UCDAVIS
VETERINARY MEDICINE
California Animal Health and Food Safety Laboratory System

Enteric clostridial diseases

Part A

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The genus *Clostridium*

***Anaerobic (more or less strict)**

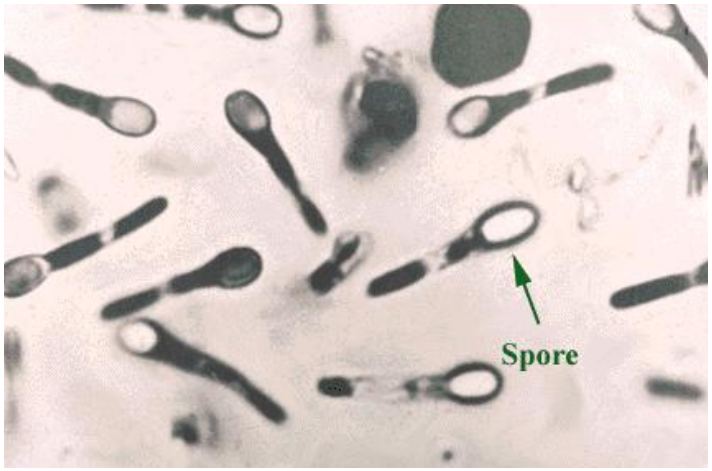
***Gram positive (most of them; exception?)**

***Rods**

***Sporulated (heat resistant endospores)**

***Ubiquitous (some of them)**

***Pathogenesis involves toxins**



***Clostridium tetani* spores – J.G. Songer**

GROUP	DISEASE	ORGANISM	HUMANS	OTHER ANIMALS
Enteric				
Histotoxic				
Neurotoxic				

GROUP	DISEASE	ORGANISM	HUMANS	OTHER ANIMALS
Enteric	Enterotoxemias/ enteritis	*C. perfringens*	✓	✓
		C. difficile	✓	✓
		C. piliforme	--	✓
		C. sordellii	--	✓
		C. colinum	--	✓
		C. spiroforme	--	✓
Histotoxic	Black leg	*C. chauvoei*	--	✓
	Gas gangrene	*C. septicum*	✓	✓
		C. chauvoei	--	✓
		C. perfringens	✓	✓
		C. sordellii	✓	✓
		C. novyi	✓	✓
	Hepatitis	*C. novyi*	--	✓
		C. haemolyticum	--	✓
		C. piliforme	--	✓
Neurotoxic	Tetanus	*C. tetani*	✓	✓
	Botulism	*C. botulinum*	✓	✓

GROUP	DISEASE	ORGANISM	HUMANS	OTHER ANIMALS
Enteric	**Enterotoxemias/ enteritis**	*C. perfringens*	✓	✓
		C. difficile	✓	✓
		C. piliforme	--	✓
		C. sordellii	--	✓
		C. colinum	--	✓
		C. spiroforme	--	✓
Histotoxic				
Neurotoxic				

GROUP	DISEASE	ORGANISM	HUMANS	OTHER ANIMALS
Enteric	Enterotoxemias/ enteritis	*C. perfringens*	✓	✓
Histotoxic				
Neurotoxic				

Enterotoxemia:

toxins generated in intestine

absorbed to circulation; act in distant organs

Take-home message!!!

Up to 20 toxins

* “major” (typing) toxins
* beta2
* delta
* lamda
* NetF
* Tpel
* etc.

The classic toxinotyping of *C. perfringens*

Toxinotype	α-toxin (CPA)	β-toxin (CPB)	ε-toxin (ETX)	ι-toxin (ITX)
A	+	-	-	-
B	+	+	+	-
C	+	+	-	-
D	+	-	+	-
E	+	-	-	+

The 2018 *C. perfringens* toxin-based typing scheme

Toxinotype	α-toxin (CPA)	β-toxin (CPB)	ε-toxin (ETX)	ι-toxin (ITX)	enterot (CPE)	NetB
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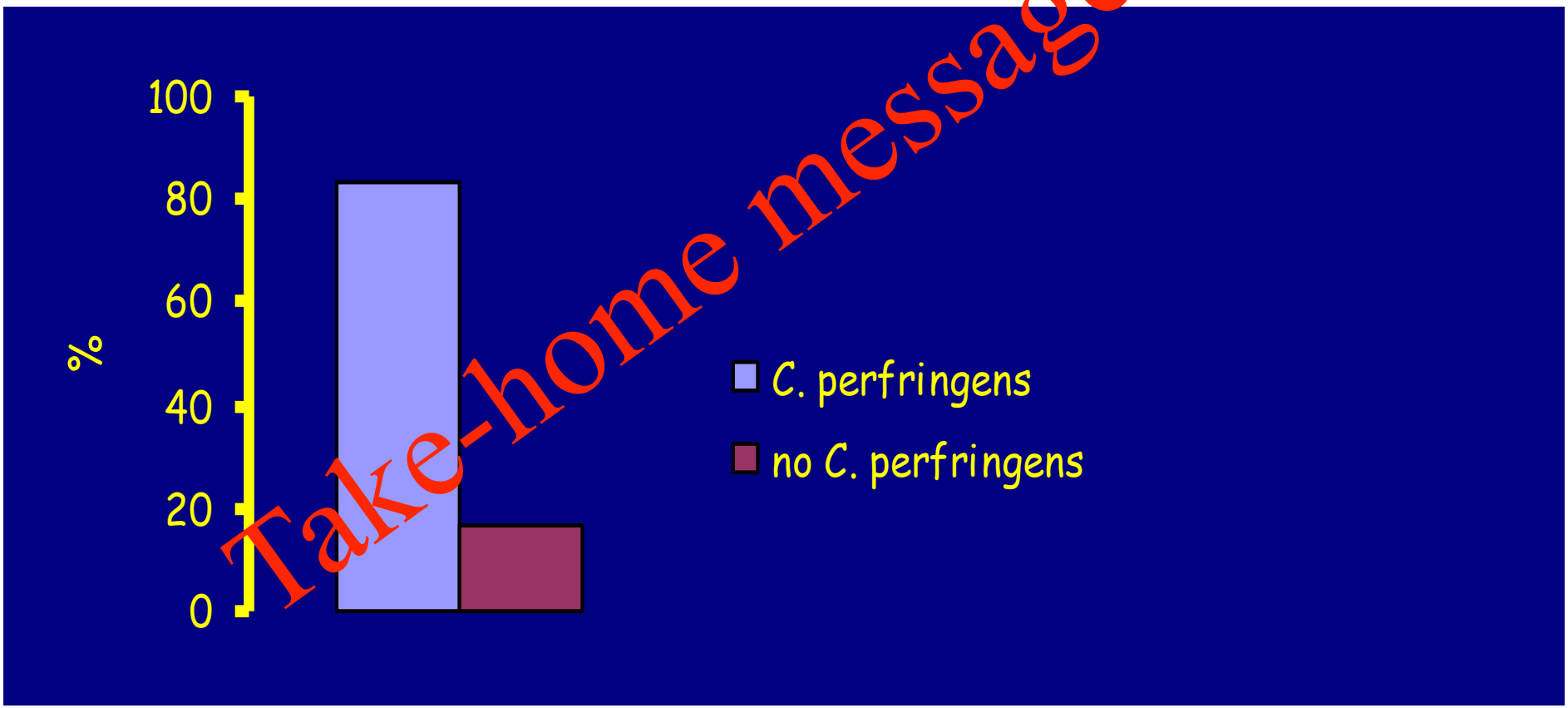
B	+	+	+	-	-	-
C	+	+	-	-	+/-	-
D	+	-	+	-	+/-	-
E	+	-	-	+	+/-	-
F	+	-	-	-	+	-
G	+	-	-	-	-	+

Rood et al, Anaerobe 2018

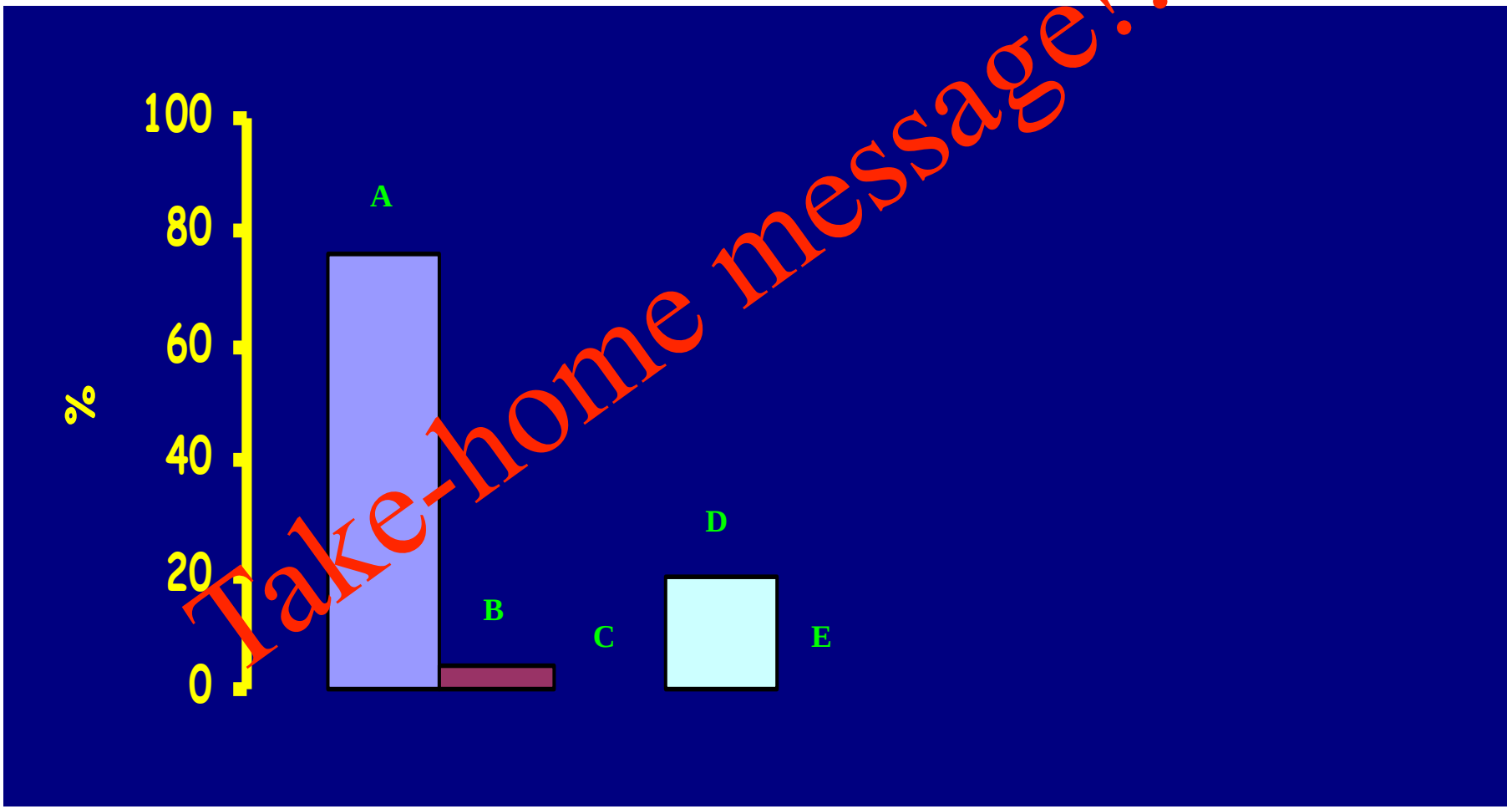
Type	Main virulence factors	Diseases
A	CPA; others?	Suggested to be involved in enterocolitis in pigs and horses, enterotoxemia in sheep and cattle and abomasitis of cattle, sheep and goats. Definitive proof lacking
B	CPB; ETX	Hemorrhagic enteritis of sheep
C	CPB	Necro-hemorrhagic enteritis of neonatal pigs, sheep, goats, horses, others
D	ETX	Enterotoxemia of sheep, goats and cattle
E	ITX	Suggested to be involved in enteritis of sheep, cattle and rabbits
F	CPE	Suggested to be involved in canine hemorrhagic gastroenteritis and colitis of horses
G	NetB	Necrotic enteritis of poultry

C. perfringens in s.i. of healthy sheep (lambs and adults) n=113



Take-home message!!!

C. perfringens in s.i.of healthy sheep (lambs and adults) n=113



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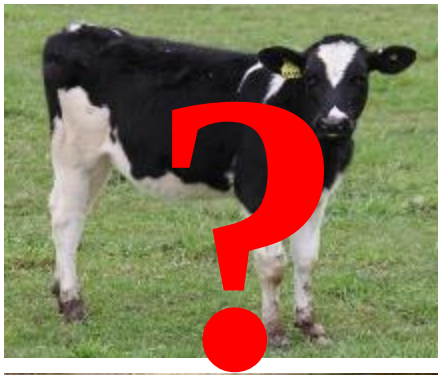
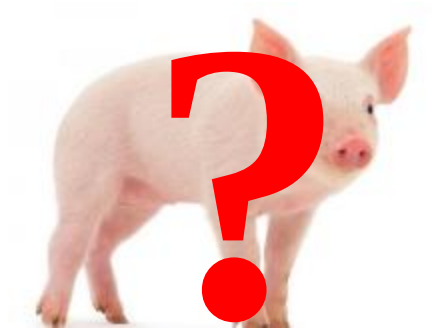
Rood et al, Anaerobe 2018





Yellow lamb disease

Pathogenesis

CPA: highly hemolytic

Pathogenesis:

High CPA expression

intravascular hemolysis

1-Anemia → **Hepatic necrosis**

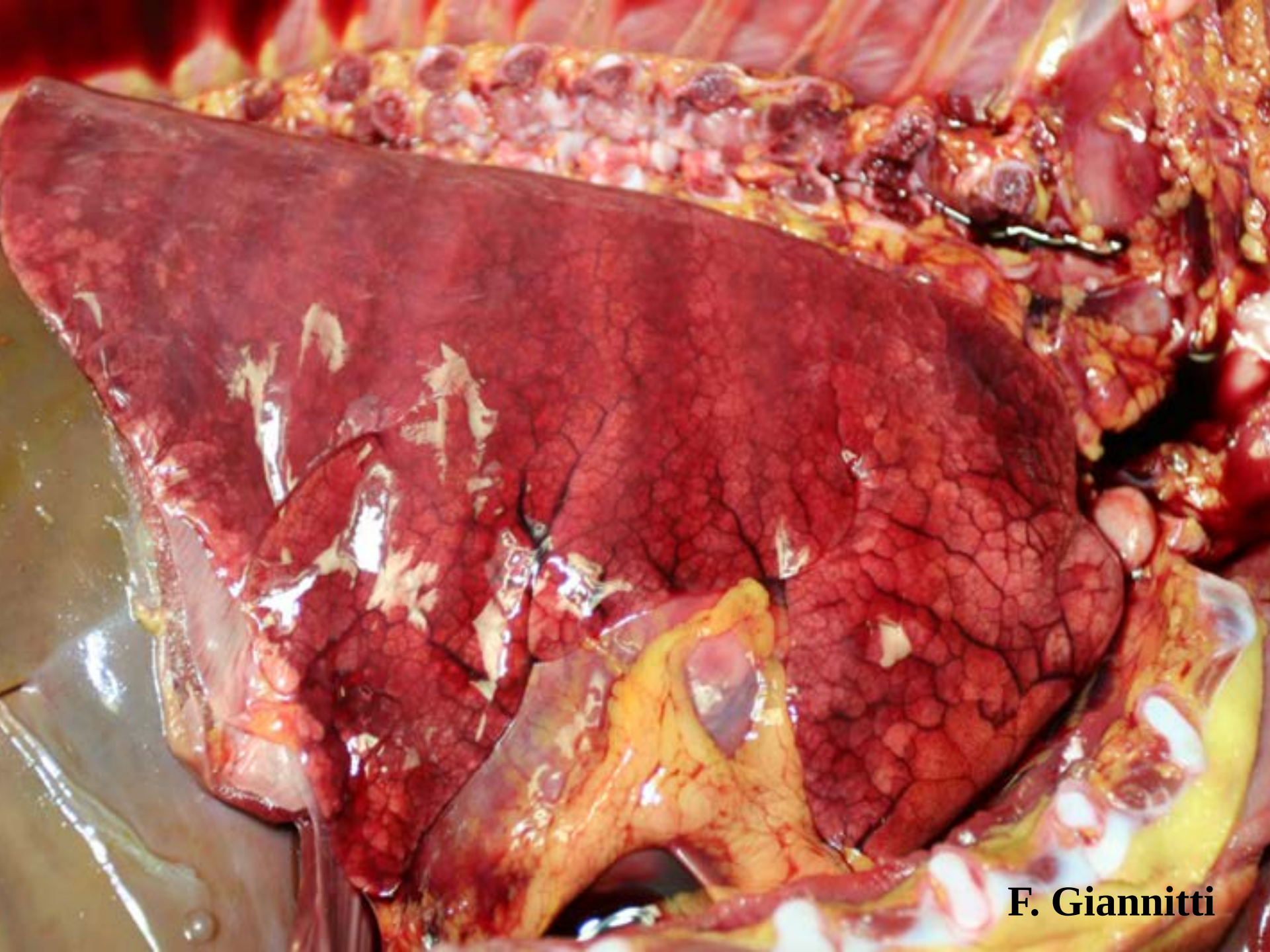
→ **Hypoxia**

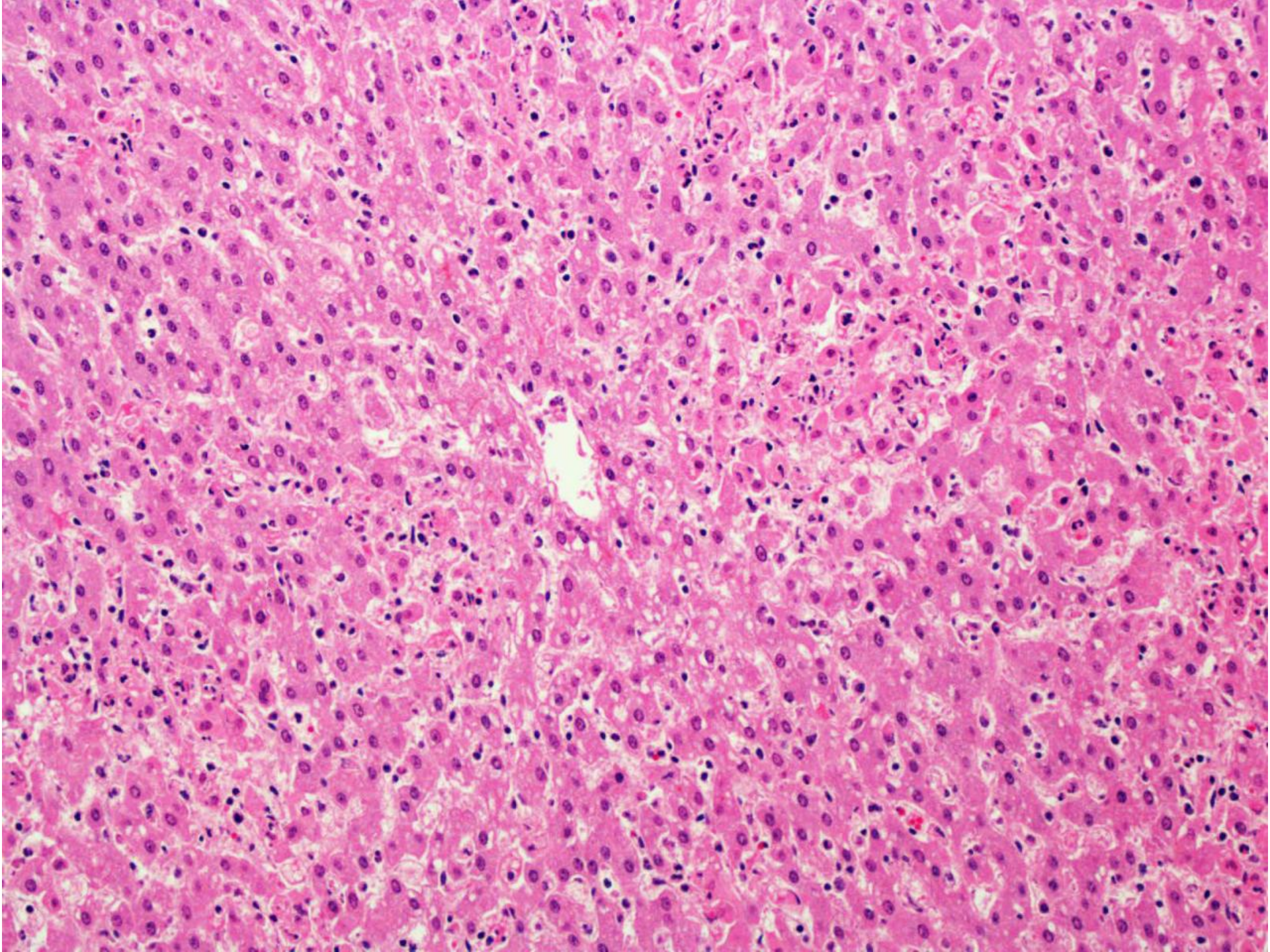
2-Free hemoglobin → **Nephrosis**

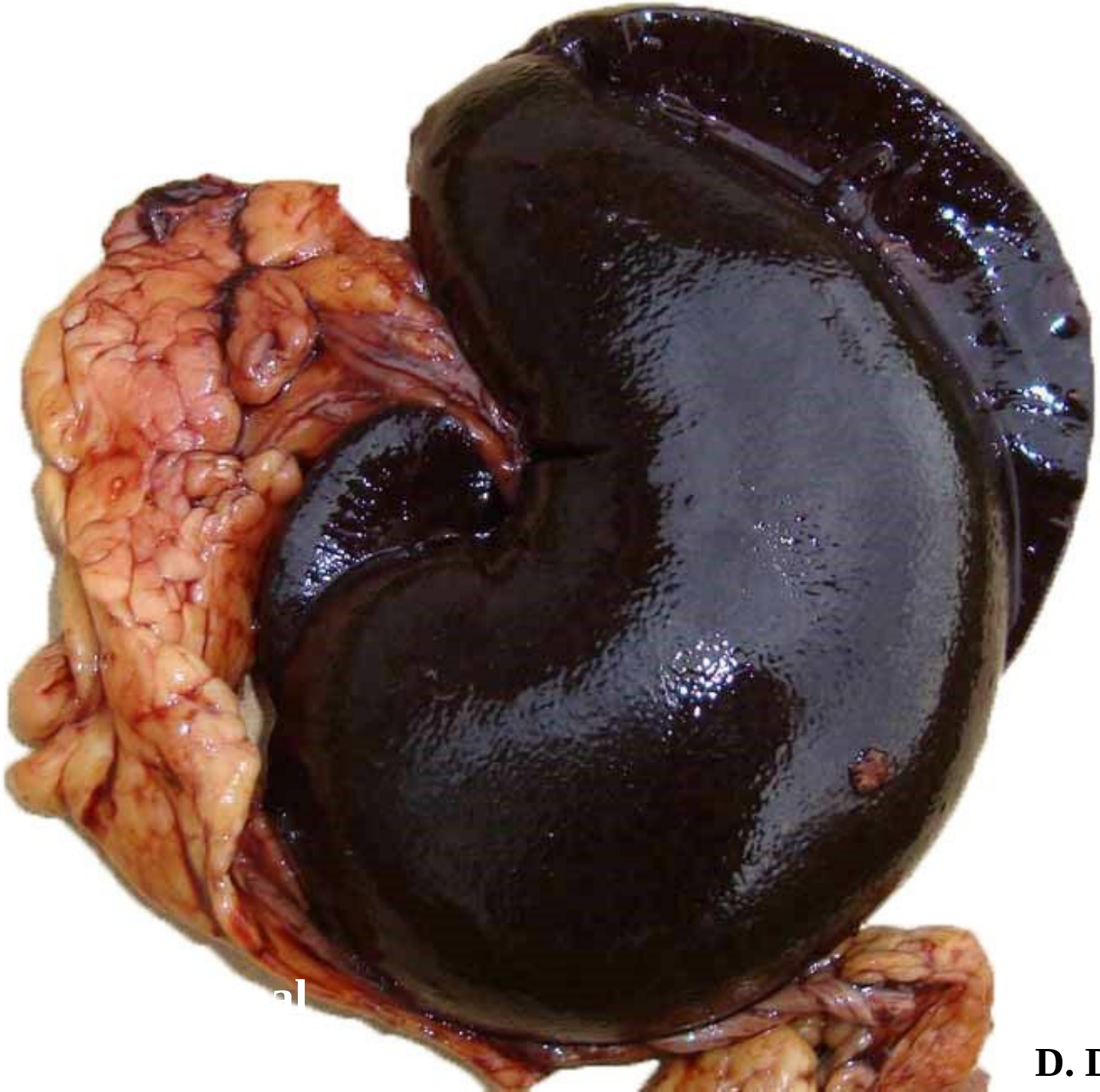
→ **Icterus**

Clinical signs

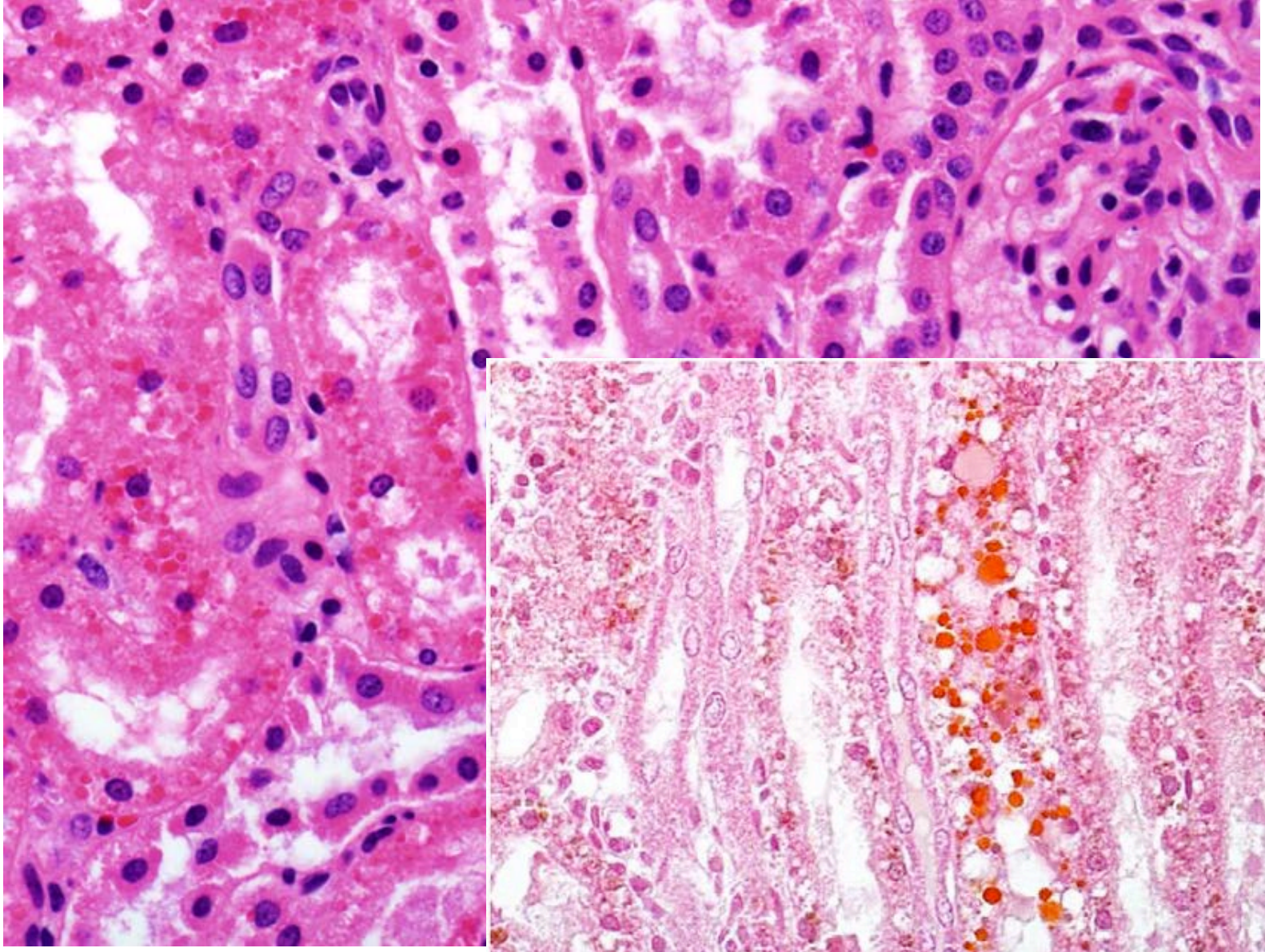
Depression
Anemia
Icterus
Diarrhea
Sudden death

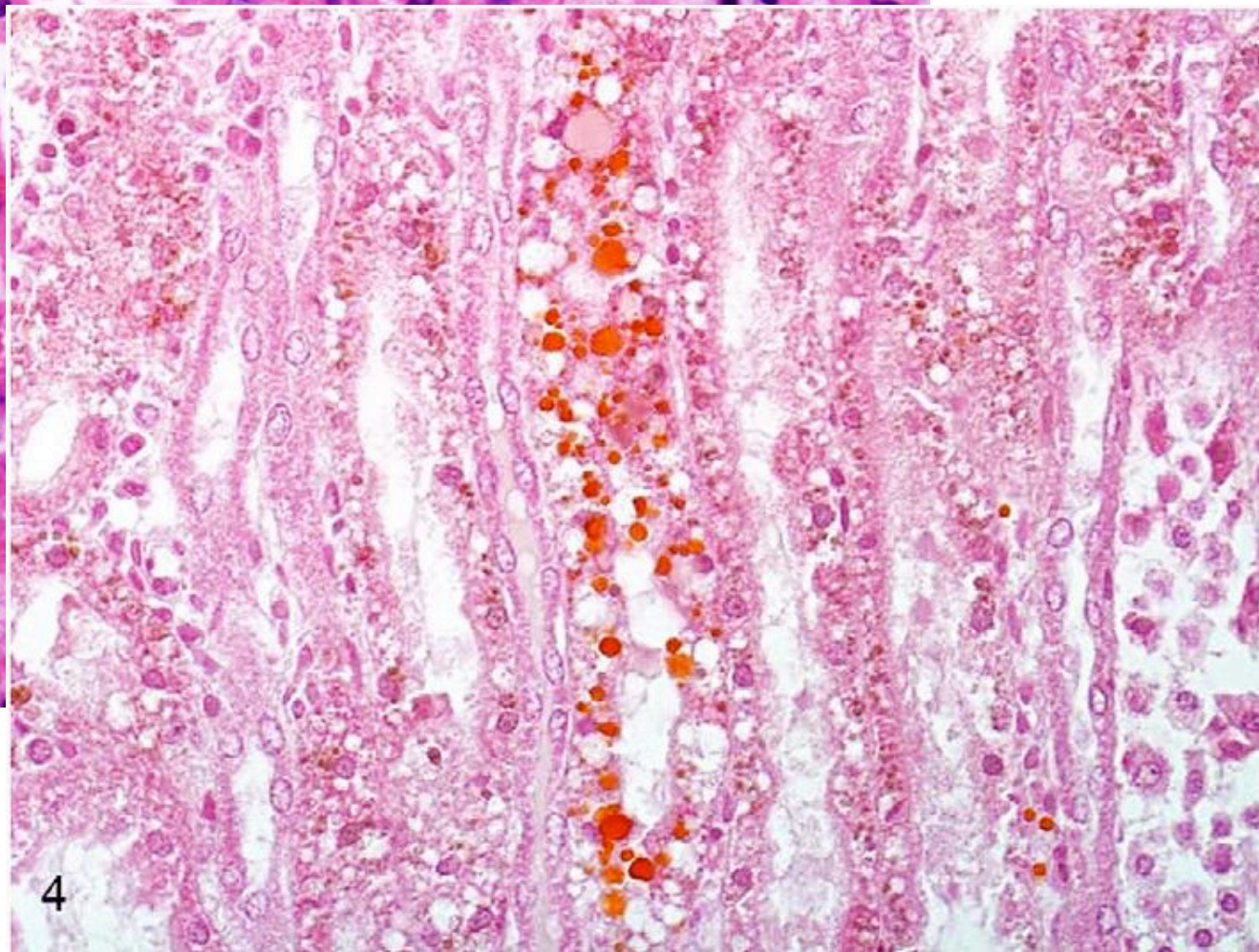

F. Giannitti





D. Dinev





4

Giannitti et al. Vet. Path
2014, 51: 624-627

Diagnostic criteria

1-Clinics/gross

2-Histo

} **Suggestive**

3-Ancillary: **Culture (+ typing)**
(colony count)

} **Confirmatory?**

> 10^6/gr

Also: type D high CPA isolates

Domestic Animals

Veterinary Pathology
2014, Vol. 51(3) 624-627

DOI: 10.1177/0300985813501339
vet.sagepub.com

Diagnostic Exercise: Hemolysis and Sudden Death in Lambs

F. Giannitti[1], M. Macias Rioseco[2], J. P. García[1], J. Beingesser[1], L. W. Woods[1], B. Puschner[1], and F. A. Uzal[1]

Abstract

Within a 24-hour period, 7 out of 200 three- to four-week-old pastured Katahdin lambs died after showing clinical signs of hemoglobinuria, red-tinged feces, weakness, and recumbency. One of the lambs that was examined clinically before natural death also had abdominal pain, trembling, tachycardia, and severe anemia with a packed cell volume of 4%. Pathologic findings included icterus, hemoglobinuric nephrosis, dark red urine, pulmonary edema, hydrothorax, splenomegaly, and acute centrilobular to midzonal hepatocellular degeneration and necrosis with cholestasis. The differential diagnoses and diagnostic

Differential diagnoses

* Copper intoxication
* Hemoparasites
* Leptospirosis
* Oak intoxication
* Others….

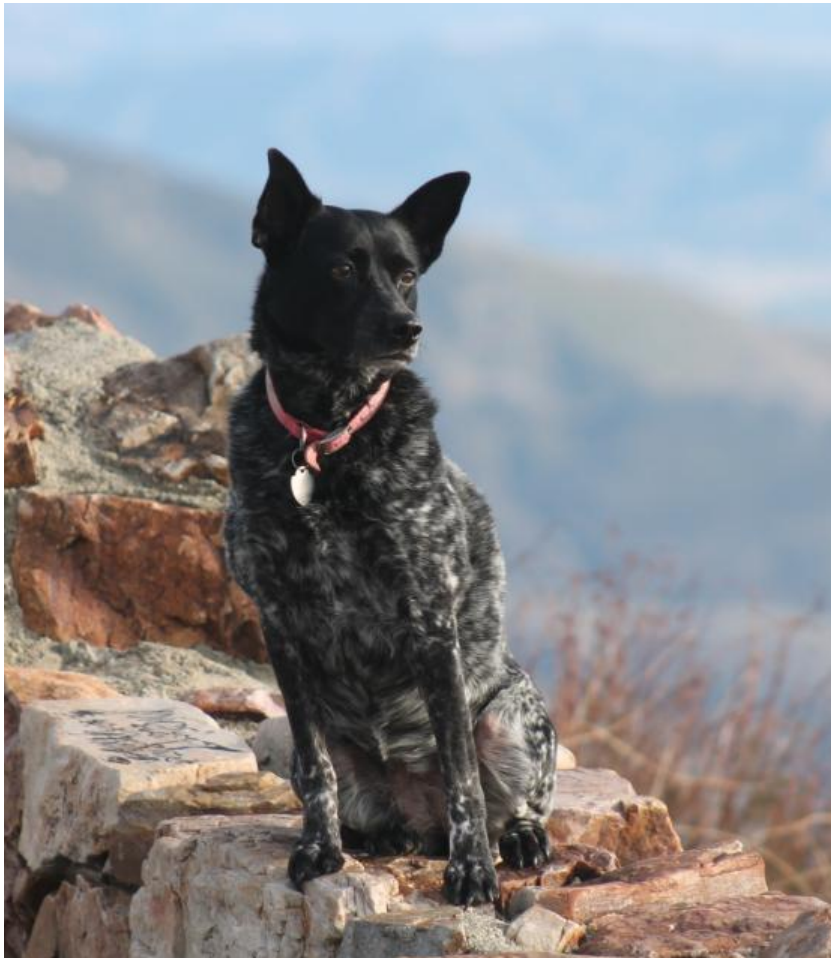
Canine hemorrhagic gastroenteritis
Necrotizing enteritis of foals

C. perfringens type A NetF + isolates

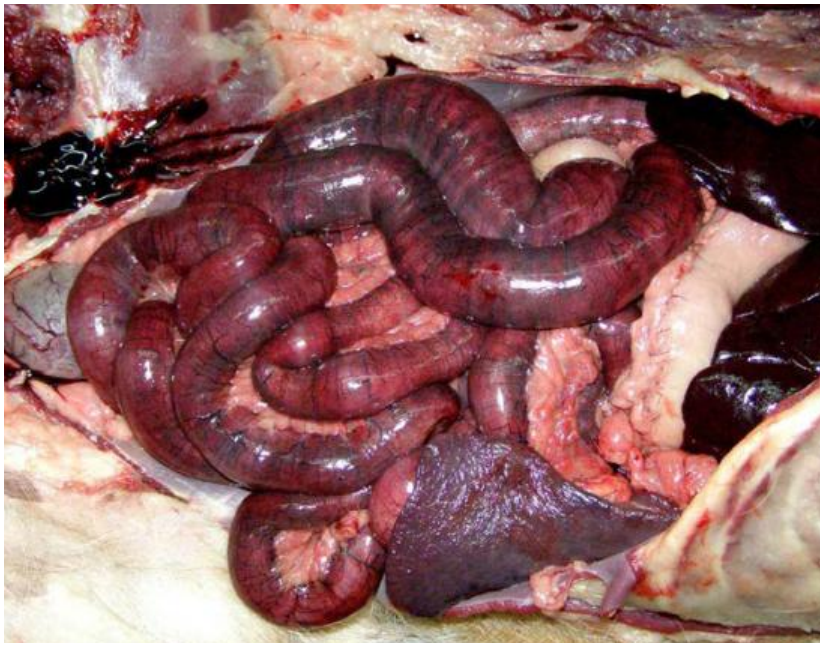
High correlation isolation/disease

A novel pore-forming toxin in type A *Clostridium perfringens* is associated with both fatal canine hemorrhagic gastroenteritis and fatal foal necrotizing enterocolitis. Mehdizadeh Gohari I, Parreira VR, Nowell VJ, Nicholson VM, Oliphant K, Prescott JF. PLoS One. 2015 Apr 8;10(4):e0122684

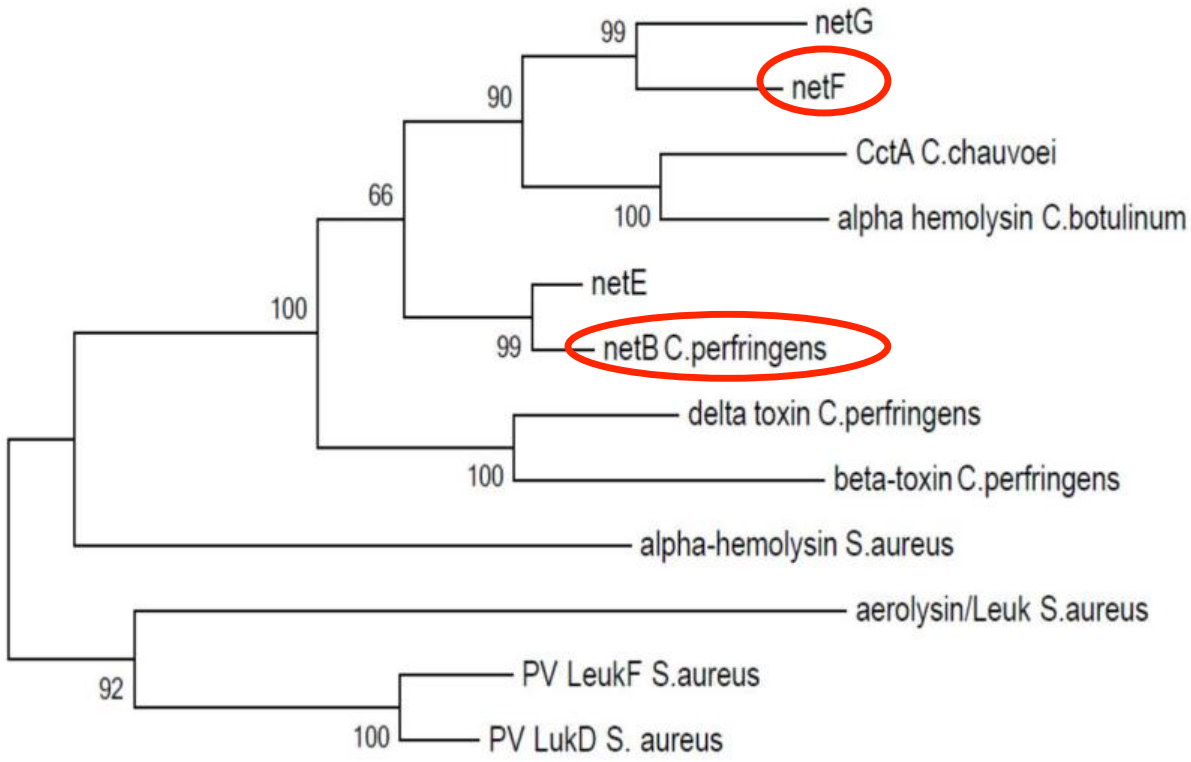
Necrotizing enteritis of foals
Canine hemorrhagic gastroenteritis



Gohari et al, 2016



L. Minatel



Gohari et al, 2016

C. perfringens type A frequently blamed for enteritis, abomasitis and/or enterotoxemia in cattle

Is this true……..?

* *C. perfringens* type A in intestinal content of healthy cattle (Uzal et al, 2006; Uzal et al, 2016; many more….)

* Isolation of *C. perfrinngens* type A from intestinal content of sick animals:

no diagnostic relevance

* Large amounts of CPA in feces of healthy cattle (Niilo et al, 1963; Timoney et al,1988; Uzal et al, 2016)

Detection of alpha toxin in intestinal content of sick animals:

no diagnostic relevance

Diagnostic criteria

Who knows…….

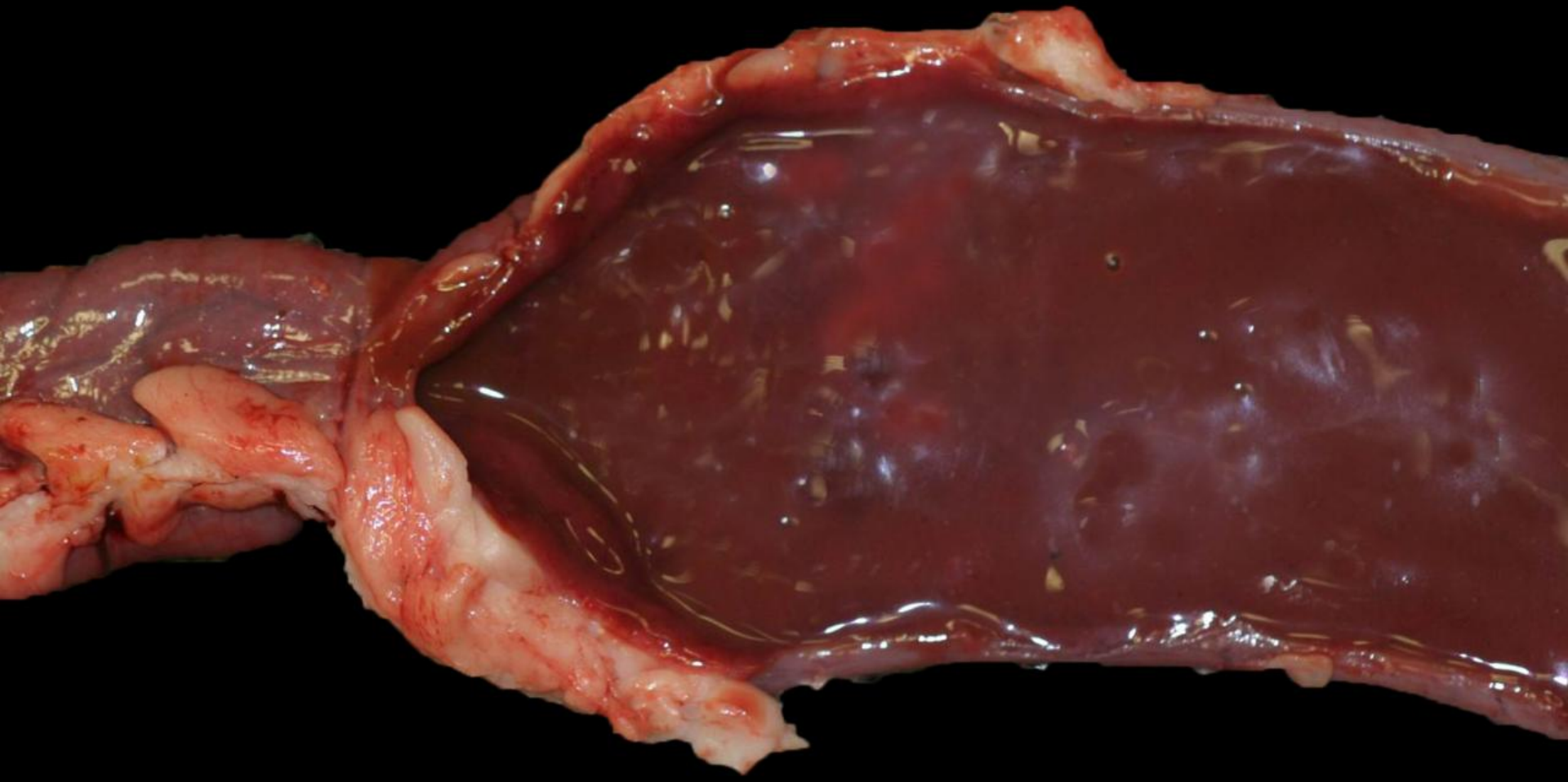
Role of *C. perfringens* type A in enteric disease of cattle:

*** No Koch postulates fullfilled**
*** No disease definition**
*** No diagnostic criteria**

Diferential diagnoses

* **Viral: BVD**
* **Bacterial: Salmonella sp.**
* **Parasitic: Coccidios**
* **Metabolic: Acidosis, tympanism**
* **Toxic: nitrates, oleander, others**

Type A enterotoxemia?



No: Oleander intoxication

The 2018 *C. perfringens* toxin-based typing scheme

Toxinotype	α-toxin (CPA)	β-toxin (CPB)	ε-toxin (ETX)	ι-toxin (ITX)	enterot (CPE)	NetB
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E	+	-	-	+	+/-	-
F	+	-	-	-	+	-
G	+	-	-	-	-	+

Rood et al, Anaerobe 2018

***C. perfringens* type B**

Rare: Mostly Middle East

Pathogenesis

CPB: necrotizing

ETX: neurotoxin

Take-home message!!!

