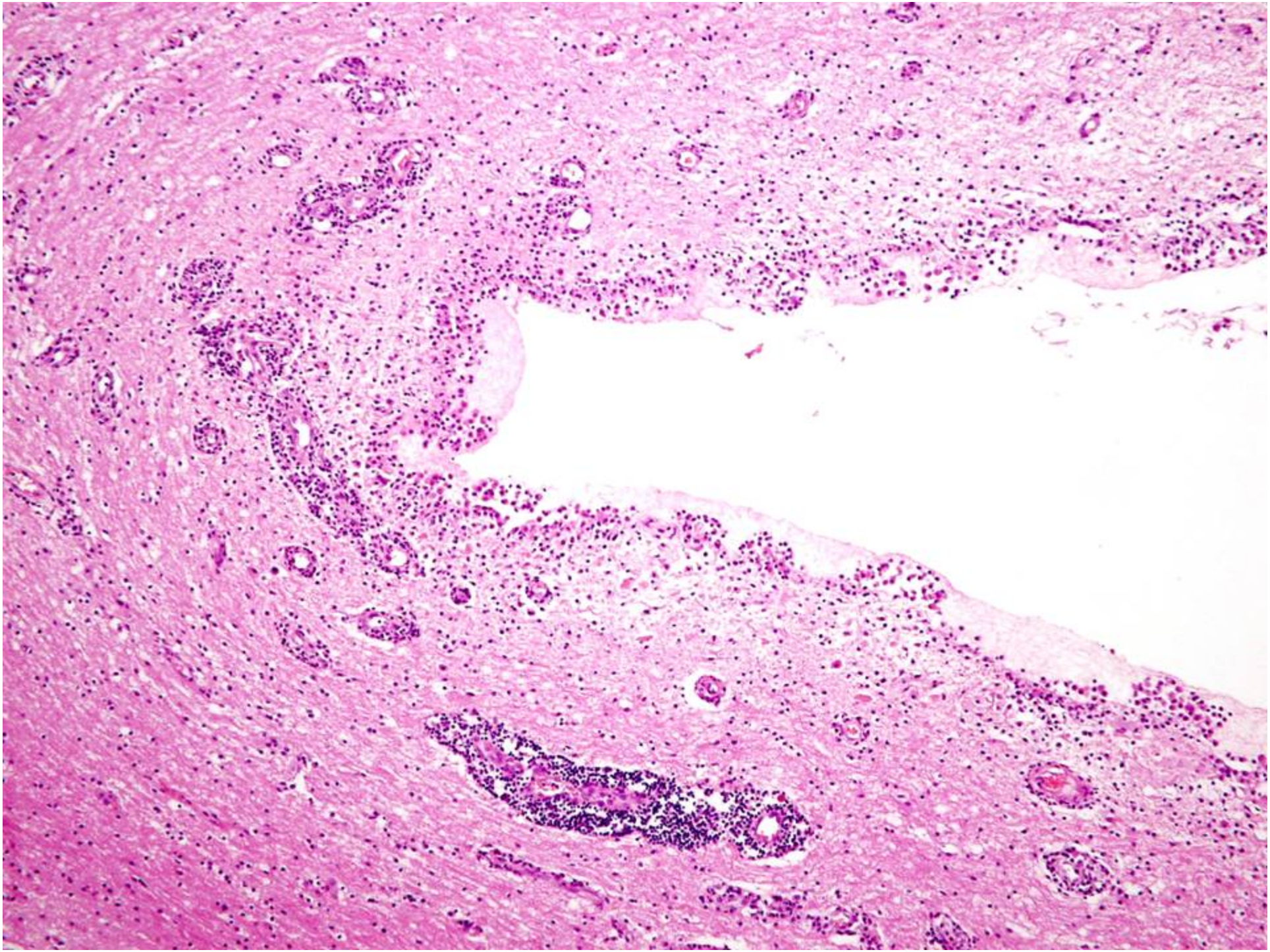


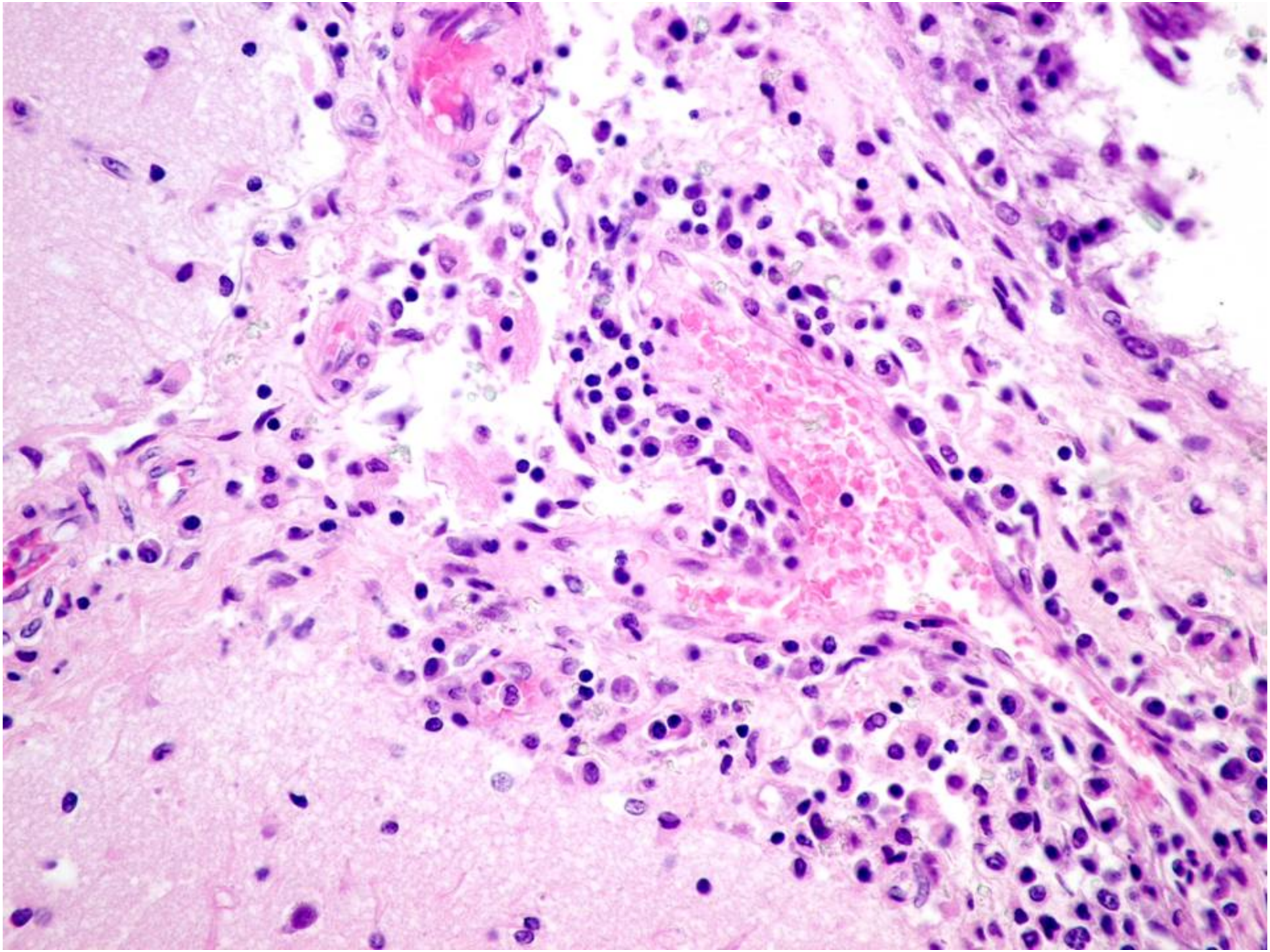






2011 Symposium International Ferret Congress





