Enteric clostridial diseases

Part B

Francisco A. Uzal

California Animal Health and Food Safety Laboratory
University of California, San Bernardino

Experimental disease

Natural disease

Experimental disease

Natural disease

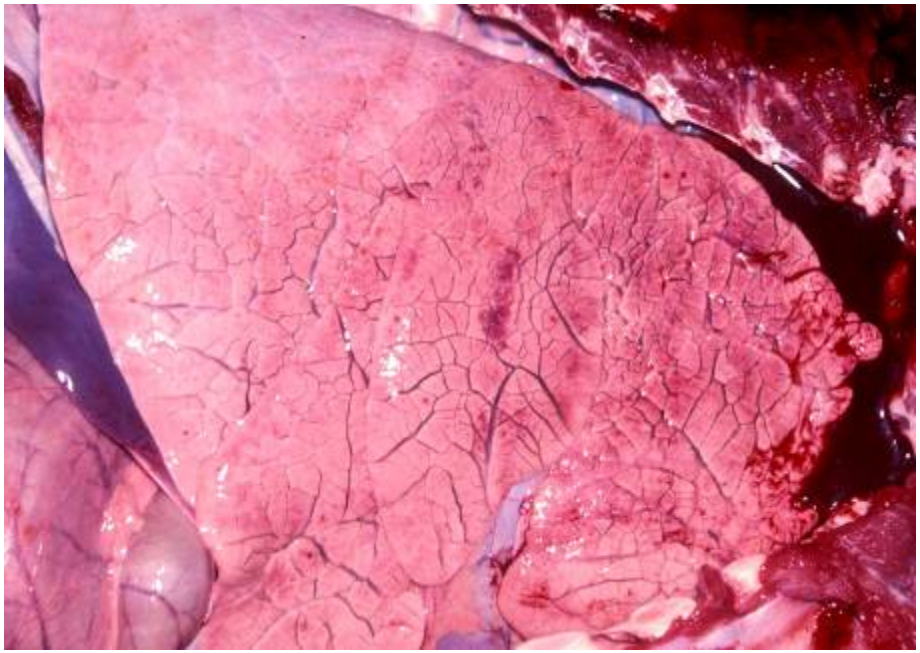
J. Comp. Path. 2002, Vol. 126, 71–75
doi:10.1053/jcpa.2001.0514, available online at http://www.idealibrary.com on IDEAL®

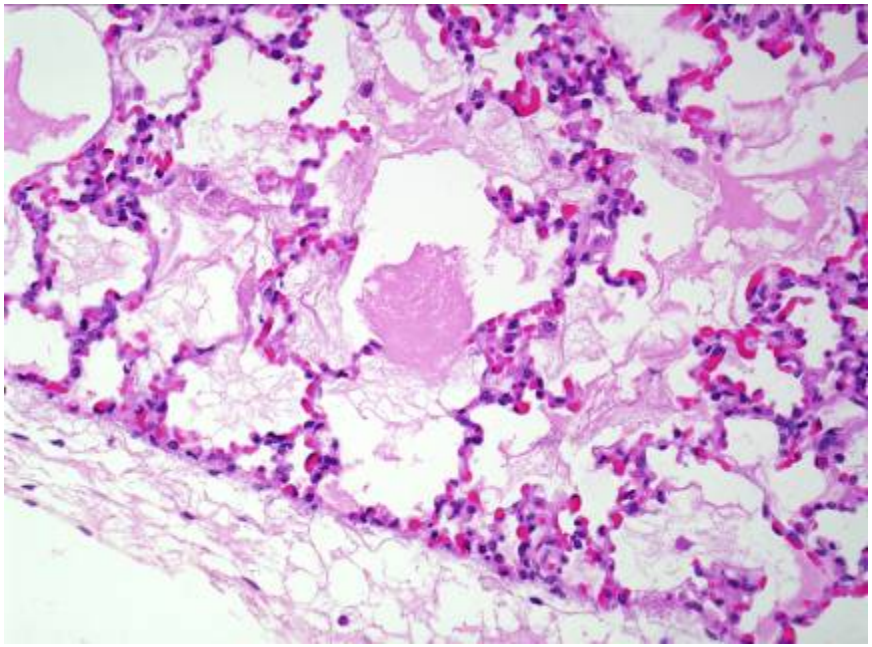
SHORT PAPER

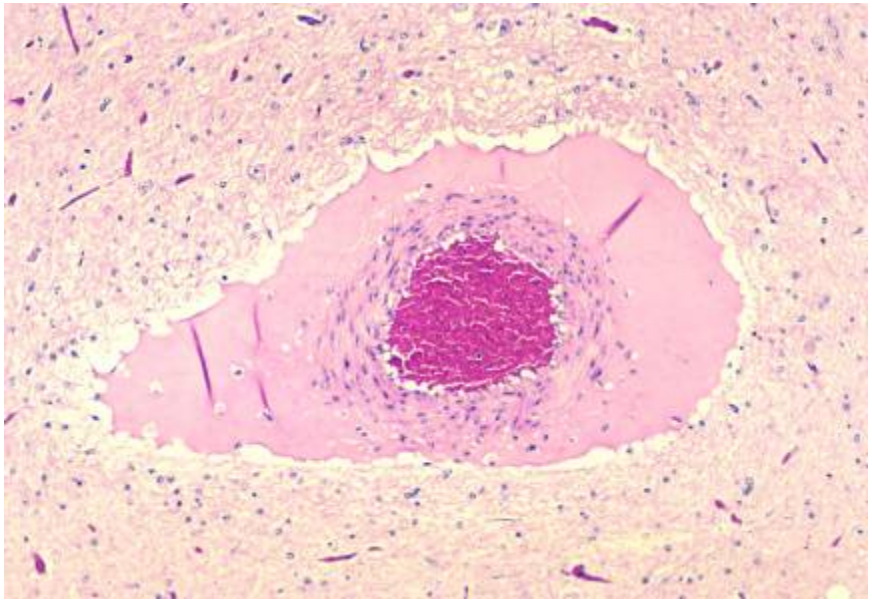
Effects of Intravenous Injection of *Clostridium perfringens* Type D Epsilon Toxin in Calves

F. A. Uzal, W. R. Kelly*, W. E. Morris and R. A. Assis†

*Animal Health Unit, The National Institute of Agricultural Technology, CC 277 (8400) Bariloche, Argentina, *School of Veterinary Sciences and Animal Production, The University of Queensland, Brisbane, Qld 4072, Australia, and †Veterinary School, Federal University of Minas Gerais, Belo Horizonte, Brazil*







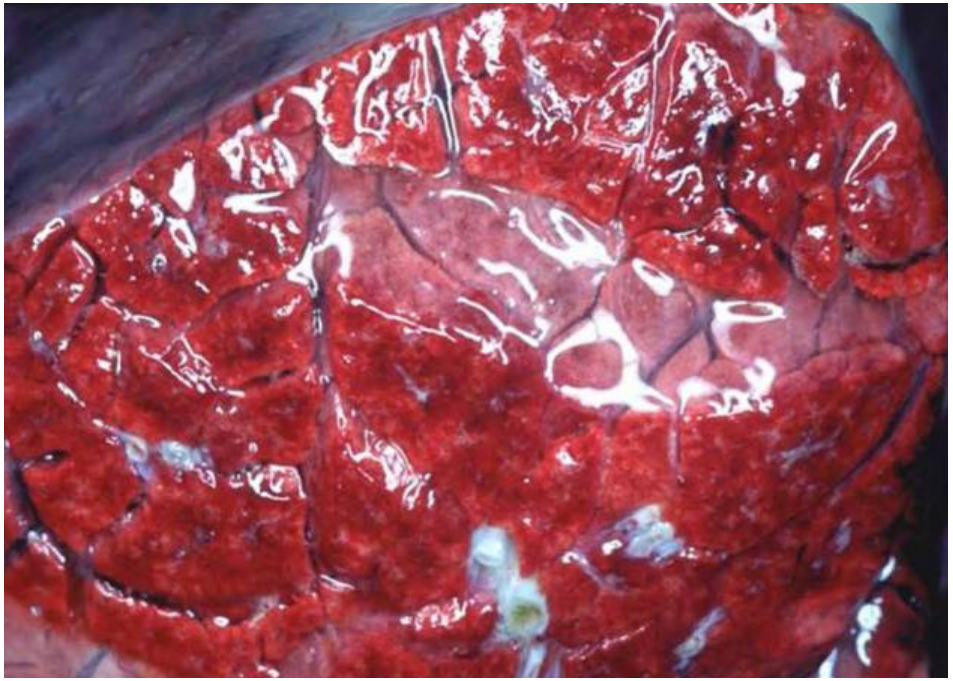
Vet Pathol 46:1213–1220 (2009)
DOI: 10.1354/vp.08-VP-0304-U-FL

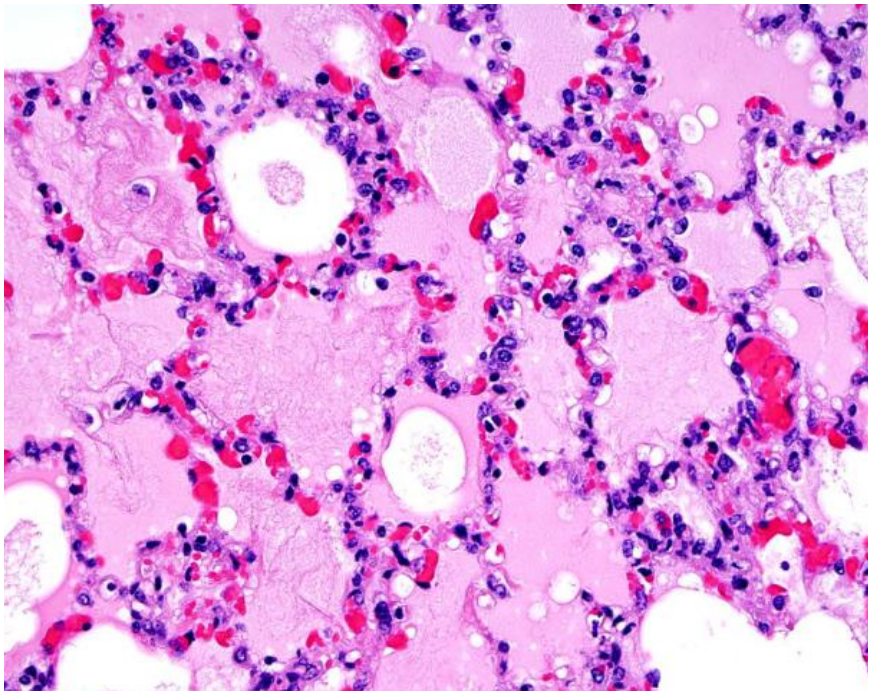
Clinicopathologic Features of Experimental *Clostridium perfringens* Type D Enterotoxemia in Cattle

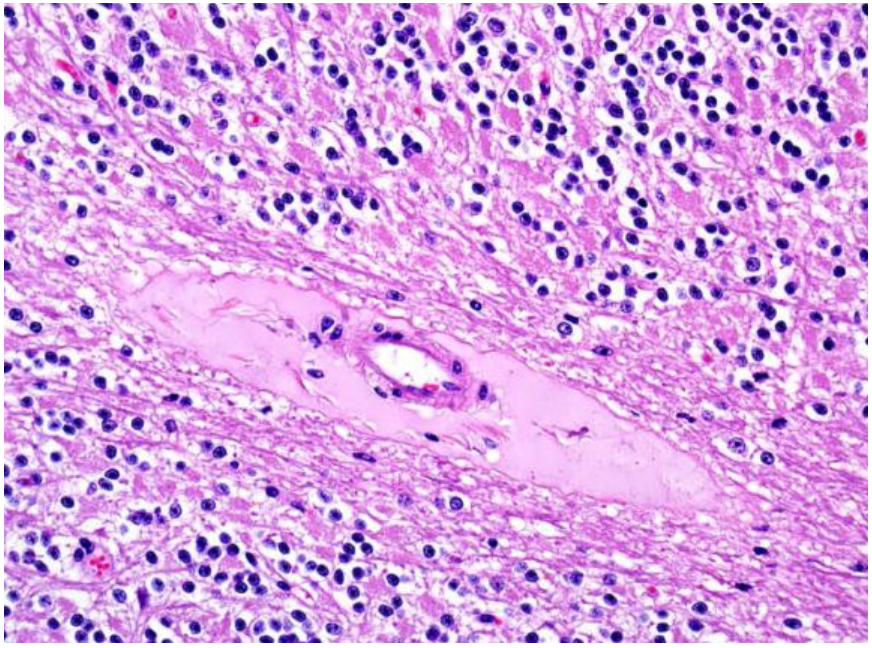
E. J. F. Filho, A. U. Carvalho, R. A. Assis, F. F. Lobato, M. A. Rachid, A. A. Carvalho, P. M. Ferreira, R. A. Nascimento, A. A. Fernandes, J. E. Vidal, and F. A. Uzal

Department of Veterinary Clinics and Surgery (EJFF, AUC, MAR, PMF) and Department of Preventive Veterinary Medicine (FFL, AAF), Veterinary School, Federal University of Minas Gerais, Belo Horizonte, Brazil; LANAGRO, Ministry of Agriculture and Provisioning, Pedro Leopoldo, Minas Gerais, Brazil (RAA, AAC, RAN); Department of Microbiology and Molecular Genetics, University of Pittsburgh, School of Medicine, Pittsburgh, PA (JEV); and California Animal Health and Food Safety Laboratory, San Bernardino Branch, University of California, Davis, Davis, CA (FAU)


Photo Facury Filho







***C. perfringens* type D may produce disease in cattle very similar to sheep enterotoxmia, ….experimentally**

Does this occur naturally…….?

Experimental disease

Natural disease

Clostridium perfringens type D epsilon one-day-

P. J. Watson, S. F. I

Clostridium perfringens t
is a very common caus
numerous scientific pu
enterotoxaemia in she
type D enterotoxaemia
reports of naturally occ
and others 1981, Fairle
cases of C *perfringens* ty
that was notable for the
tion of both character
in the intestinal conte

The affected calves
hill sheep farm in the I
housed in traditional s
in February while the
February 28, 2004, in
born unassisted in the
and was seen to feed
discovered 'flat out' in
21.30 on the same day.

The carcase was su
(VLA) – Penrith for di
weighed 38 kg and was

Domestic Mammal Disease

Veterinary Pathology
50(5) 765-768

DOI: 10.1177/0300985813476058
vet.sagepub.com

Brain Lesions Associated With *Clostridium perfringens* Type D Epsilon Toxin in a Holstein Heifer Calf

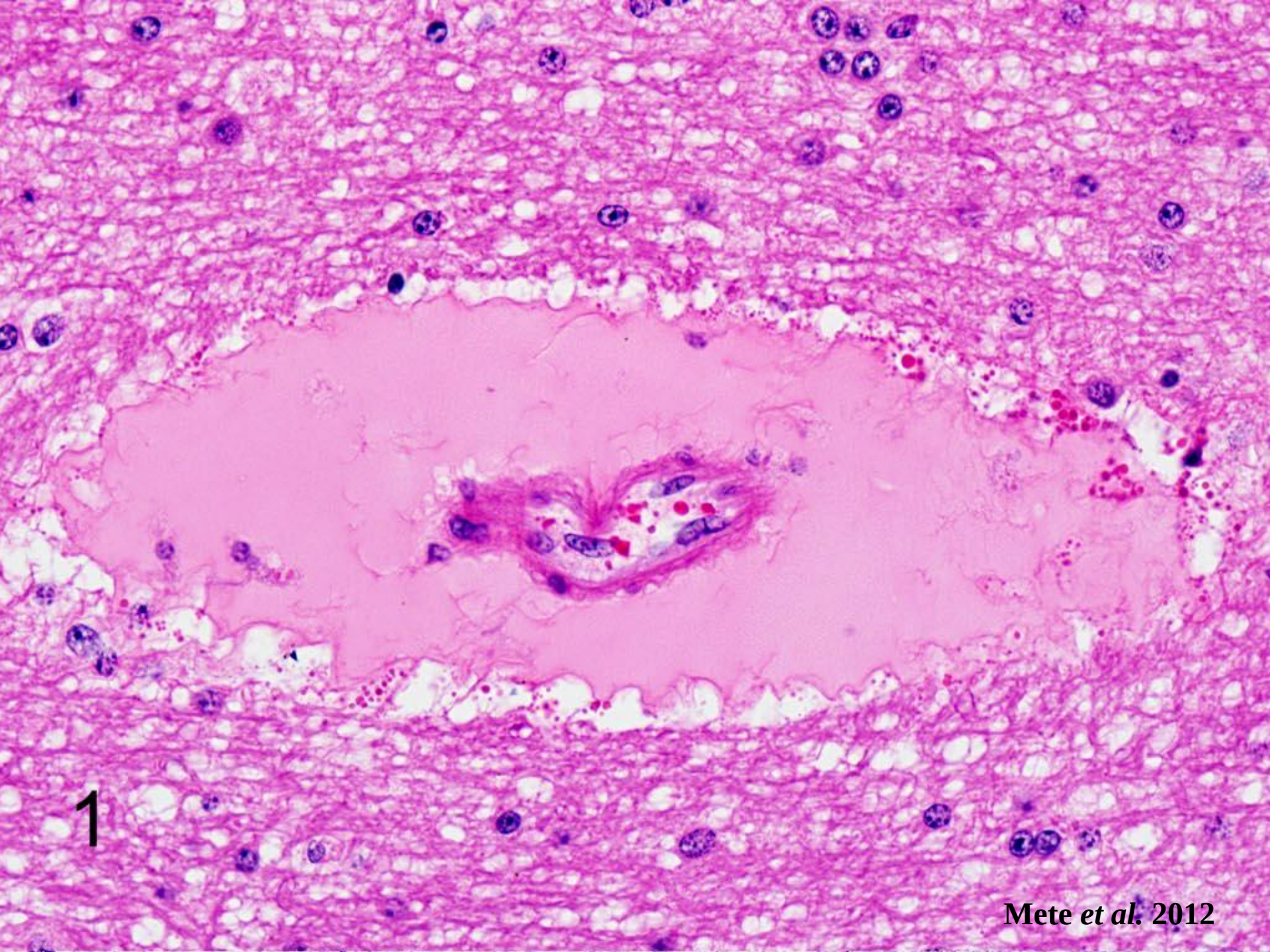
A. Mete[1], J. Garcia[2], J. Ortega[3], M. Lane[4], S. Scholes[5], and F. A. Uzal[2]

Abstract

A 6-month-old dairy heifer calf with no premonitory signs was acutely down after the morning feeding and could not rise. On presentation, the heifer was in right lateral recumbency and moribund with opisthotonus and left hind limb paddling. Following euthanasia, gross examination of the brain revealed multifocal loss of gray-white matter distinction and extensive petechiae throughout the brainstem. On histopathological examination, there was striking white matter edema and marked perivascular proteinaceous edema surrounding many arterioles and venules (microangiopathy), mainly in the white matter of the internal capsule, thalamus, midbrain, cerebellum, and cerebellar peduncles. The perivascular neuropil was strongly positive for Alzheimer precursor protein A4. *Clostridium perfringens* epsilon toxin was detected in the intestinal contents. This is the first report of microangiopathy in postneonatal cattle associated with the detection of epsilon toxin in the intestinal contents.