Beta toxin (CPB)

* 35 kDa

* pore-forming

* necrotizing

* trypsin-sensitive

Epsilon toxin (ETX)

* 30 KD toxin ~~(USDA/CDC list B select agent)~~

* pore forming

* neurotoxin

* trypsin-activation required

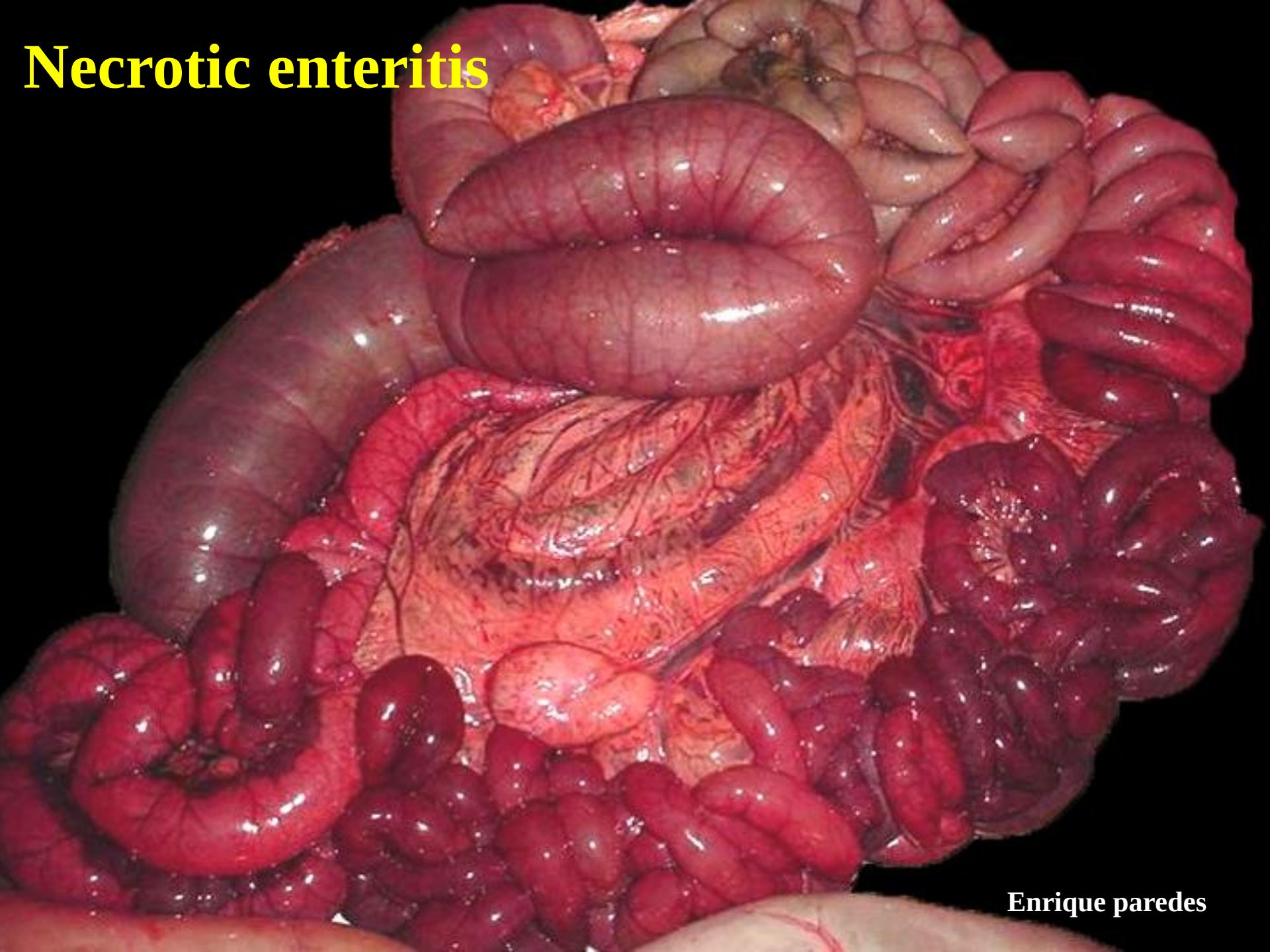
Pathogenesis

Beta: necrotizing

Epsilon: neurotoxin

Take-home message!!!

Necrotic enteritis



Enrique paredes

FSE: ETX

Keith Thompson

Diagnostic criteria

1-Clinics/gross

2-Histopathology

} **Suggestive**

3-Ancillary: **Culture (+ typing)**

} **Suggestive +**

Toxins
beta
epsilon

} **Confirmatory**

The 2018 *C. perfringens* toxin-based typing scheme

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E	+	-	-	+	+/-	-
F	+	-	-	-	+	-
G	+	-	-	-	-	+

Rood et al, Anaerobe 2018

Beta toxin (CPB)

* 35 kDa

* pore-forming

* necrotizing

* trypsin-sensitive

Due to this……

1-Intestinal trypsin: natural defense against type C disease

2-Type C disease:

-neonates

-pancreatic disease

-trypsin inhibitors (sweet potato; soybean)

Clostridium perfringens type C

1-enteritis necroticans: humans
2-enterotoxemias: animals

Clostridium perfringens type C

1-enteritis necroticans: humans

2-enterotoxemias: animals

The pigbel story……, 1960s……

Photo: Greg Lawrance

Photo Greg Lawrance

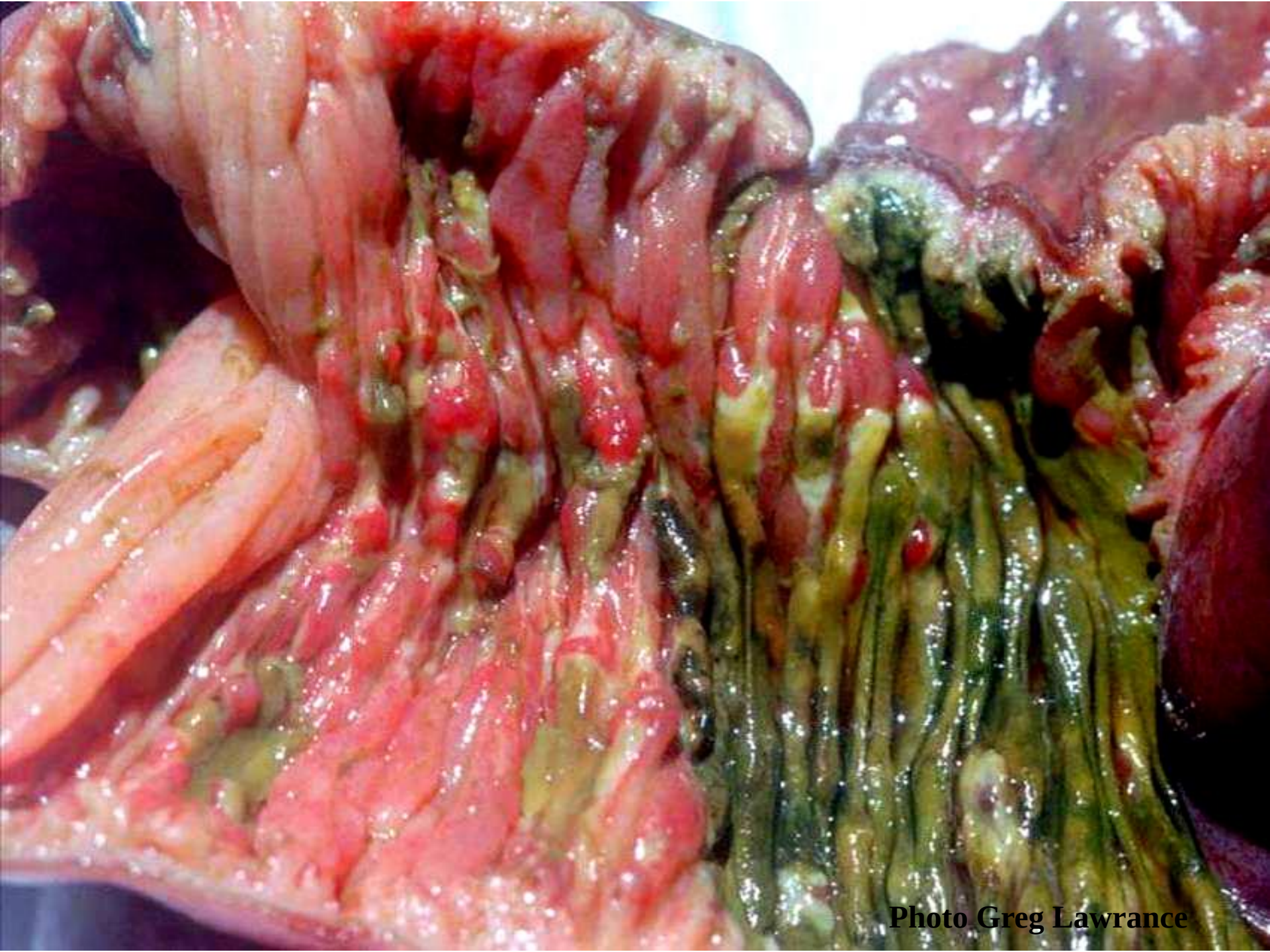
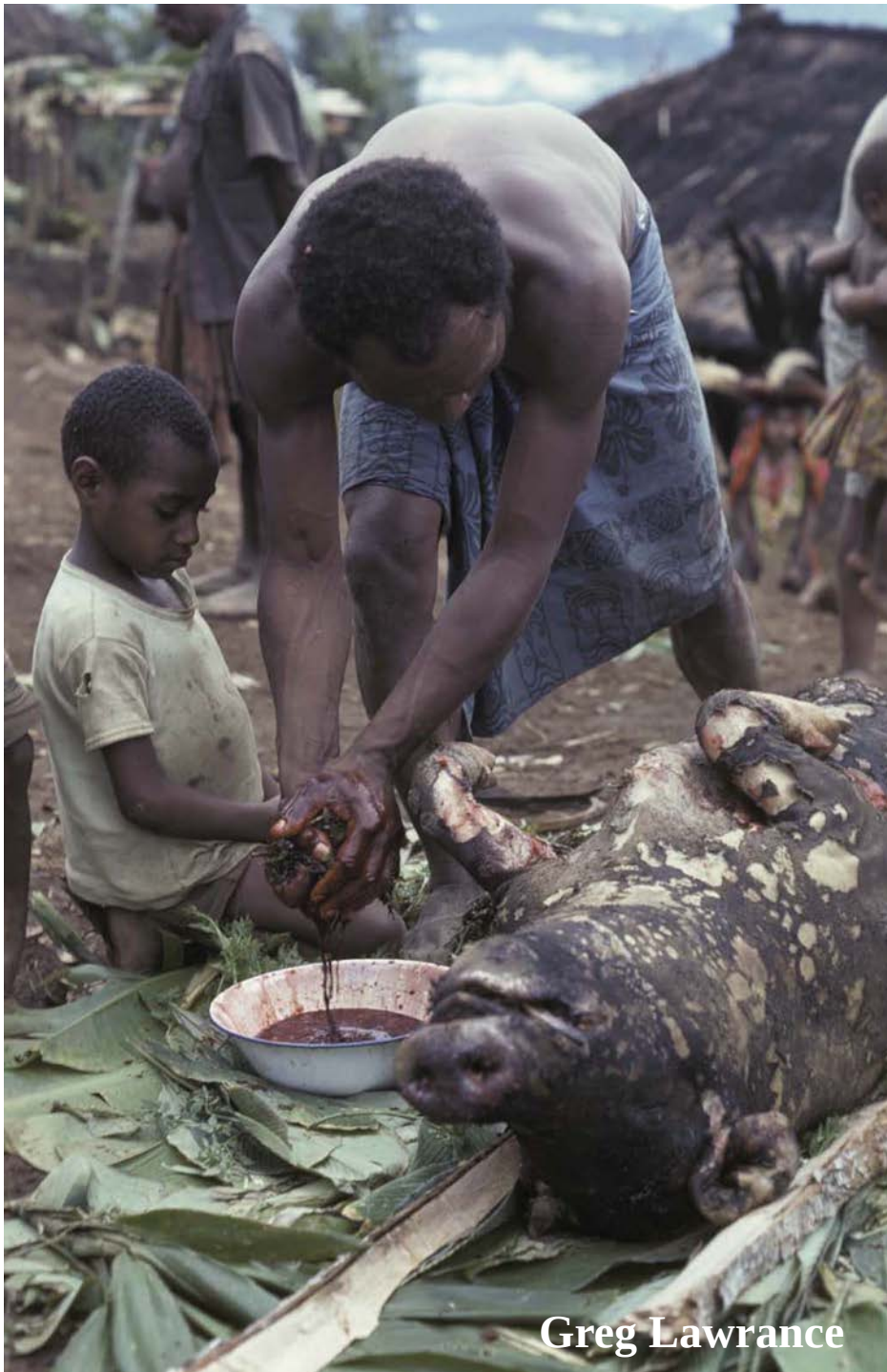


Photo Greg Lawrance

Frequent carrier of
C. perfringens type C

Photo Greg Lawrance

Fecal contamination of meat




Sweet potatoes:
Trypsin inhibitor!!!!
Photo Greg Lawrance

Photo Greg Lawrance

Clostridium perfringens type C

1-enteritis necroticans: humans

2-enterotoxemias: animals

Clinical signs

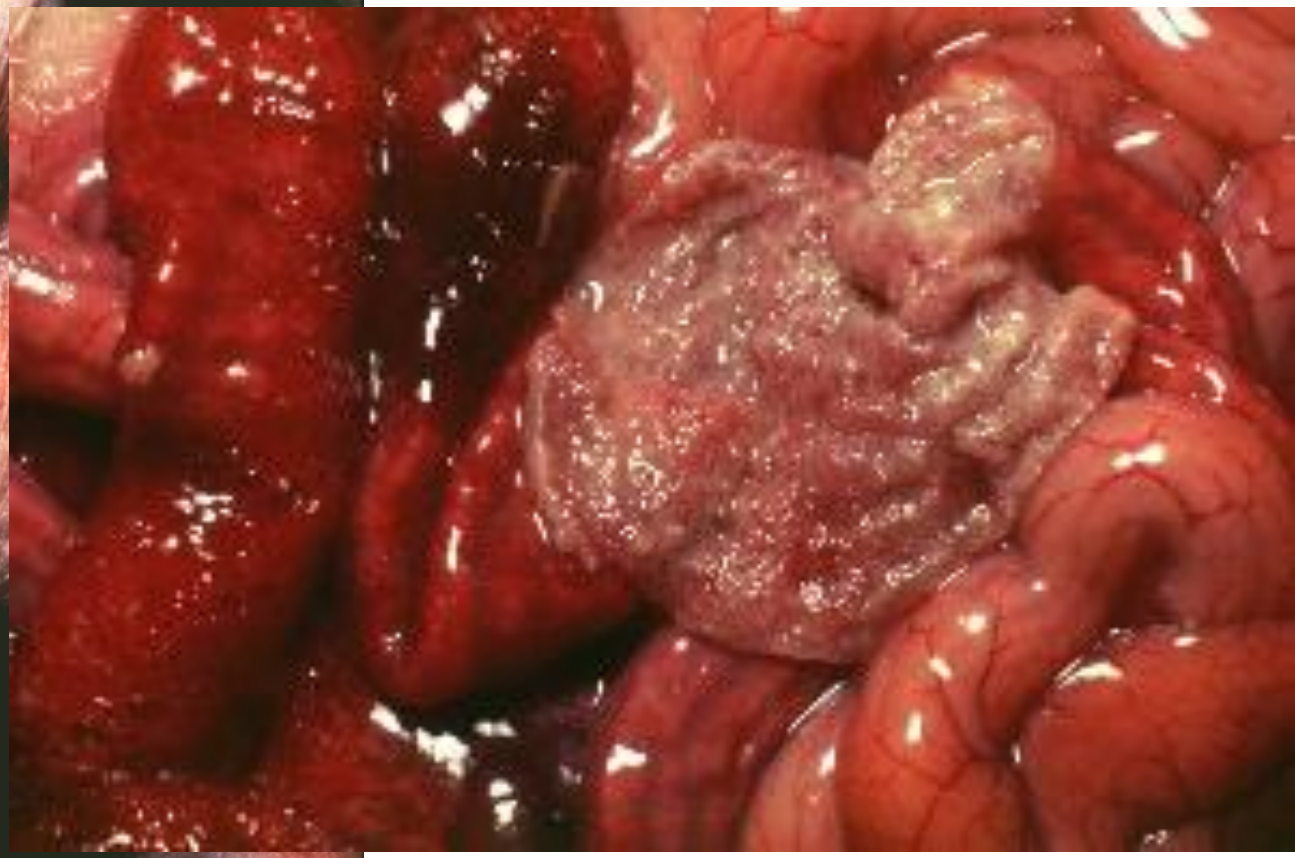
NEONATES

hemorrhagic diarrhea

neurologic signs

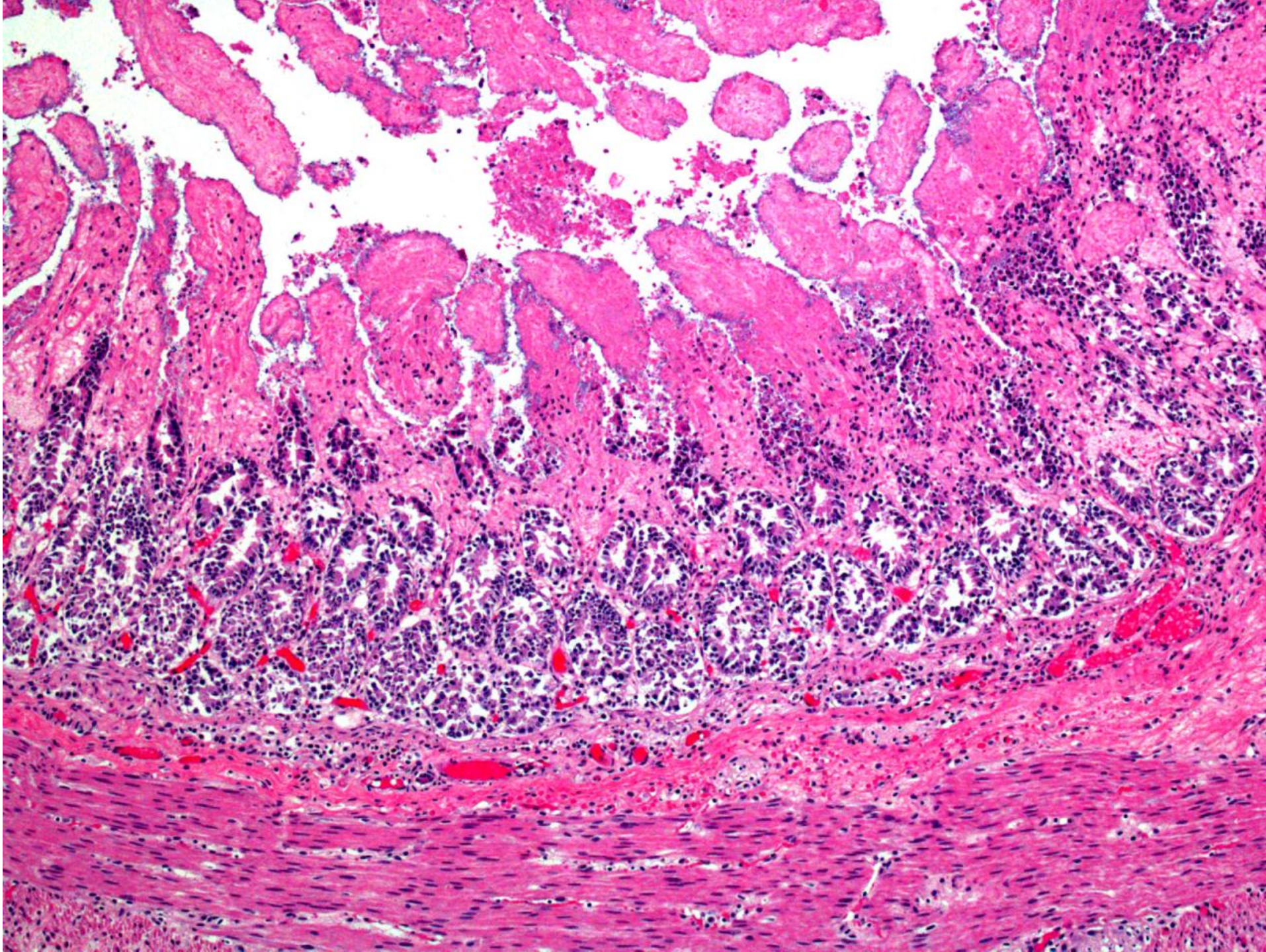
sudden death

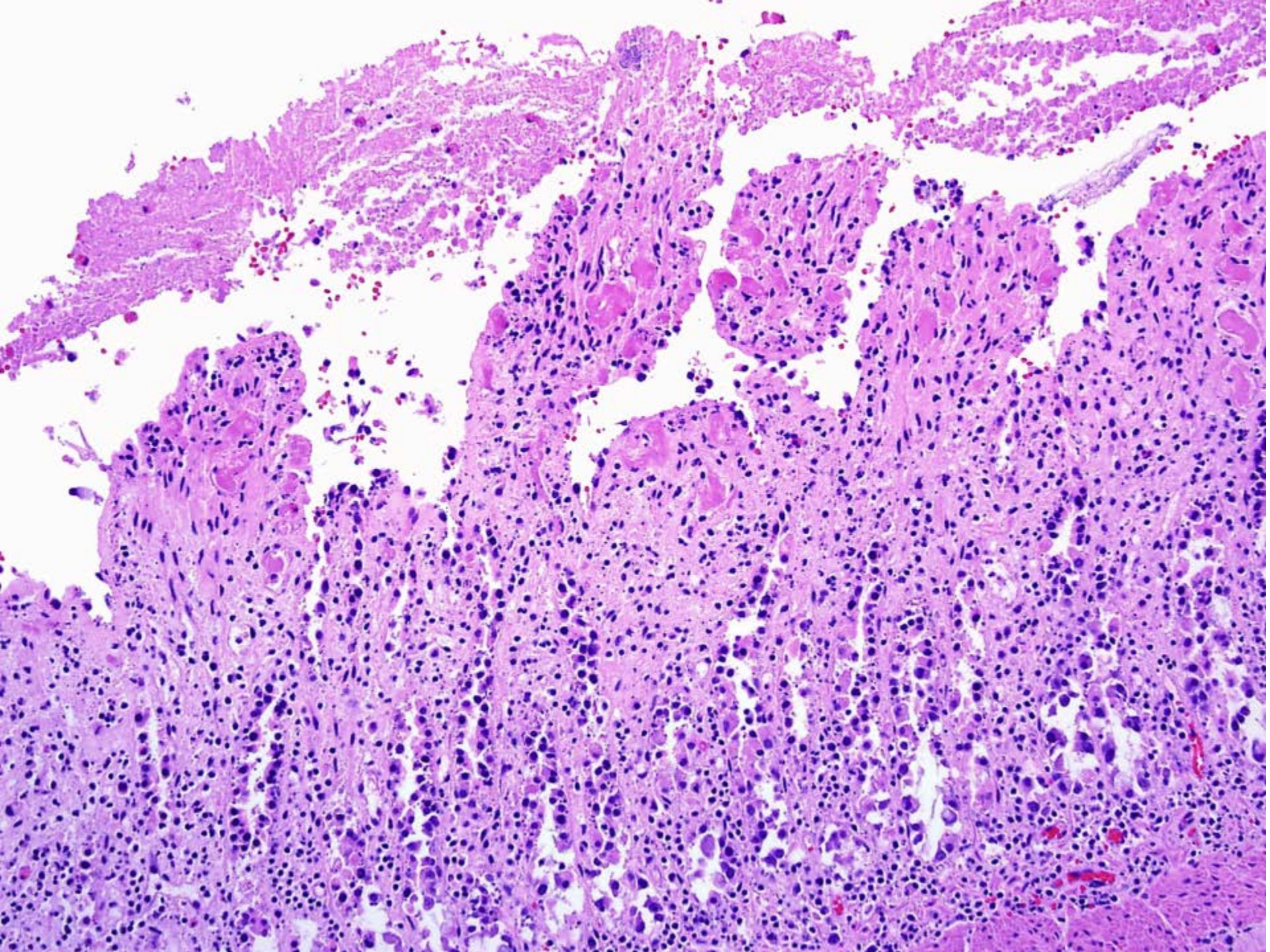
Take-home message!!!



