Emerging Diseases Impacted by Chemotherapeutic Restrictions

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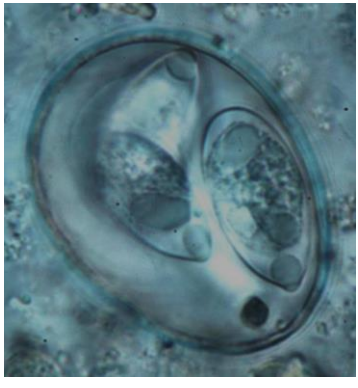
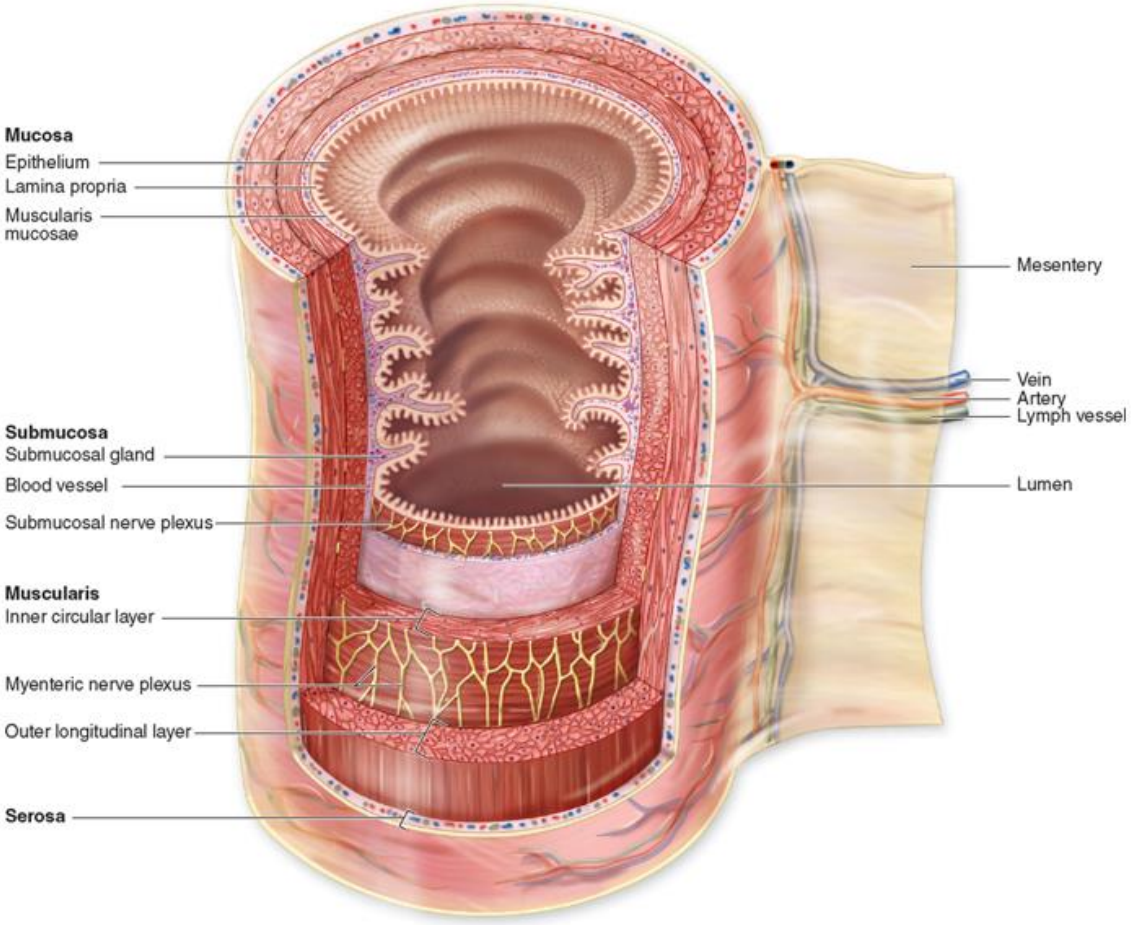
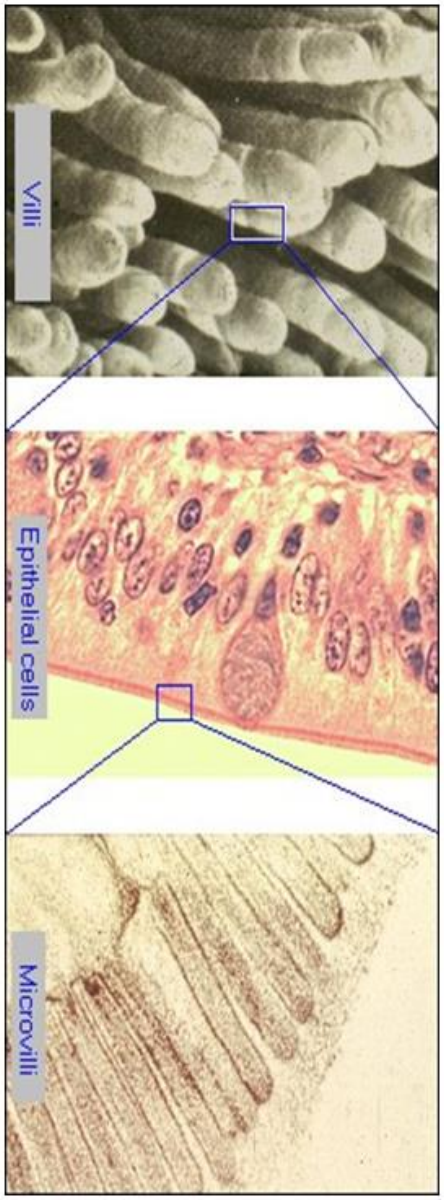
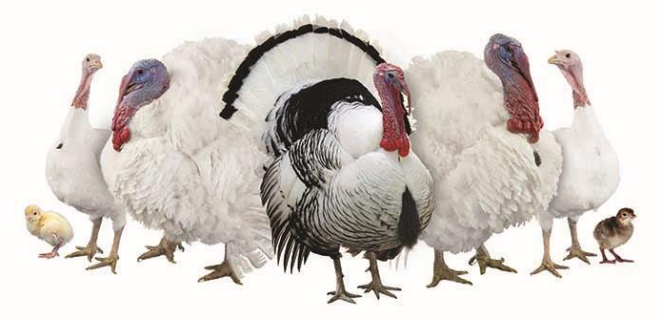