Keywords

brain, cattle, *Clostridium perfringens*, enterotoxemia, epsilon toxin, ETX, microangiopathy, Alzheimer precursor protein, APP



Mete *et al.* 2012

ETX

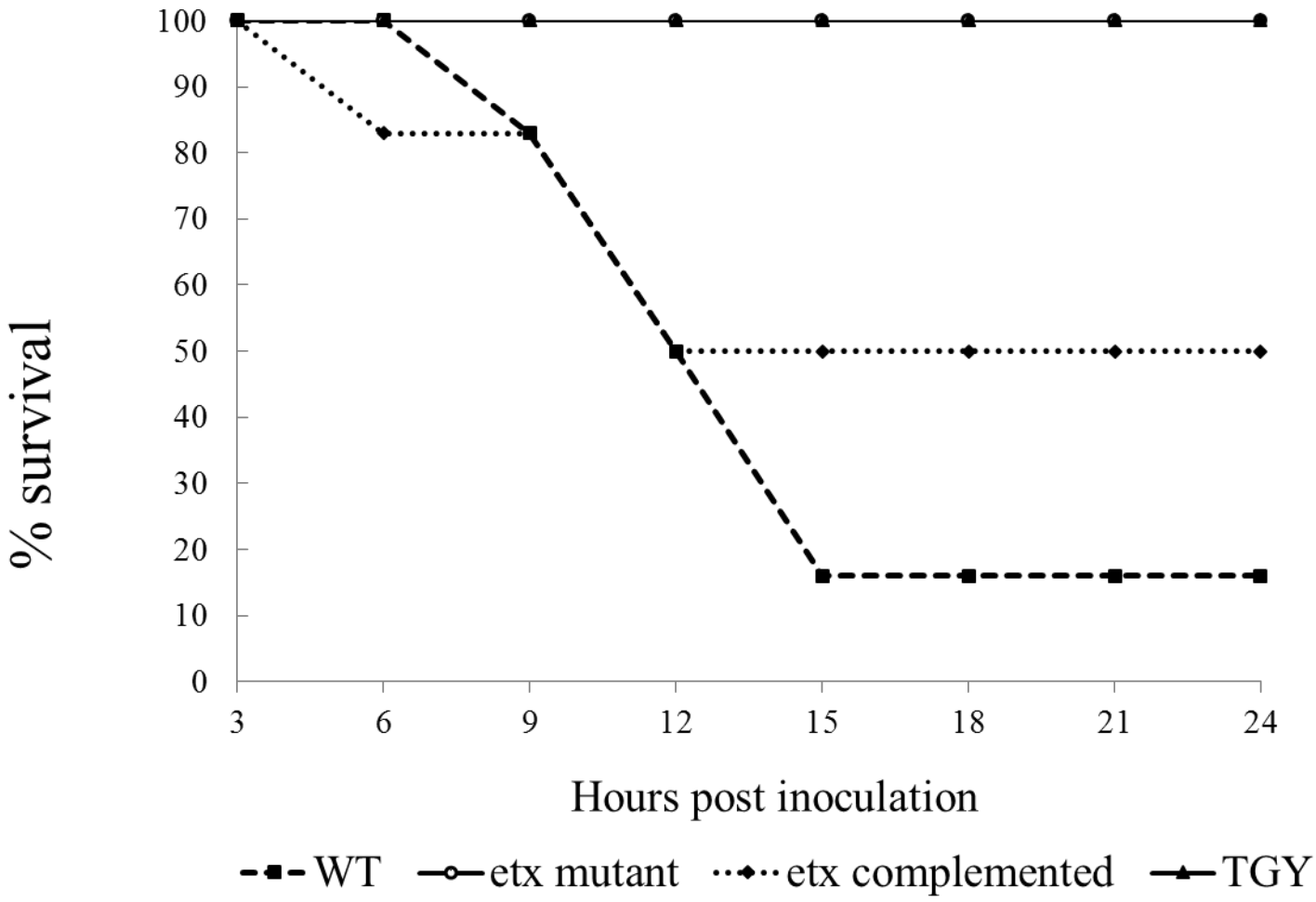
Confirmation of role in animal/ human (?) disease

Reverse genetics in animal models (sheep, goats, mice)

Type D mutants

* **Wild type**
* **ETX KO**
* **ETX Complement**

SHEEP


% survival
100
90
80
70
60
50
40
30
20
10
0
3
6
9
12
15
18
21
24
Hours post inoculation
WT
etx mutant
etx complemented
TGY

Conclusions:

1-Molecular Koch's postulates fullfilled (almost….)

2-ETX necessary and sufficient for lethality/ disease: key role in pathogenesis

3-Clinical signs, gross and histological lesions type D enterotoxemia different in sheep and goats

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

Toxinotype	α-toxin	β-toxin	ε-toxin	ι-toxin	CPE	NetB
A	+	-	-	-	-	-
B	+	+	+	-	-	-
C	+	+	-	-	+/-	-
D	+	-	+	-	+/-	-
E	+	-	-	+	+/-	-
F	+	-	-	-	+	-
G	+	-	-	-	-	+

Rood et al, Anaerobe 2018

Main virulence factors

Alpha toxin
Iota toxin

Iota:

binary toxin (A & B)

cross reaction: *C. spiroforme* toxin

Cases published in……

diagnoses mostly based on isolation……

ELSEVIER

Anaerobe 10 (2004) 239–242

Anaerobe

www.elsevier.com/locate/anaerobe

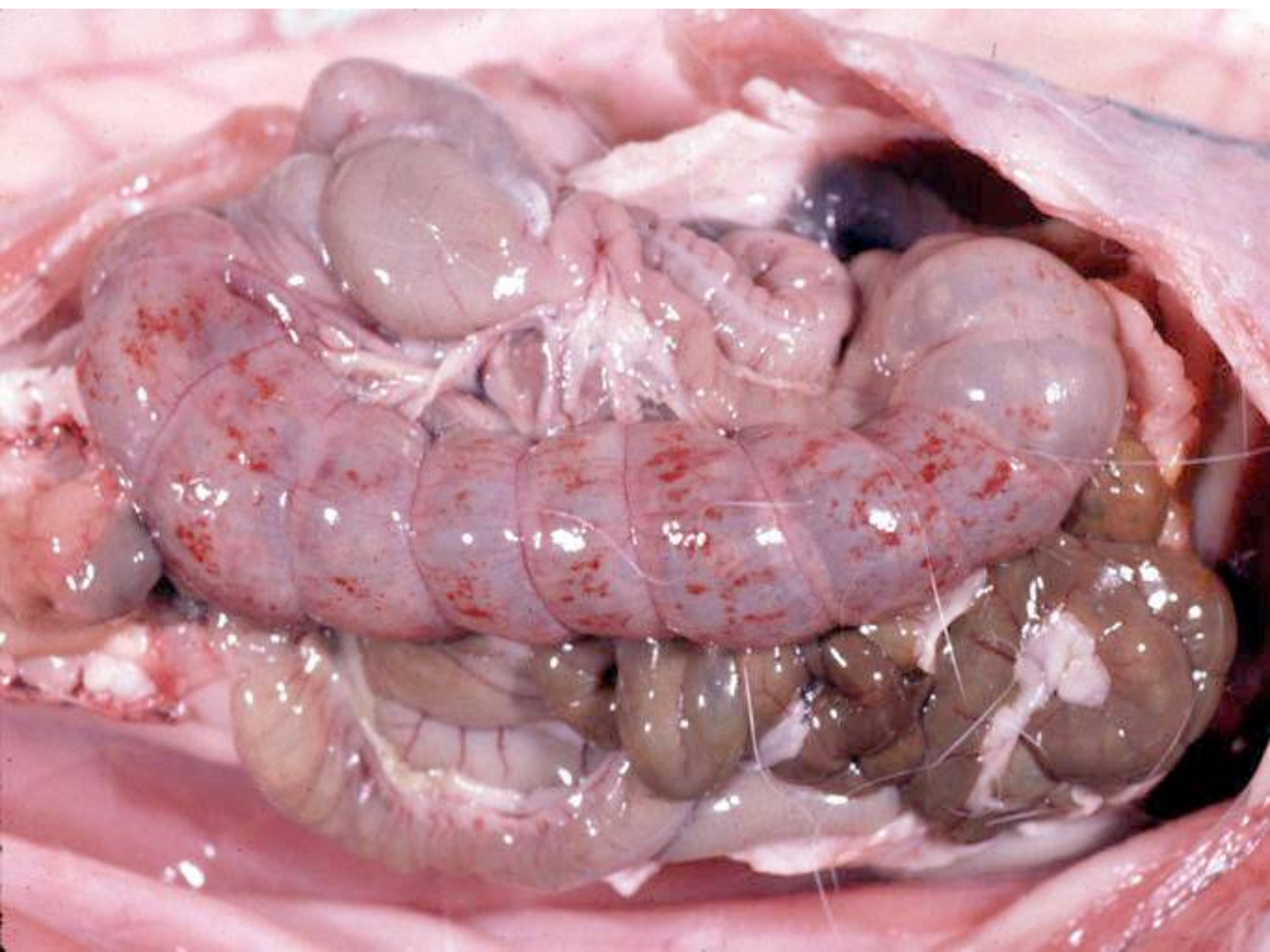
Veterinary anaerobes and diseases

Clostridium perfringens type E enteritis in calves: two cases and a brief review of the literature

J. Glenn Songer[a,*], Dale W. Miskimmins[b]

[a] *Department of Veterinary Science and Microbiology, The University of Arizona, Tucson, AZ 85721, USA*
[b] *Department of Veterinary Science, South Dakota State University, Brookings, SD 57007, USA*

Received 5 April 2004; accepted 5 May 2004



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C	+	+	-	-	+/-	-
D	+	-	+	-	+/-	-
E	+	-	-	+	+/-	-
F	+	-	-	-	+	-
G	+	-	-	-	-	+

Rood et al, Anaerobe 2018

Enterotoxin of *C. perfringens* (CPE)

C. perfringens type F

Humans: **Food poisoning**

Animals: **Enteritis (?)**

Enterotoxin (CPE):

*** *C. perfringens* type A food poisoning**

*** *C. perfringens* antibiotic-associated diarrhea**

C. perfringens type F food poisoning

- **Second most commonly identified cause of bacterial food poisoning cases in the USA (~400,000/year).**

- **Fourth most common cause of deaths from bacterial food poisoning in the USA (mostly in the elderly or debilitated).**

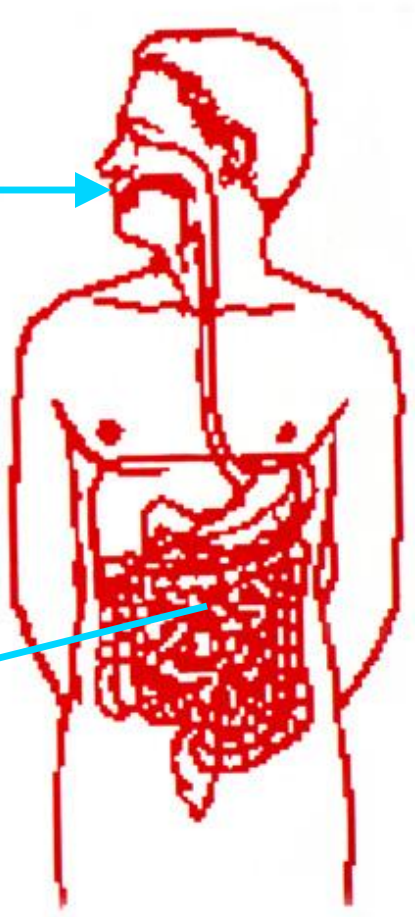
C. perfringens type F food poisoning

- **~12 hour incubation, illness 12-24 hours.**
- **Diarrhea and abdominal cramps.**