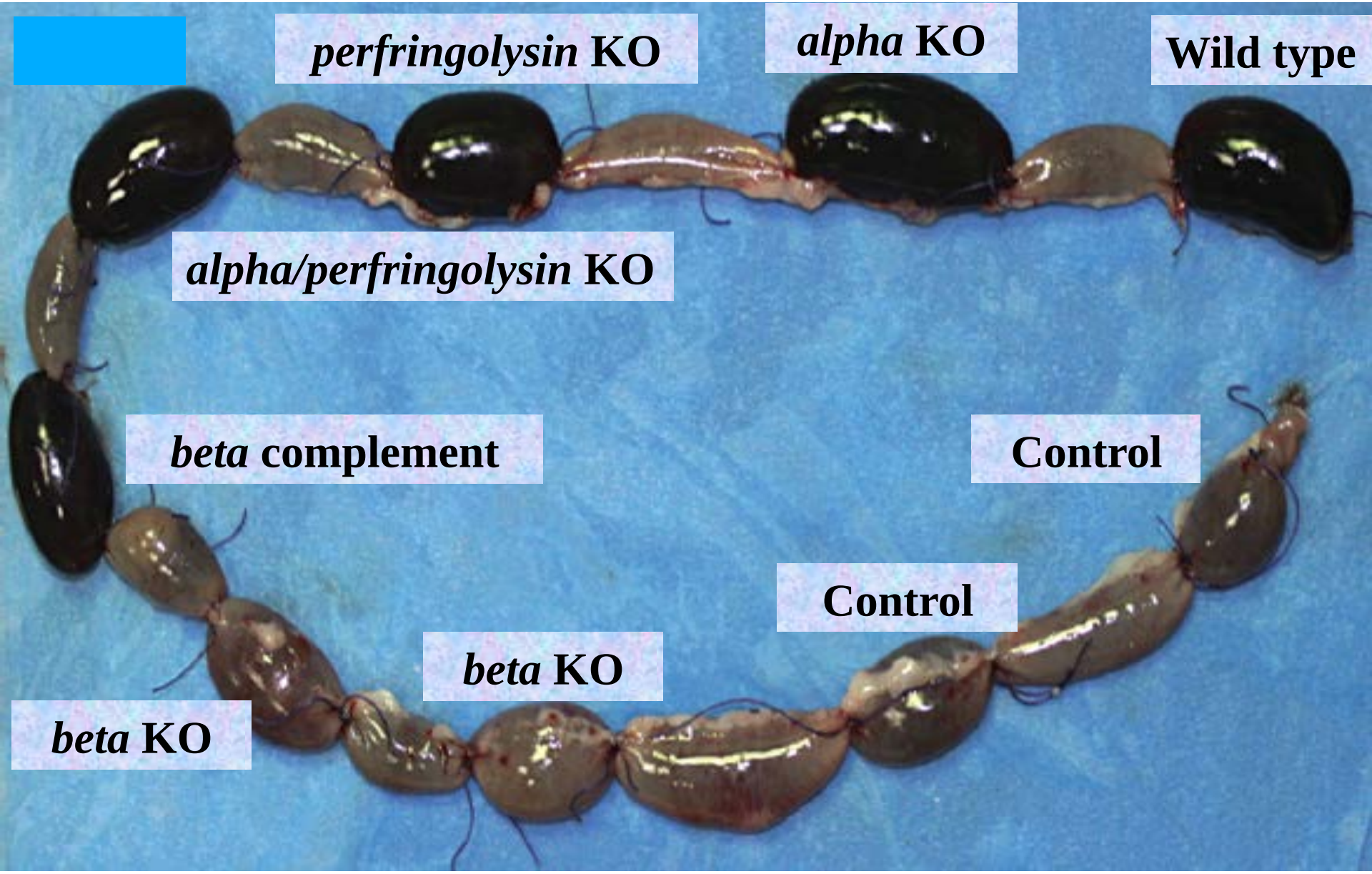
G. Stevenson

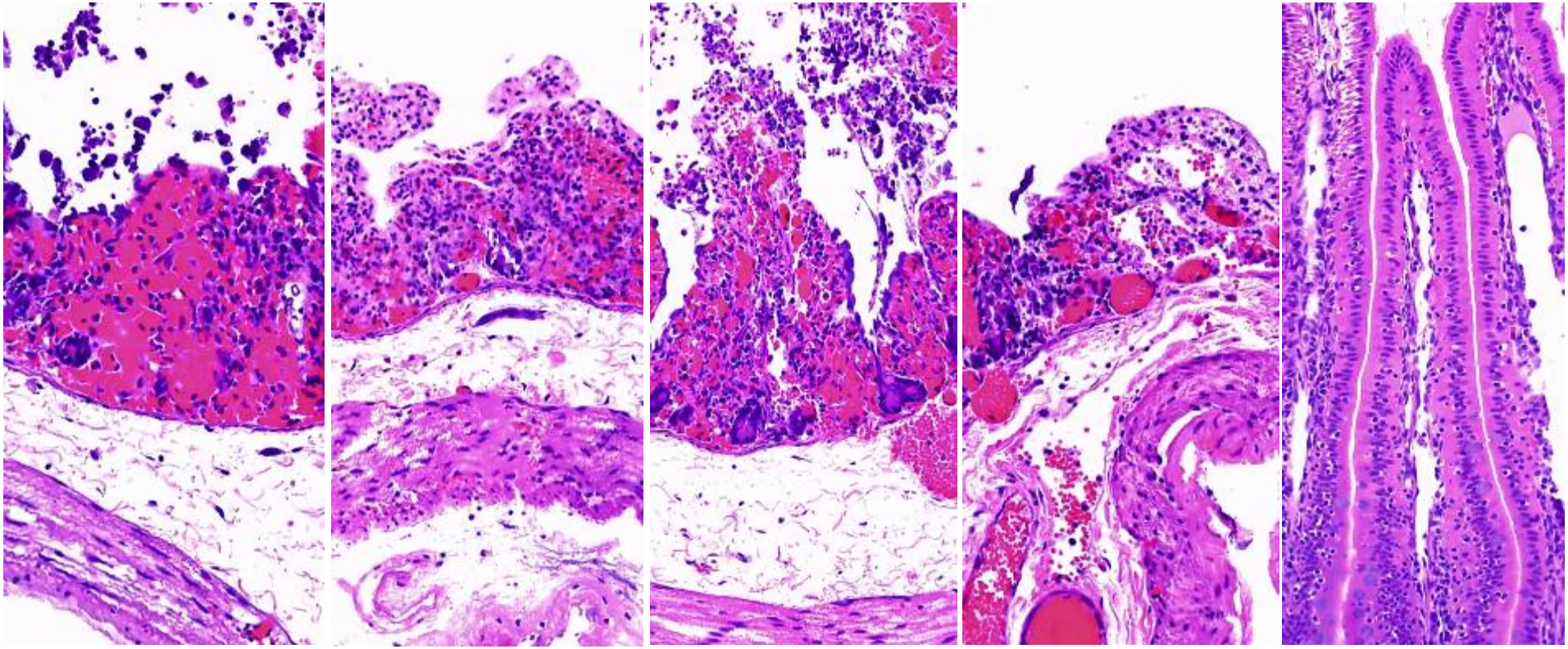

D. O’Toole



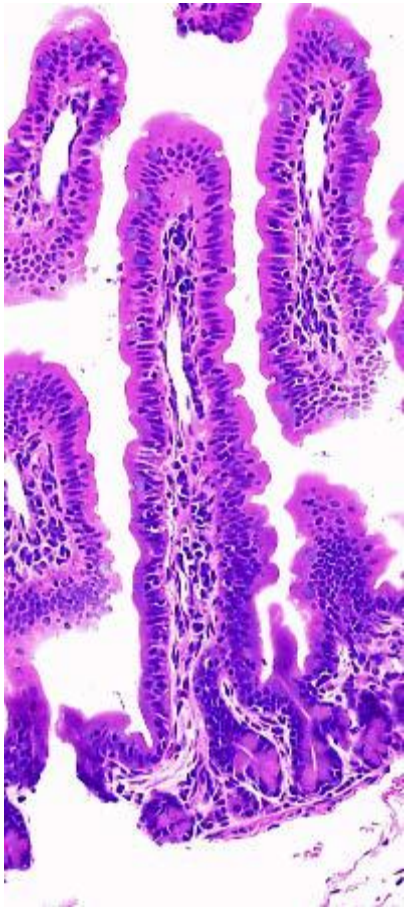


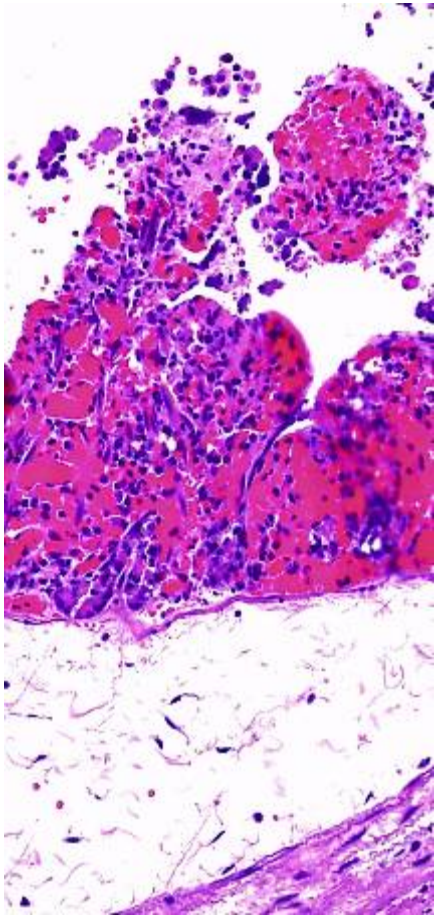
Molecular Kock's postulates fulfilled!!! (rabbits, mice, goats)


perfringolysin KO
alpha KO
Wild type
alpha/perfringolysin KO
beta complement
Control
Control
beta KO
beta KO



wild type ***alpha* KO** ***perfringolysin* KO** ***alpha/pfo A* KO** ***beta* KO**





***beta* KO** ***beta* complement**

Diagnostic criteria

1-Clinics/Gross

2-Histo

} **Suggestive**

3-Ancillary: Culture (+ typing)

} **Suggestive +**

CPB toxin
(intestinal content)

} **Confirmatory**

The 2018 *C. perfringens* toxin-based typing scheme

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E	+	-	-	+	+/-	-
F	+	-	-	-	+	-
G	+	-	-	-	-	+

Rood et al, Anaerobe 2018

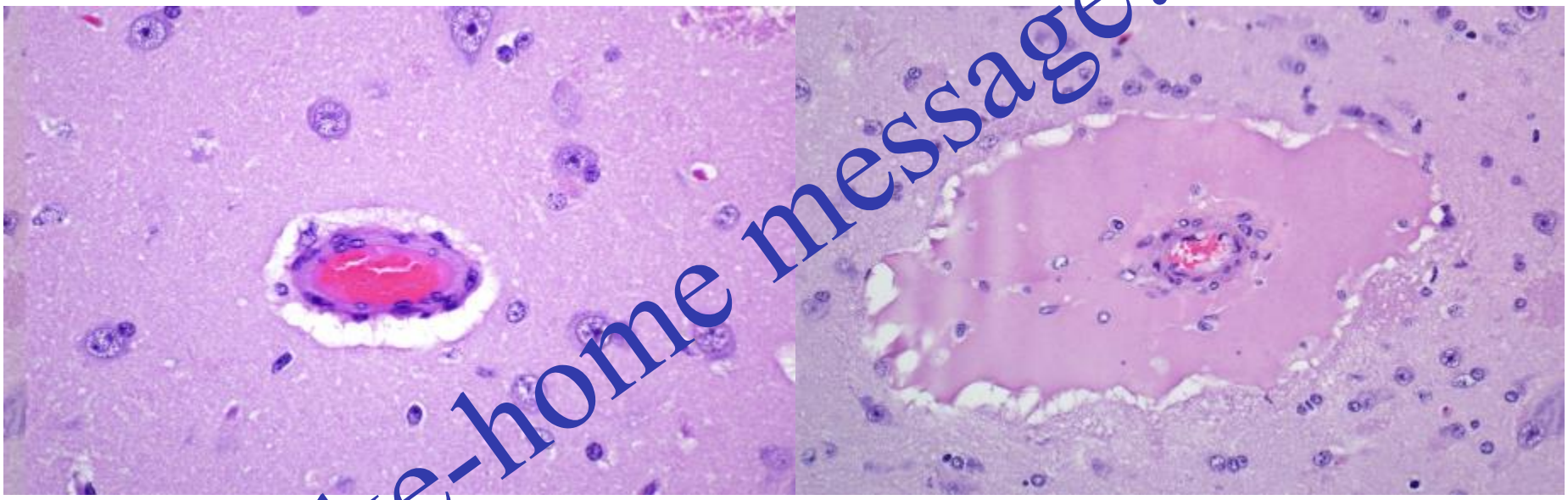
Epsilon toxin (ETX)

* 30 KD toxin ~~(USDA/CDC list B select agent)~~

* pore forming

* neurotoxin

* trypsin-activation required

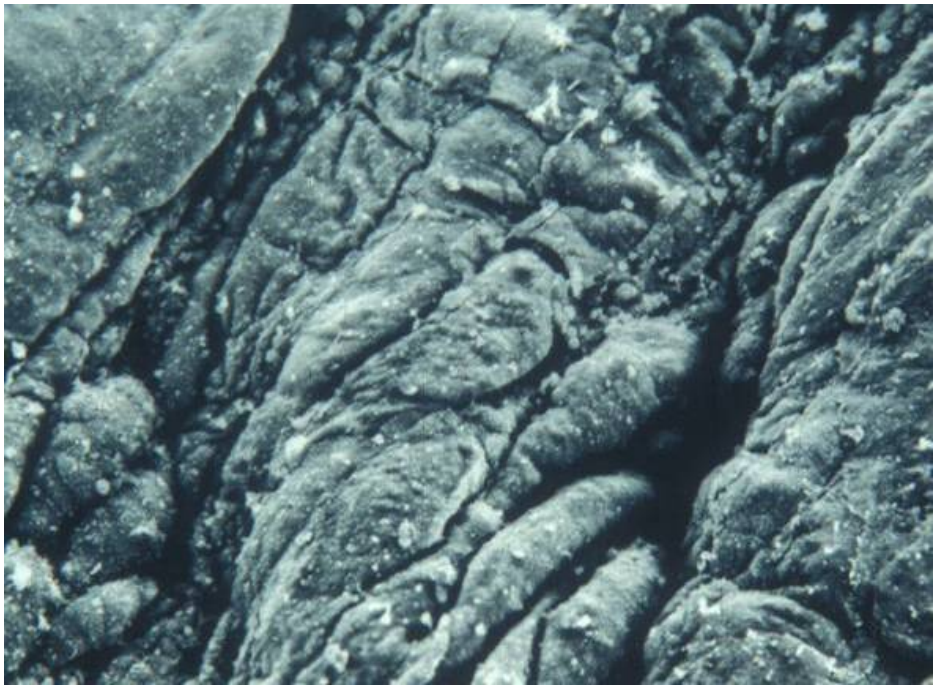


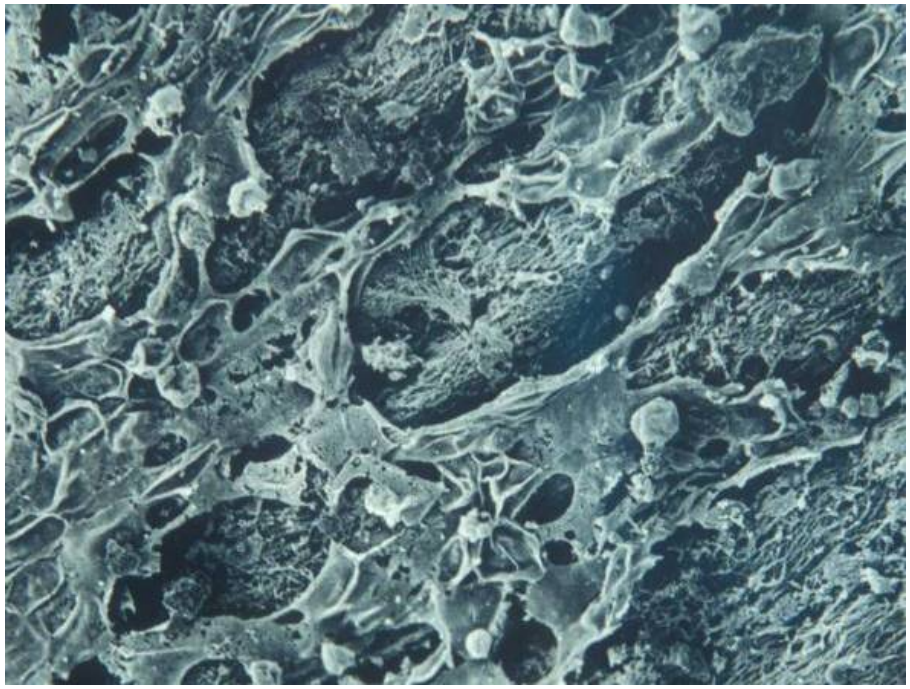
Take-home message!!!

HE

Photo Jorge Garcia

Ex vivo endothelial treatment with ETX





control

ETX

Epsilon toxin

*** *Clostridium perfringens* types B and D**

*** Enterotoxemia of sheep, goats and cattle**

*** Human disease: multiple sclerosis**
association suggested

OPEN ACCESS Freely available online

PLOS ONE

Isolation of *Clostridium perfringens* Type B in an Individual at First Clinical Presentation of Multiple Sclerosis Provides Clues for Environmental Triggers of the Disease

Kareem Rashid Rumah[1,2,3], Jennifer Linden[2], Vincent A. Fischetti[3], Timothy Vartanian[2]*

1 Tri-Institutional M.D.-Ph.D. Program of Weill Cornell Medical College, Rockefeller University and Memorial Sloan-Kettering Hospital, New York, New York, United States of America, **2** The Brain and Mind Research Institute and the Department of Neurology, Weill Cornell Medical College, New York, New York, United States of America, **3** The Laboratory of Bacterial Pathogenesis and Immunology, Rockefeller University, New York, New York, United States of America

Abstract

We have isolated *Clostridium perfringens* type B, an epsilon toxin-secreting bacillus, from a young woman at clinical presentation of Multiple Sclerosis (MS) with actively enhancing lesions on brain MRI. This finding represents the first time that *C. perfringens* type B has been detected in a human. Epsilon toxin's tropism for the blood-brain barrier (BBB) and binding to oligodendrocytes/myelin makes it a provocative candidate for nascent lesion formation in MS. We examined a well-characterized population of MS patients and healthy controls for carriage of *C. perfringens* toxinotypes in the

MULTIPLE SCLEROSIS JOURNAL | MSJ

Original Research Paper

Evidence of *Clostridium perfringens* epsilon toxin associated with multiple sclerosis

Sariqa Wagley, Monika Bokori-Brown, Helen Morcrette, Andrea Malaspina, Caroline D'Arcy, Sharmilee Gnanapavan, Nicholas Lewis, Michel R Popoff, Dominika Raciborska, Richard Nicholas, Ben Turner and Richard W Titball

Multiple Sclerosis Journal

1–8

DOI: 10.1177/1352458518767327

Correspondence to:
RW Titball
College of Life and Environmental Sciences, University of Exeter, Exeter EX4 4QD, Devon, UK.

Abstract

Background: It was recently reported that, using Western blotting, some multiple sclerosis (MS) patients in the United States had antibodies against epsilon toxin (Etx) from *Clostridium perfringens*, suggesting that the toxin may play a role in the disease.

epsilon toxin

vascular permeability

Take-home message!!!

Type D disease

Natural hosts

* Sheep
* Goats
* Cattle
* May be others….

Type D disease

Natural hosts

* Sheep
* Goats
* Cattle
* May be others….

Clinical signs

Neurological disease

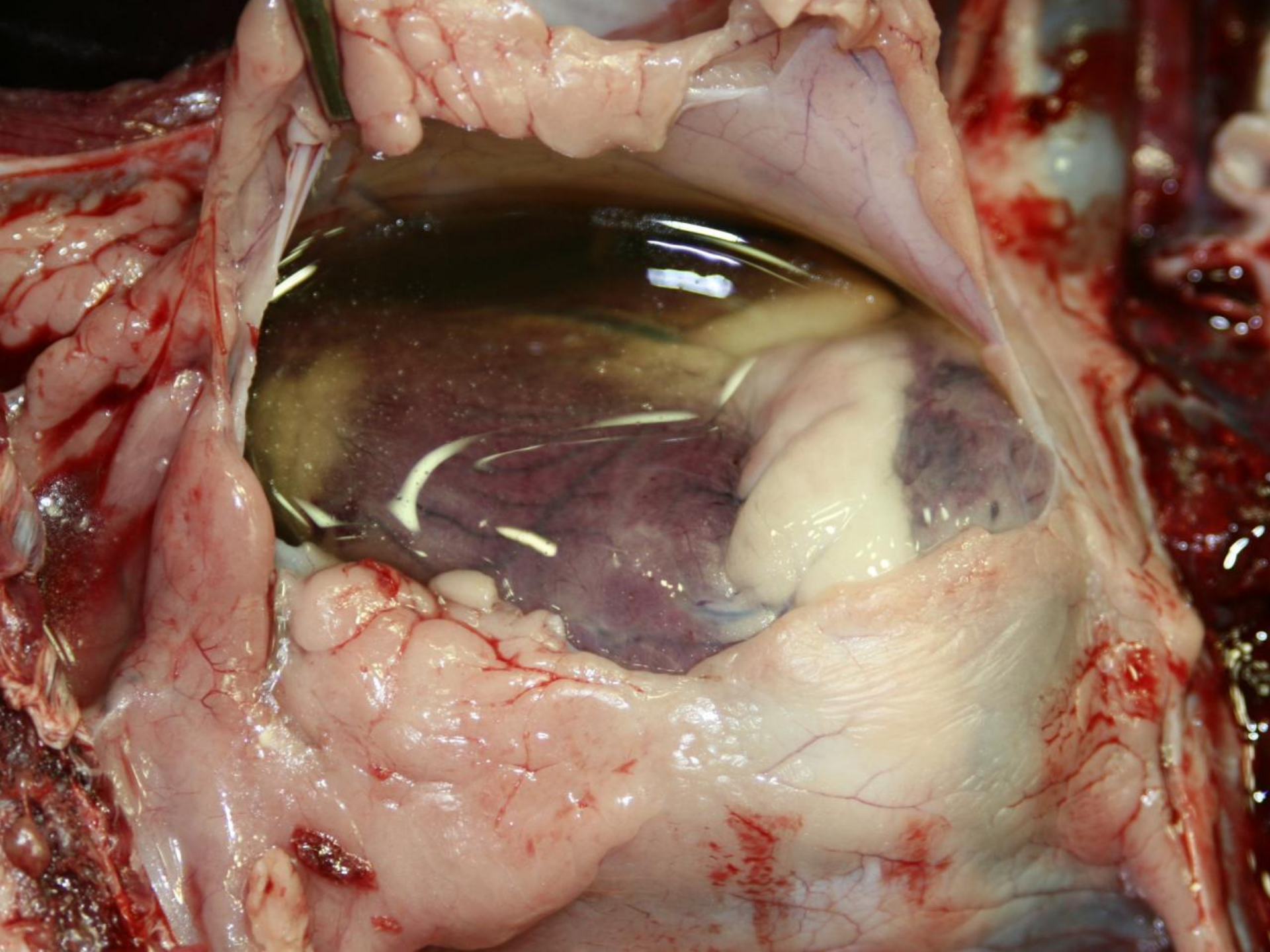
(leaky disease)