Deceptively Simple





Decreasing Acceptance of AGP



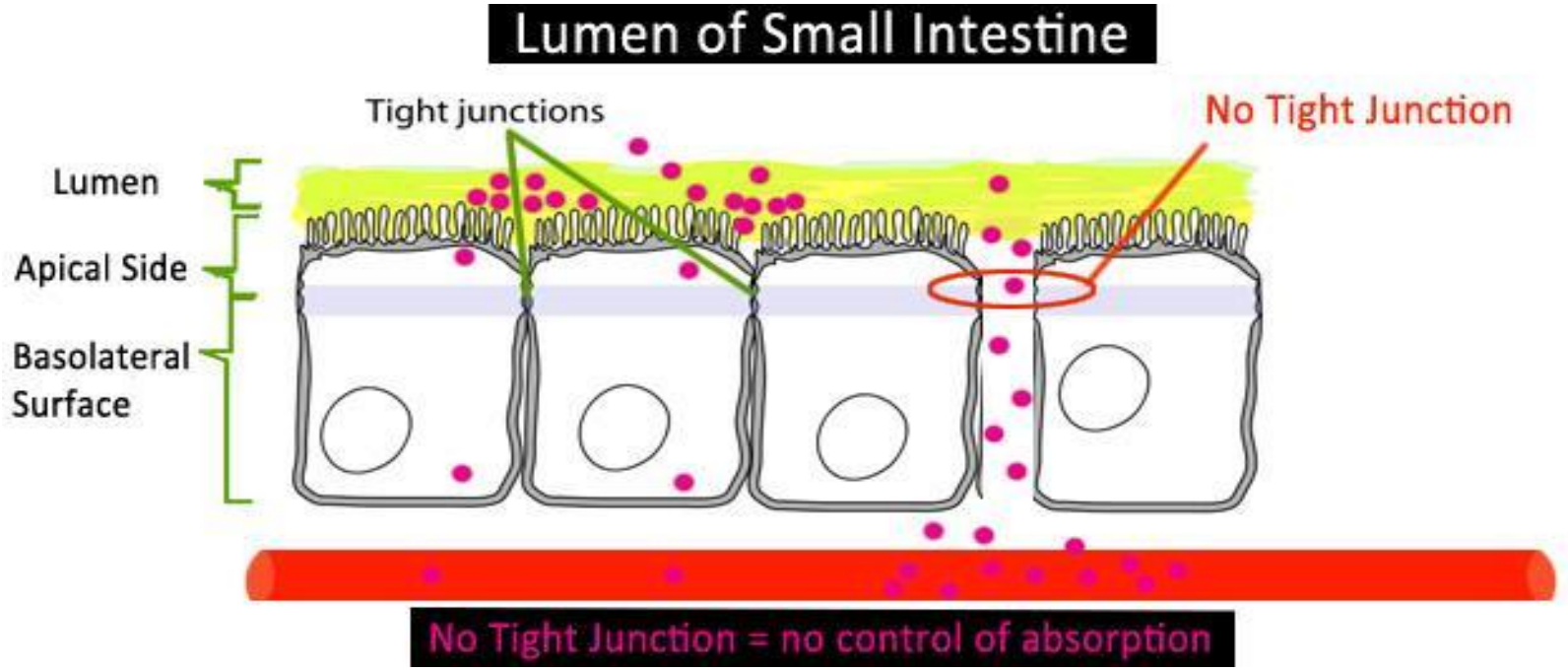
- Banned in EU & some Asian countries
- Consumer demand in USA
- USA ban in near future?
- We have already lost valuable therapeutics
- Economical and sustainable alternatives are imperative – **Development of poultry models to evaluate Gut Health parameters**

https://jamanetwork.com
http://www.zootecnicainternational.com

Major Issues

- Enteric inflammation from any cause – dietary, dysbiosis, viral, specific bacterial pathogens, protozoa, other parasites
- Coccidiosis control without ionophores
- Necrotic Enteritis due to poor coccidiosis control and loss of anti-inflammatory AGPs
- Re-emergence of Histomoniasis (Blackhead) and other protozoal diseases – with loss of organic arsenicals

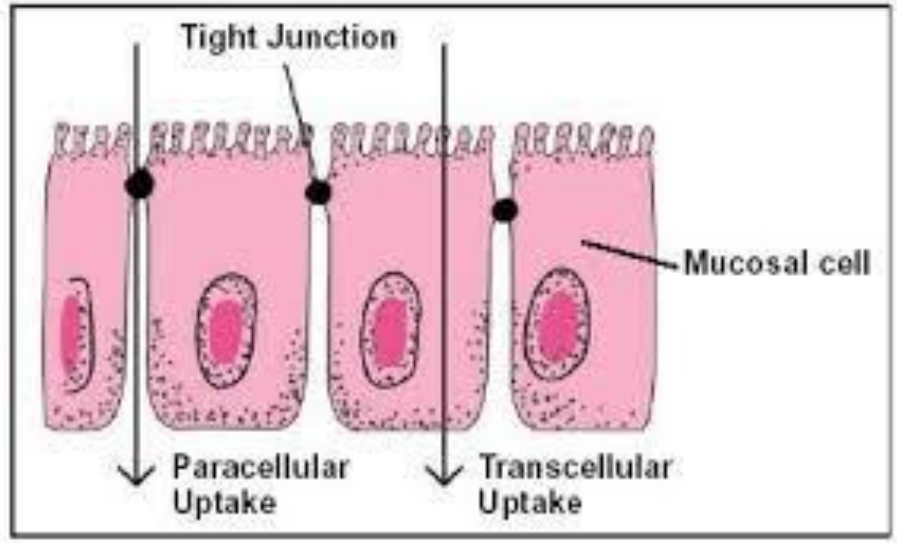
Integrity of GIT Barrier Through Tight Junctions



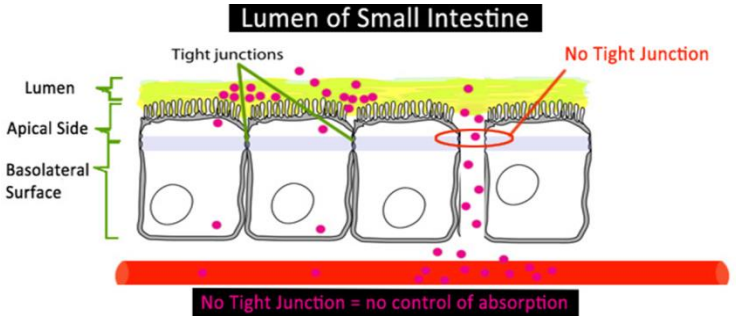
http://www.dbriers.com/tutorials/2012/12/junctions-between-cells-simplified/

Inflammation Leads to Bacterial Translocation

- Normal closure of tight junctions
- Normalized by commensal bacteria
- Stress, low digestibility feed, feed restriction, therapeutic antibiotics cause leaky gut