Clostridium perfringens food poisoning

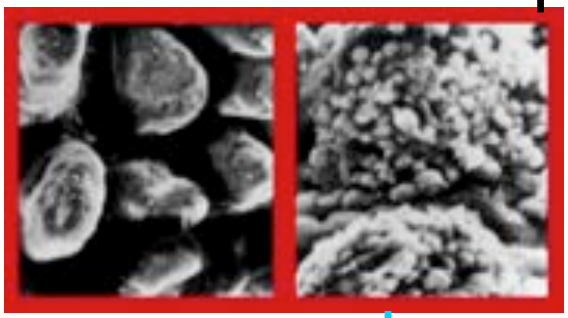
Vegetative
C. perfringens

Food Source



INTESTINAL VILLI

CPE - **CPE +**



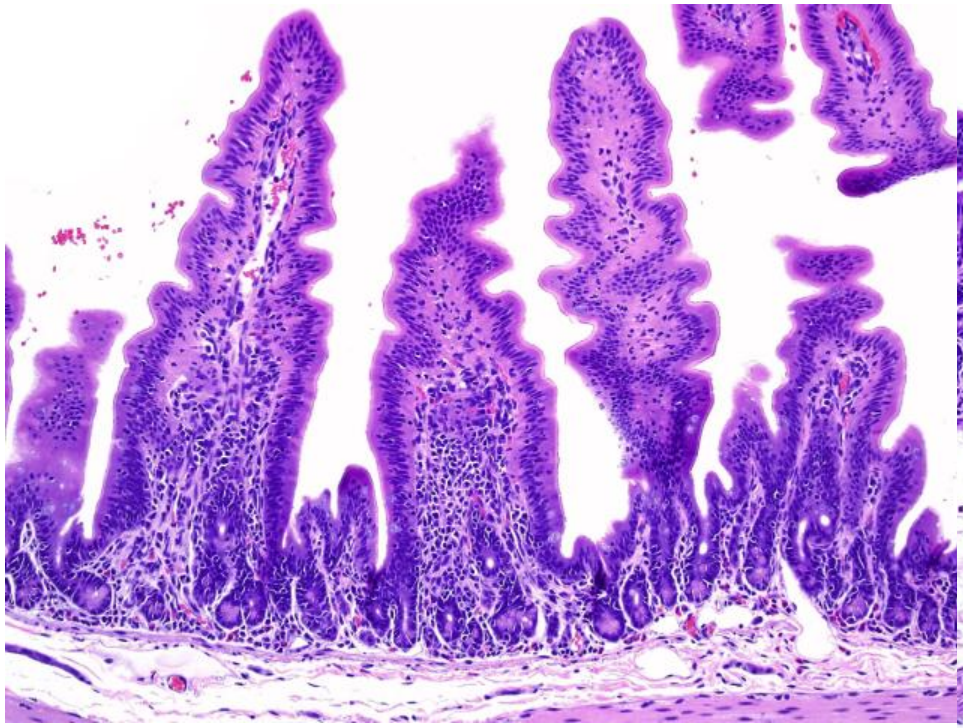
CPE

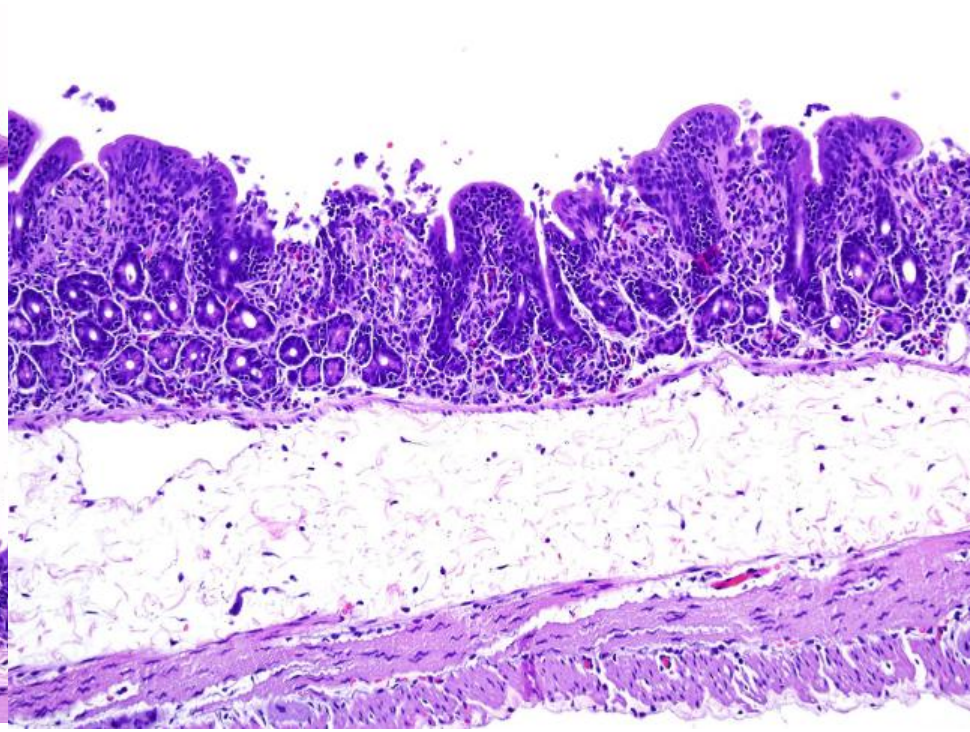
Sporulation of
C. perfringens

DIARRHEA and CRAMPS

Rabbit intestinal loops 6 hours

Buffer **CPE**





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B	+	+	+	-	-	-
C	+	+	-	-	+/-	-
D	+	-	+	-	+/-	-
E	+	-	-	+	+/-	-
F	+	-	-	-	+	-
G	+	-	-	-	-	+

Rood et al, Anaerobe 2018

Necrotic enteritis

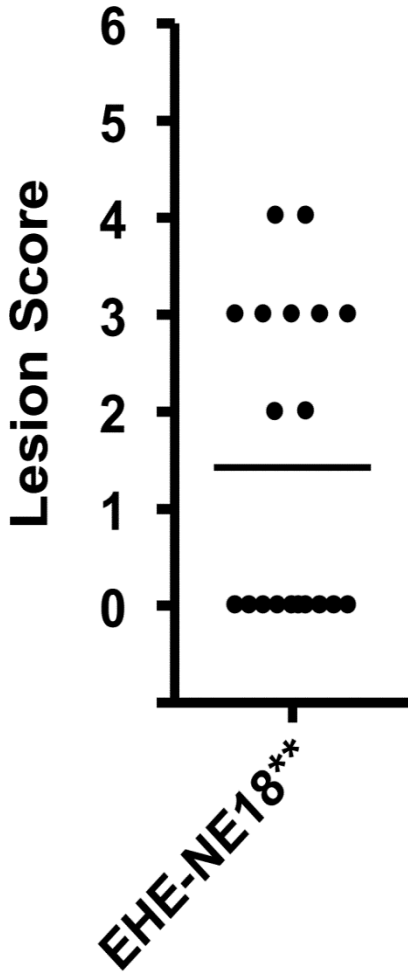
Virulence factors

NetB

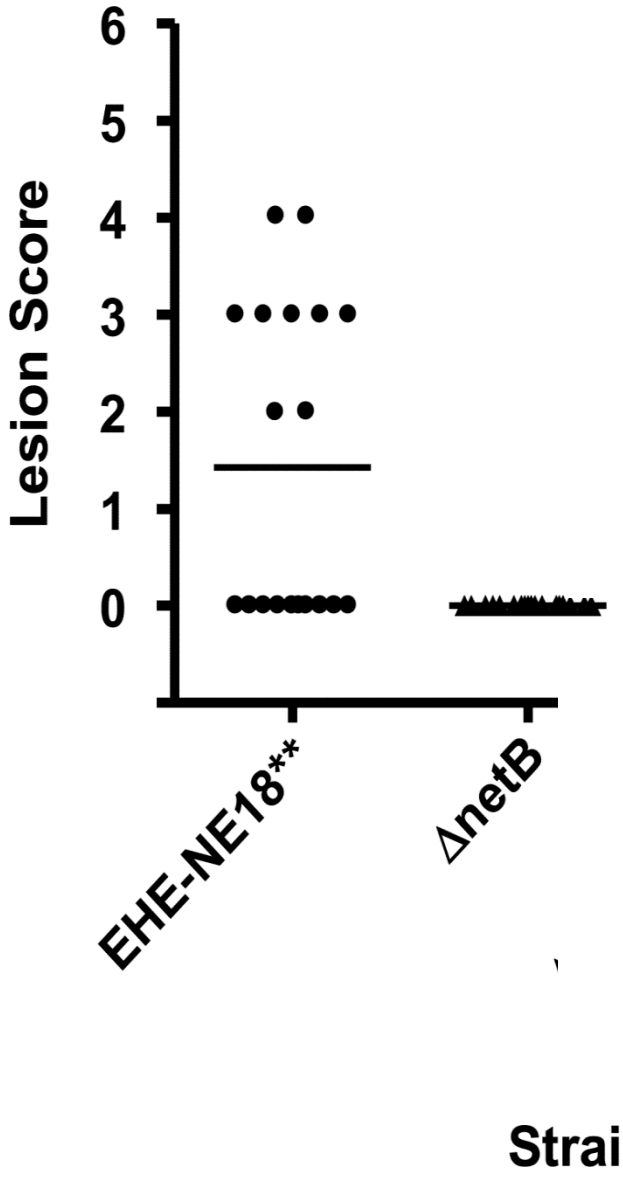
Alpha?

Others?

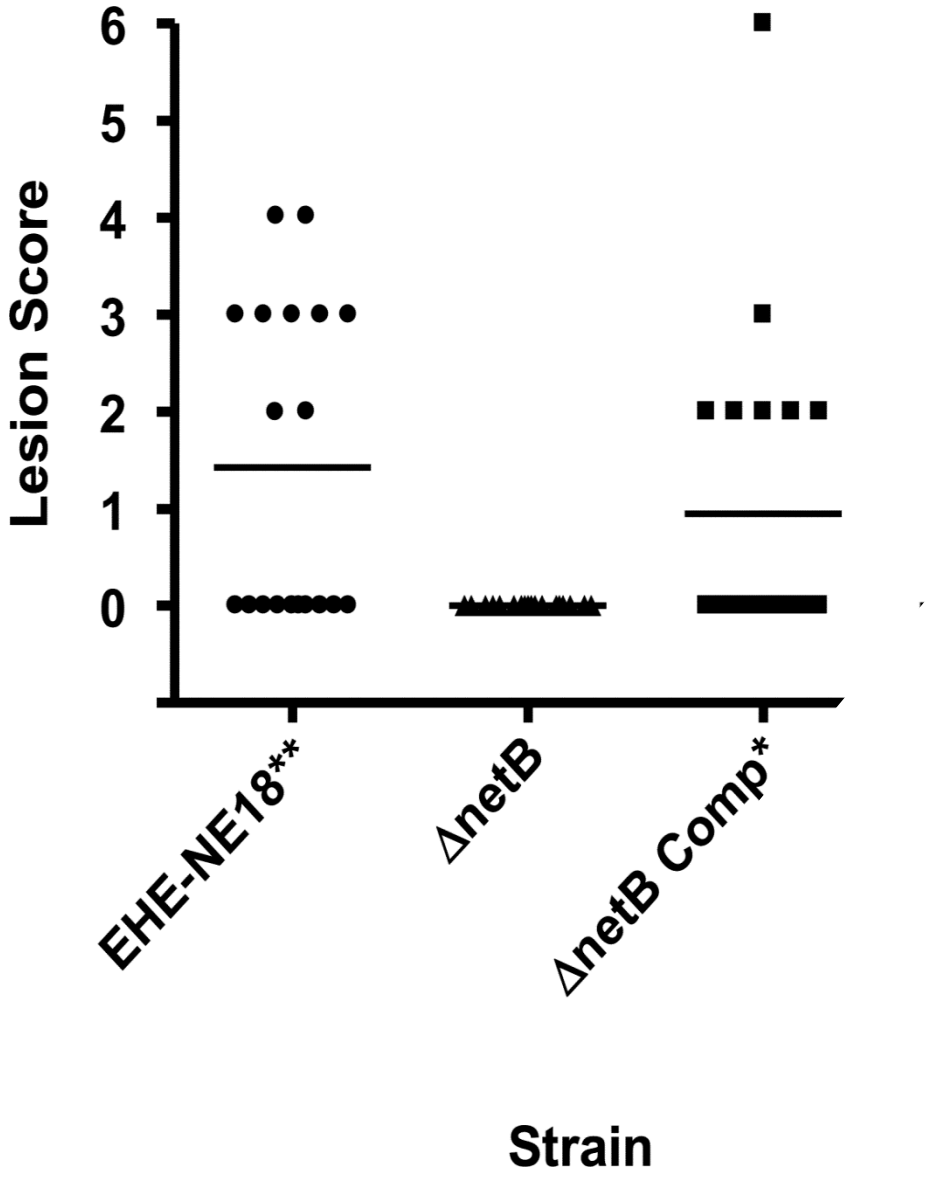
Take-home message!!!



Keyburn et al , 2008



Keyburn et al , 2008



Keyburn et al , 2008

Re-emerging disease

* Restrictive use of antibiotics

* High-density housing

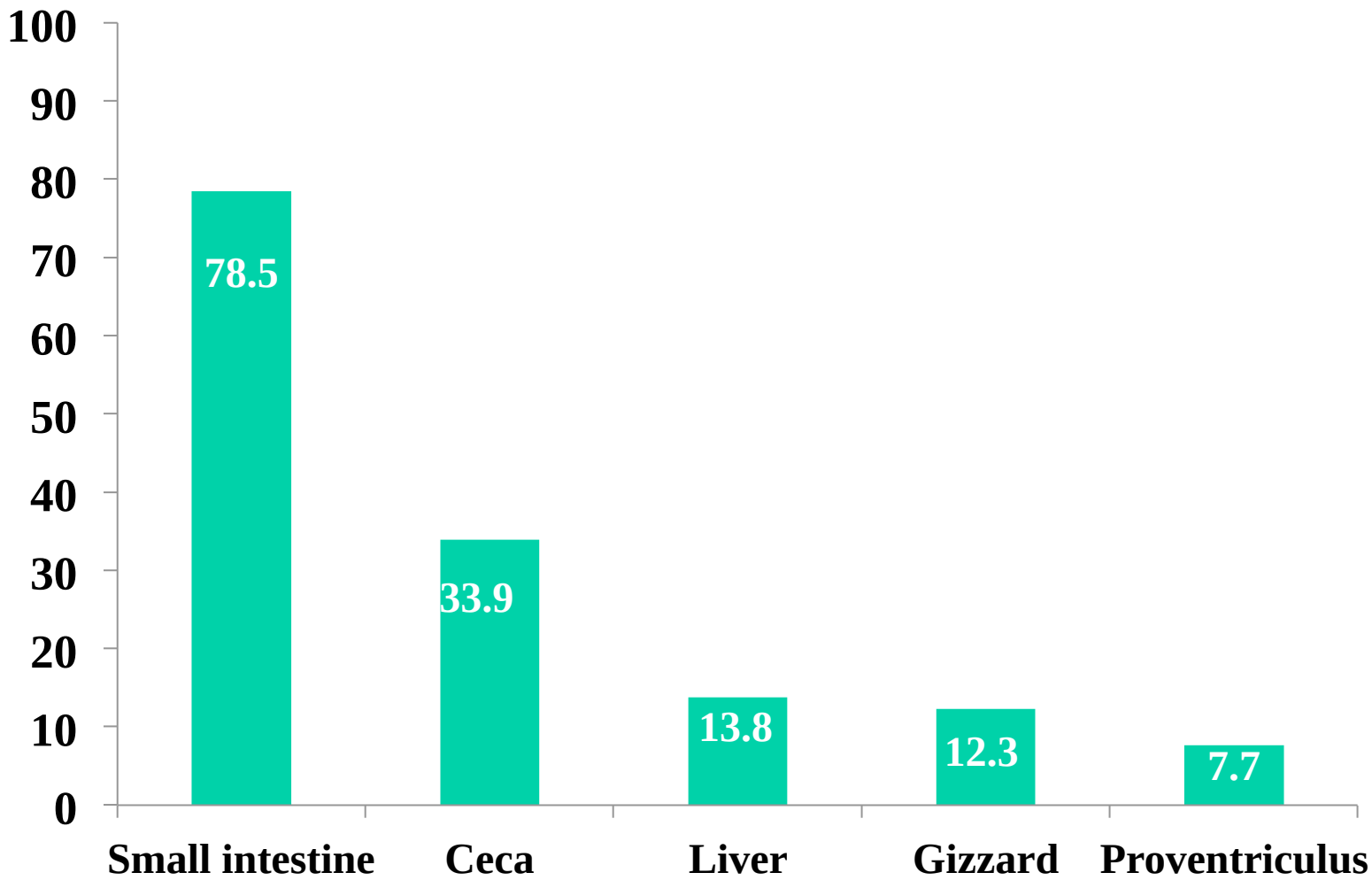
* Re-use of litter

Main predisposing factor: *Eimeria spp.*

Take-home message!!!