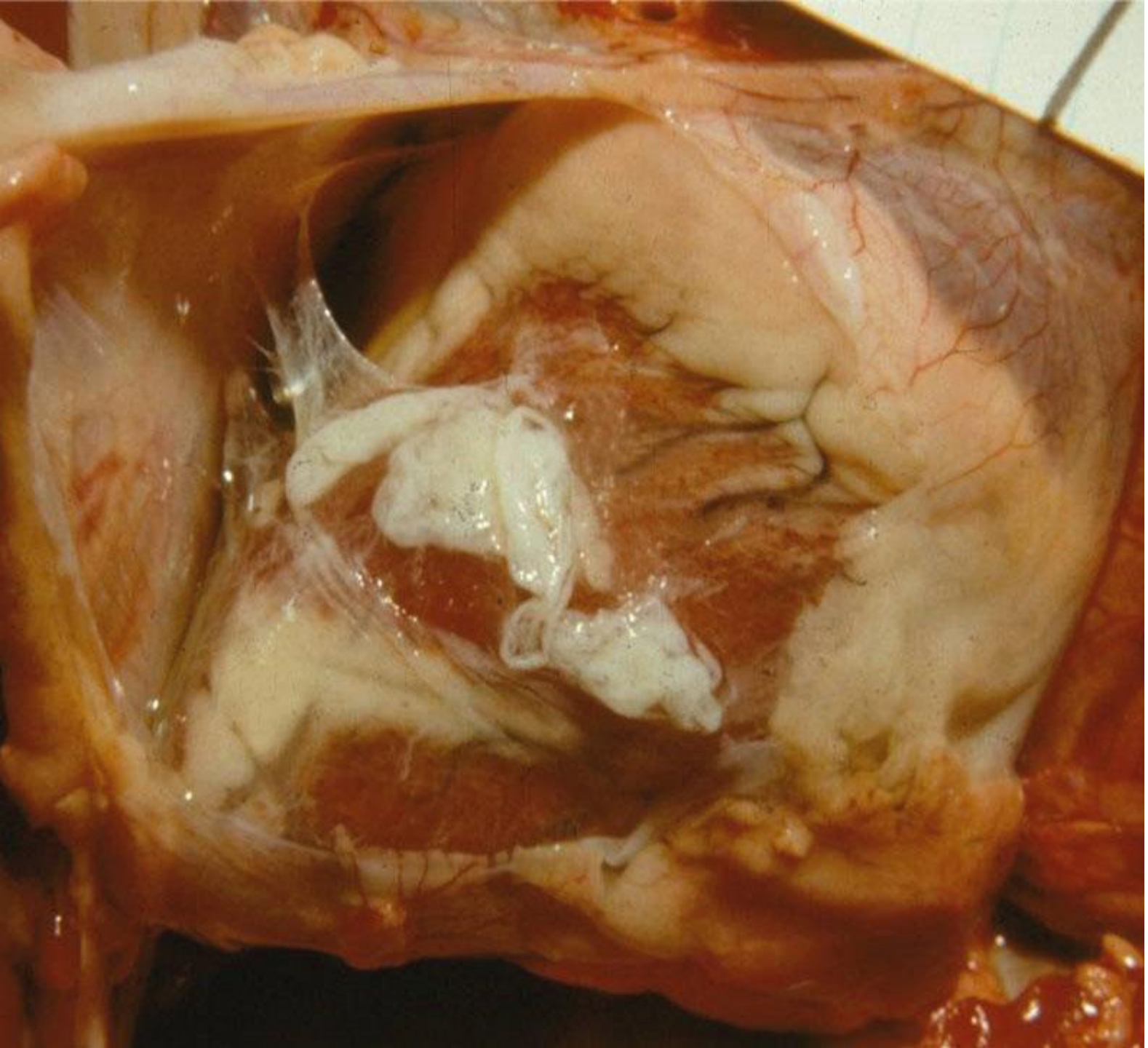
Respiratory difficulty

Sudden death

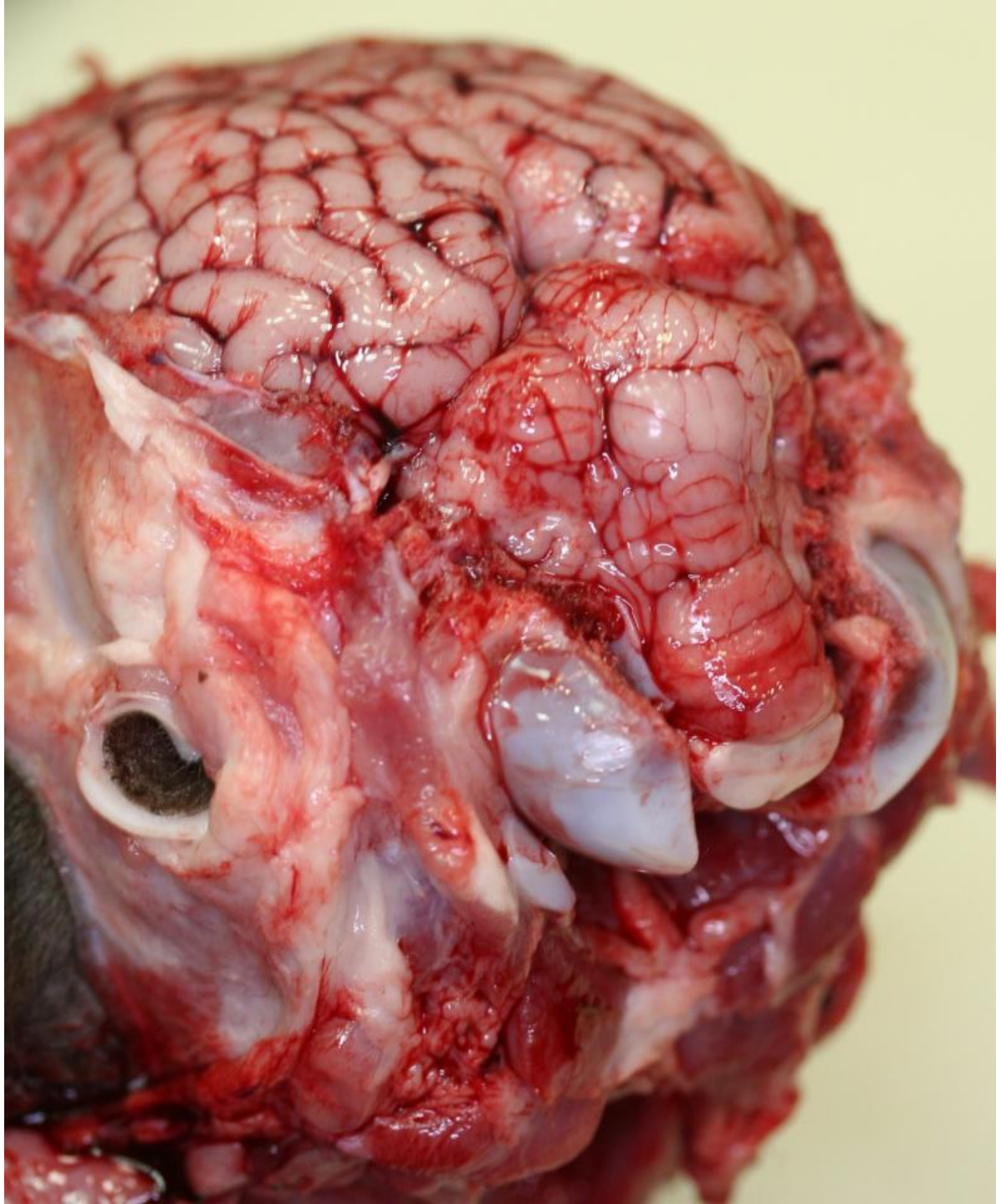
NO DIARRHEA (usually)

Take-home message!!!