http://allnaturaladvantage.com.au/how_gastrointestinal_health_affe.htm



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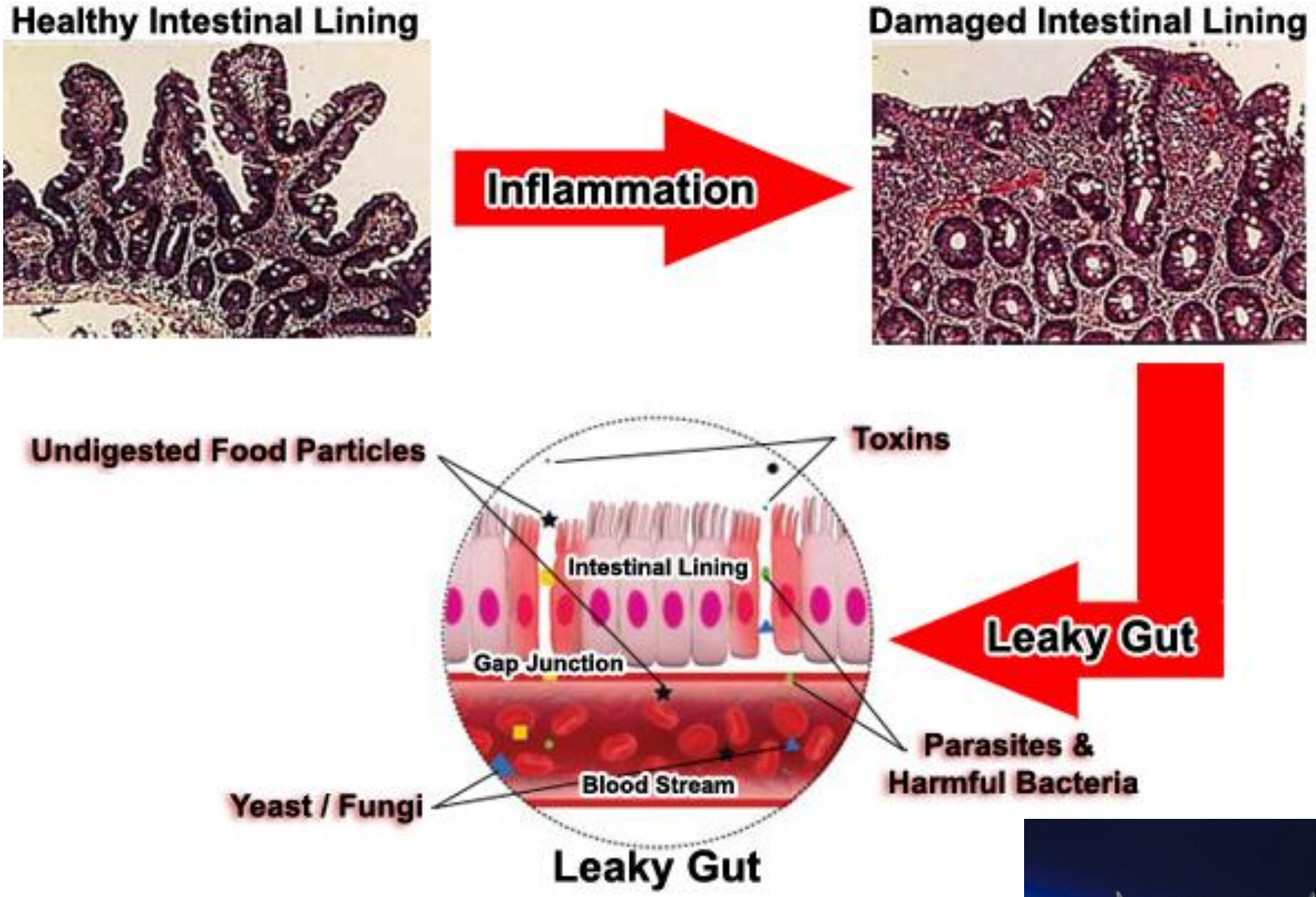


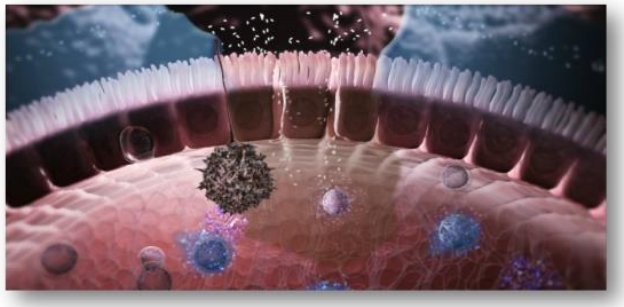
http://scdlifestyle.com/2010/03/the-scd-diet-and-leaky-gut-syndrome/

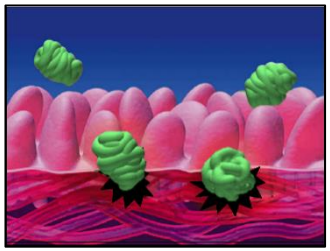
Serum FITC-d levels

Marker
Fluorescein isothiocyanate dextran (FITC-d)
3,000-5,000 Da; Green fluorescent dye

FITC-d oral gavage (8.34mg/kg)
1 h before taking blood samples

Gut leakage





Serum FITC-d levels

Serum FITC-d level:
Excitation wavelength of 485nm and emission wavelength of 528nm

Kuttappan et al. 2015
Vicuña et al. 2015

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http://www.nature.com/ni/multimedia/mucosal/animation/index.html

Gut Inflammation Model: Rye

- Rye (*Secale cereale*)is a cereal member of wheat tribe
  - 152 g of total NSP per kg of dry matter (Antoniou et al., 1981; Bach Knudsen,1997)
  - Used in poultry feed when the price of corn is high

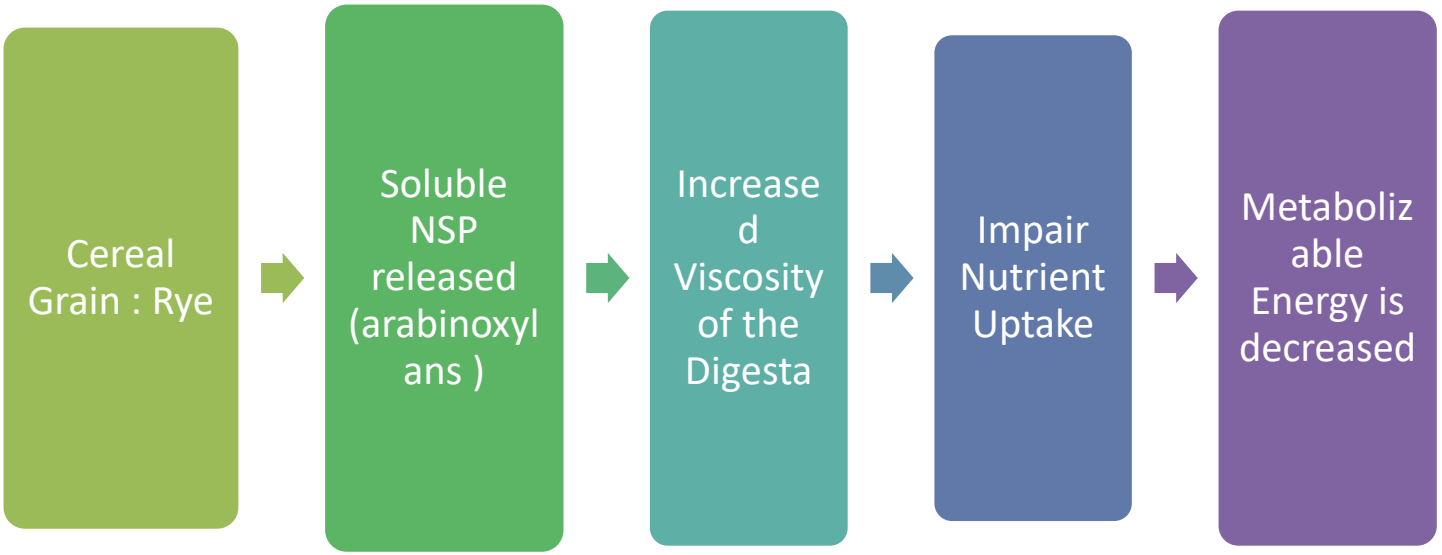

Diagram adapted from Chesson, 2001

Background: The Gut

- Increase digesta viscosity →
  - Increases mucin secretion(Chesson, 2001)
  - Reduces absorption of sodium, calcium, and phosphorus (Fengler and Marquardt, 1988;
  - Reduces total bone ash and strength in poults and chicks (Tellez et al., 2015; Tellez et al., 2014)
  - Reduces conjugated bile acid, lipid digestibility (Langhout et al., 1997).
  - Promotes dysbiosis in the lower intestinal tract (Annison and Choct, 1991)
  - Causes enteric inflammation: higher bacterial translocation and enteric leakage (Tellez et al., 2014: Tellez et al., 2015; Vicuna et al., 2014)