* **Clinical**

* **Sub-clinical**


100
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70
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50
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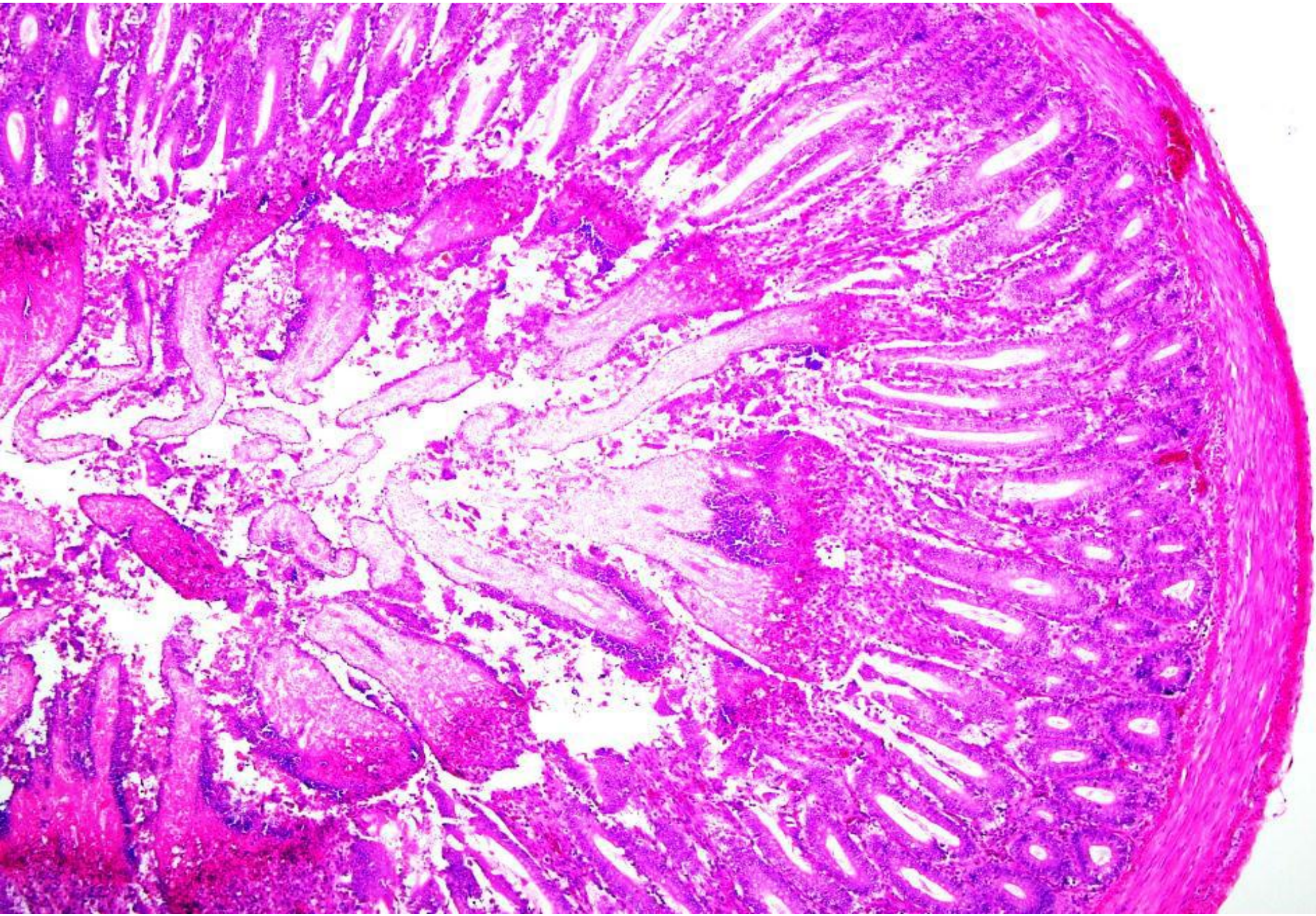
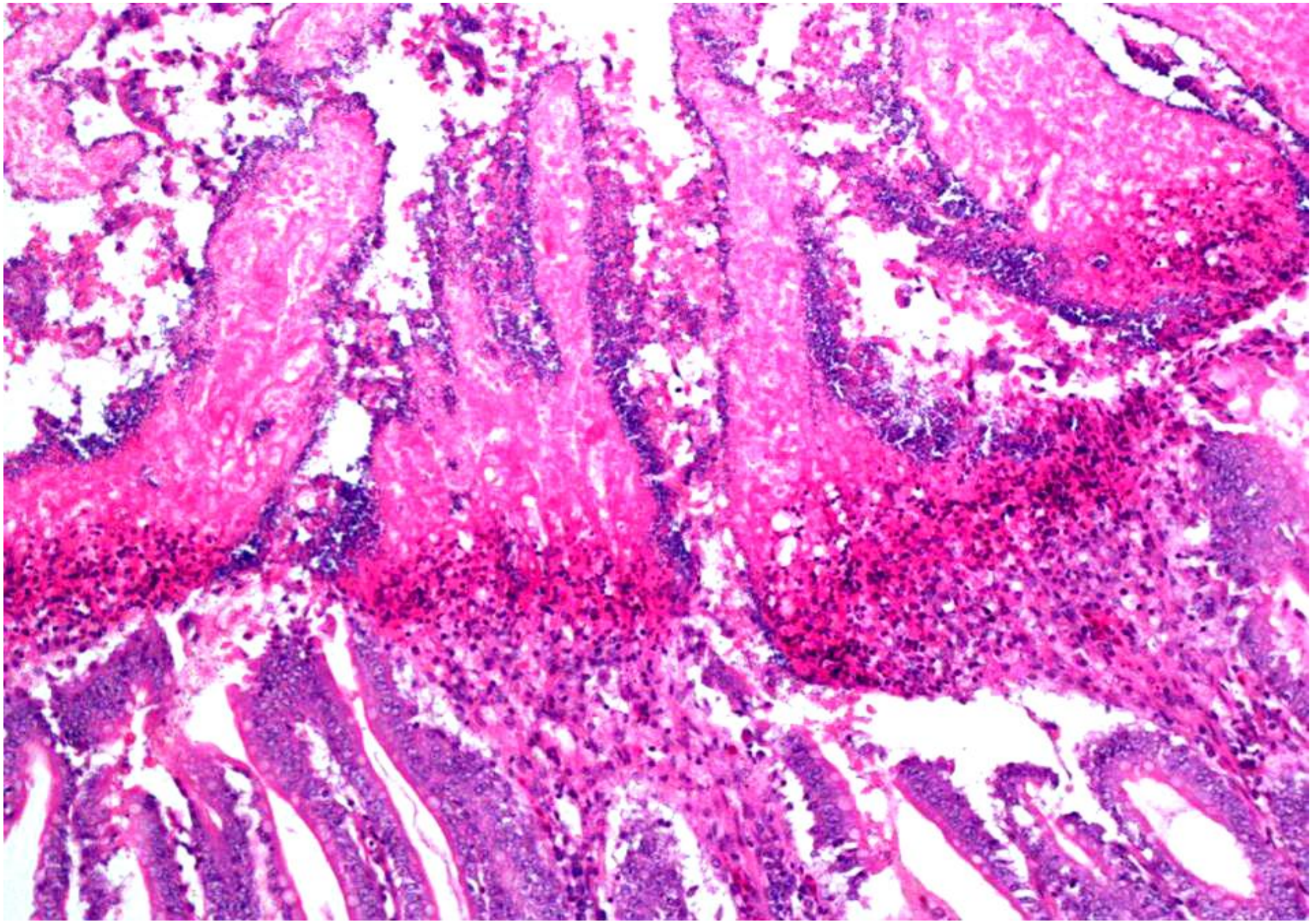
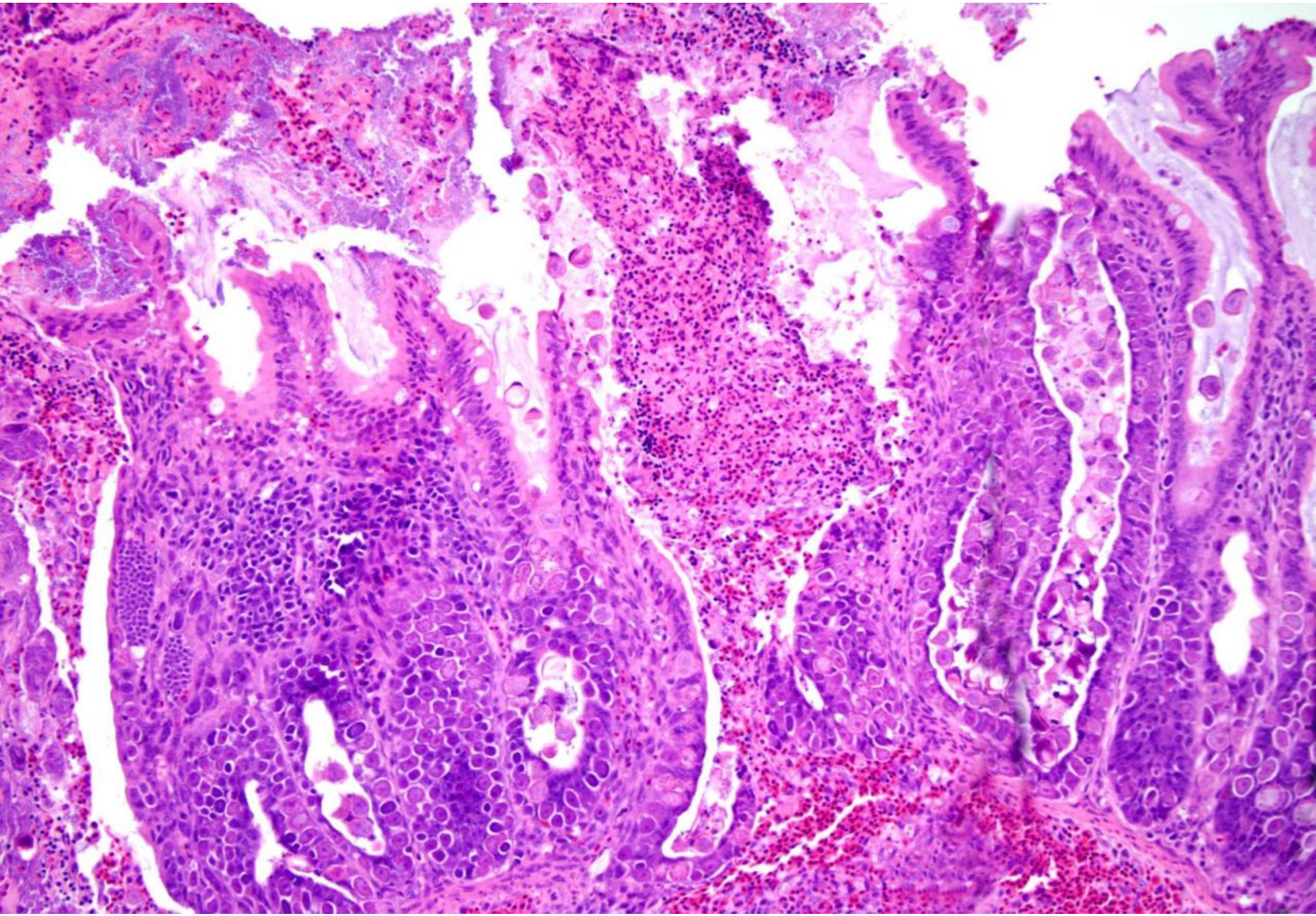
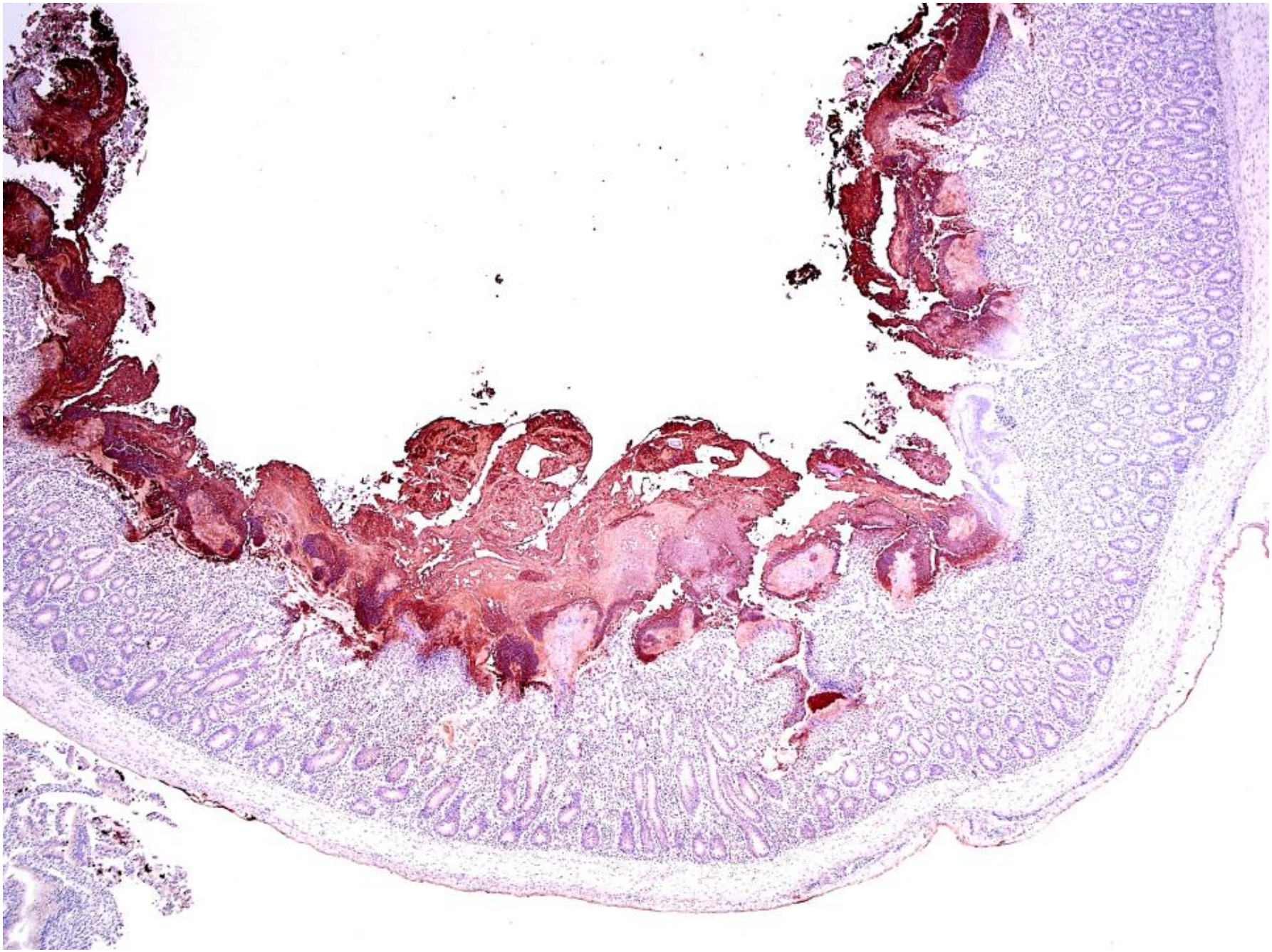
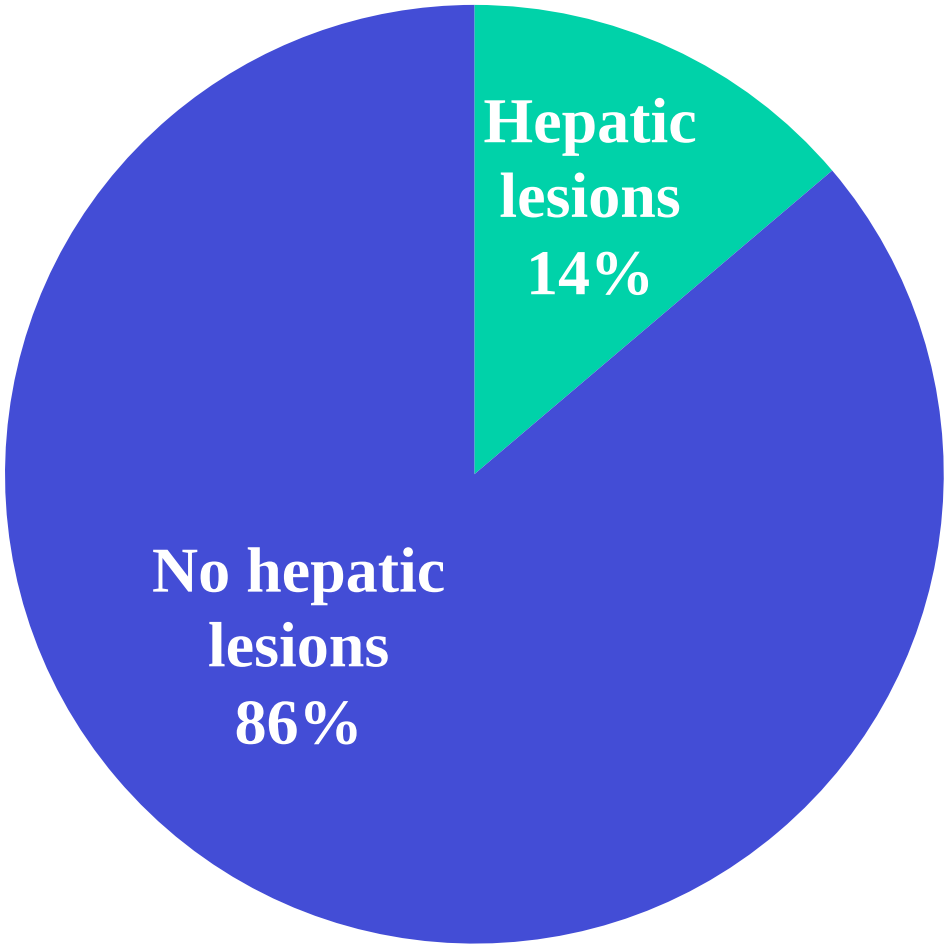
20
10
0
78.5
33.9
13.8
12.3
7.7
Small intestine
Ceca
Liver
Gizzard
Proventriculus