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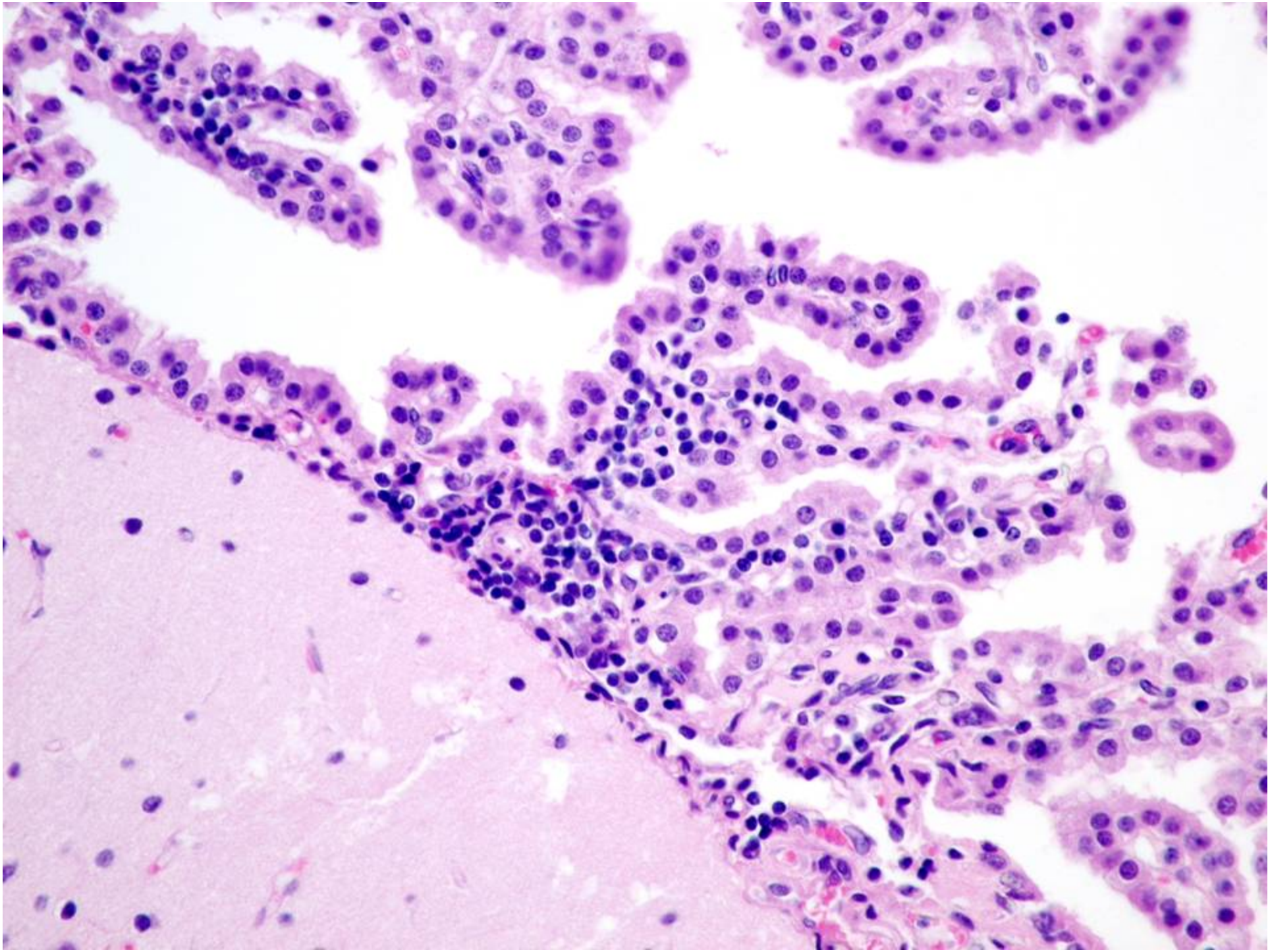
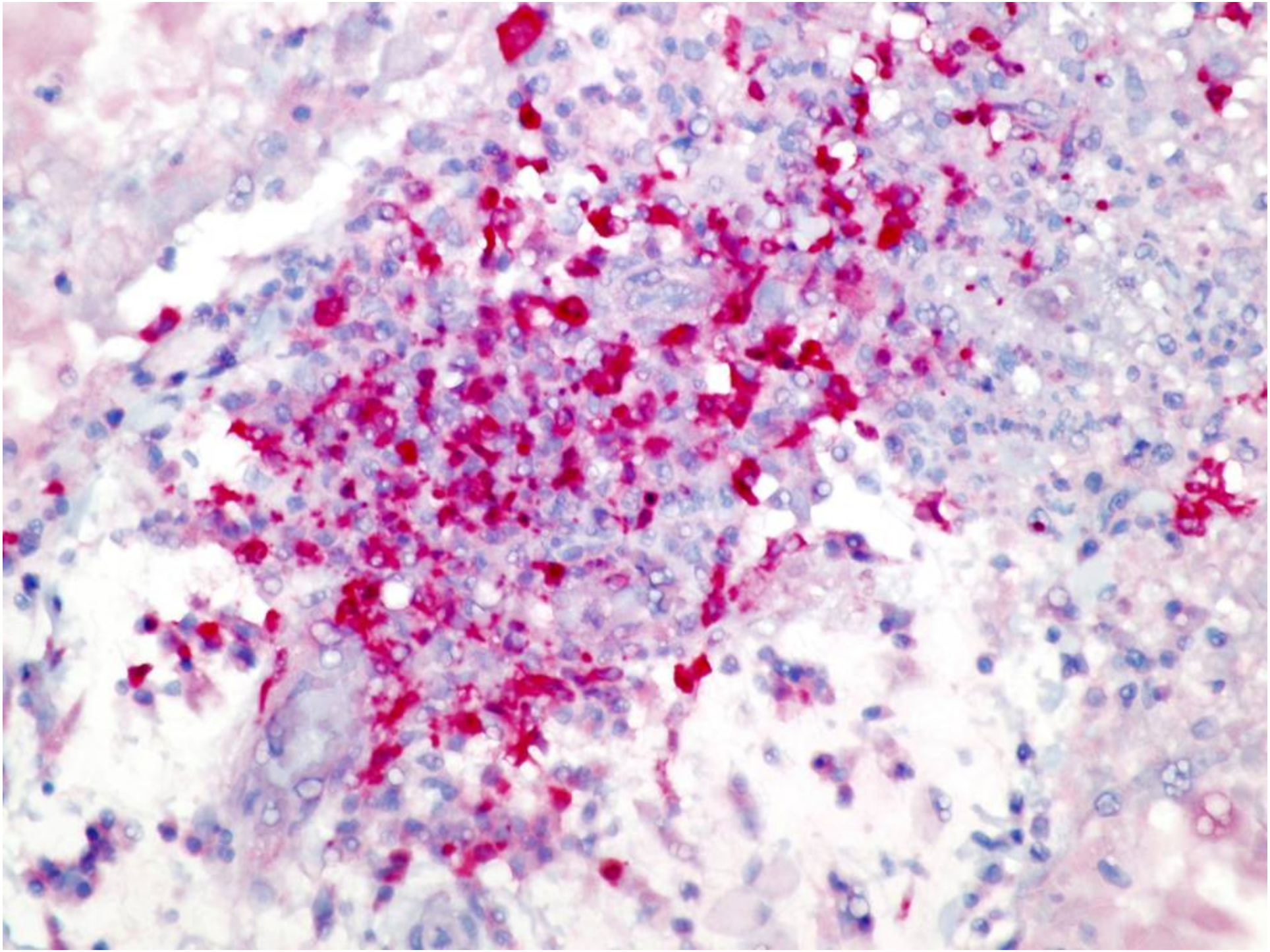
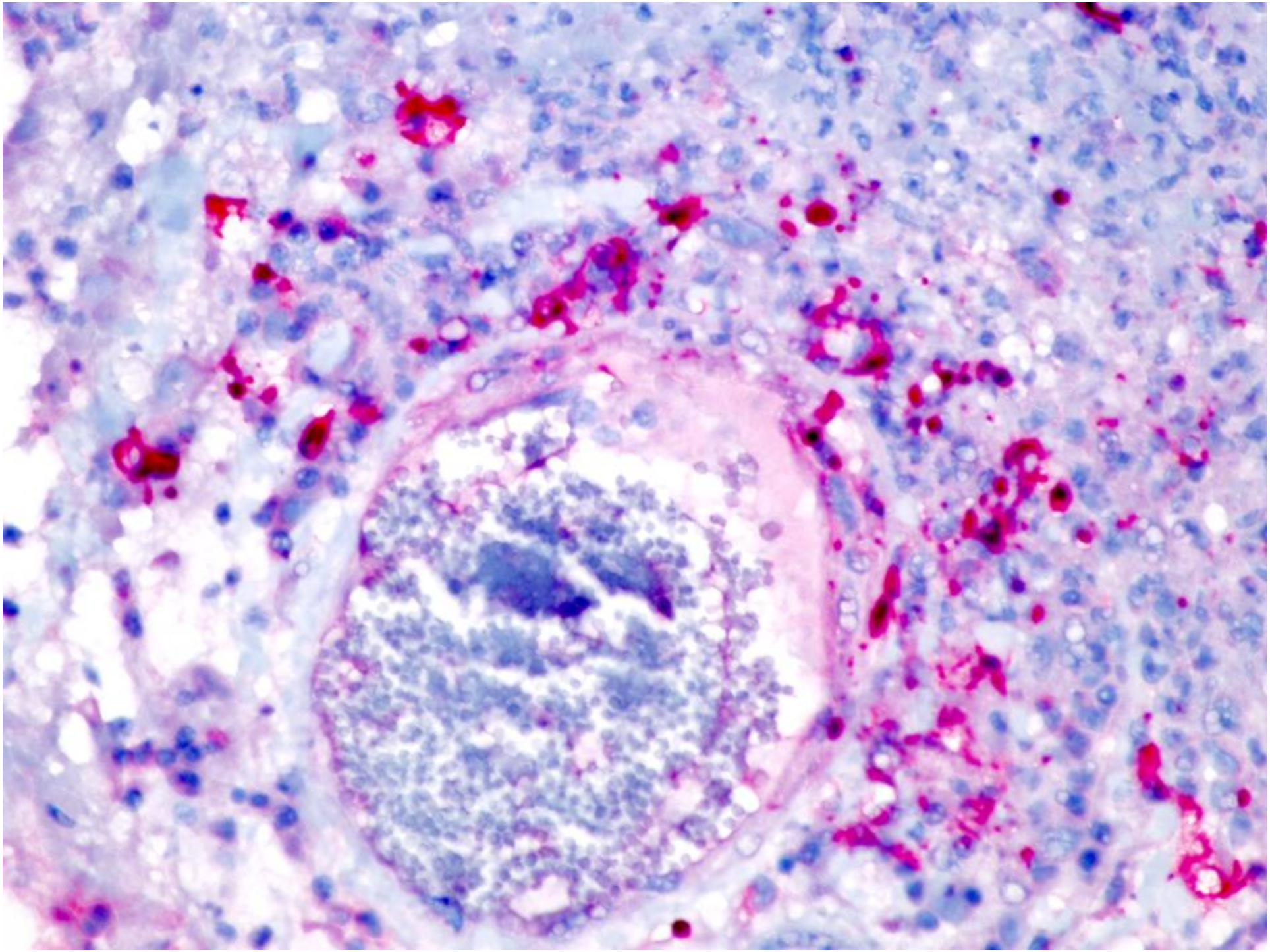