Cost of Coccidiosis

- Malabsorption – disruption of epithelial integrity
- Local and systemic inflammation
- Promotion of necrotic enteritis
- Cost of vaccines or anticoccidials
- Litter management, ammonia control, welfare issues

Topics

- coccidiosis as an **“artificial”** disease (a by-product of poultry domestication)
- coccidian life cycle
- *Eimeria* species in chickens
- coccidiosis in the gut of a single bird
- coccidiosis in a flock (transmission)

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Which came first?
The chickens or the coccidiosis

Gallus varius
(Green Jungle Fowl)

Gallus gallus
(Red Jungle Fowl)

Gallus sonneratii
(Grey Jungle Fowl)

Gallus lafayetii
(Ceylon Jungle fowl)

Example subspecies
Gallus gallus domesticus

Types of domesticated birds

Broiler (meat) production

Layer (egg) production

Exhibition, traditional and general purpose production

Genetic Resistance?

Little pressure prior to domestication

Coccidiosis in Jungle Fowl?

- small numbers of birds over a broad area
- mixed ages and genetic backgrounds
- one or a few generations of birds per year with infections occurring in the young birds

- virtually no clinical coccidiosis -- little morbidity or mortality because of small infectious doses over a extended period of time

Plenty of infection – little disease

Coccidiosis - An Artificial Disease

- number of oocysts ingested and the immune status of the host defines whether or not clinical disease will occur

- during poultry production, immunologically naïve birds, crowding and huge numbers of oocysts result in massive challenge ⟹ **disease**

- once exposed, *surviving birds normally immune to subsequent challenge with the same species and/or strain of parasite*

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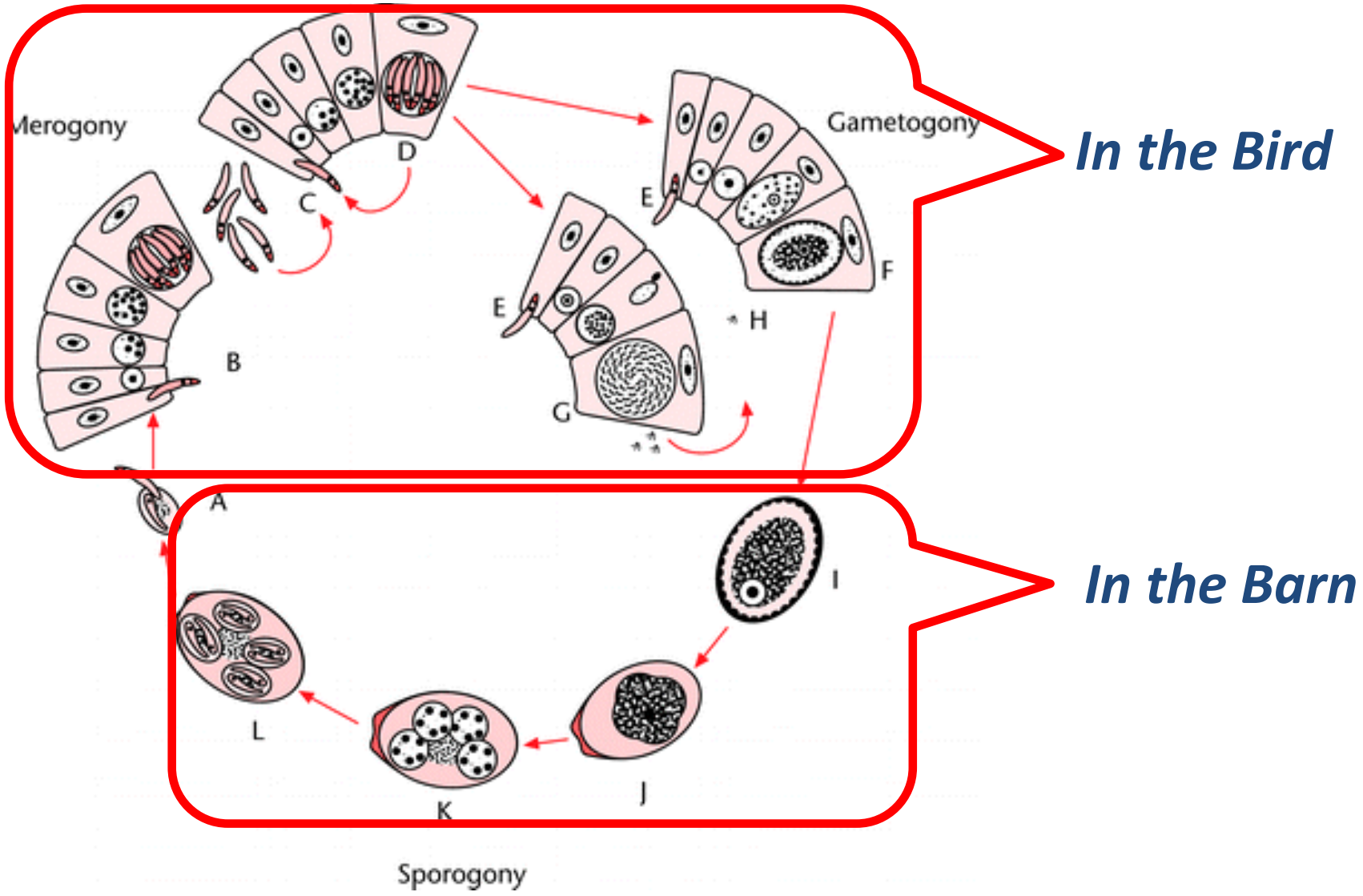
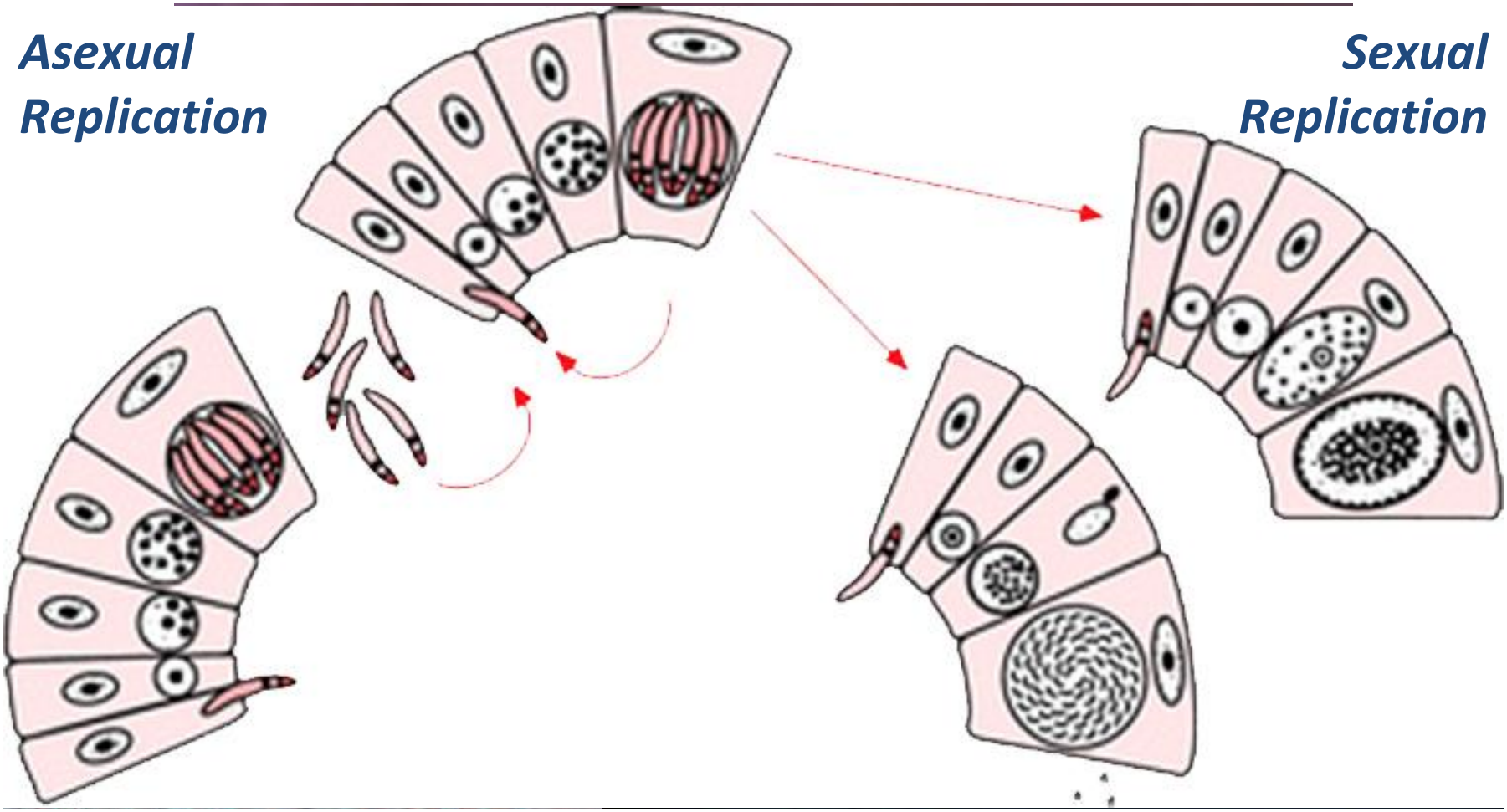
Eimeria species – Life Cycle