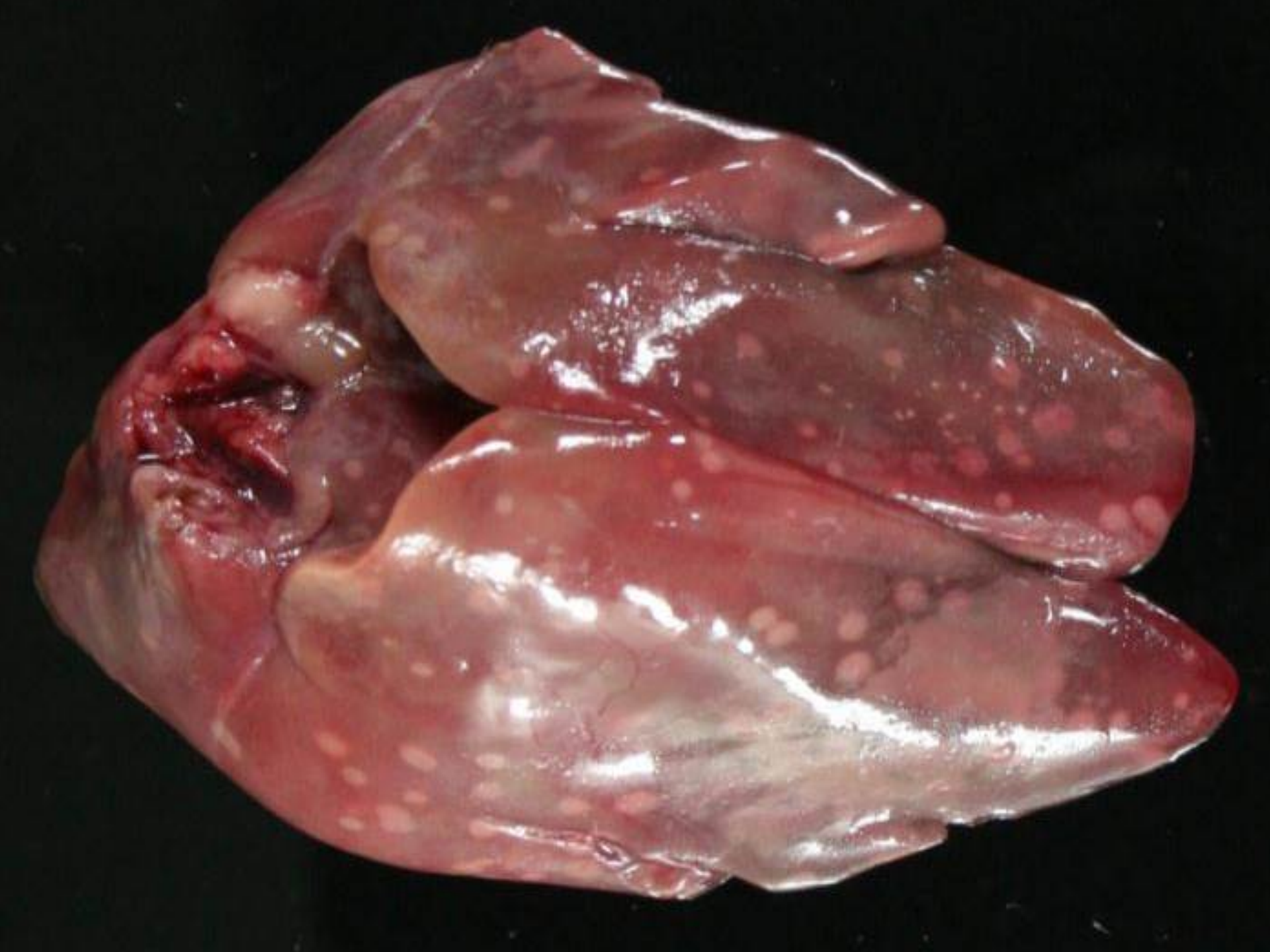


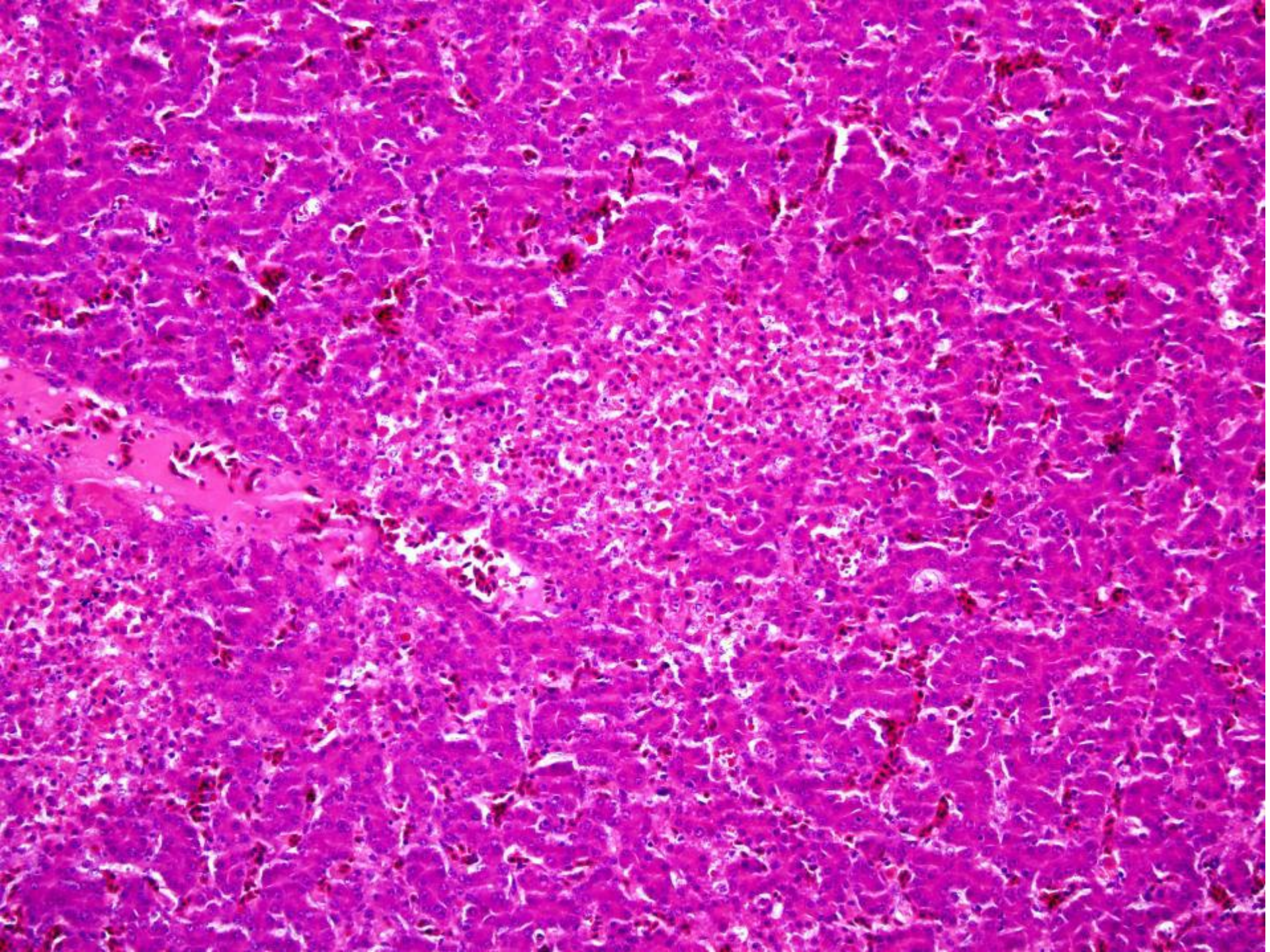







Hepatic lesions 14%
No hepatic lesions 86%





*** Diagnosis based on gross/histo examination alone: CHALLENGING**

*** Increased level of certainty: combining several diagnostic tests**

Diagnostic criteria

1-Clinics/gross

2-Histo

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

} **Suggestive**

4- 1+2+3

} **Confirmatory?**

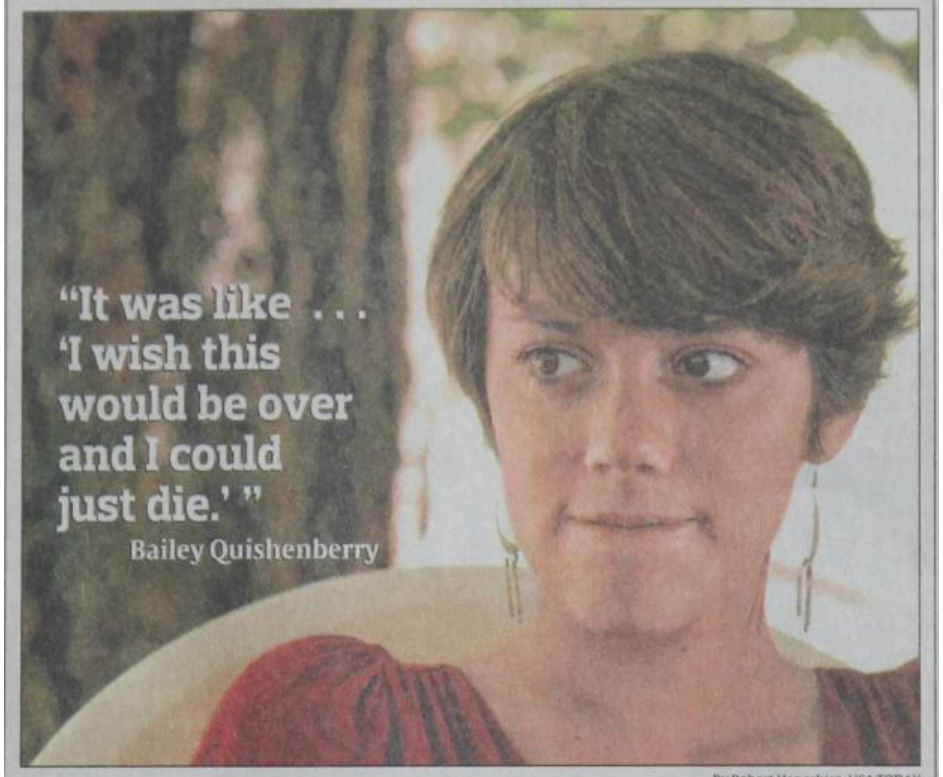
GROUP	DISEASE	ORGANISM	HUMANS	OTHER ANIMALS
Enteric	Enterotoxemias/ enteritis	*C. perfringens*	✓	✓
		C. difficile	✓	✓
Histotoxic				
Neurotoxic				

Clostridium difficile

Main cause of ATB associated diarrhea in humans and animals

NOT ATB IN PIGS or FOALS!!!!

A USA TODAY INVESTIGATION



By Robert Hanashiro, USA TODAY

Survivor: Bailey, now 16, contracted C. diff after brain surgery.

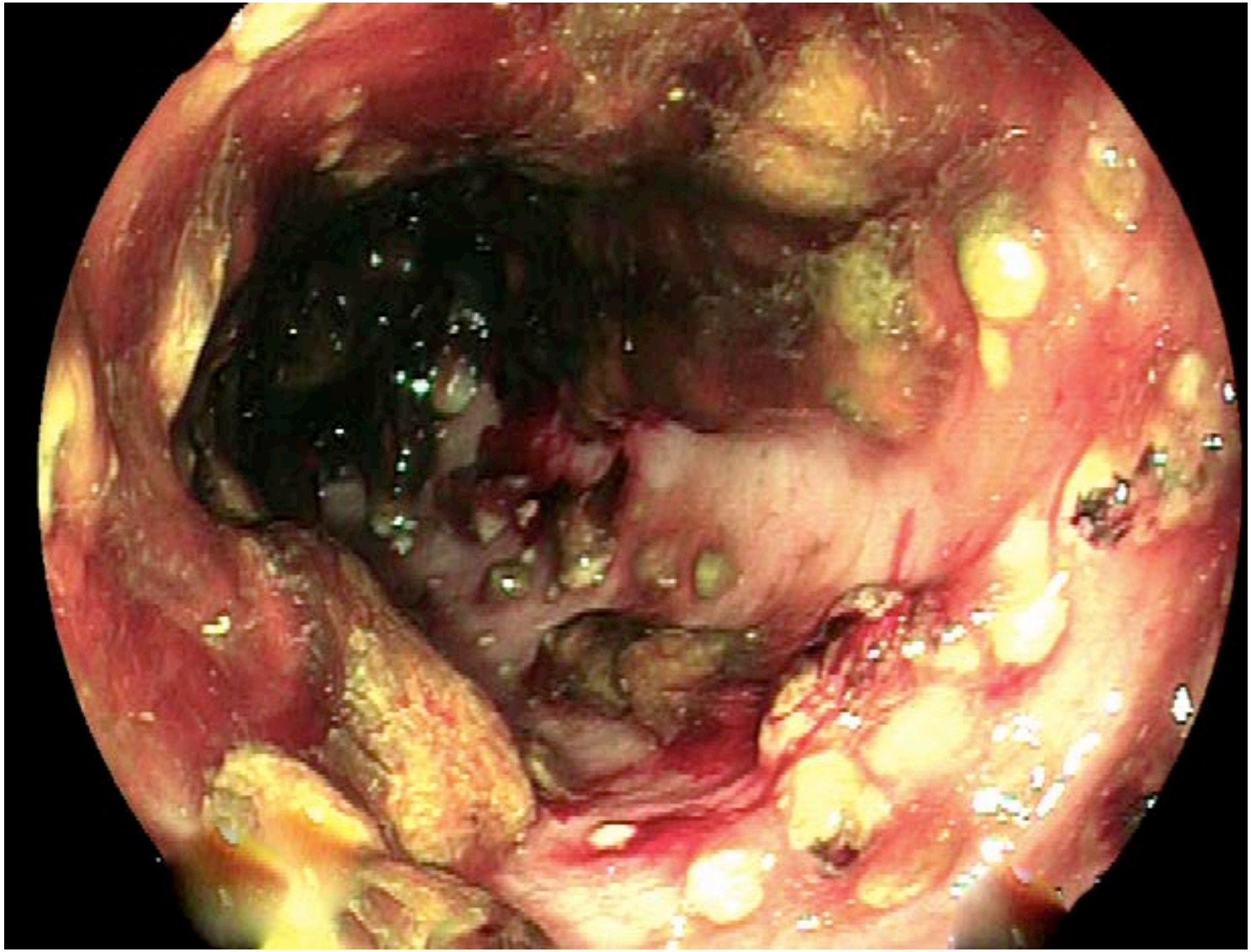
One bacteria, 30,000 deaths

An infection called C. diff is wreaking havoc in the USA's hospitals, nursing homes and other medical facilities — and officials could be doing far more to stop it

By Peter Eisler
USA TODAY

WHEN

8/16/2012



Stephen Sontag

Predisposing factors:

* antibiotic therapy (virtually any antibiotic)

* hospitalization

Take-home message!!!

Sturdy environmental contaminant

UBIQUITOUS:

- Hospitals, households, etc.
- Intestinal content and feces
- Meat and vegetables

Vegetarians warning

C. difficile detected in:

*** Ready to eat organic/nonorganic salads**

*** Lettuce, green peppers and eggplant**

(overall prevalence in vegetables: up to 7.5%)

INITIALLY

TcdA: key virulence factor

TcdB: no effect

LETTER

doi:10.1038/nature09397

The role of toxin A and toxin B in *Clostridium difficile* infection

Sarah A. Kuehne[1]*, Stephen T. Cartman[1]*, John T. Heap[1], Michelle L. Kelly[1], Alan Cockayne[1] & Nigel P. Minton[1]

7 OCTOBER 2010 | VOL 467 | NATURE | 711

Toxin B is essential for virulence of *Clostridium difficile*

Dena Lyras[1], Jennifer R. O'Connor[1,3], Pauline M. Howarth[1], Susan P. Sambol[3], Glen P. Carter[1], Tongted Phumoonna[1], Rachael Poon[1,2], Vicki Adams[1], Gayatri Vedantam[3], Stuart Johnson[3], Dale N. Gerding[3], and Julian I. Rood[1,2,†]

[1]Australian Bacterial Pathogenesis Program, Monash University, VIC 3800, Australia

[2]Australian Research Council Centre of Excellence in Structural and Functional Microbial Genomics, Department of Microbiology, Monash University, VIC 3800, Australia

[3]Medical Service and Research Service, Hines VA Hospital, Hines, IL, U.S.A. and Infectious Disease Section, Stritch School of Medicine, Loyola University Chicago, Maywood, IL, U.S.A.

Published in final edited form as:
Nature. 2009 April 30; 458(7242): 1176–1179. doi:10.1038/nature07822.

CURRENTLY

TcdA and TcdB might act synergistically

TcdA disrupts epithelial integrity

TcdB enters cell >> mediates toxic effects

Is CDAD a zoonosis?