What’s is going on
?

Morphological Diagnosis

Multiple organs: Severe chronic multifocal to coalescing granulomatous to pyogranulomatous perivasculits and serositis





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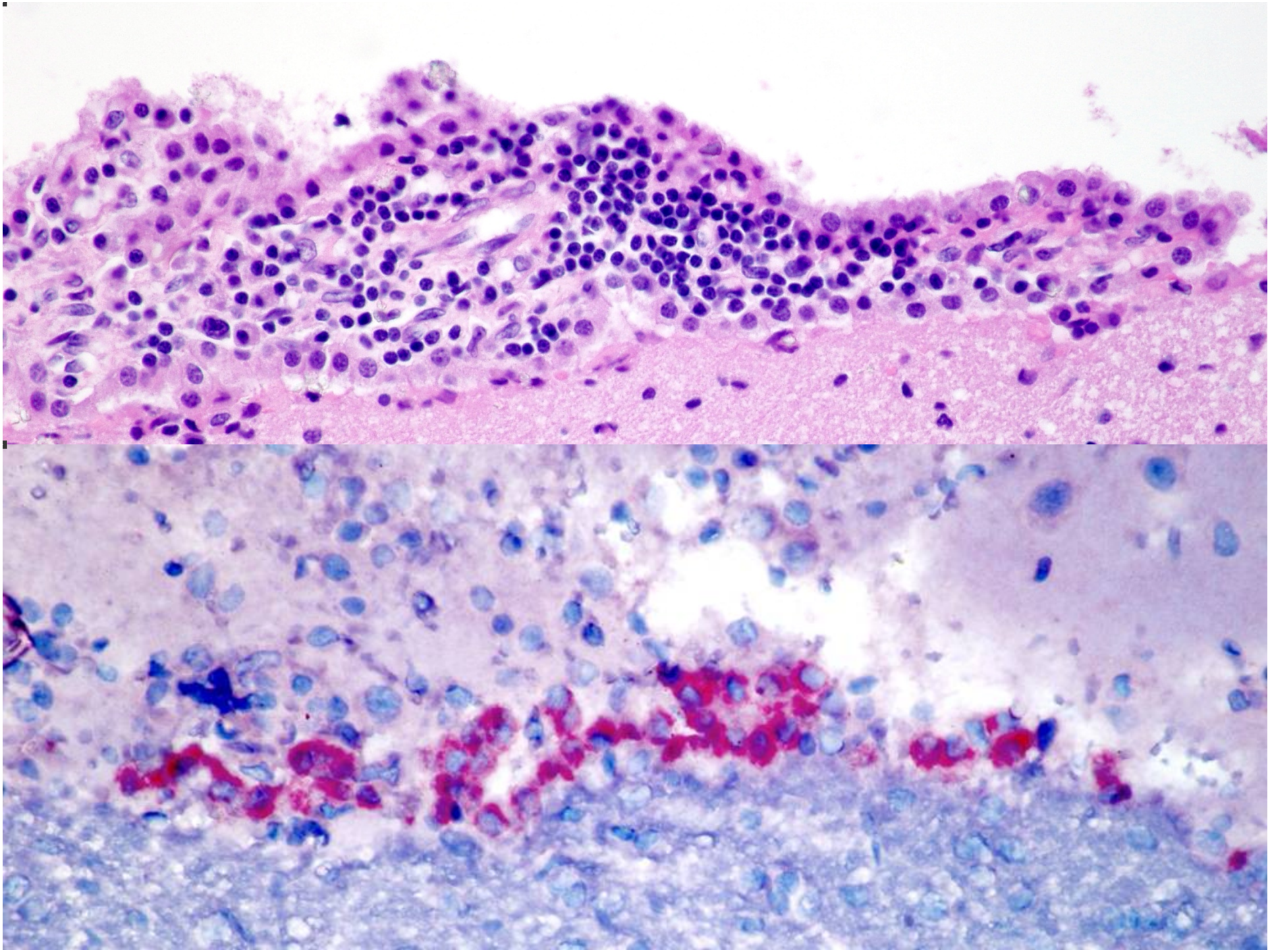