Eimeria species – Life Cycle
Endogenous development



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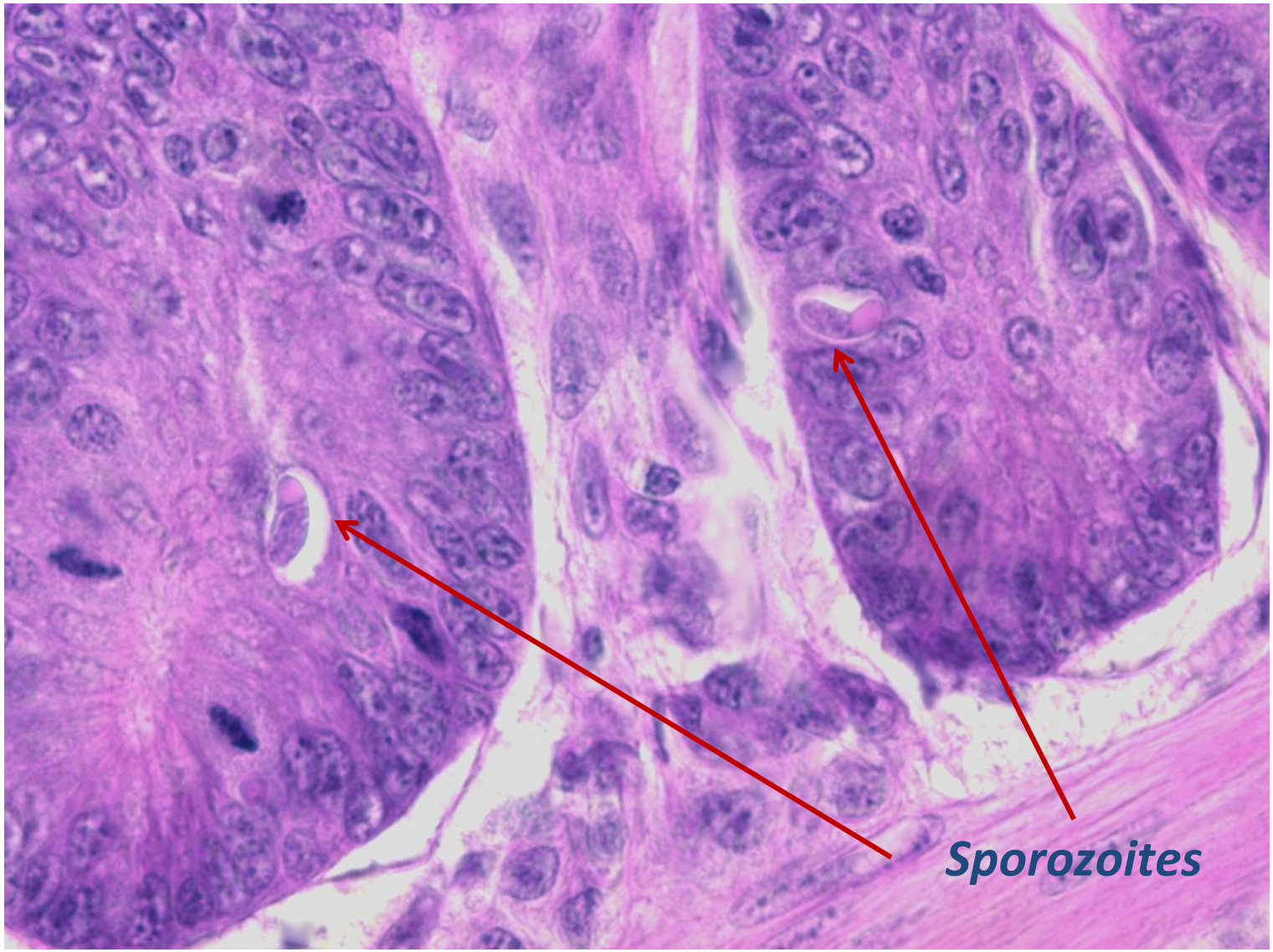