Transmission of *C. difficile* from animals and food to people

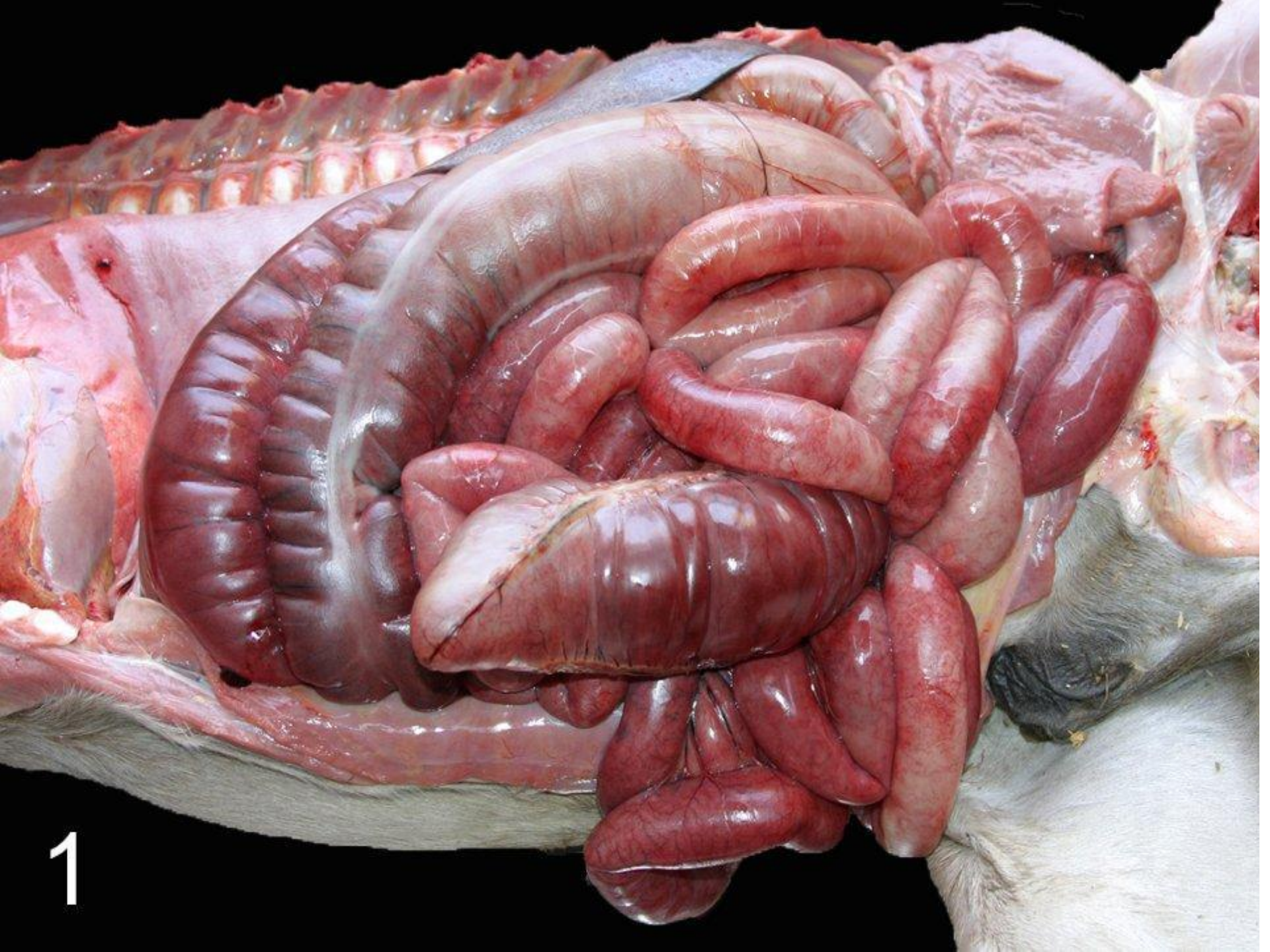
* **Very likely**

* **Not confirmed**

Increased incidence (“hypervirulent” strains)

* Ribotype 027

* Ribotype 078 (predominantly animal)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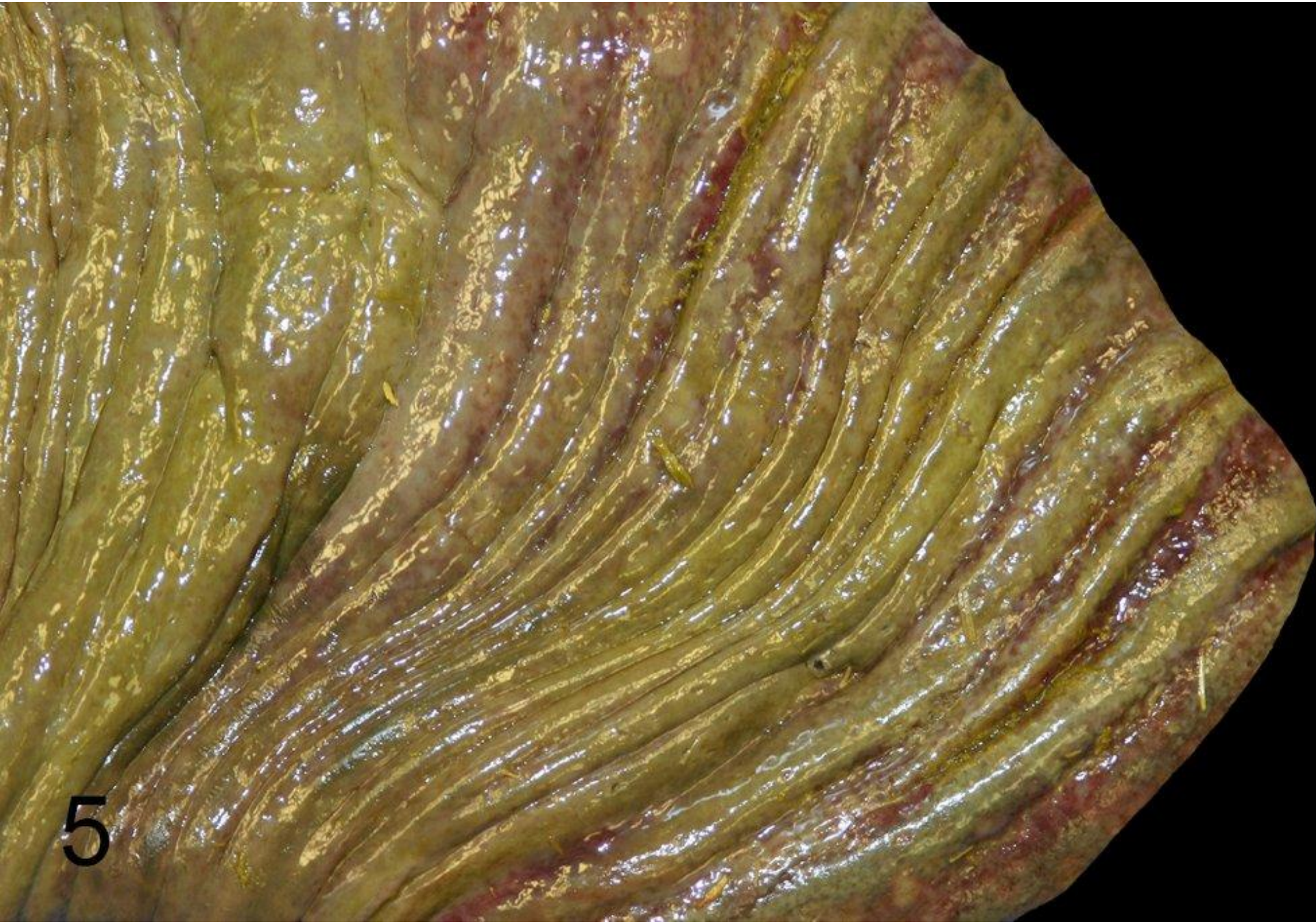
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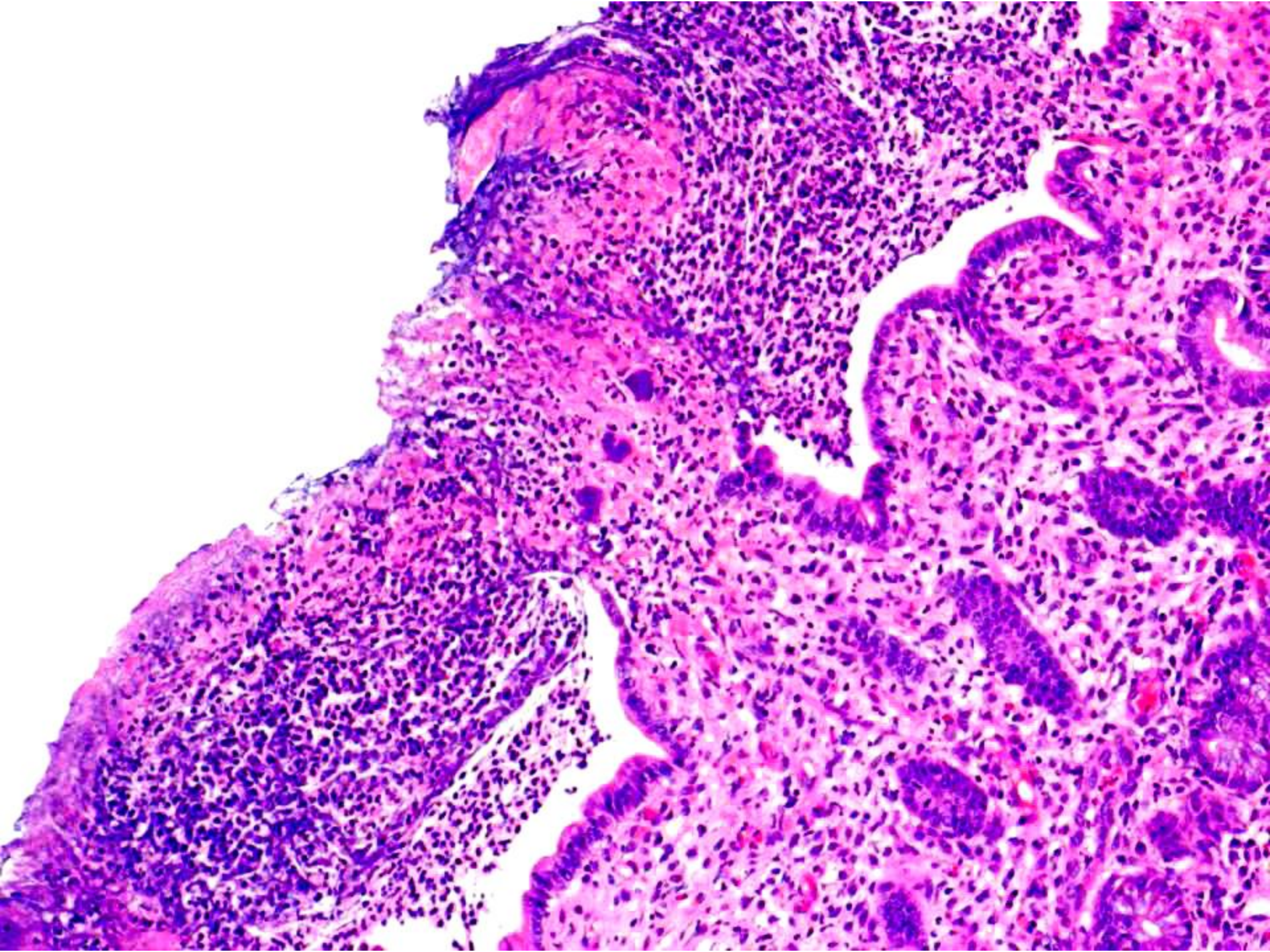