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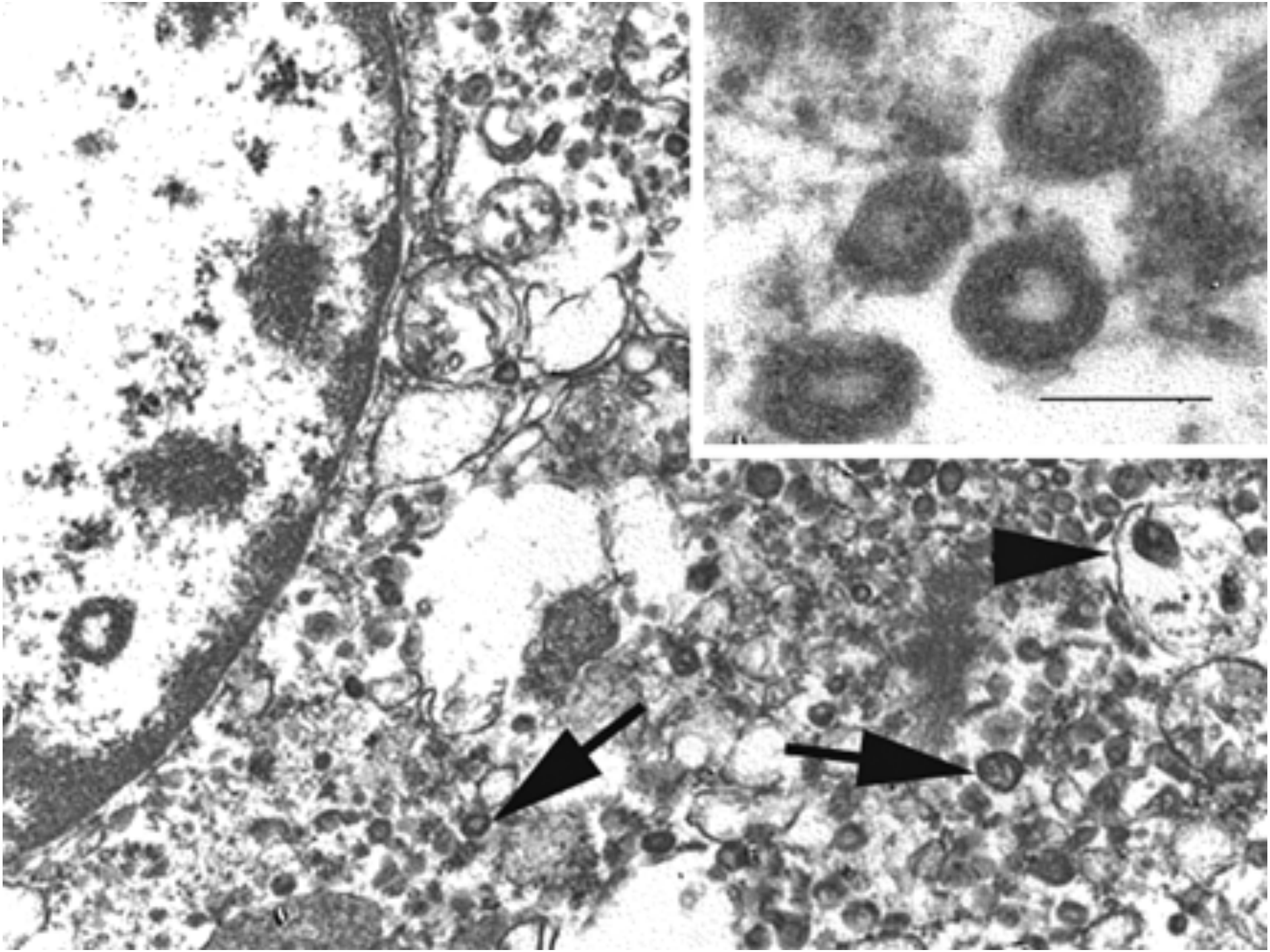
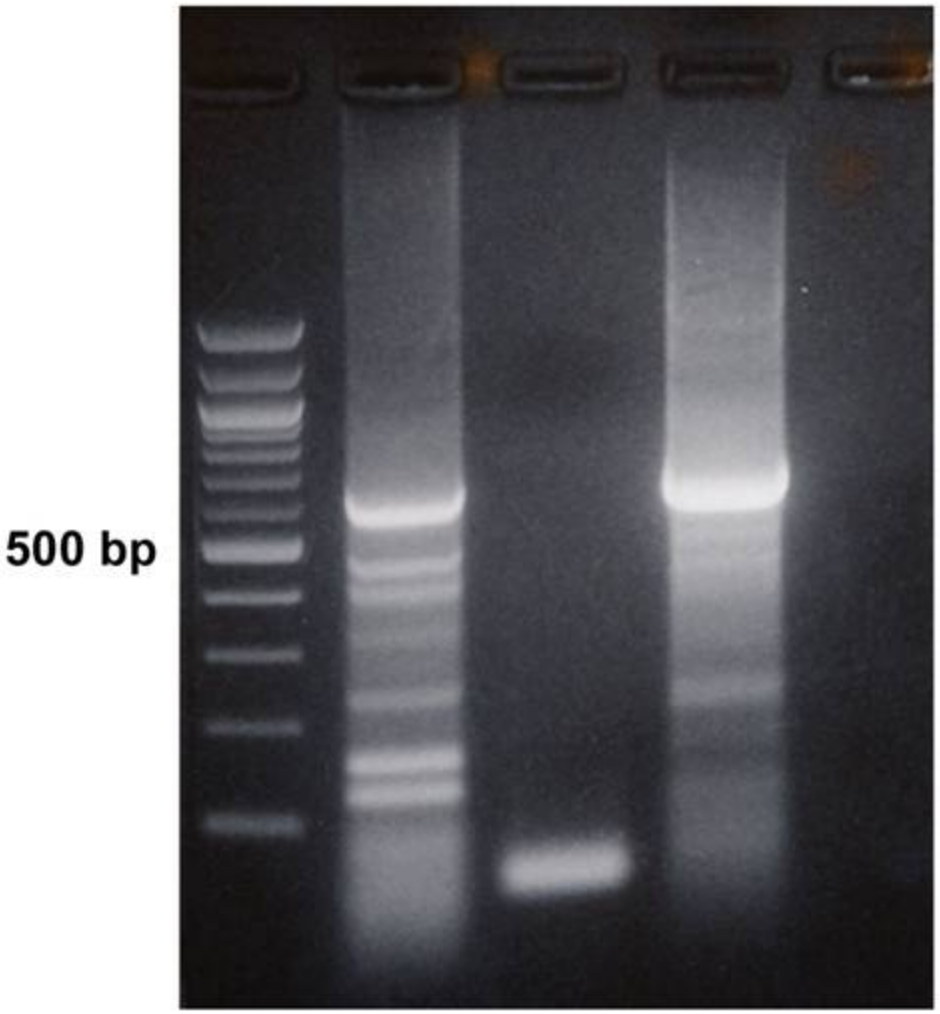


RT-PCR

- PCR for corona virus yielded 650bp fragment
- Sequencing showed amplified viral genome was distinct from FCoV and most closely related to FRECV (cause of ECE, epizootic catarrhal enteritis)



Outcome

- Not aware of positive cases that are still alive and doing well
- Prognosis probably similar to FIP

Vet Pathol **45**:236–246 (2008)

Clinicopathologic Features of a Systemic Coronavirus-Associated Disease Resembling Feline Infectious Peritonitis in the Domestic Ferret (*Mustela putorius*)

M. M. Garner, K. Ramsell, N. Morera, C. Juan-Sallés, J. Jiménez, M. Ardiaca, A. Montesinos, J. P. Teifke, C. V. Löhr, J. F. Evermann, T. V. Baszler, R. W. Nordhausen, A. G. Wise, R. K. Maes, and M. Kiupel

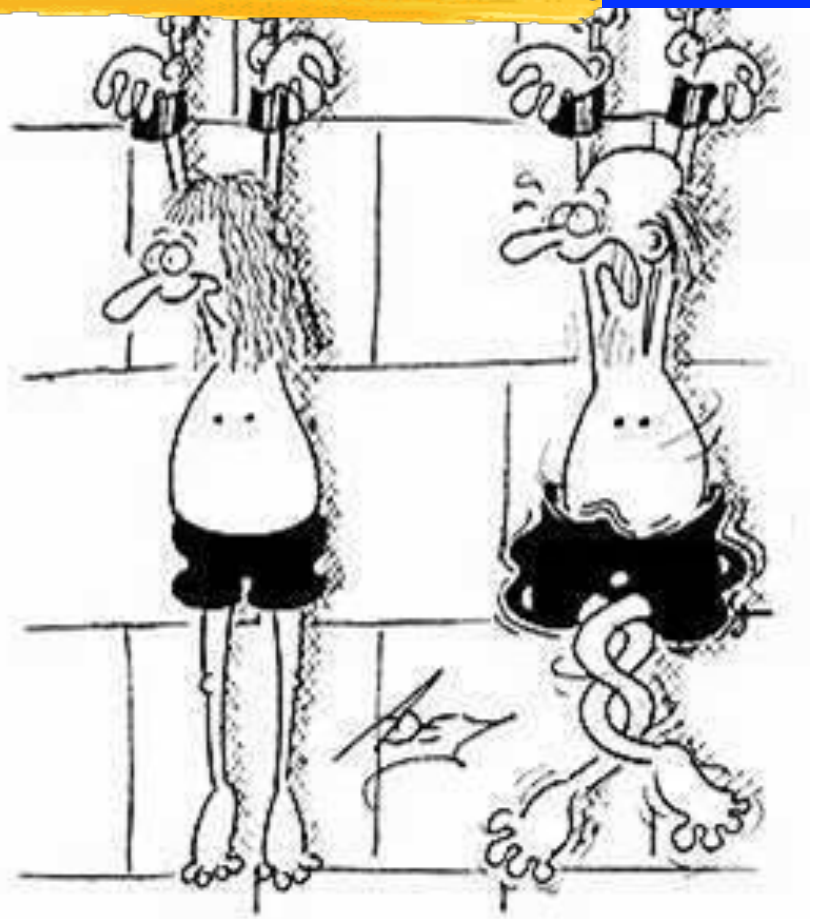
Northwest ZooPath, Monroe, WA (MMG); Southwest Animal Hospital, Beaverton, OR (KR); ConZOOlting Wildlife Management, Samalüs, Spain (CJS); Washington Animal Disease Diagnostic Laboratory, Pullman, WA (JFE, TVB); Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Isle of Riems, Germany (JPT); Department of Biomedical Sciences, College of Veterinary Medicine, Oregon State University, Corvallis, OR (CVL); California Animal Heath and Food Safety Laboratory, Davis, CA (RWN); and Diagnostic Center for Population and Animal Health, Lansing, MI (AGW, RKM, MK)