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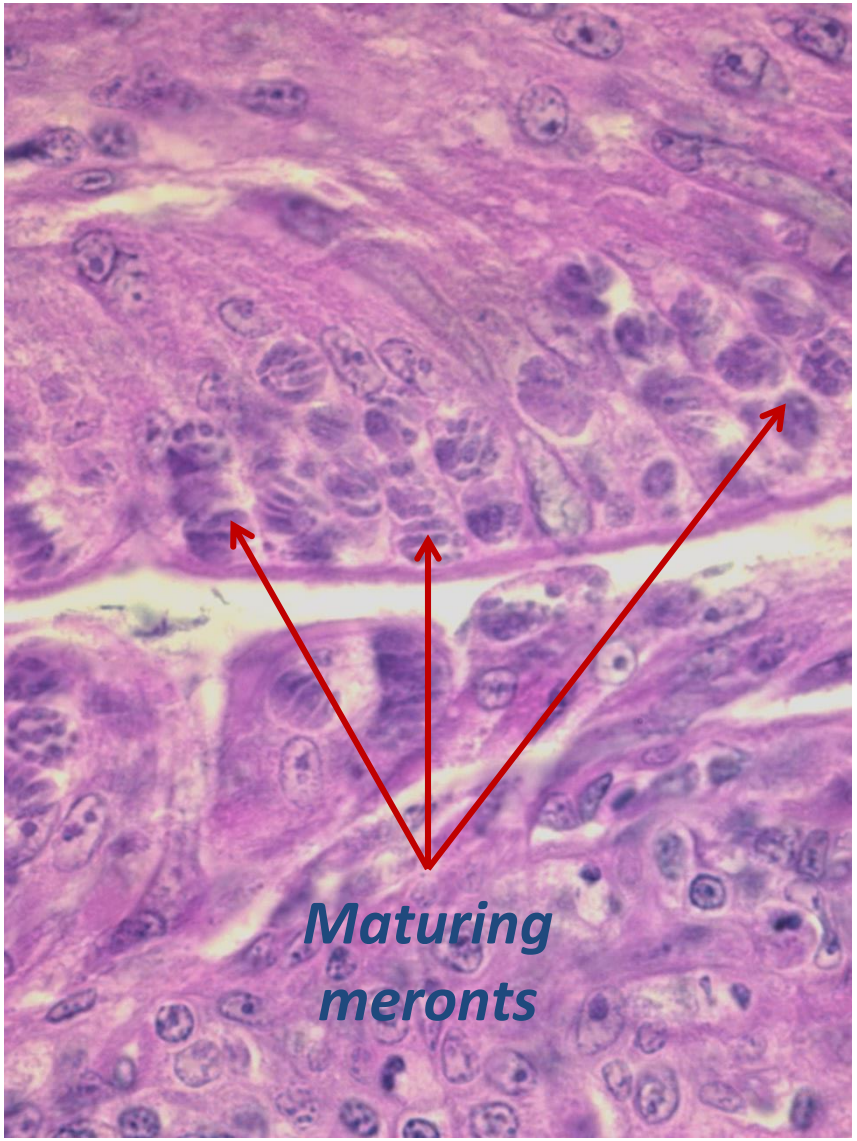
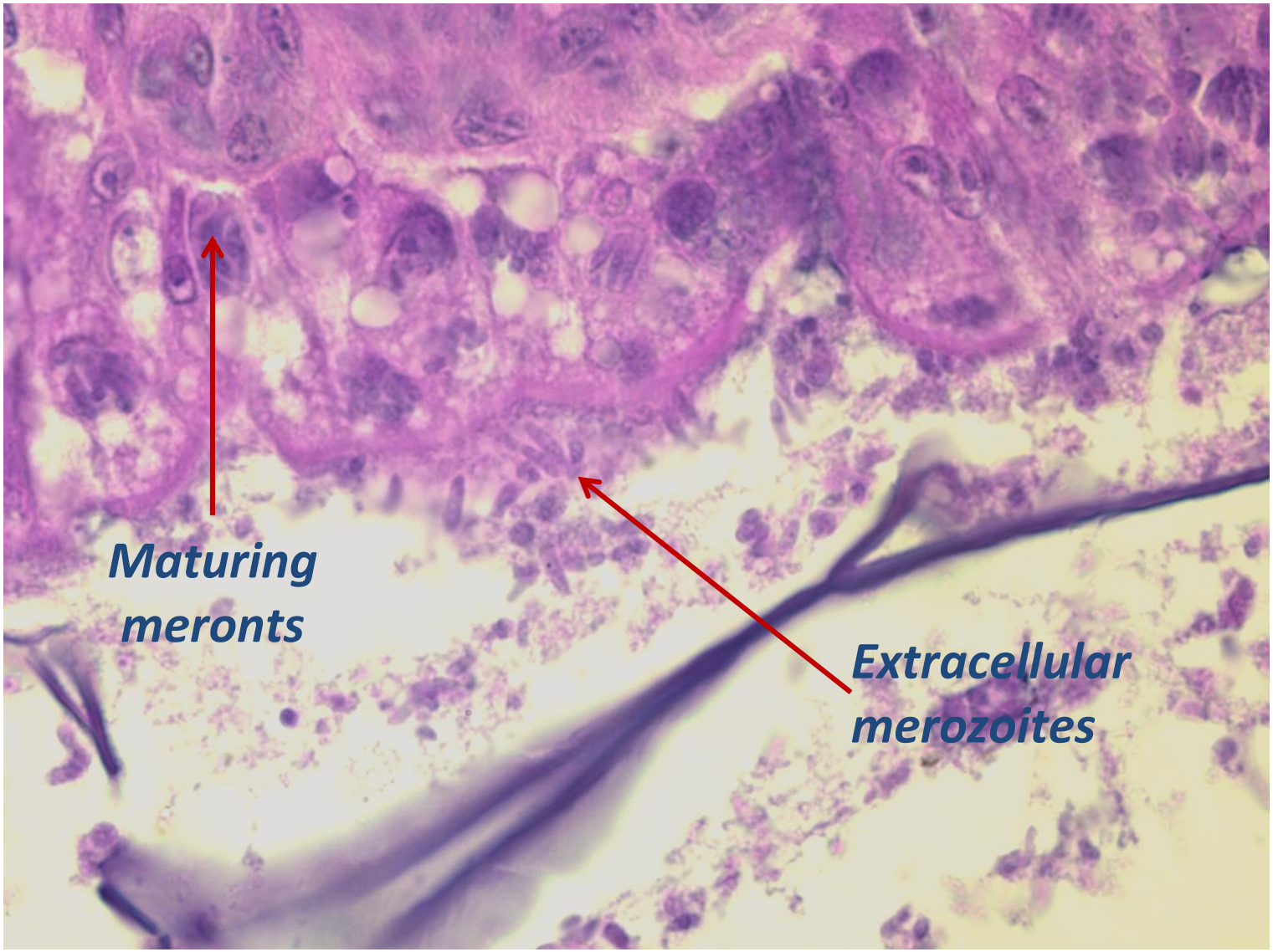
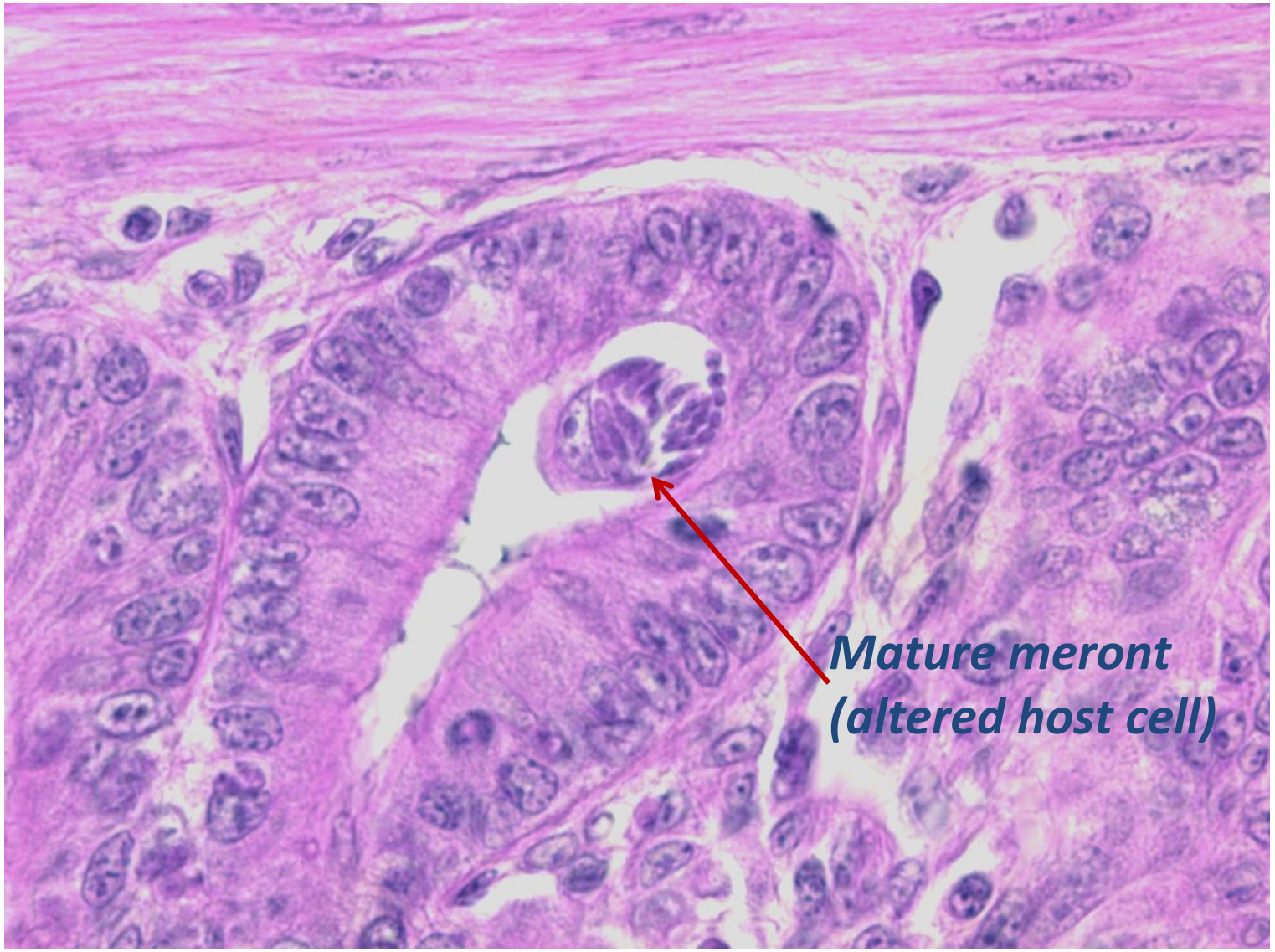
Endogenous development