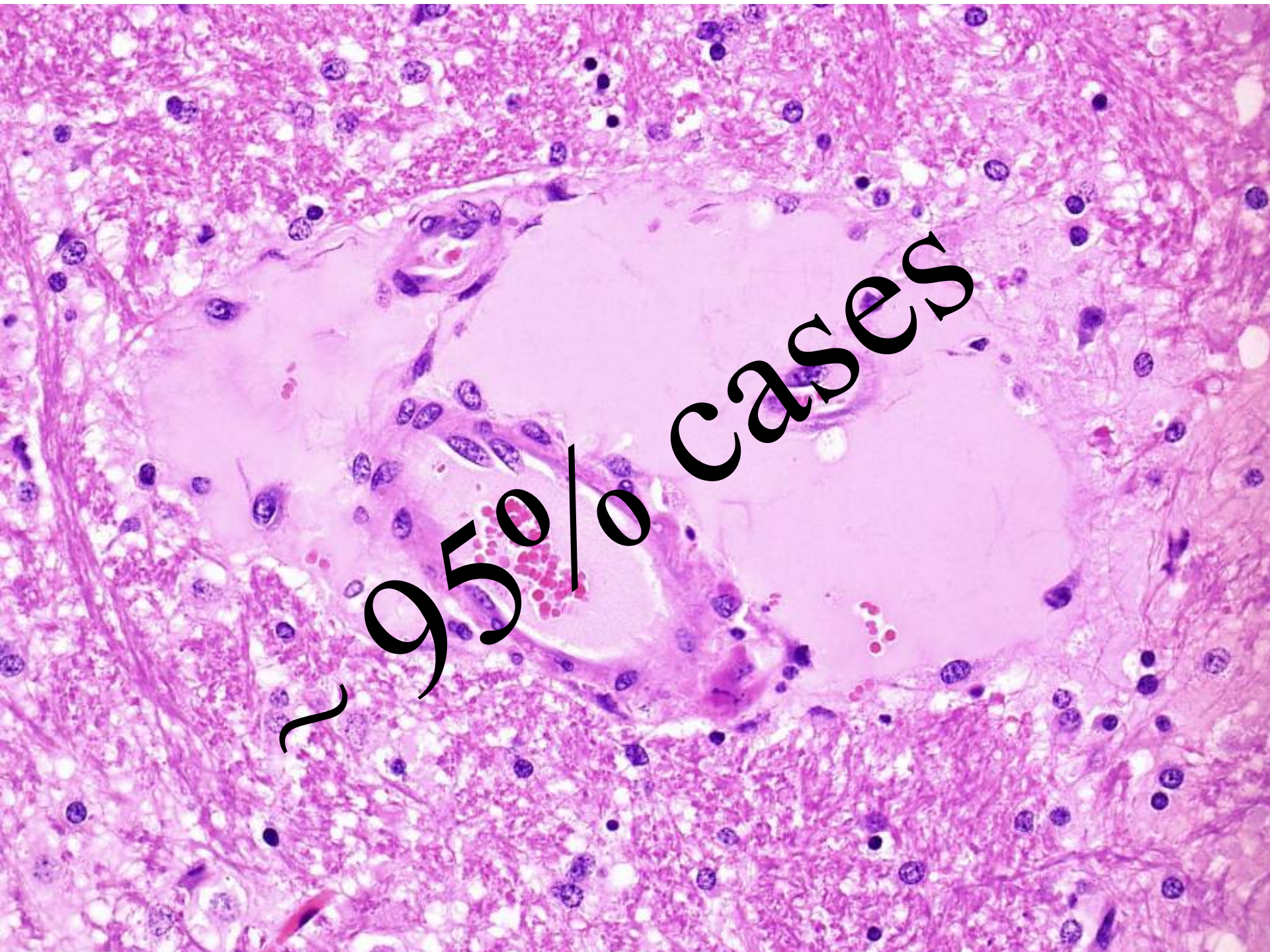


Bill Hartley

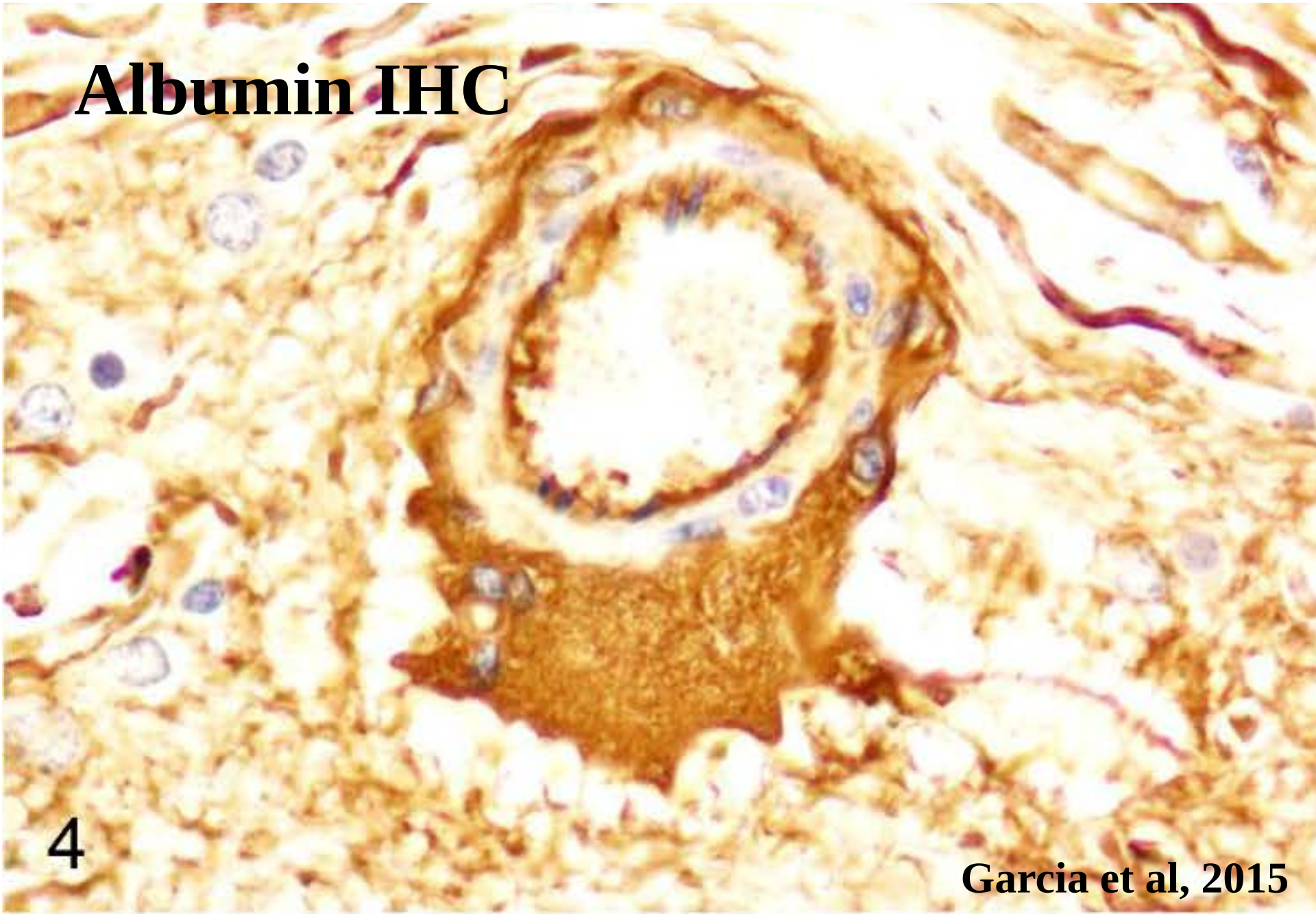


Bradd Barr


UCDavis

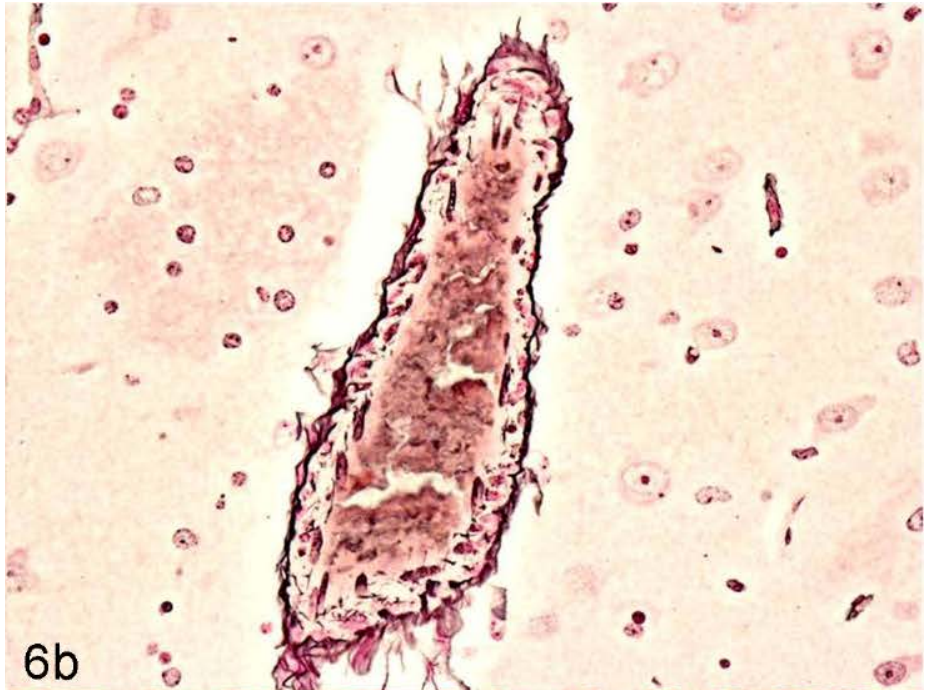

~95% cases

Albumin IHC



Garcia et al, 2015

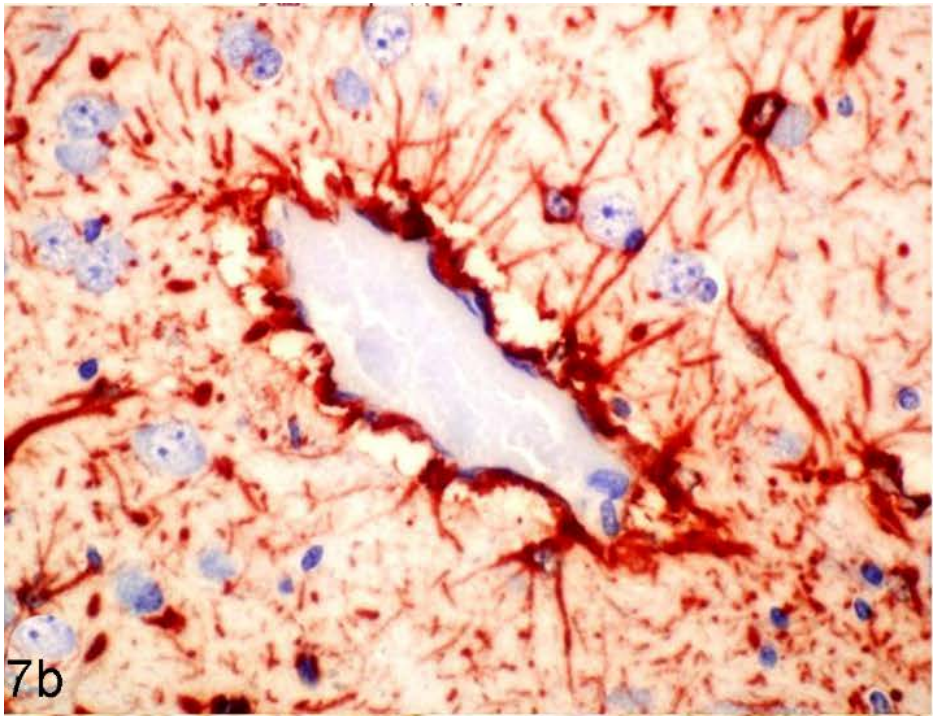
Gomori

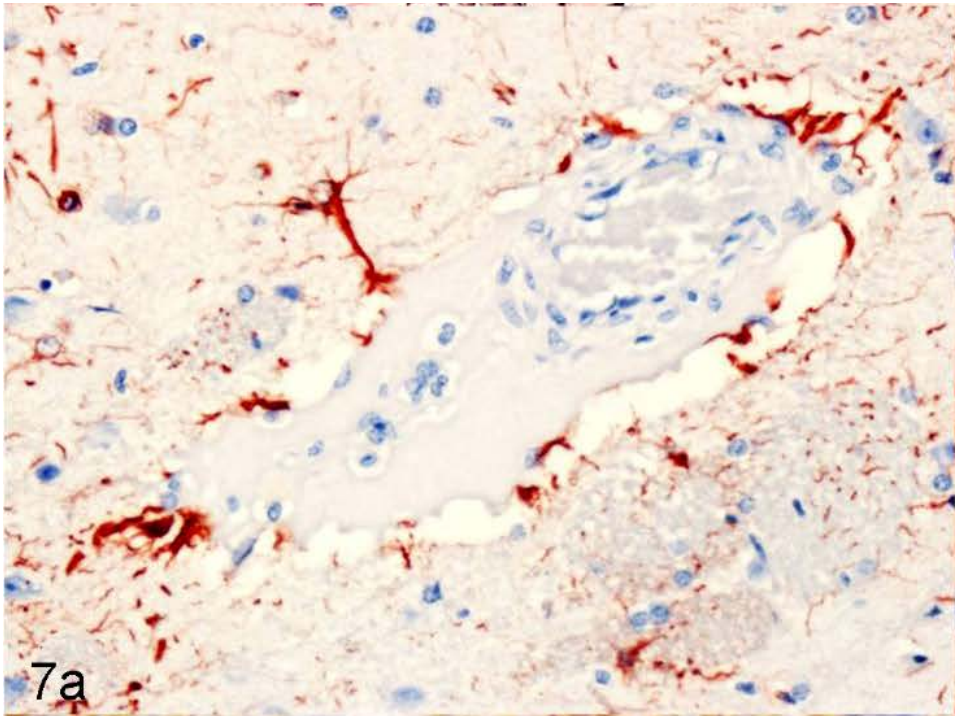


Control **ETX**

Garcia et al, 2015

GFAP





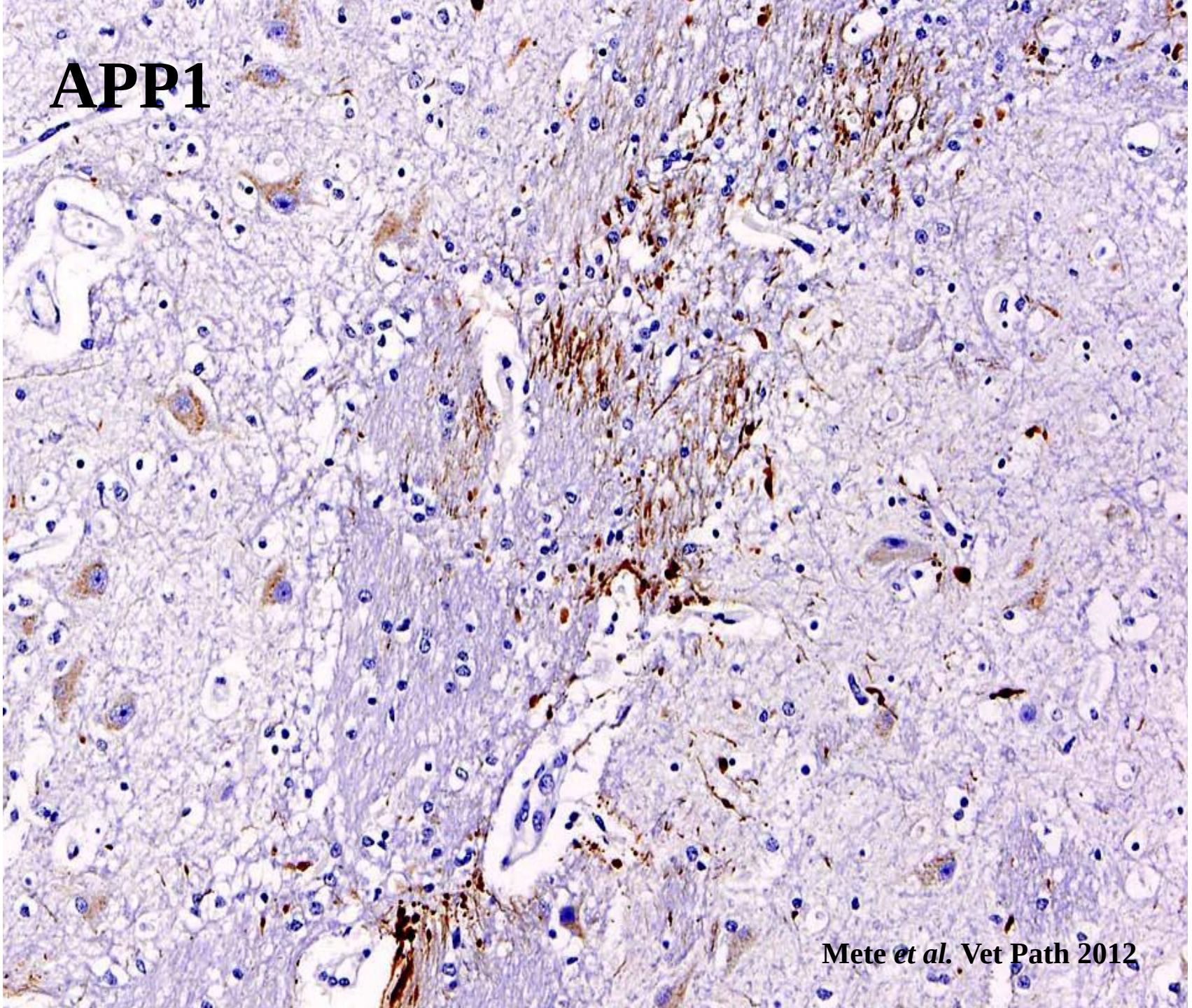
Control **ETX**

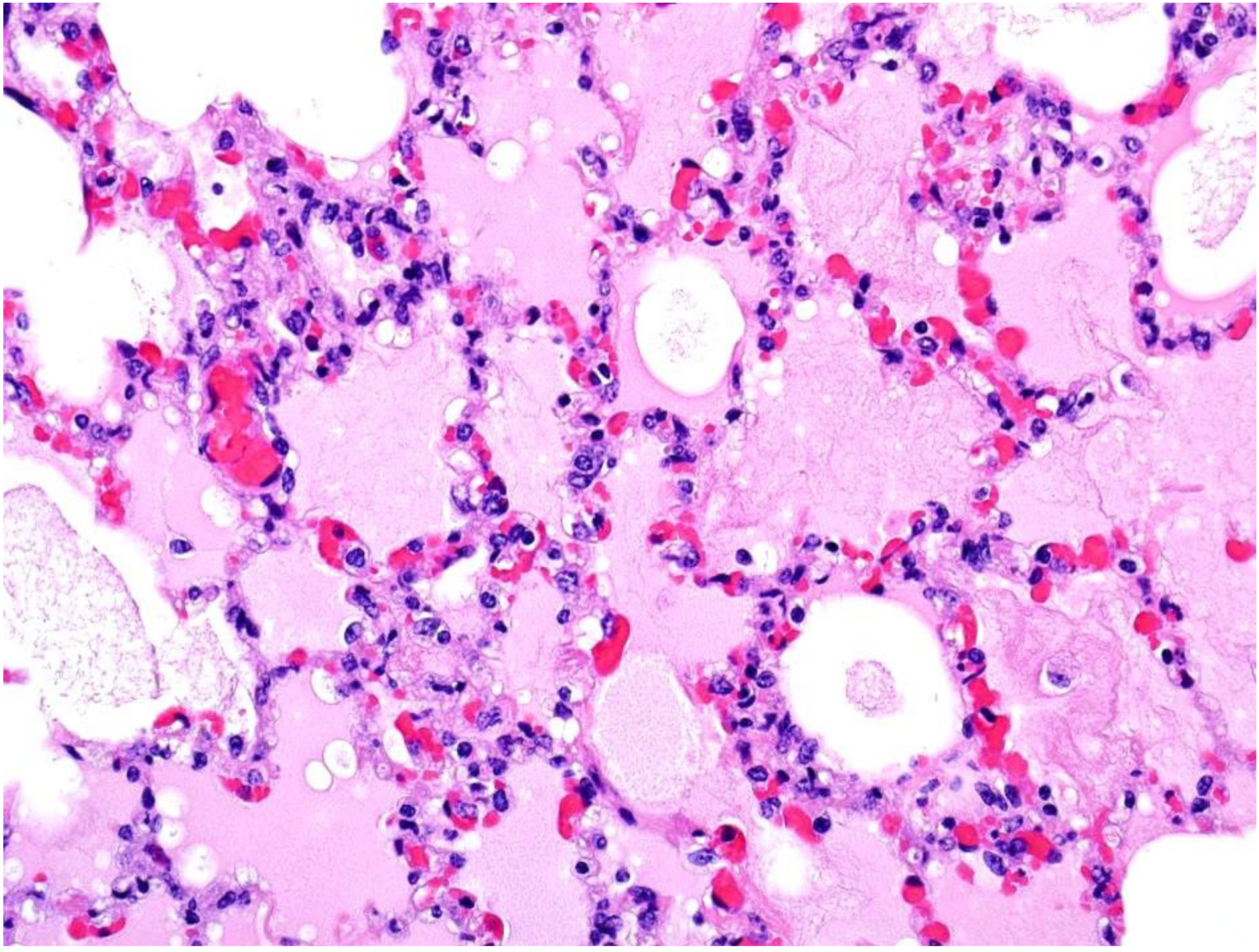
Garcia et al, 2015

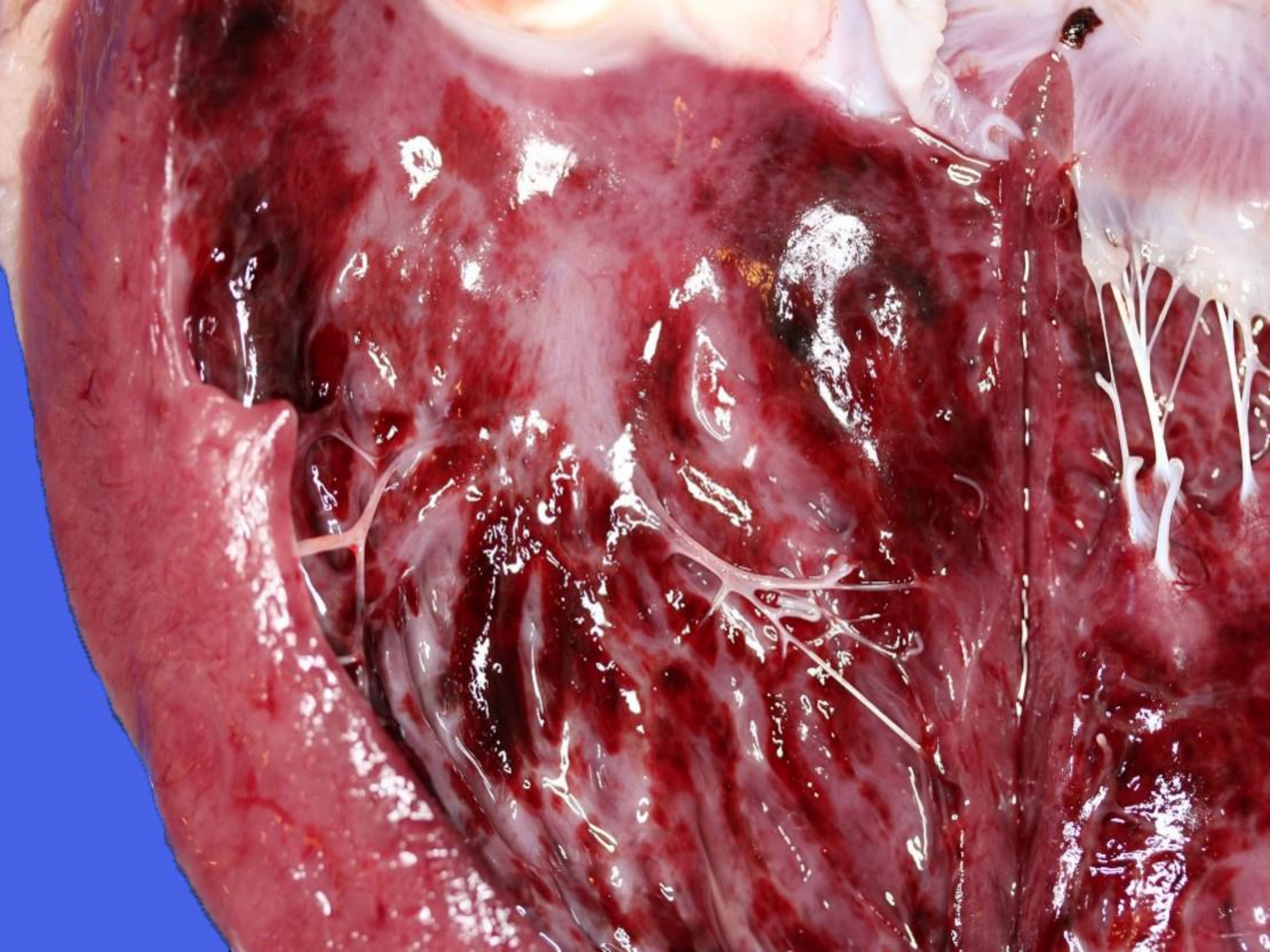
Intra-MURAL

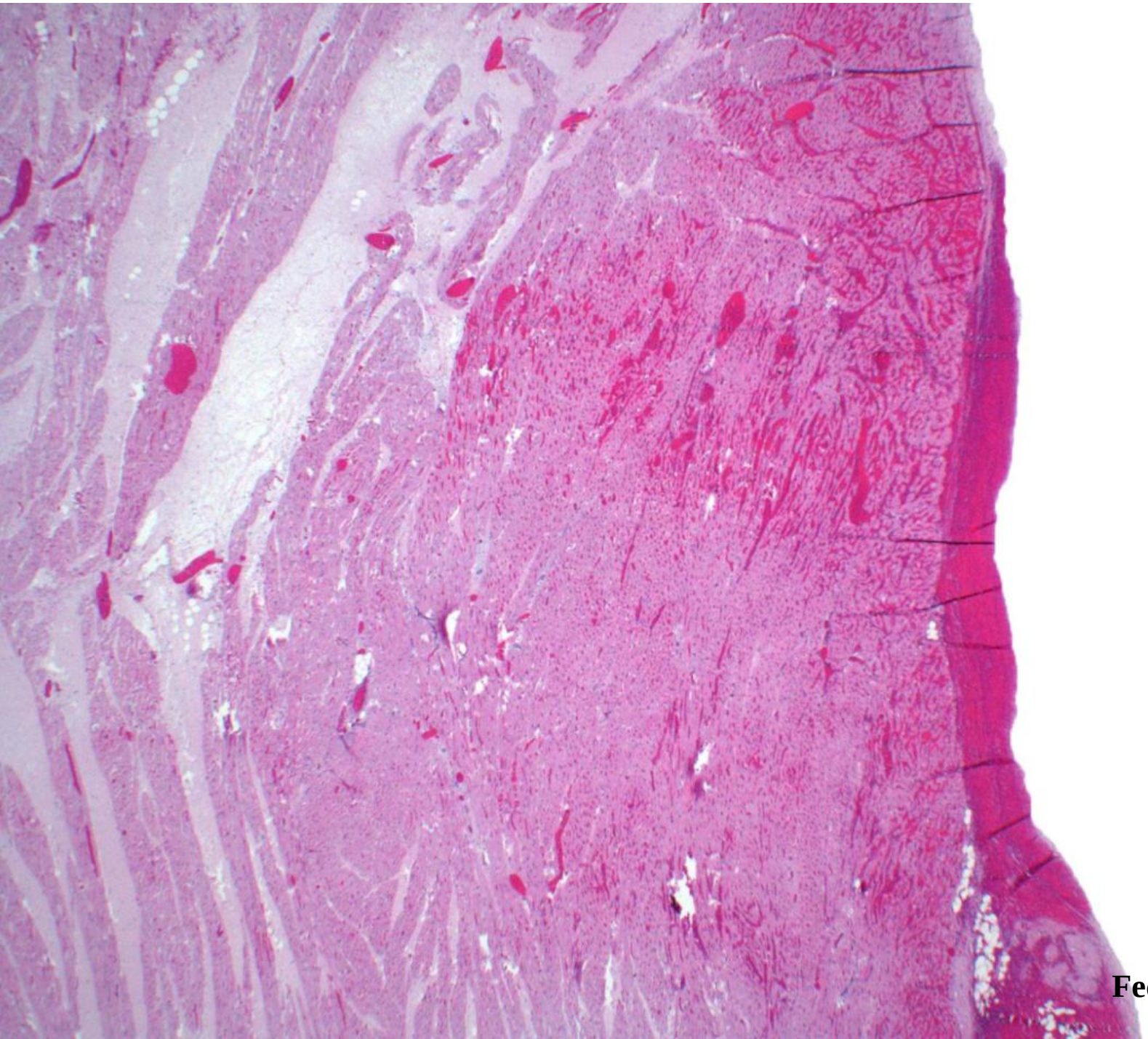
vs

Peri-VASCULAR

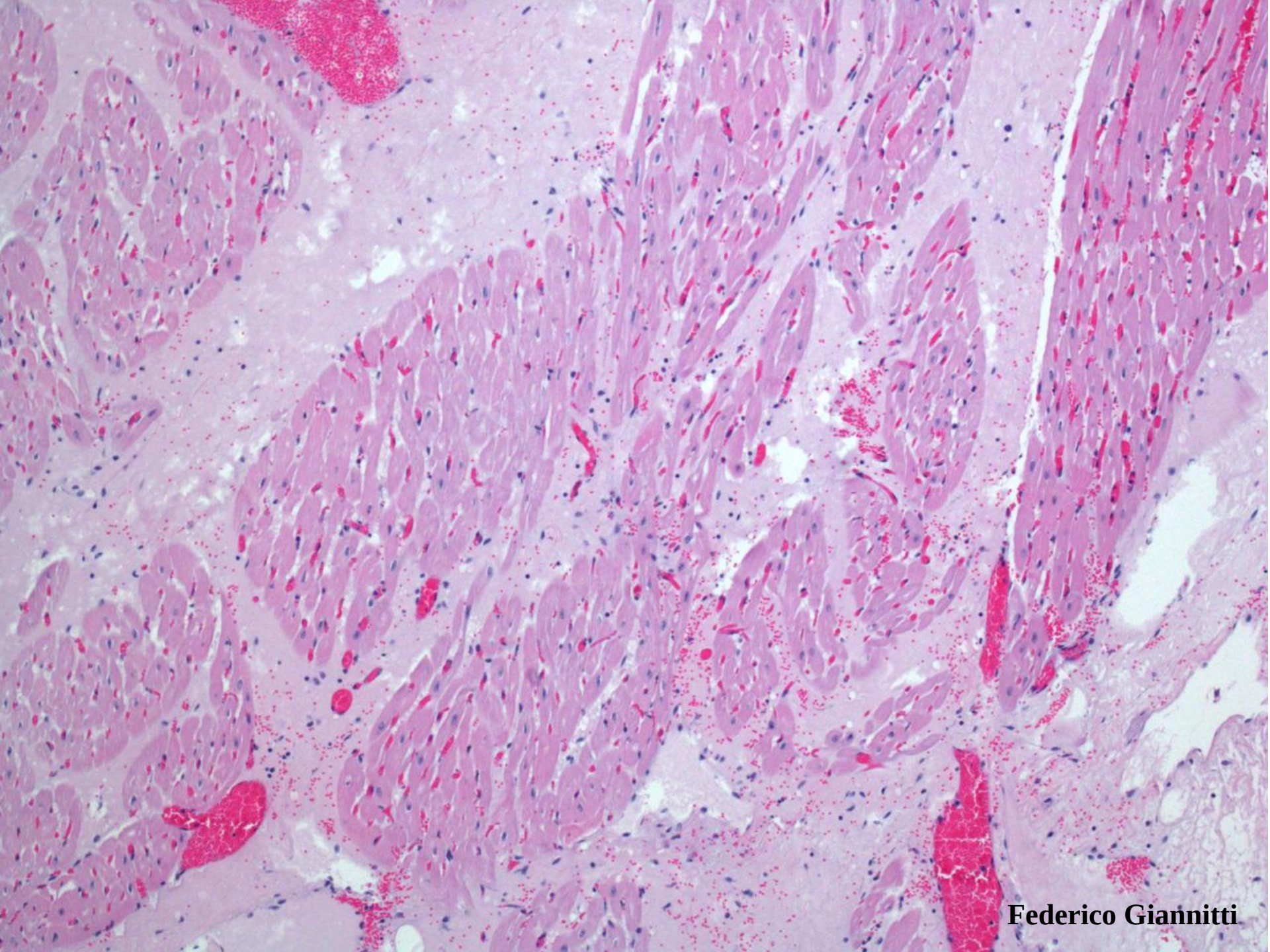

APP1
Mete *et al.* Vet Path 2012

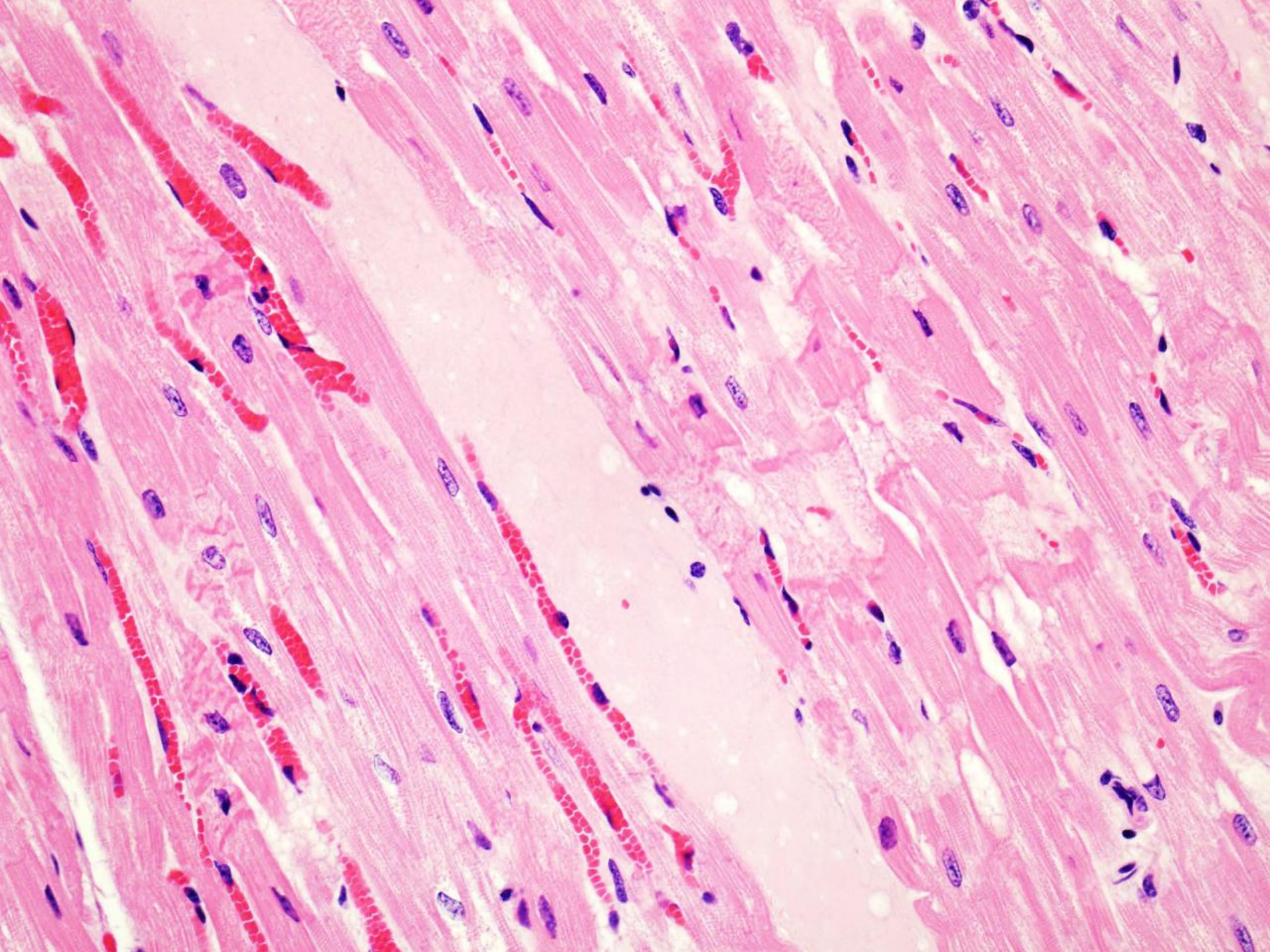


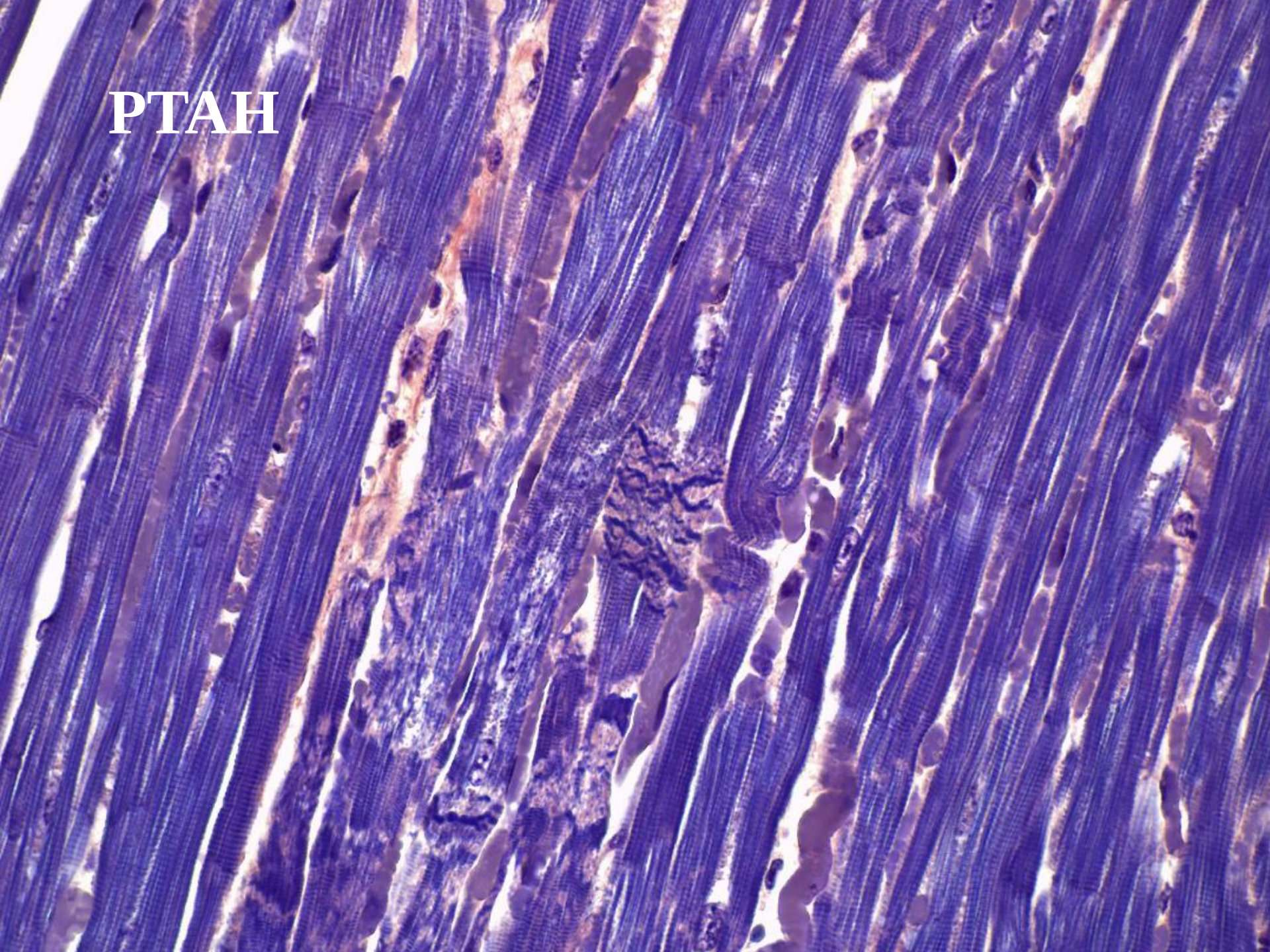


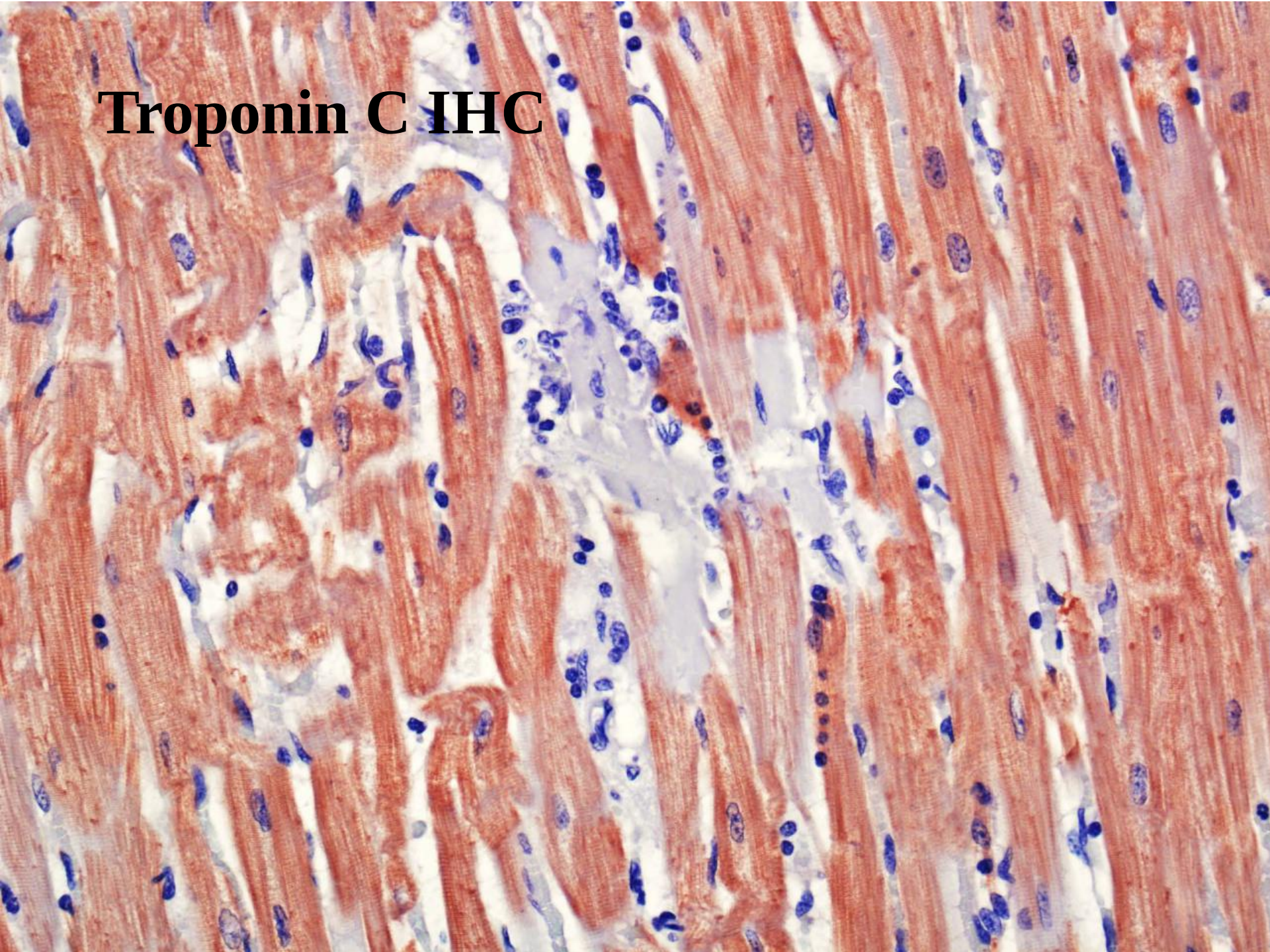


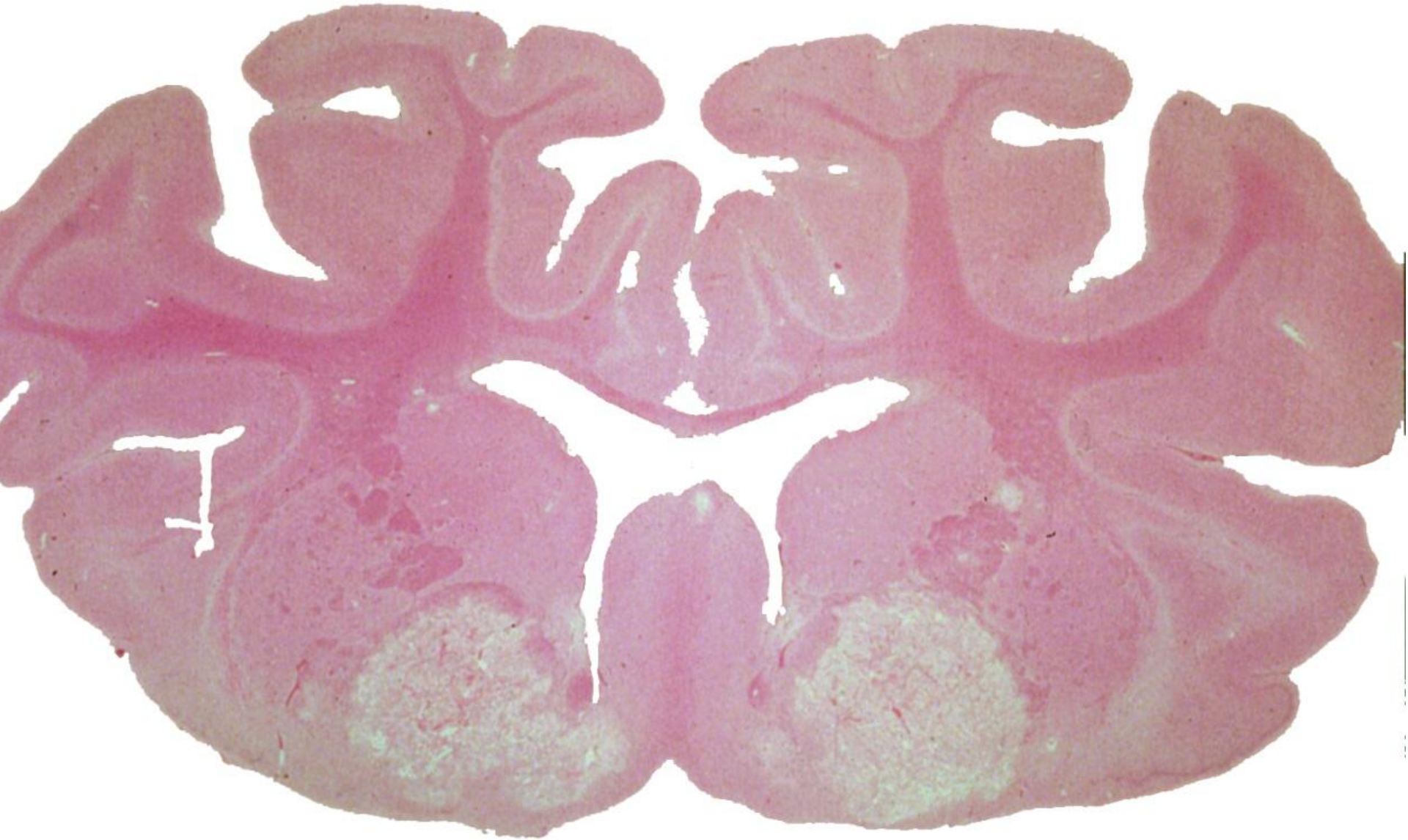
Federico Giannitti


Federico Giannitti



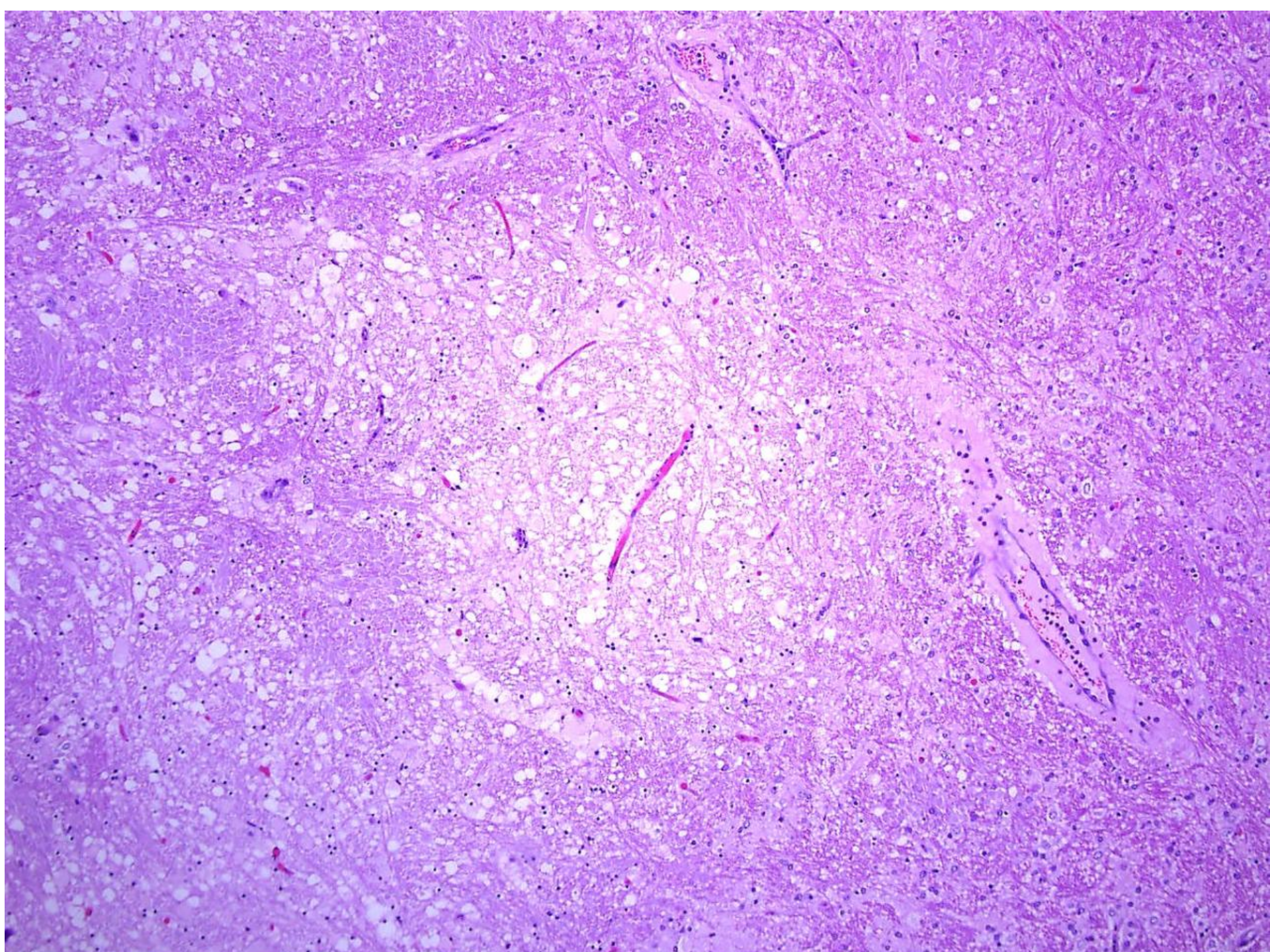

РТАН