Abstract. From 2002 to 2007, 23 ferrets from Europe and the United States were diagnosed with systemic pyogranulomatous inflammation resembling feline infectious peritonitis (FIP). The average age at the time of diagnosis was 11 months. The disease was progressive in all cases, and average duration of clinical illness was 67 days. Common clinical findings were anorexia, weight loss, diarrhea, and large, palpable intra-abdominal masses; less frequent findings included hind limb paresis, central nervous system signs, vomiting, and dyspnea. Frequent hematologic findings were mild anemia, thrombocytopenia, and hypergammaglobulinemia. Grossly, whitish nodules were found in numerous tissues, most frequently the mesenteric adipose tissue and lymph nodes, visceral peritoneum, liver, kidneys, spleen, and lungs. One ferret had a serous abdominal effusion. Microscopically, pyogranulomatous inflammation involved especially the visceral peritoneum, mesenteric adipose tissue, liver, lungs, kidneys, lymph nodes, spleen, pancreas, adrenal glands, and/or blood vessels. Immunohistochemically, all cases were positive for coronavirus antigen using monoclonal antibody FIPV3-70. Electron microscopic examination of inflammatory lesions identified particles with coronavirus morphology in the cytoplasm of macrophages. Partial sequencing of the coronavirus spike gene obtained from frozen tissue indicates that the virus is related to ferret enteric coronavirus.

Key words: Coronavirus; feline infectious peritonitis; ferrets; immunohistochemistry; PCR.

The Main Question

- Novel ferret coronavirus?
- Strain variation of FRECV
- Mutated FRECV
  - Within the animal
  - Circulating strain



"Gosh, I think that guard who put his pet ferret down your pants really likes you."

The Objective

Investigate the genetic relatedness of FRECV and FRSCV

2 ferret coronaviruses: FRECV and FRSCV

2 phenotypes: enteric and systemic

2 genotypes: FRECV-like and FRSCV-like

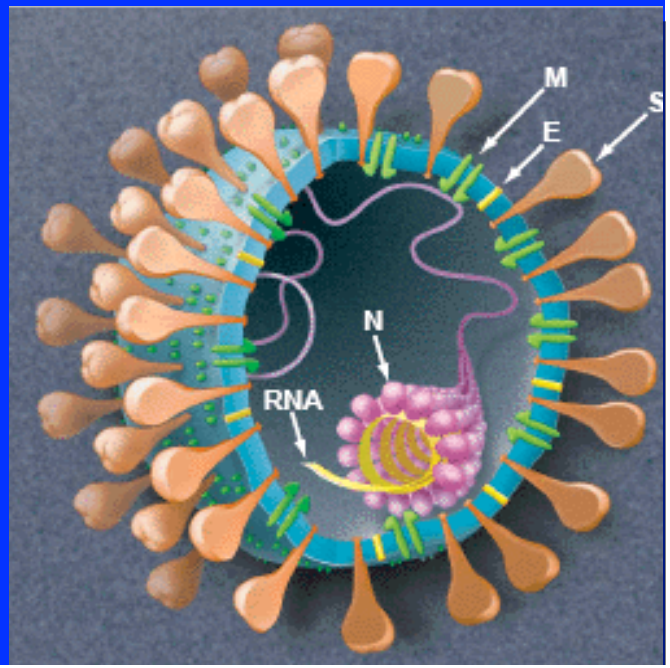
Is genotype consistently associated with phenotype?

3’ Genomic End Sequencing Strategy

Group 1 coronavirus genome (TGEV)

◄ - 8.6 kb - ►

- Consensus RT-PCR assays for coronavirus:
  - S ~600 bp
  - M to N ~700 bp
- 3’ RACE (N to 3’ UTR)
- S to M RT-PCR
- CODEHOP (consensus degenerate hybrid oligo primers):
  - 3’ pol 1b to S, in 3 overlapping fragments



Genomic Organization of FRSCV and FRECV

Group 1 coronavirus genome (TGEV)

- Consistent with coronavirus genome organization
- Non-structural gene 3 region has a single ORF (3c-like)
- FCoVs and CCVs have 3 ORFs in gene 3 region = 3a, 3b, 3c
- 3x-like ORF found instead of ORF 7a in gene 7 region
- ORF 3x is located within the ORF 3abc cluster of CCV and FCoV

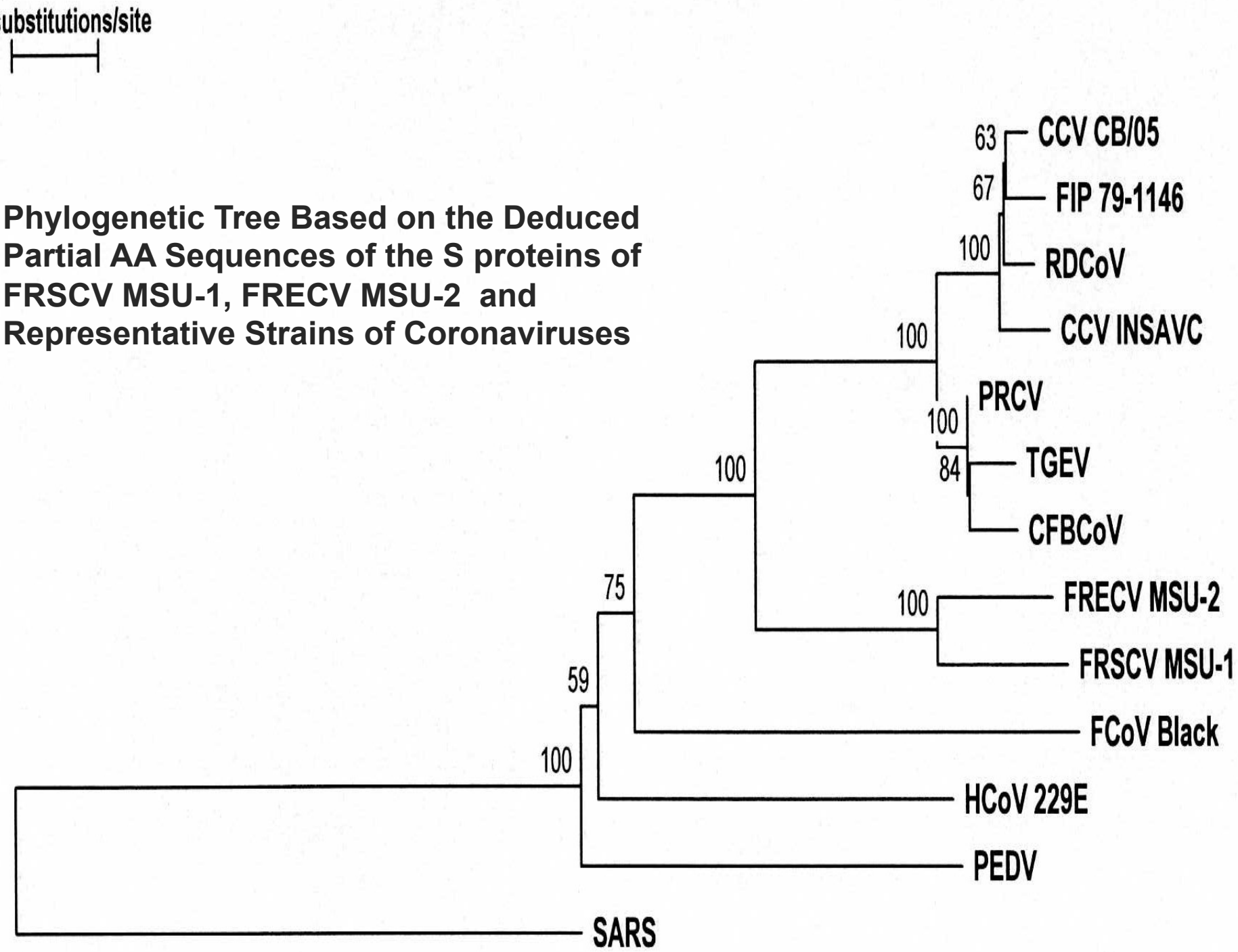
Comparison of the 3'Genomic Ends of FRSCV MSU-1 and FRECV MSU-2