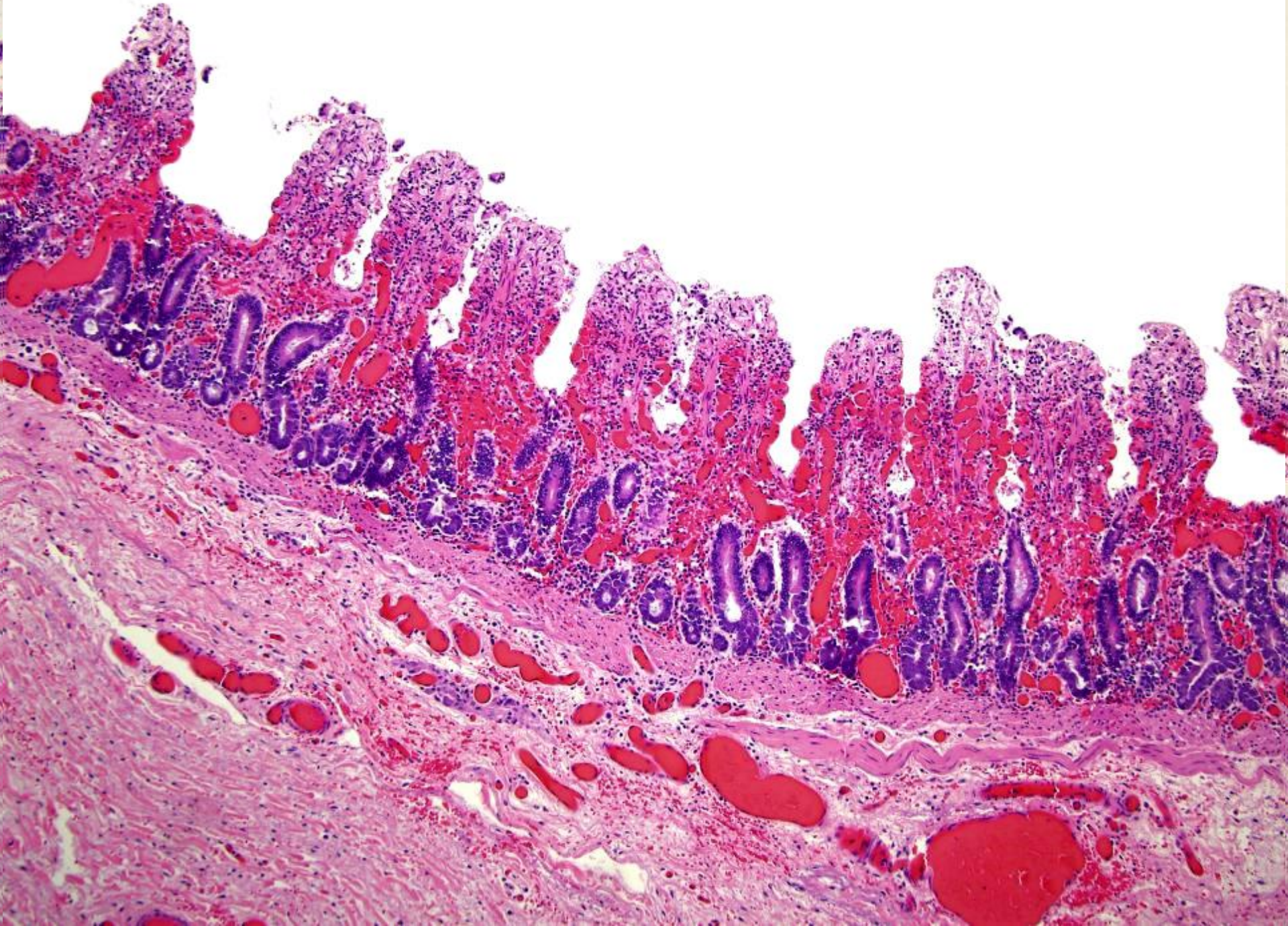

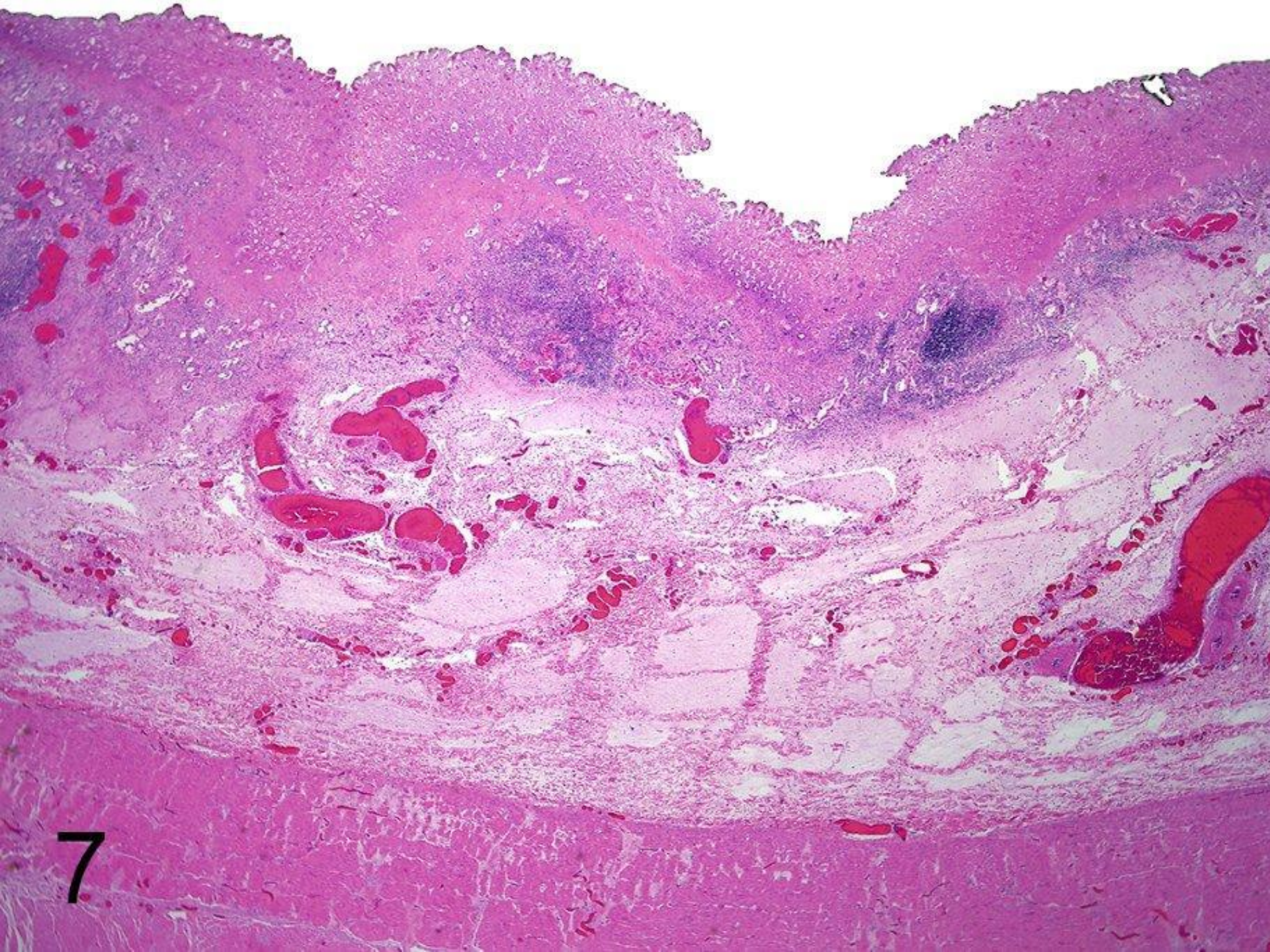
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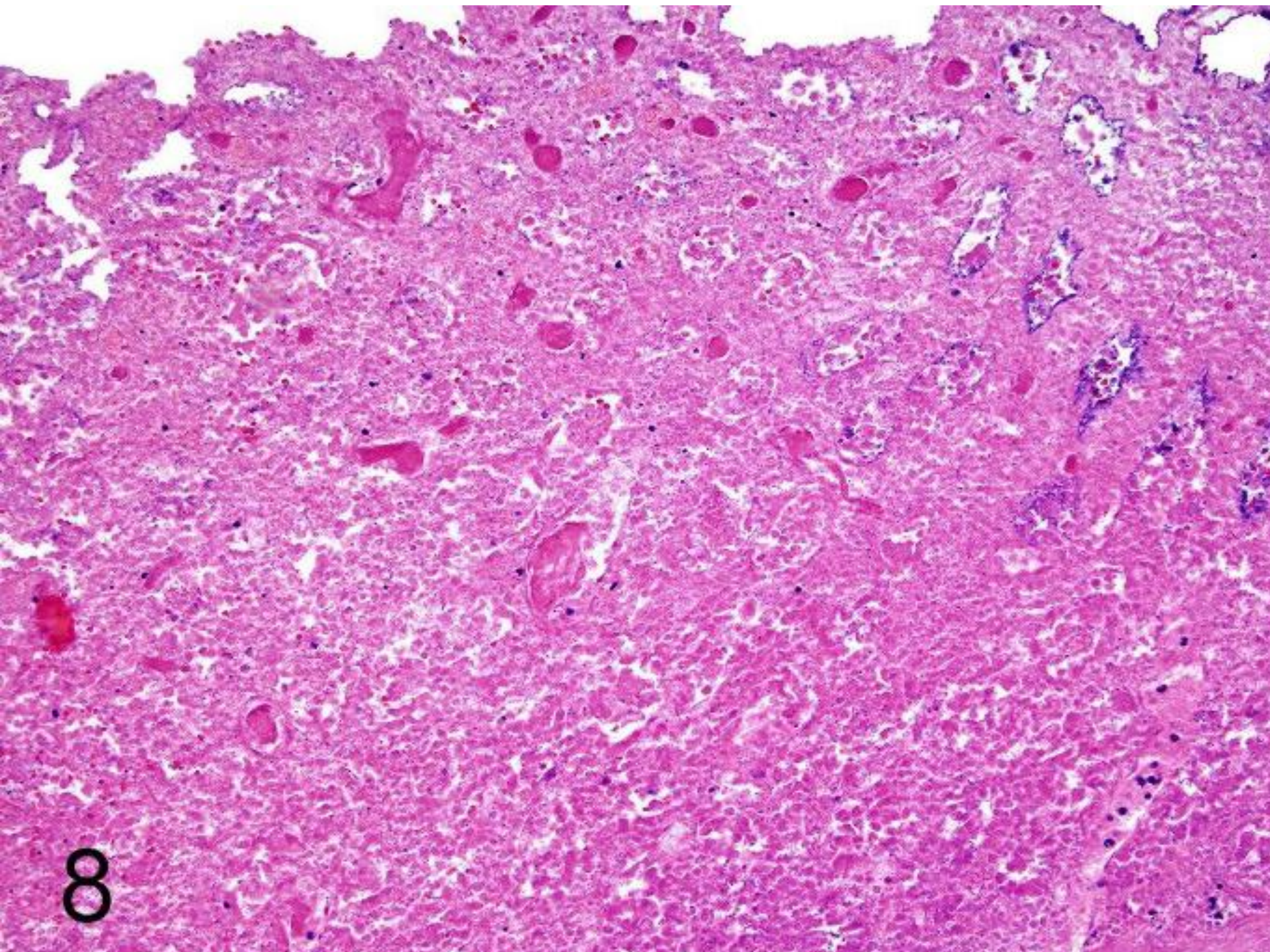

8


Mesocolonic edema
Take-home message!!!!

Causes of mesocolonic edema in pigs:

1-*C. difficile*
2-*E. coli* (edema disease)
3-PCV-2
4-PRSS virus

CATTLE

C. difficile

potential role in neonatal calf diarrhea

C. difficile prevalence (calves):

1-Rodriguez-Palacios et al. (2006)

Culture:

*** diarrheic calves:** **7.6% (11/144)**

*** control calves:** **15% (20/134)**

Toxins:

*** diarrheic calves:** **39.6% (57/144)**

*** control calves:** **20.9% (28/134)**

C. difficile prevalence (calves):

2-Hammitt et al. (2008):

Culture

* Diarrheic: 25.3% (64/253)
* Control: 13% (7/53)

Toxin

* Diarrheic: 22.9% (58/253)
* Controls: 30.2% (16/53)

Risk factors

* younger age
* antibiotis

C. difficile in calves

*** Accompanying BCV, BRV, Crypto, Salmonella, AEEC, others**

*** role in diarrhea: not fully determined**

SHEEP AND GOATS

Prevalence: 0–8.5%

(Knight and Riley, 2013; Avbersˇek et al., 2015; Rodriguez et al., 2016)

No evidence of role in disease

Diagnostic criteria

1-Clinics/gross } **Suggestive**

2-Histology } **Suggestive**

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

4-Ancillary: Toxins A/B } **Confirmatory**

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

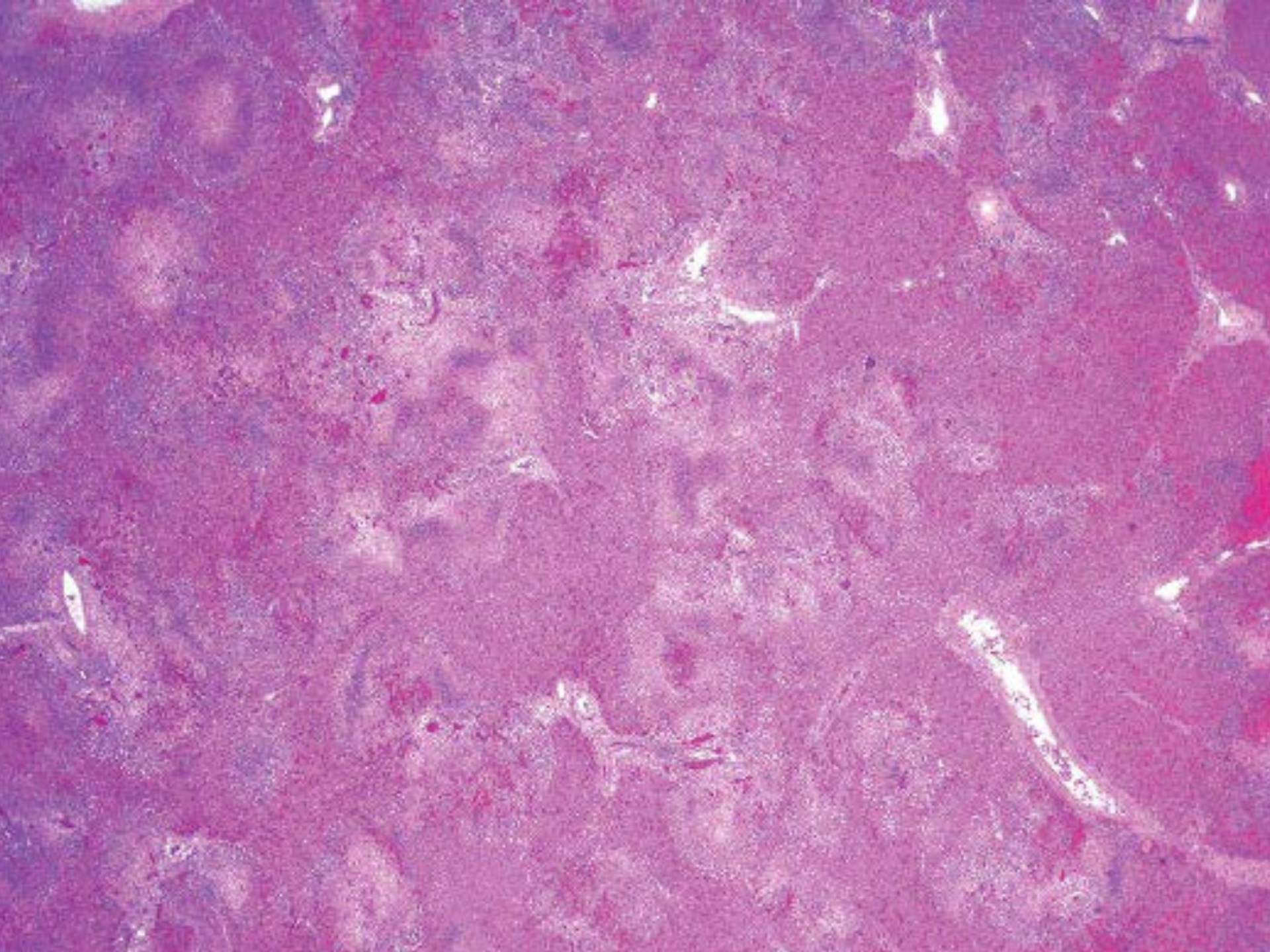
Tizzer’s disease

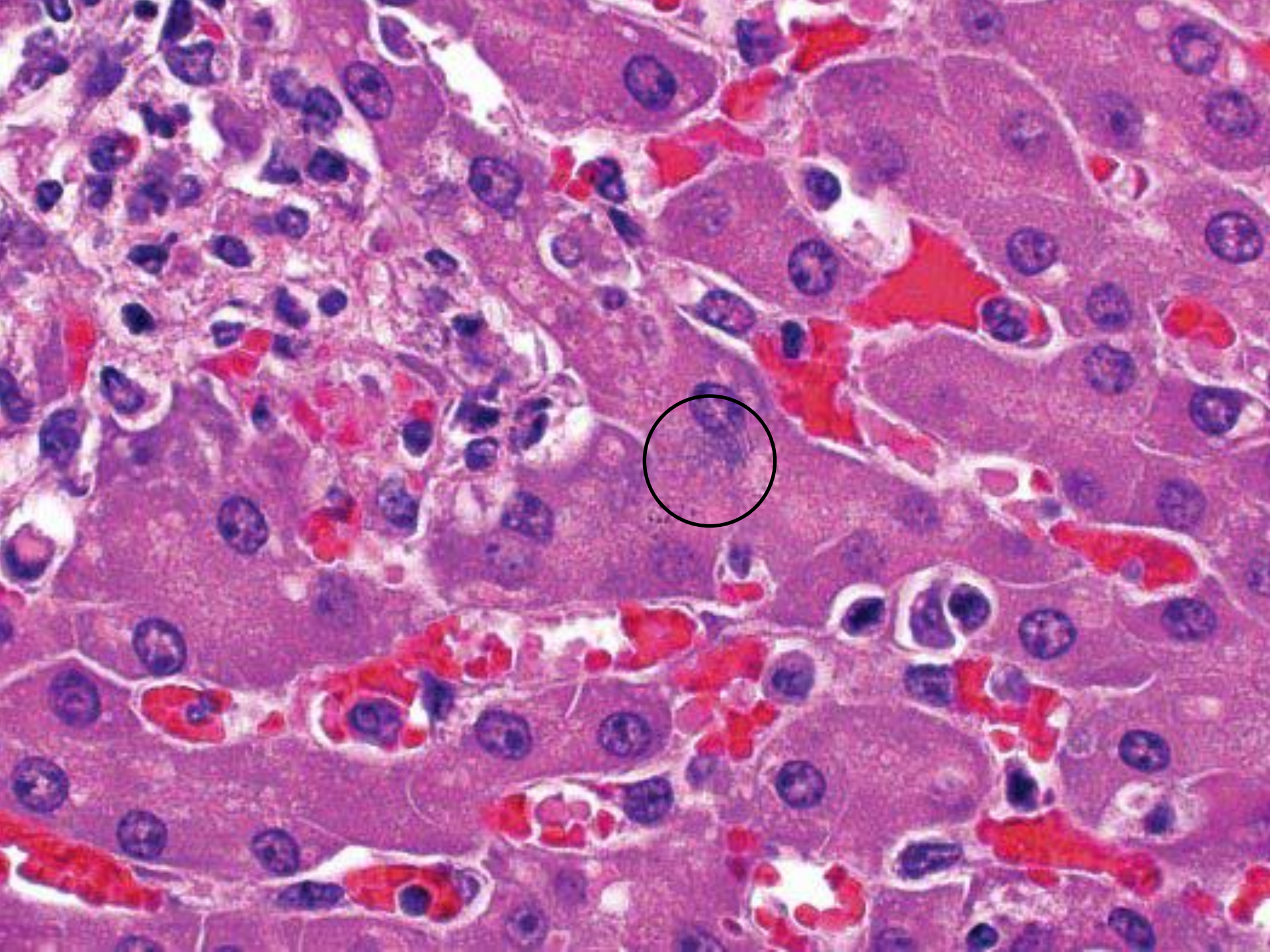
Horse
Rabbit
Rat
Hamster
Cats
Others

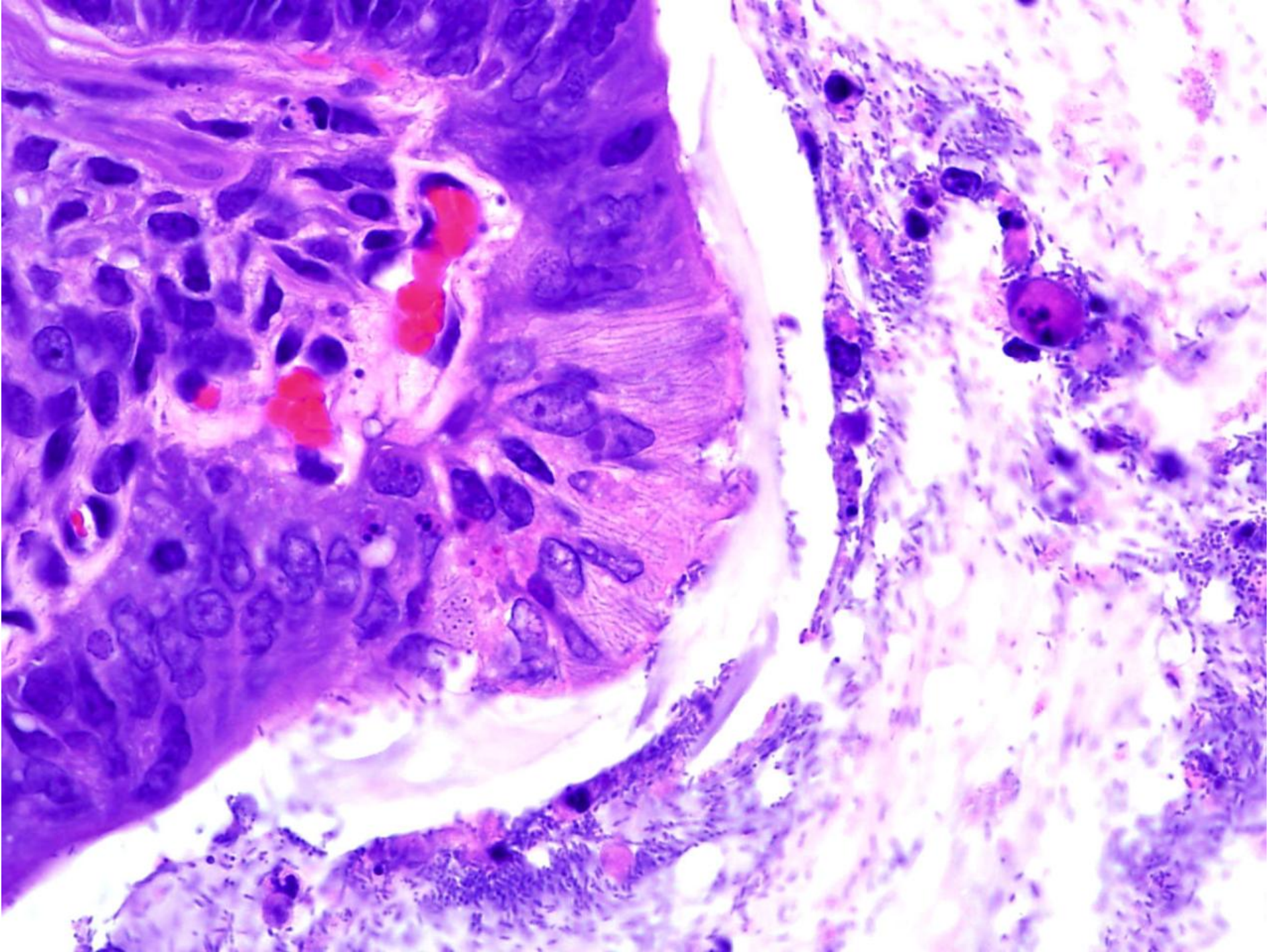
Triad of lesions

Hepatitis
Colitis
Myocarditis

Take-home message!!!