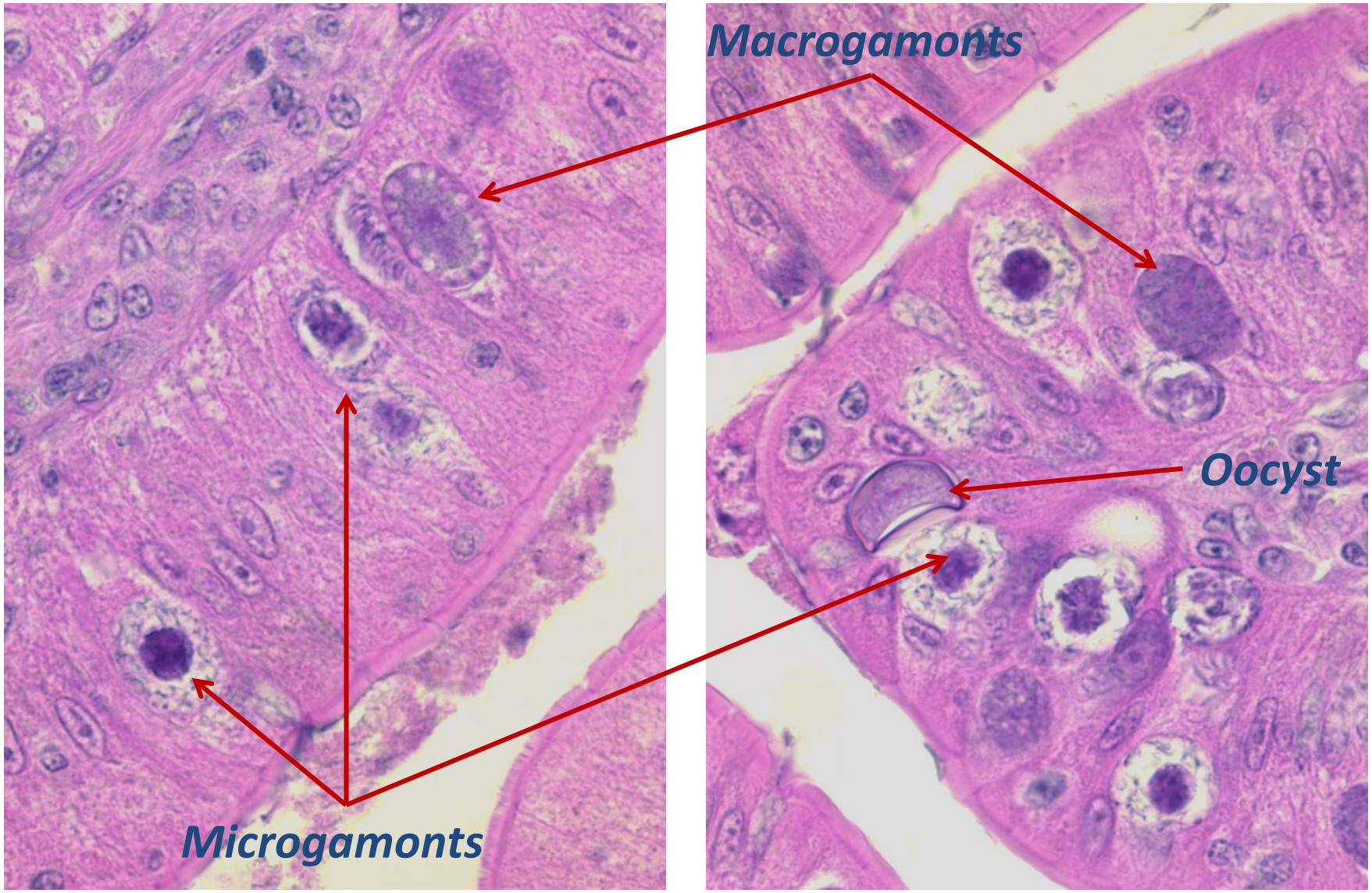
Eimeria species – Life Cycle
Endogenous development