Gene/Protein Name	ORF length in bp		No of amino acids		% amino acid similarity	% nucleotide similarity
	FRSCV	FRECV	FRSCV	FRECV		
Pol 1b*	360	360	119	119	96.6	97.5
S	4,374	4,350	1457	1449	80.3	79.5
ORF 3	252	744	83	247	96.3**	96.4**
E	249	249	82	82	87.8	91.2
M	792	792	263	263	96.6	97.7
N	1,125	1,125	374	374	95.2	96.5
3x-like	225	225	74	74	91.9	96.9
7b	615	615	204	204	93.6	96.1

Percentage AA Similarities of FECV MSU-1 Proteins to FSCV MSU-1 and FECV MSU-2

Protein	% Similarity to FRSCV MSU-1	% Similarity to FRECV MSU-2
Partial S	77.4	96.4
Partial M	100	100
N	95.2	100
3x-like	90.5	98.6
7b	93.6	100

Phylogenetic Tree Based on the Deduced Partial AA Sequences of the S proteins of FRSCV MSU-1, FRECV MSU-2 and Representative Strains of Coronaviruses



Coronavirus Virulence Determinants

- **S gene C-Terminus**
  The C-terminal domain of the S protein of feline coronavirus strains determines their macrophage tropism
  Rottier et al. J. Virology 79:14122-14130 (2005)
  FRSCV and FRECV strains examined have consistent differences

- **ORF 3c**
  Deletions are associated with FIPV strains
  Pedersen J. Feline Medicine and Surgery 11:225-258 (2009)
  FRSCV strains examined had a deletion

- **ORF 7b**
  Deletions in ORF 7b are not constrained to feline enteric coronavirus
  Lin et al. J. Feline Medicine and Surgery 11:413-419 (2009)
  No deletions in FRSCV or FRECV

Sequencing of a Highly Conserved Region of the 3' end of S gene (Upstream of the Fusion Peptide)

- In feline coronaviruses (Rottier et al., 2005, J. Virol., 79): "D-to-A" substitution (contributing to macrophage tropism)

FECV 79-1683	MVLPGVAN**D**DKMTMYT
FIPV 79-1146	MVLPGVAN**A**DKMTMYT

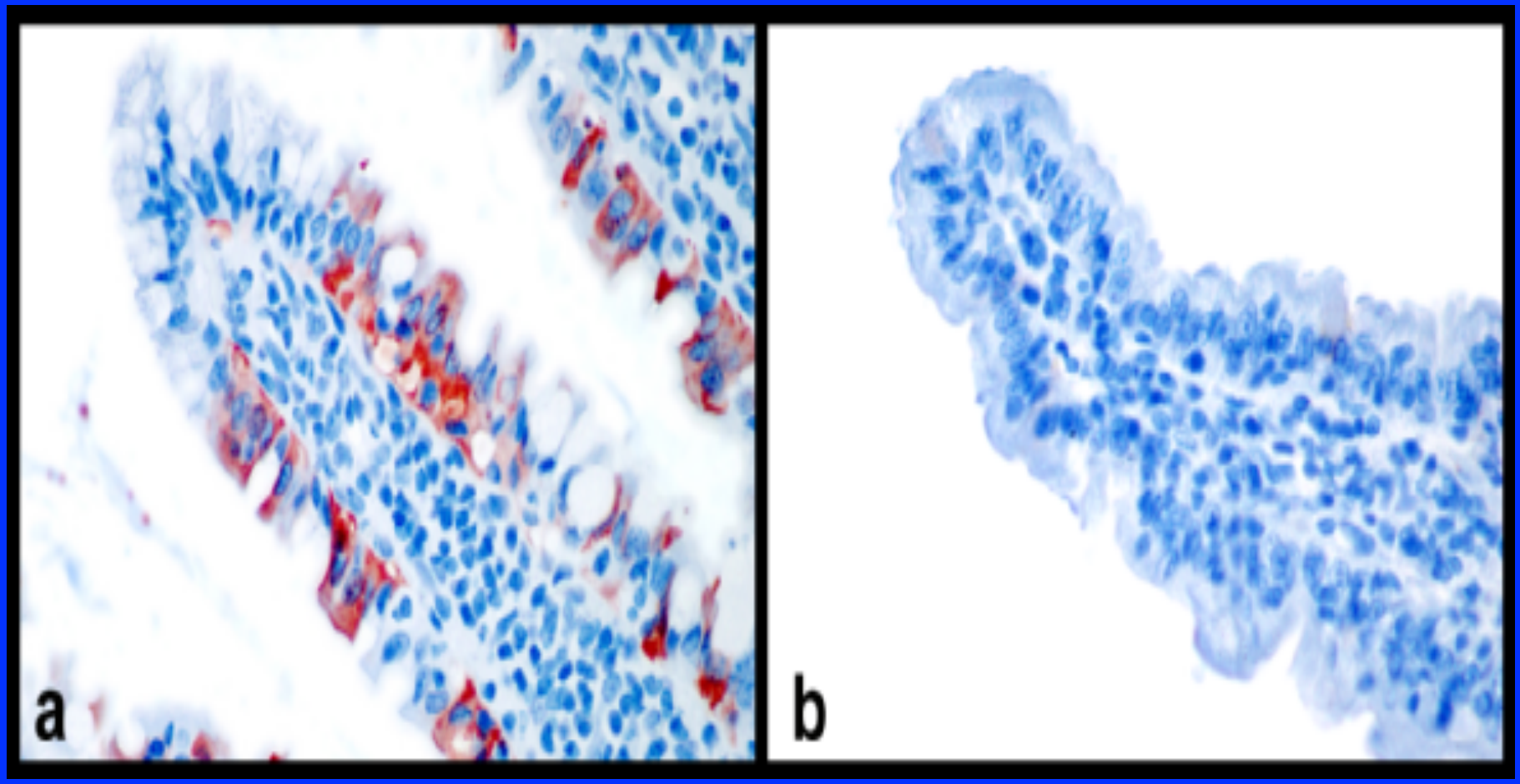
- In ferret coronaviruses: "S-to-A" substitution observed