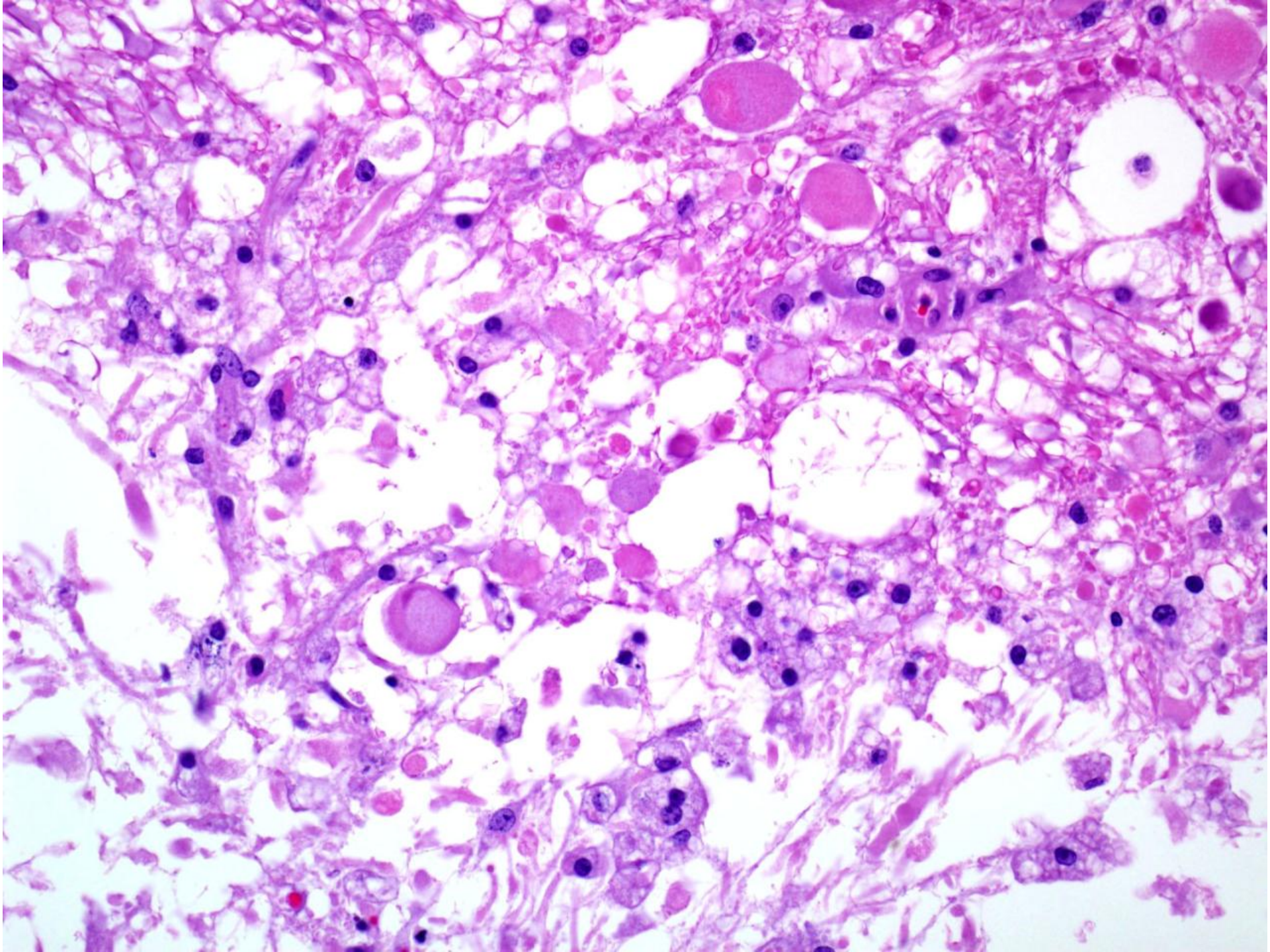

Troponin C IHC



Bill Hartley

Bill Hartley





Diagnostic criteria

1-Clinics/gross		**Suggestive/confir.***
2-Histology		**Confirmatory***
3-Ancillary:	Culture (+ typing)	**Suggestive**
4-Ancillary:	Epsilon toxin	**Confirmatory**

Other practical diagnostic tools

BAYER

Multistix® SG

Reagent Strips
for Urinalysis

- Glucose
- Bilirubin
- Ketone (Acetoacetic Acid)
- Specific Gravity
- Blood
- pH
- Protein
- Urobilinogen

For *In Vitro* Diagnostic Use

100 Strips

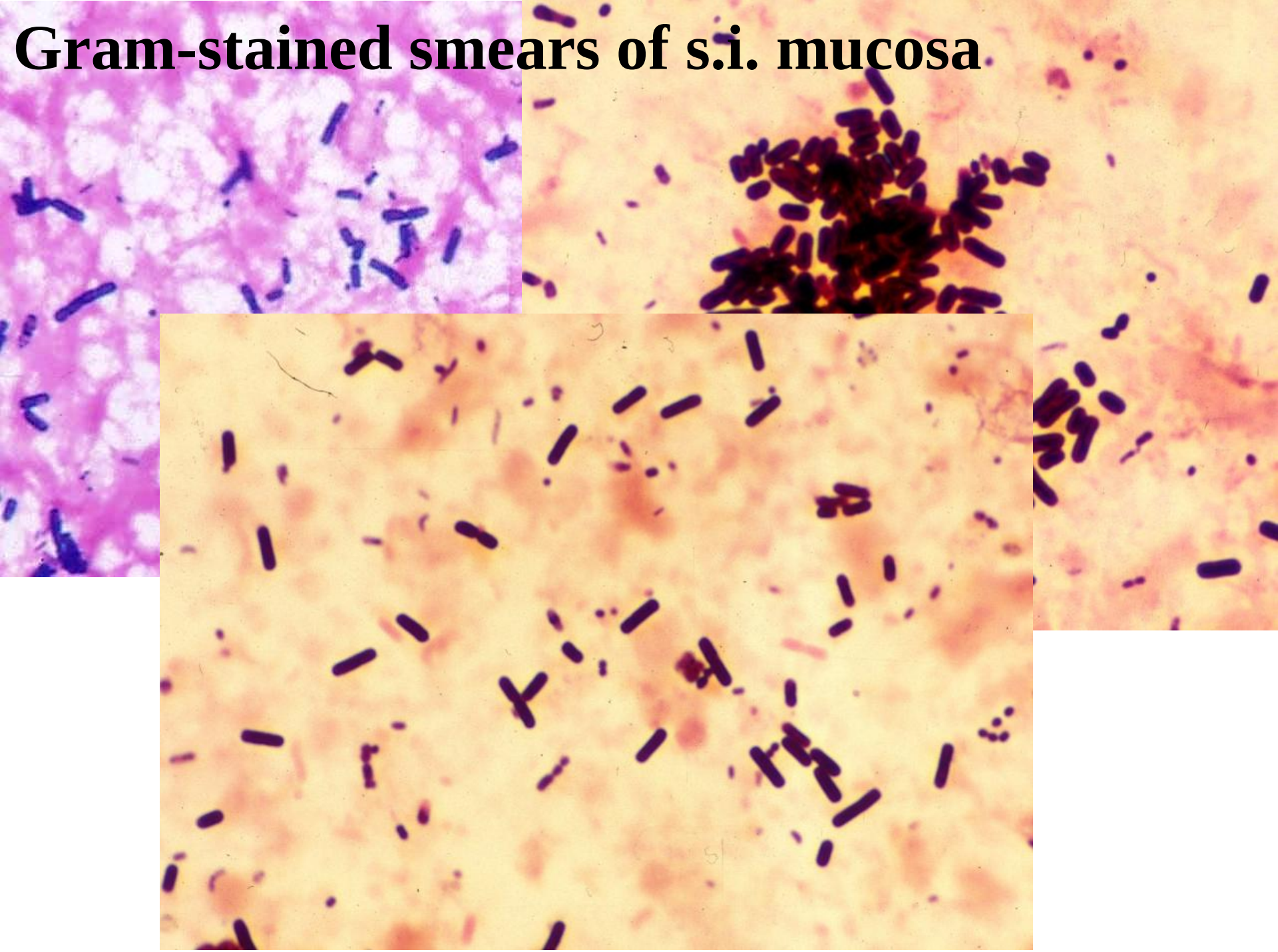
BILIRUBIN 30 seconds	NEGATIVE				SMALL +	MODERATE + +	LARGE + + +
GLUCOSE 30 seconds	NEGATIVE	g/dL (%) mg/dL	1/10 (tr.) 100	1/4 250	1/2 500	1 1000	2 or more 2000 or more

KC7515C Rev. 10/97 USA

Glucosuria:

Helpful but rare
Treatment complicates

Gram-stained smears of s.i. mucosa



Natural hosts

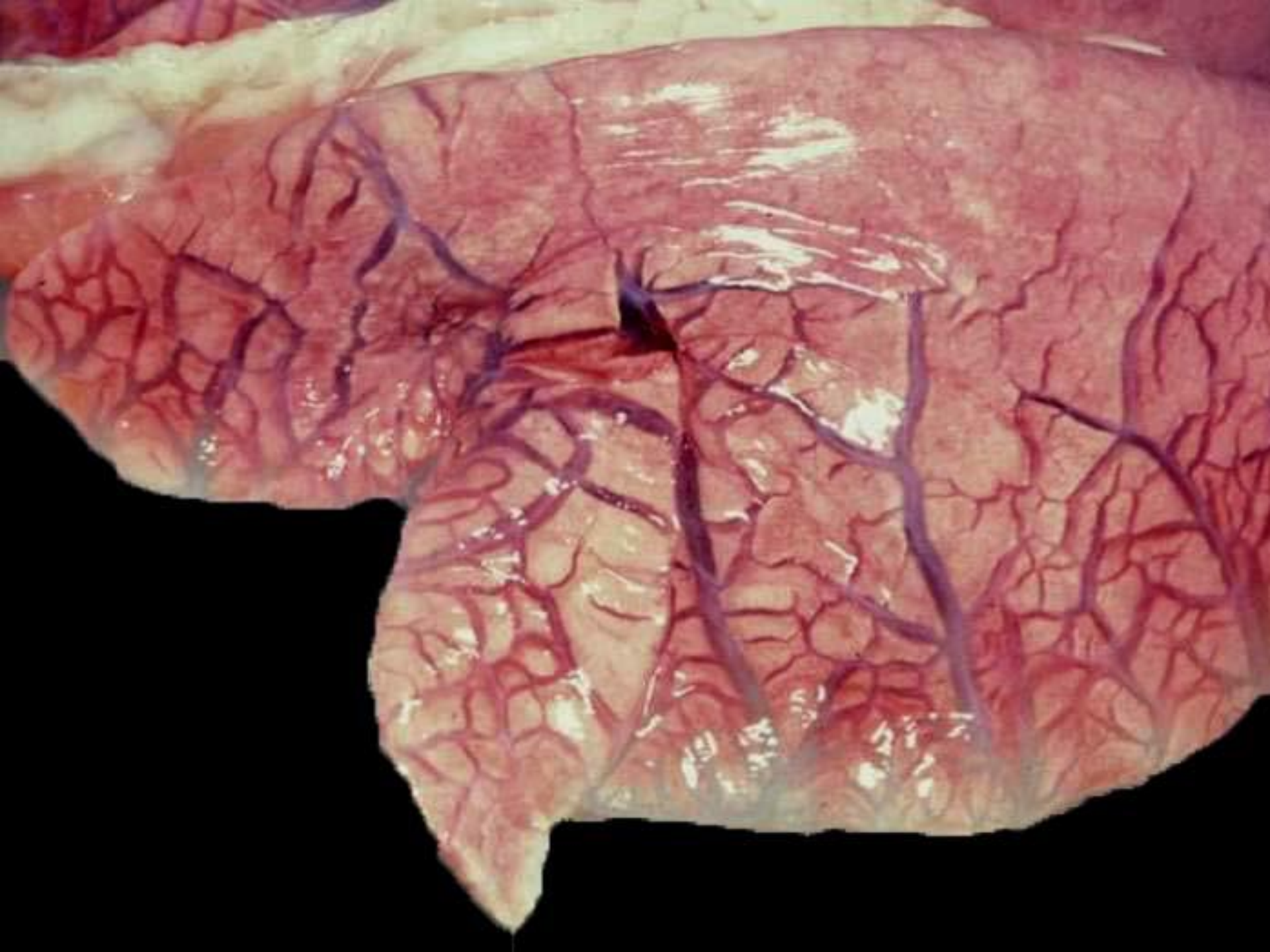
* Sheep
* **Goats**
* Cattle
* May be others….