Diagnosis:

1-Histology (HE; silver; Giemsa)
2-PCR
3-Culture (embryonated egg only)

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

- **Distribution**:
  - Soil, sewage, GI tract
  - Vagina of healthy women (5-10%)

- **Disease association**:
  - Toxic shock syndrome in humans
    - Gynecologic procedures (childbirth, abortion)
    - Intravenous drug use

  - Gas gangrene in **ruminants**, **pigs** and **horses**
  - Omphalitis in **foals**

M. Navarro

GI disease in animals:

AVIAN DISEASES 59:447–451, 2015

Case Report——

Necrotic Enteritis in Chickens Associated with *Clostridium sordellii*

Guillermo Rimoldi,[AE] Francisco Uzal,[B] R. P. Chin,[A] Enzo A. Palombo,[C] Milena Awad,[D] Dena Lyras,[D] and H. L. Shivaprasad[A]

[A]California Animal Health and Food Safety Laboratory System, Tulare Branch, 18830 Road 112, Tulare, CA 93274
[B]California Animal Health and Food Safety Laboratory System, San Bernardino Branch, 105 W. Central Avenue, San Bernardino, CA 92408-2113
[C]Department of Chemistry and Biotechnology, Swinburne University of Technology, Victoria 3122, Australia
[D]Department of Microbiology, Sub-Faculty of Biomedical and Psychological Sciences, Office 152, 19 Innovation Walk, Monash University, Victoria 3800, Australia

Received 7 April 2015; Accepted 22 April 2015; Published ahead of print 20 May 2015

AVIAN DISEASES 57:698–702, 2013

Case Report—

Ulcerative Enteritis-like Disease Associated with *Clostridium sordellii* in Quail

Rocio Crespo,[AD] Monique Franca,[B] and H. L. Shivaprasad[C]

[A]Avian Health and Food Safety Laboratory, Washington Animal Disease Diagnostic Laboratory, Washington State University, 2607 West Pioneer, Puyallup, WA 98371
[B]Poultry Diagnostic and Research Center, College of Veterinary Medicine, University of Georgia, 501 D.W. Brooks Drive, Athens, GA 30602
[C]California Animal Health and Food Safety Laboratory System, Tulare Branch, University of California–Davis, 18830 Road 112, Tulare, CA 93274

Received 17 January 2013; Accepted 27 March 2013; Published ahead of print 9 April 2013

Clostridium sordellii in Lambs with Abomasal Bloat, Haemorrhage and Ulcers

S. Vatn [a], M.A. Tranulis [a], M. Hofshagen [b]

⊞ Show more

https://doi.org/10.1053/jcpa.1999.0363

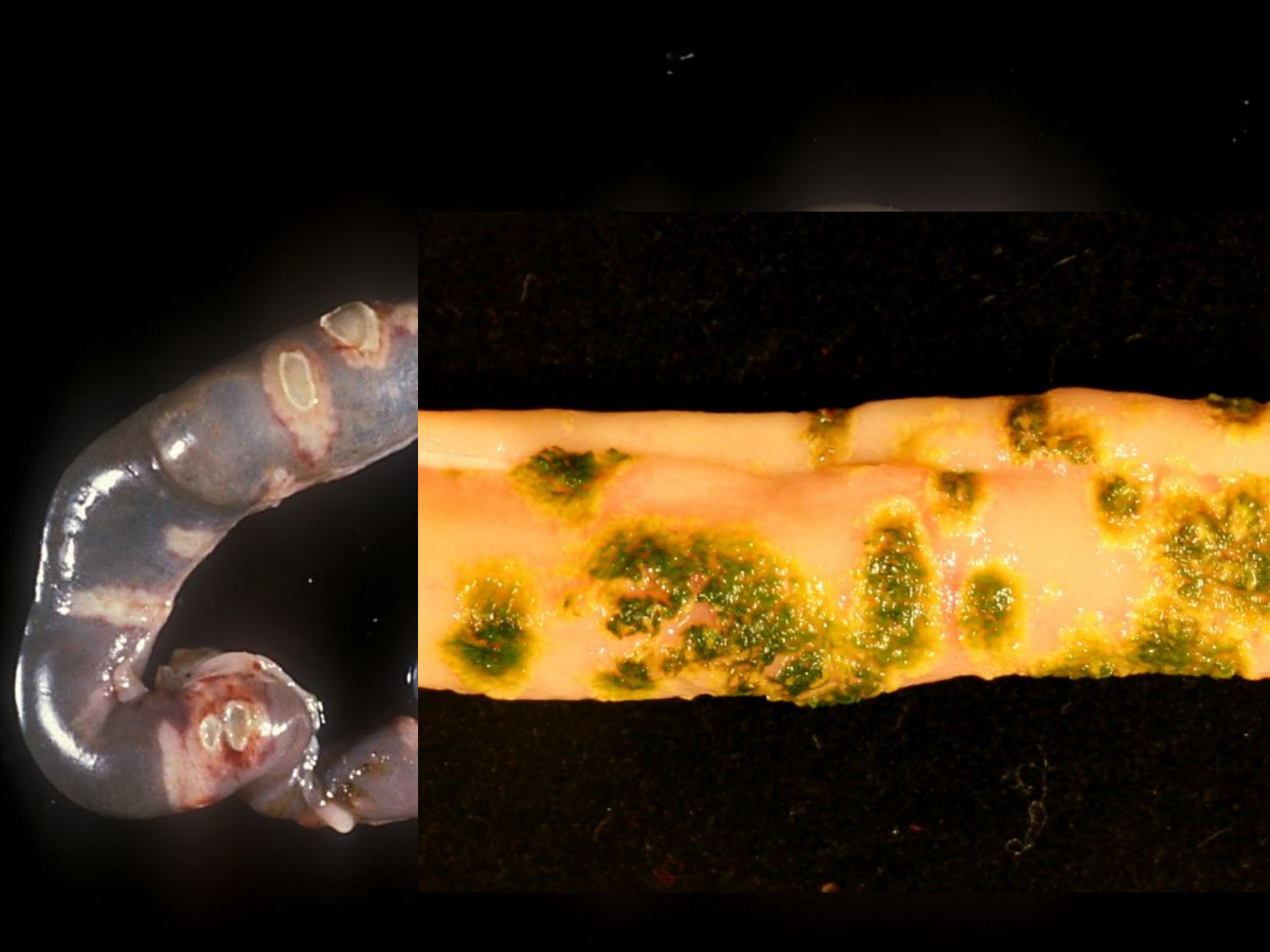
Virulence factors:

Toxin	Mechanism of Action
Lethal toxin	Inhibits signaling proteins Rac, Cdc42, Ras and Rap
Hemorrhagic toxin	Inhibits signaling proteins Rho, Rac, and Cdc42
Sordellilysin	Cholesterol-dependent hemolysin
Sialidase	Cleaves sialic acids from sialoglycoconjugates
Phospholipase C	Hydrolyzes lecithin
DNAse	Potential degeneration of host cell nuclei
Hyaluronidase	Splits hyaluronic acid, increasing permeability
Collagenase	Hydrolyzes collagen and gelatin

Aldapo et al, 2006.

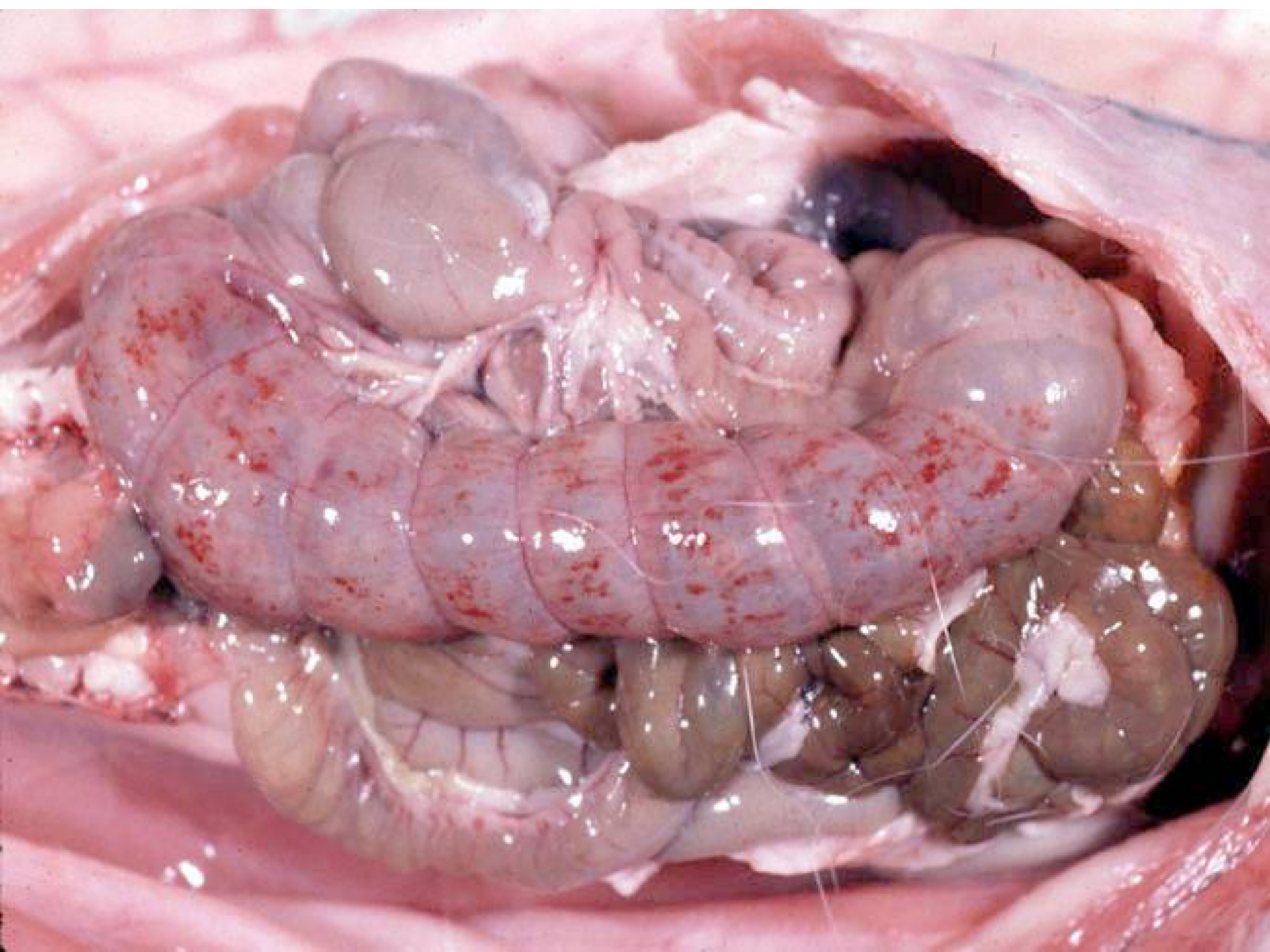
GROUP	DISEASE	ORGANISM	HUMANS	OTHER ANIMALS
Enteric	**Enterotoxemias/ enteritis**	*C. perfringens*	✓	✓
		C. difficile	✓	✓
		C. piliforme	--	✓
		C. sordellii	--	✓
		C. colinum	--	✓
Histotoxic				
Neurotoxic				

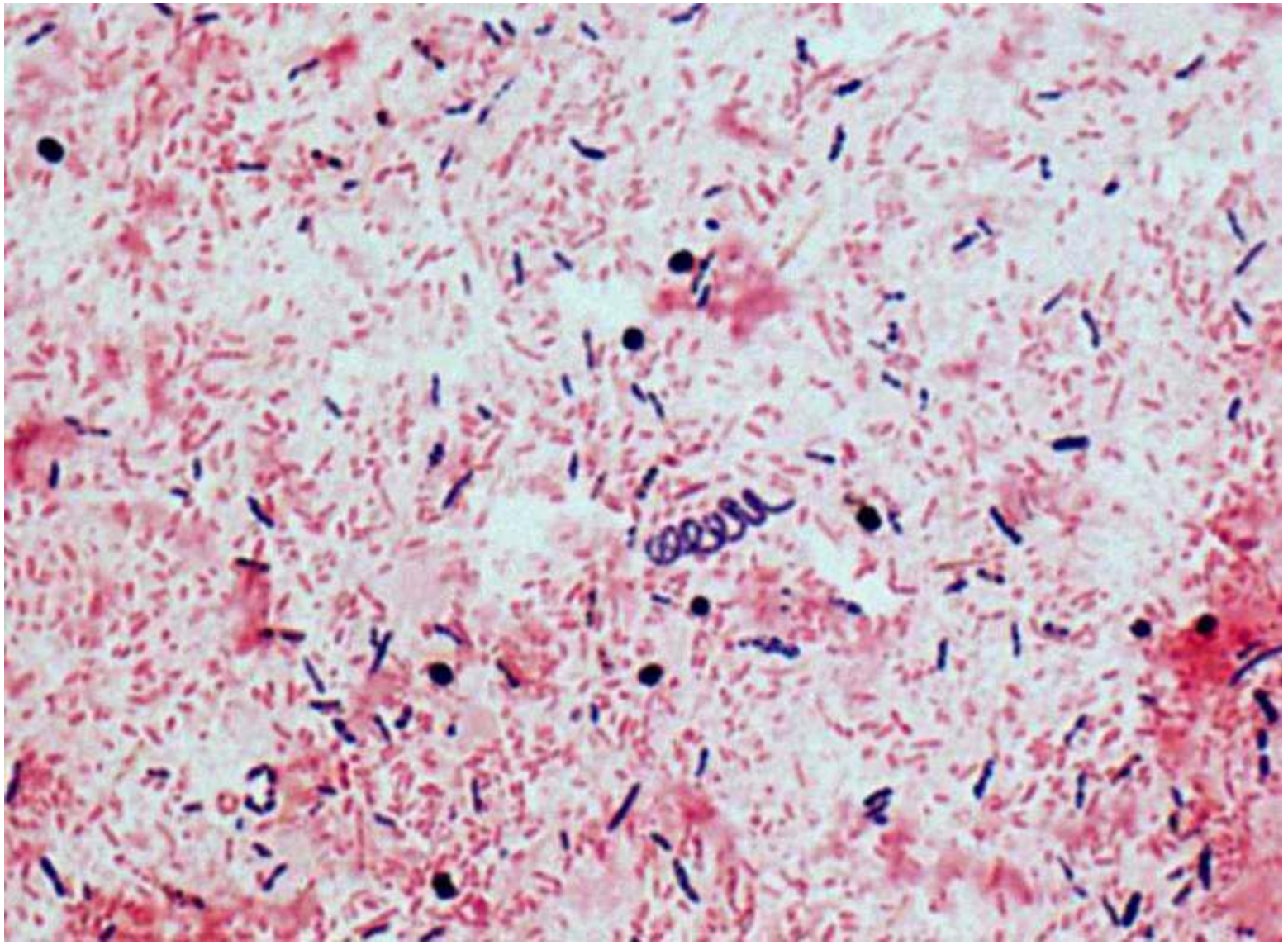
“Quail disease”



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

“Rabbit enterotoxemia”





Thank you!!!

Diseases that look clostridial

Undetermined etiology

Jejunal hematoma

Adult dairy cows


Photo Rob Moeller