14 FRECV strains	MVLPGVVN**S**DKMAMYT
5 FRSCV strains	MVLPGVVN**A**DKMAMYT

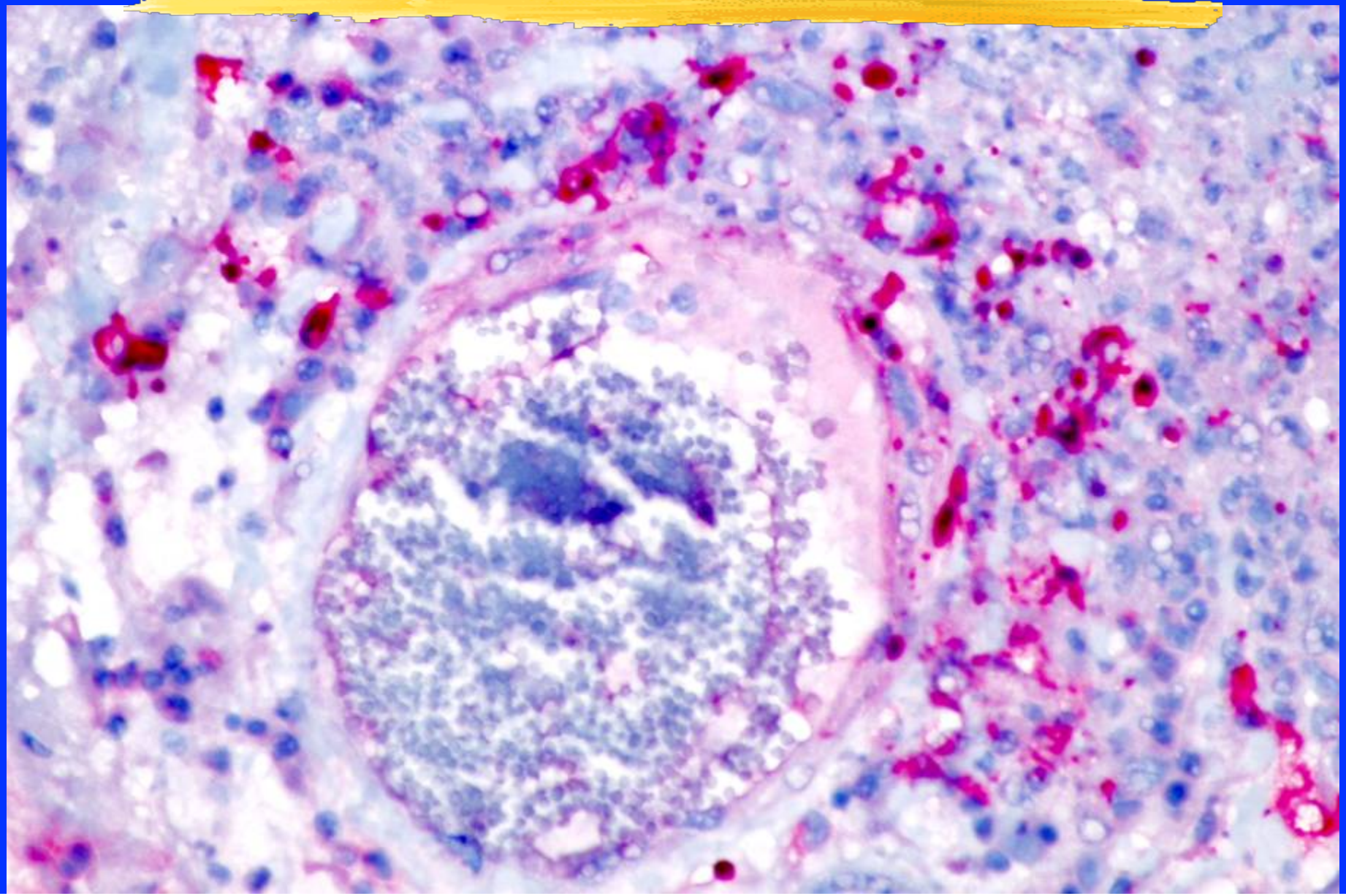
Laboratory Diagnostic Methods

- Immunohistochemistry (IHC)
- In situ hybridization (ISH)
- RT-PCR

FRECV Detection by IHC

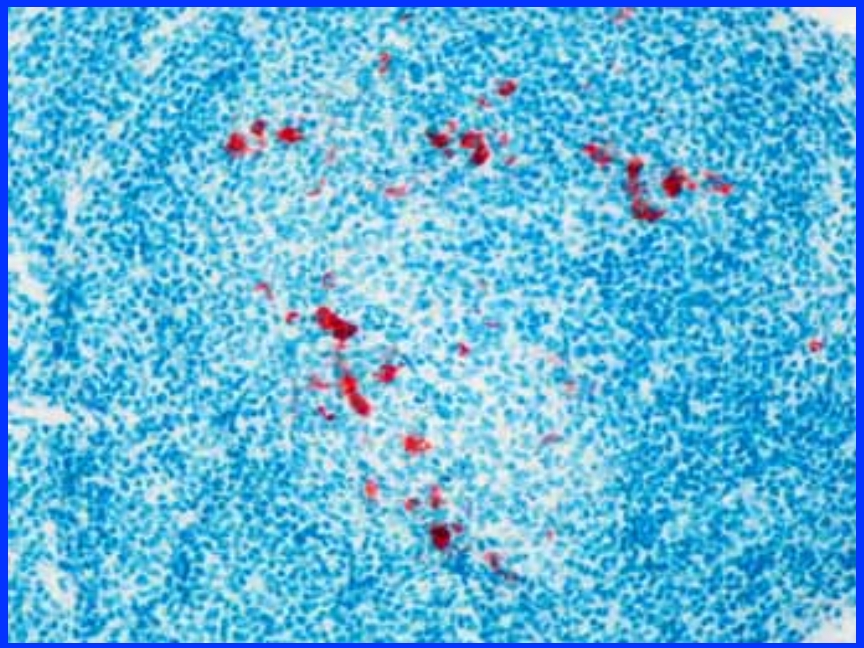


FRSCV Detection by ICH

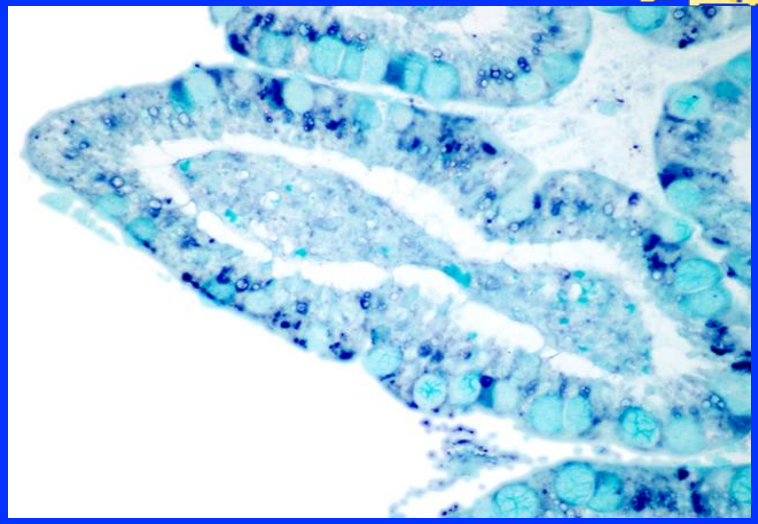


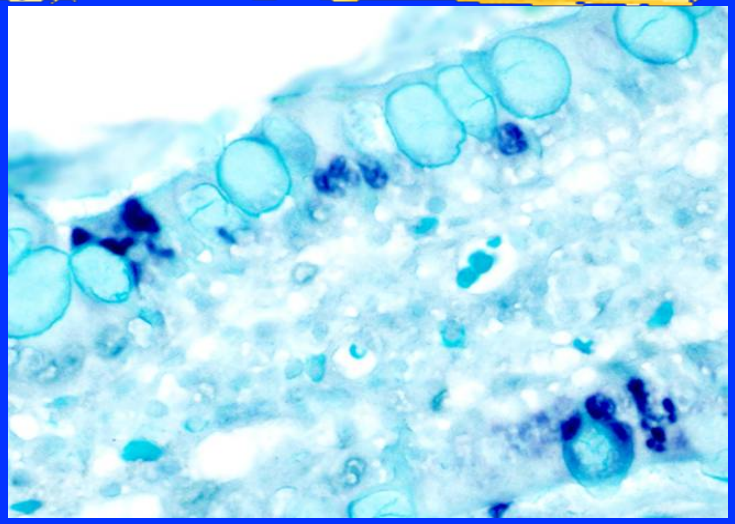
Immunocytochemistry

- ICC not working yet
  - No specific test
  - Immunocytochemistry not as clean
  - Sporadic distribution of coronavirus antigen produces false negatives
  - Cells wash off during staining procedure



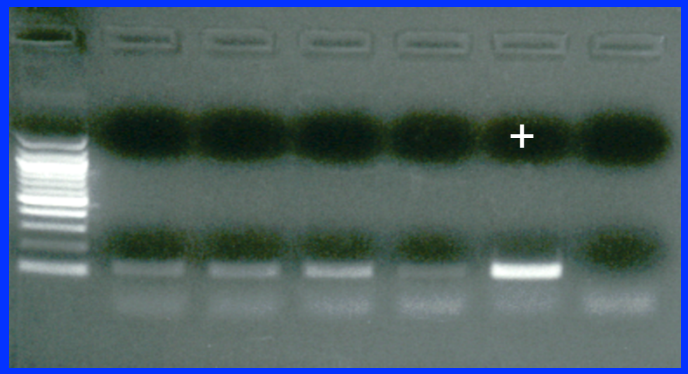
Development of Molecular Diagnostic Tests for FRECV





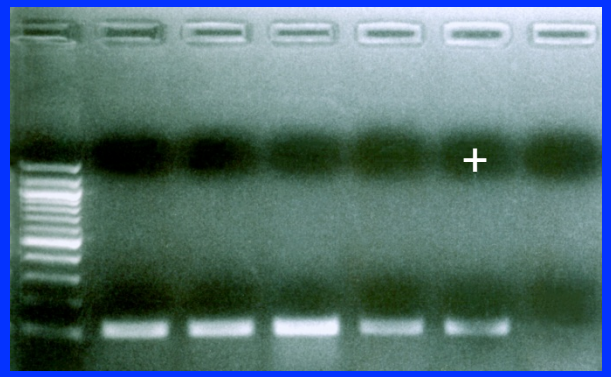
- In-situ hybridization (ISH) assay
- Digoxigenin-labelled FECV nucleocapsid gene-specific probe
- Detection system: anti-digoxigenin ab conjugated with alkaline-phosphatase and substrate NBT/X-Phos