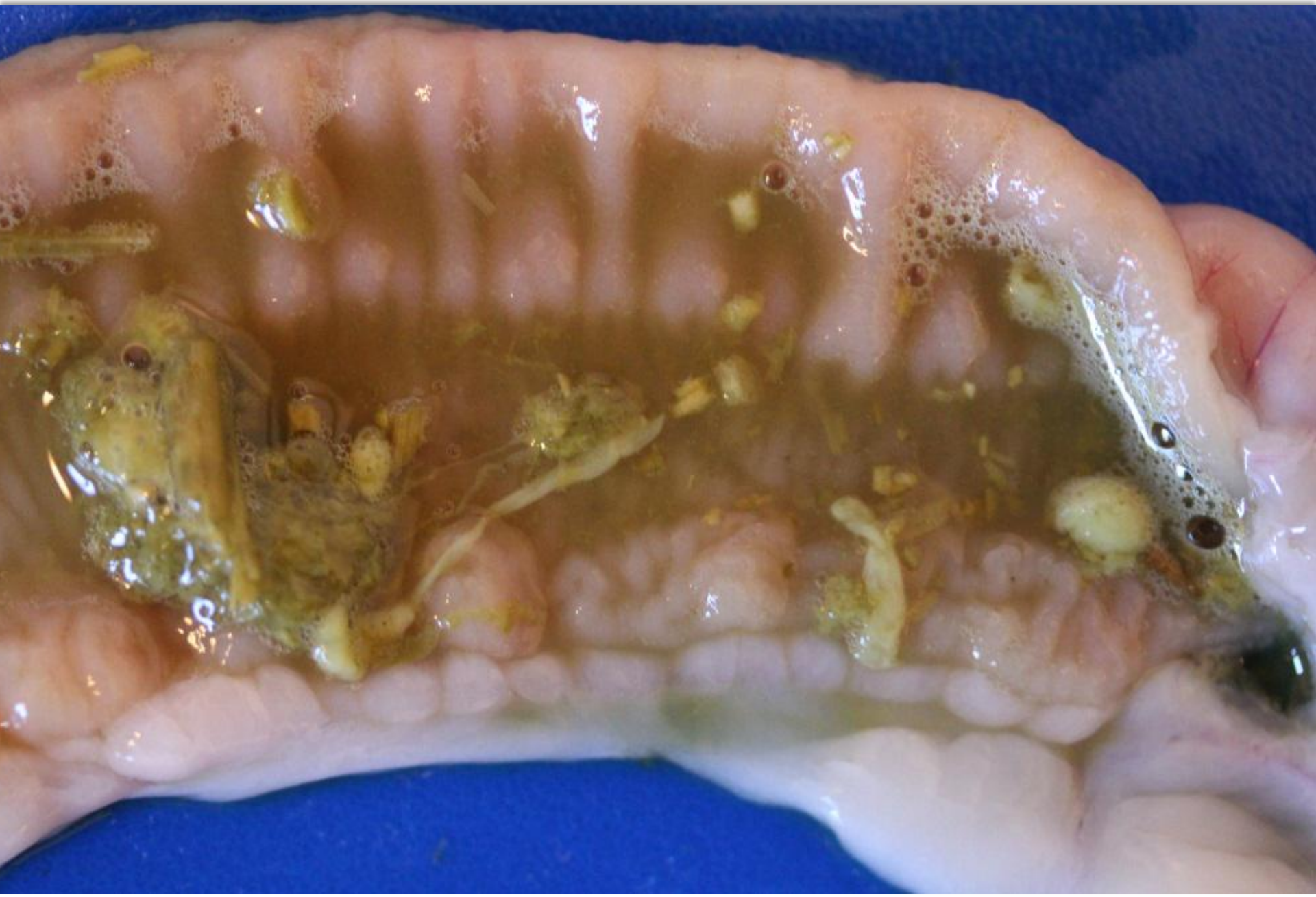
Neuro/systemic disease
or
Alimentary disease
or
Both

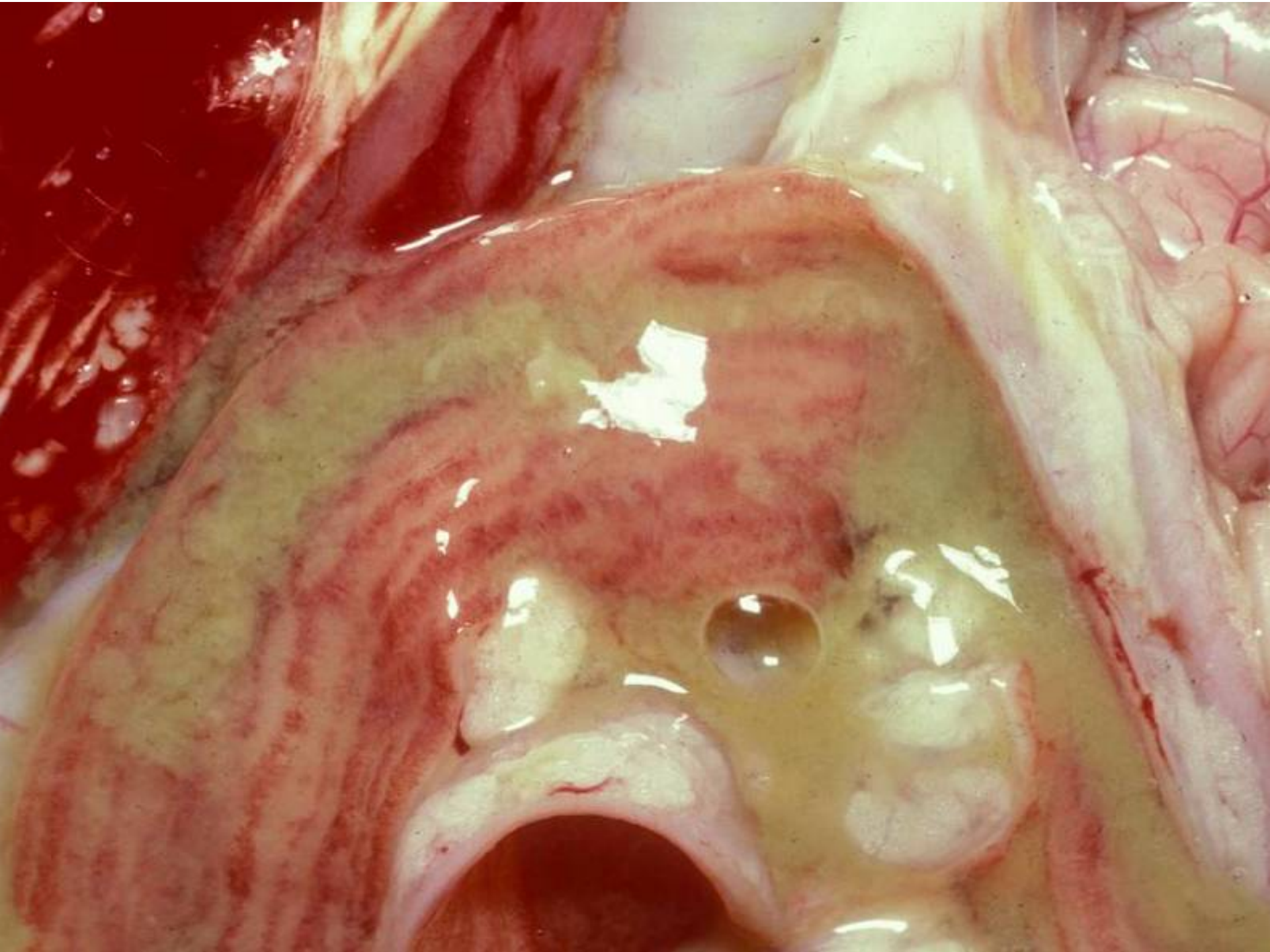
Clinical signs/lesions

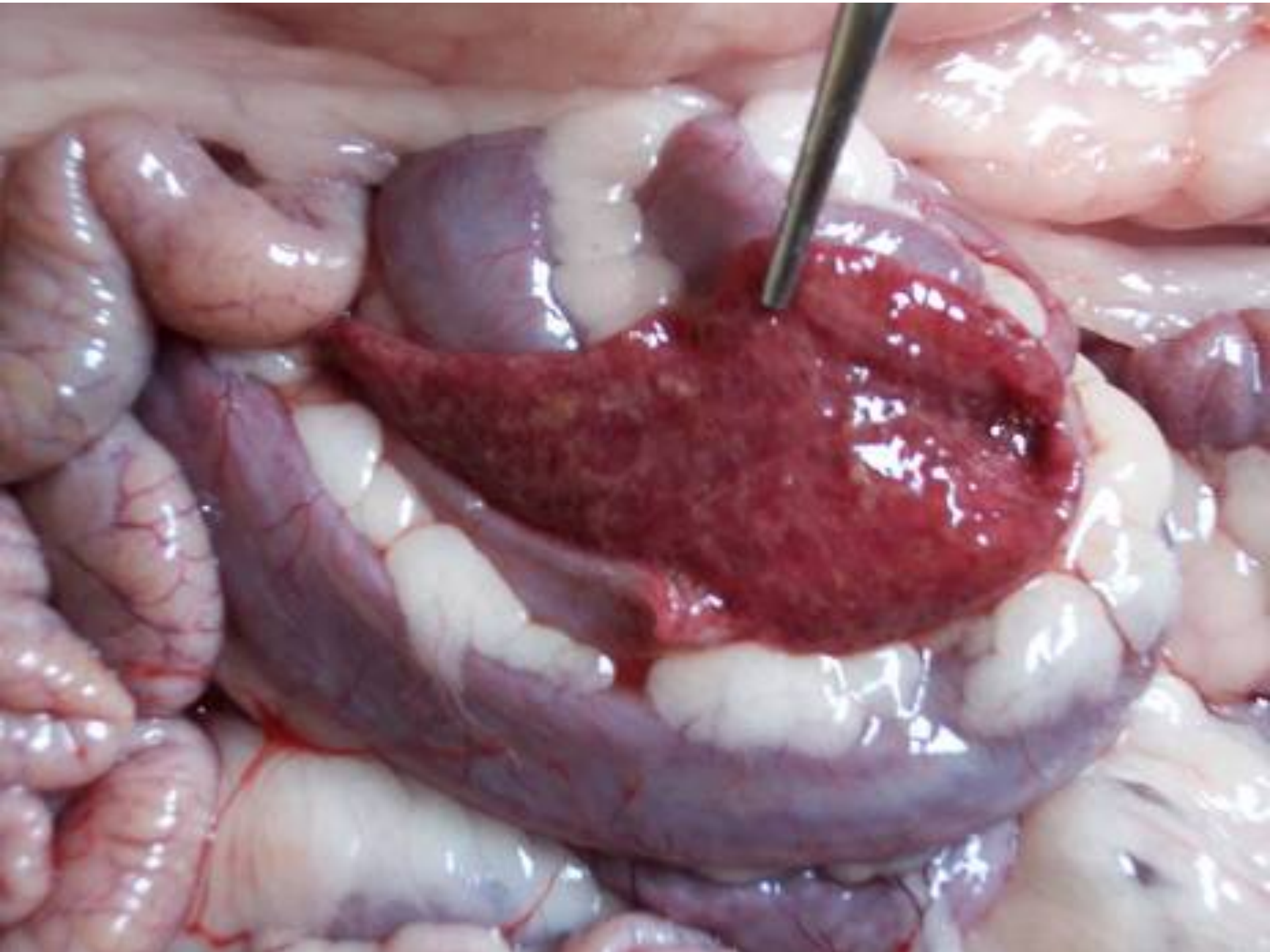
- **Acute**
- **Sub-acute**
- **Chronic**

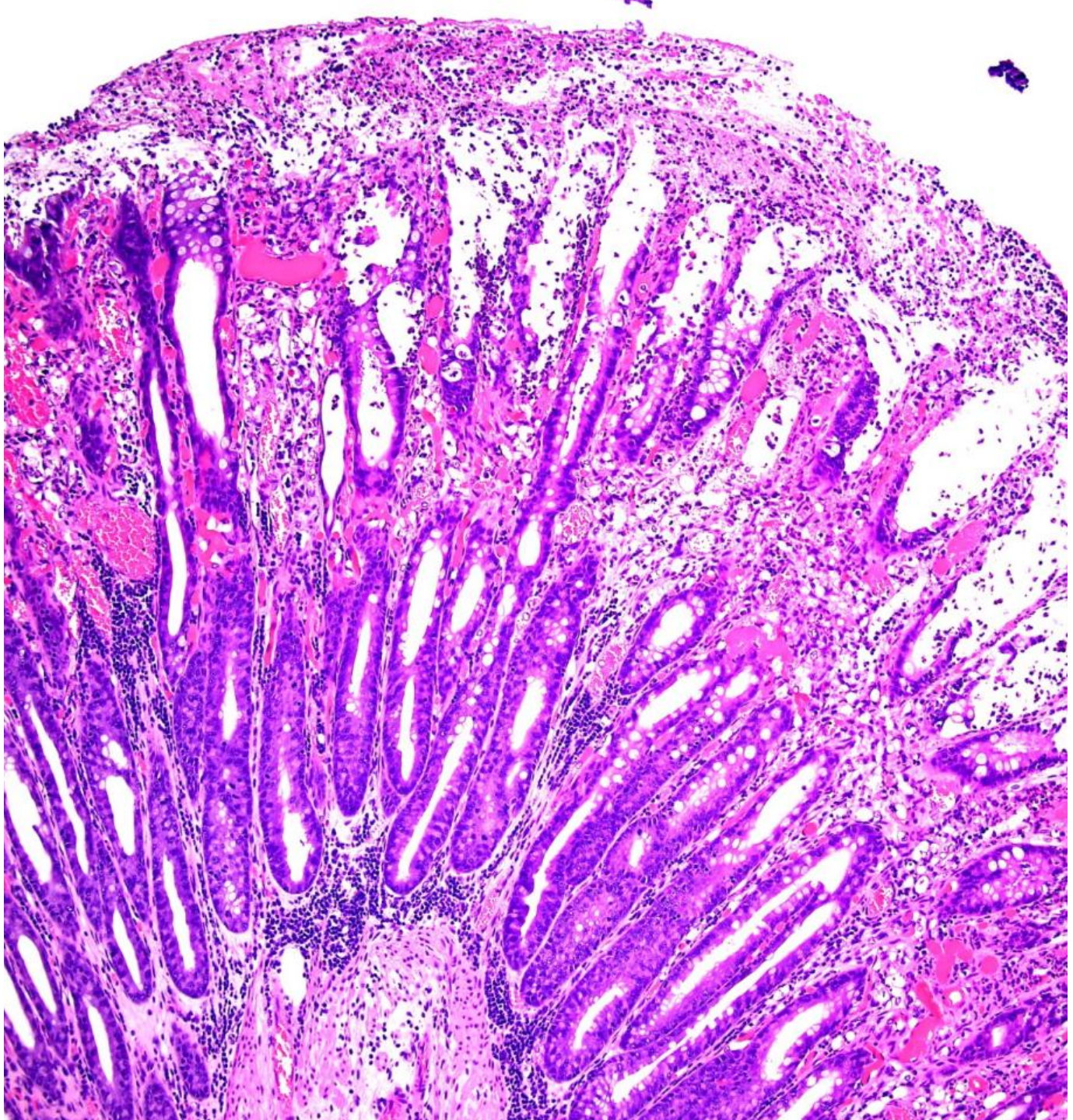
- **Acute** } **Neurologic**
- **Sub-acute** } **Neurologic or Neurologic + colitis**

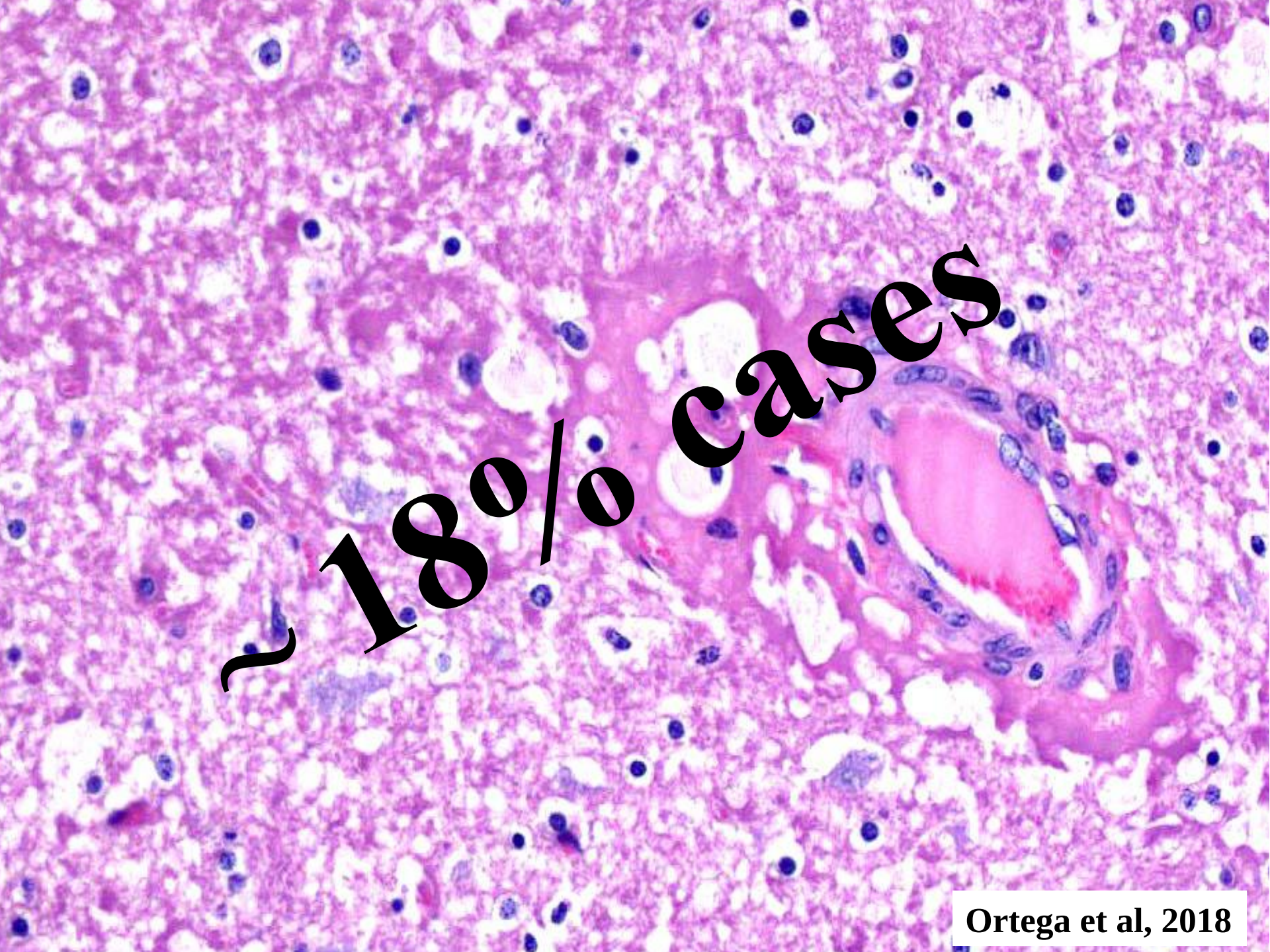






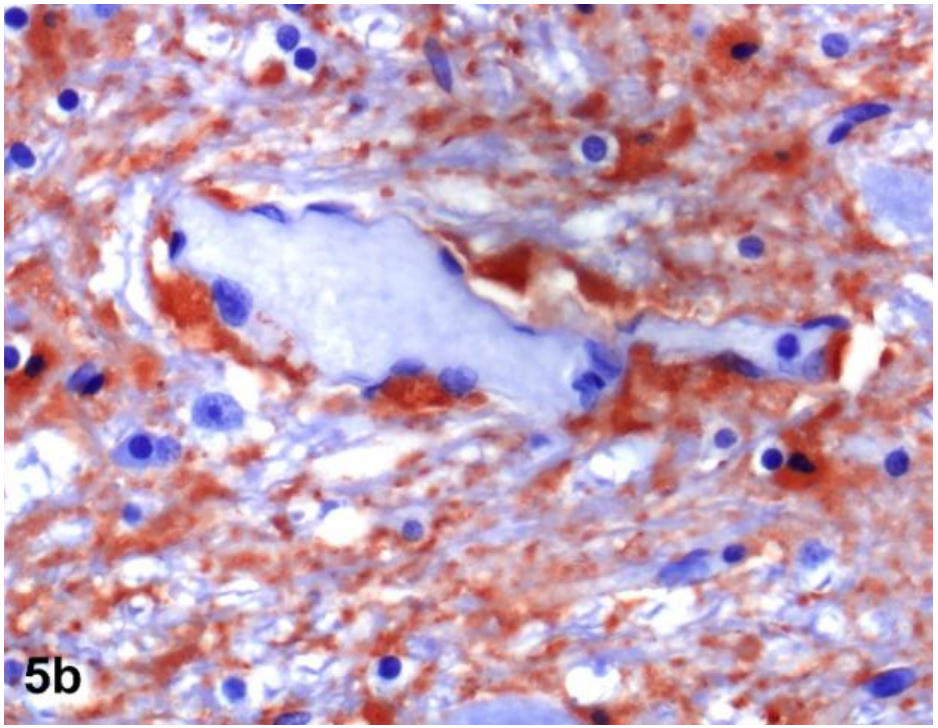




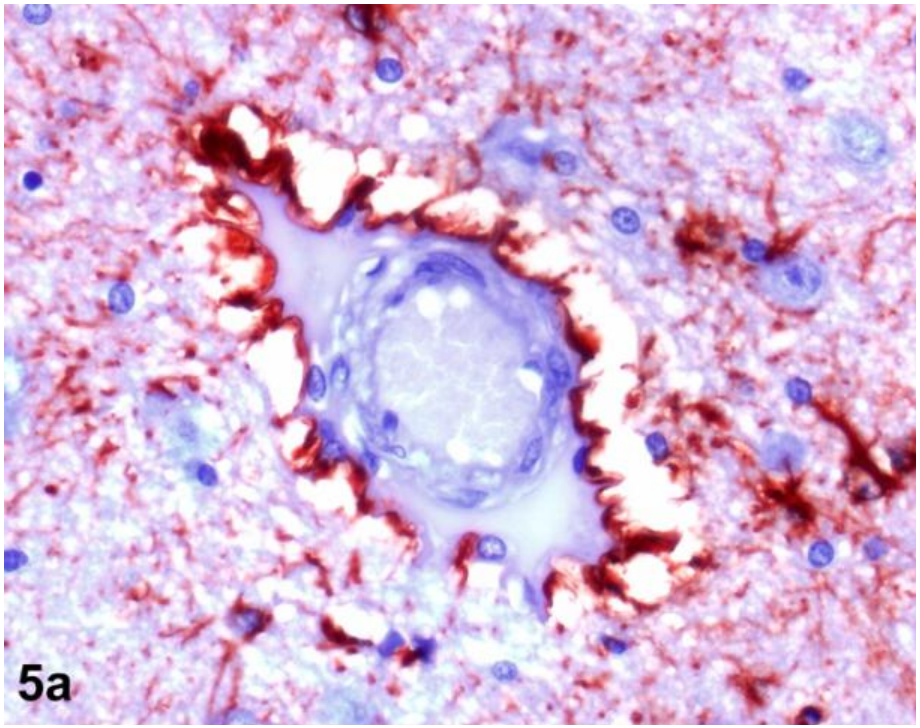

~ 18% cases
Ortega et al, 2018

GFAP IHC

Healthy control



ETX



Clinical signs

- Acute } Neurologic
- Sub-acute } Neurologic or Neurologic + colitis
- **Chronic** } **Colitis**

≠

Take home message

Natural hosts

* Sheep
* Goats
* **Cattle**
* May be others….

Brain lesions similar to those produced in sheep by *C. perfringens* type D

Buxton et al, 1981; Jeffrey, 1992; Fairley 2005


FSE
Photo Rob Fairley

No causal relationship

between *C. perfringens* type D

and

these lesions

Enterotoxemia type D:

1-One of the most frequently diagnosed diseases in cattle

2-Experimental disease: Yes!!!

3-Natural disease: very rare?