Gel-Based RT-PCR Assay to Detect FRECV or FRSCV



-113 bp

Oral swab specimens



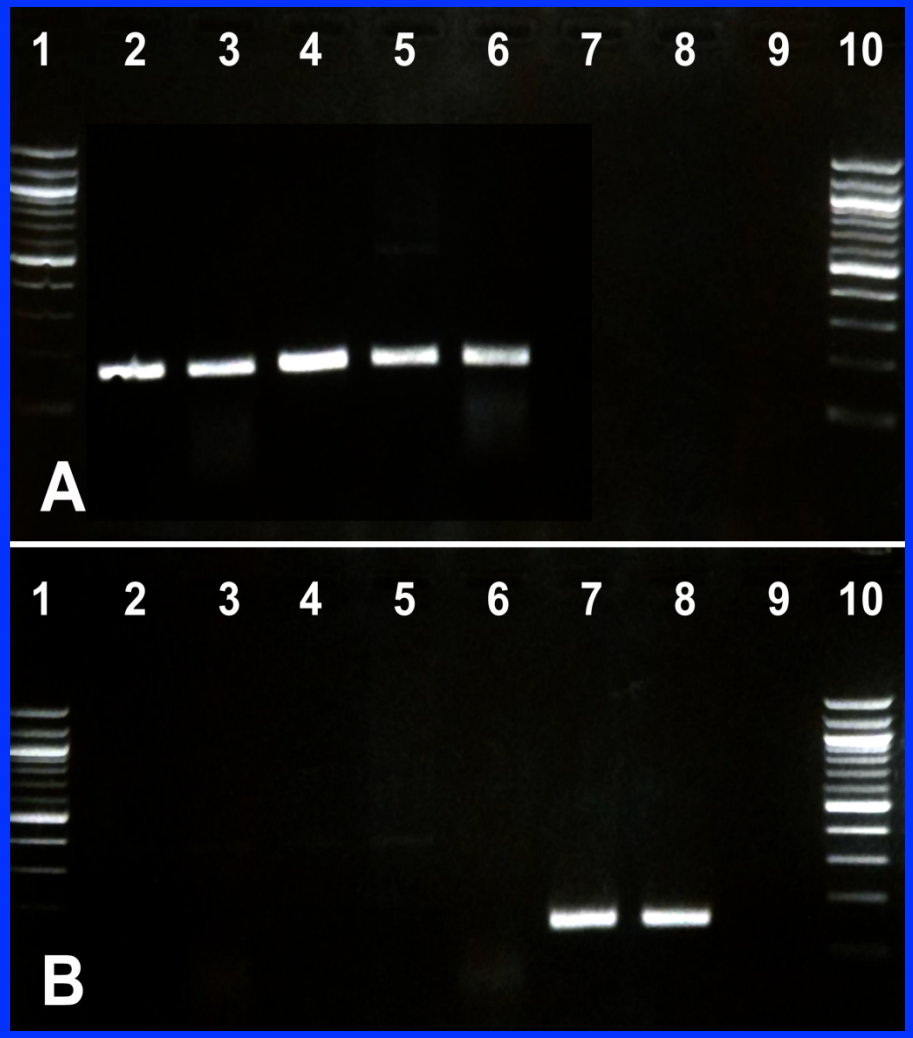
-113 bp

Fecal swab specimens

FRSCV-specific primers

Pathotype-Specific Diagnostic RT-PCR on Clinical Samples

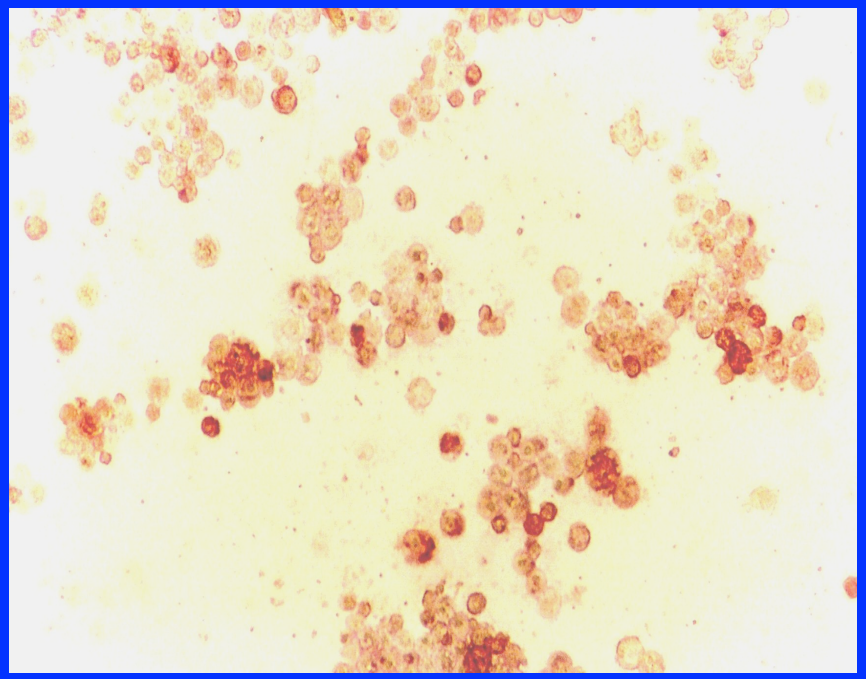
FRECV-specific primers



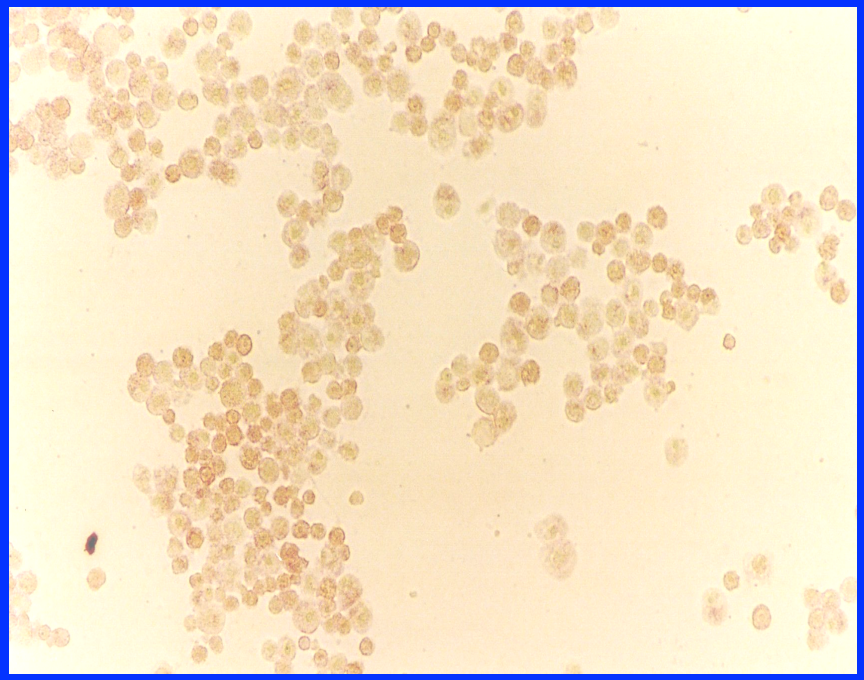
FSCV MSU-1	5'- ctggtgtttgtgcaacatctactaataaaactatgggttttattgttaaagatgtgtcgttgacgttgtttaaaaatcac	80
FECV MSU-2	5'- ...ca.....tt ----------.g.t.acg.t.....g.....c..g.....t.....c...t	68
FSCV MSU-1	gatggcaggttctacttgacaccgcgtacaatgtacgaaccgcgagttgcaactatgtctgattttgtgcaaatagaaag -3'	160
FECV MSU-2	.... at.aa..... a..... t.....t .. g..... c..g....gtg.a.....c....g...ta .t.. -3'	148

Development of a Serologic Test for ECE: Recombinant Baculovirus Technology

- FECV capsid gene cloned in baculovirus
- Expression of FECV capsid protein in insect cells (SF9) detected by IPMA (immuno-peroxidase monolayer assay)



Ferret anti-FRECV



Bovine anti-BVDV

Conclusions

- FRECV and FRSCV: two genetically distinct co-circulating group 1 coronaviruses in ferrets?
- FRECV is associated with an enteric disease in ferrets, FRECV is the cause of ECE
- FRSCV is associated with systemic disease in ferrets, FRSCV causes FIP-like disease
- Based upon IHC, FRSCV has macrophage tropism
- "S-to-A" substitution in the conserved C-terminus S region and an ORF 3 mutation may potentially play a role in systemic virulence

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These are just a few of the activities our team of researchers and clinicians participates in. They are representative of the clinical services, and research projects that your dollars help fund. We also need support to better teach our students and graduate students ferret diseases and medicine. Every gift matters, because every gift gives us the chance to enrich minds, hone skills, cultivate character, and make a difference in the community and the world.

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