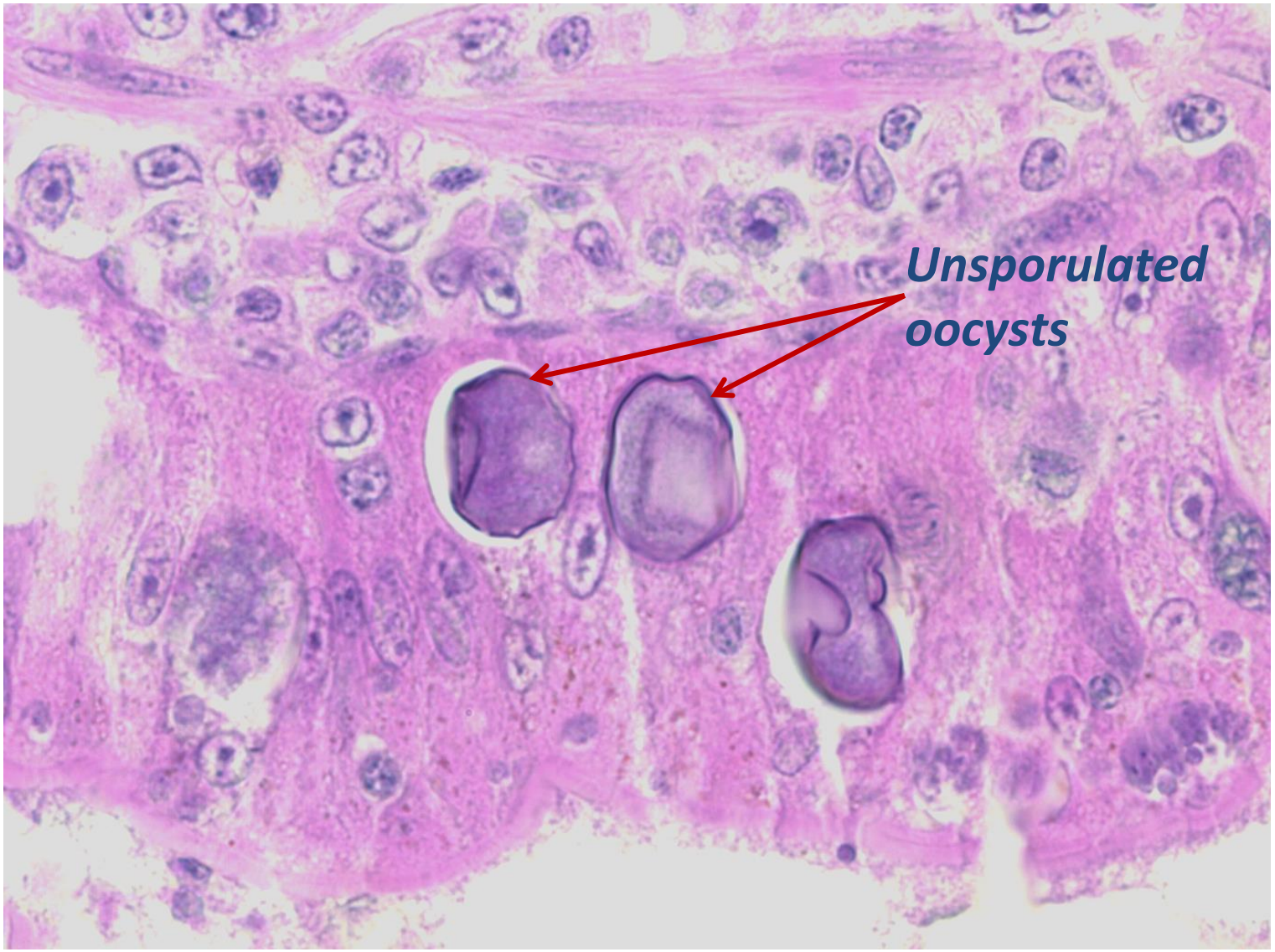
Eimeria species – Life Cycle
Endogenous development



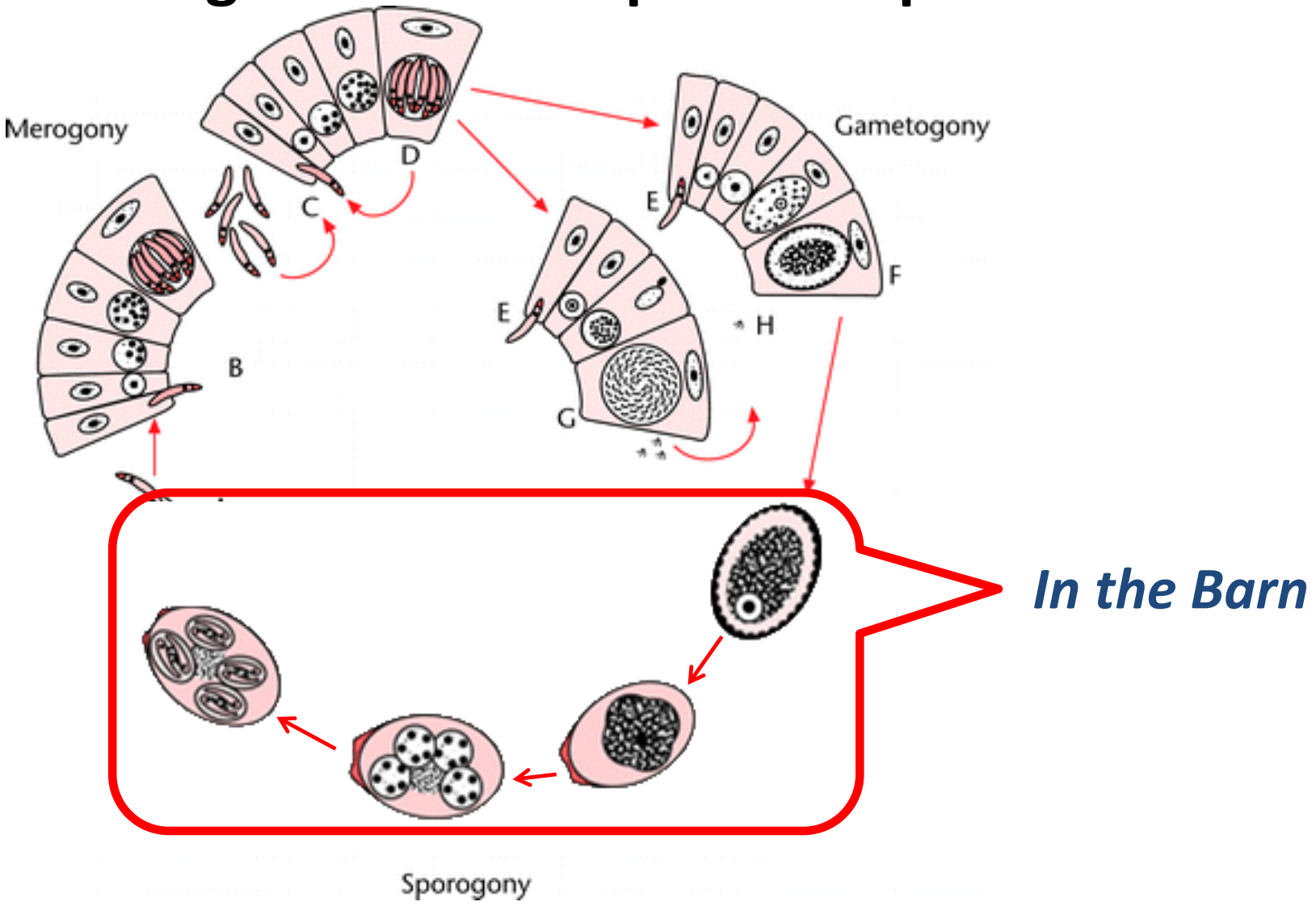
Eimeria species – Life Cycle
Endogenous development



Eimeria species – Life Cycle
Endogenous development

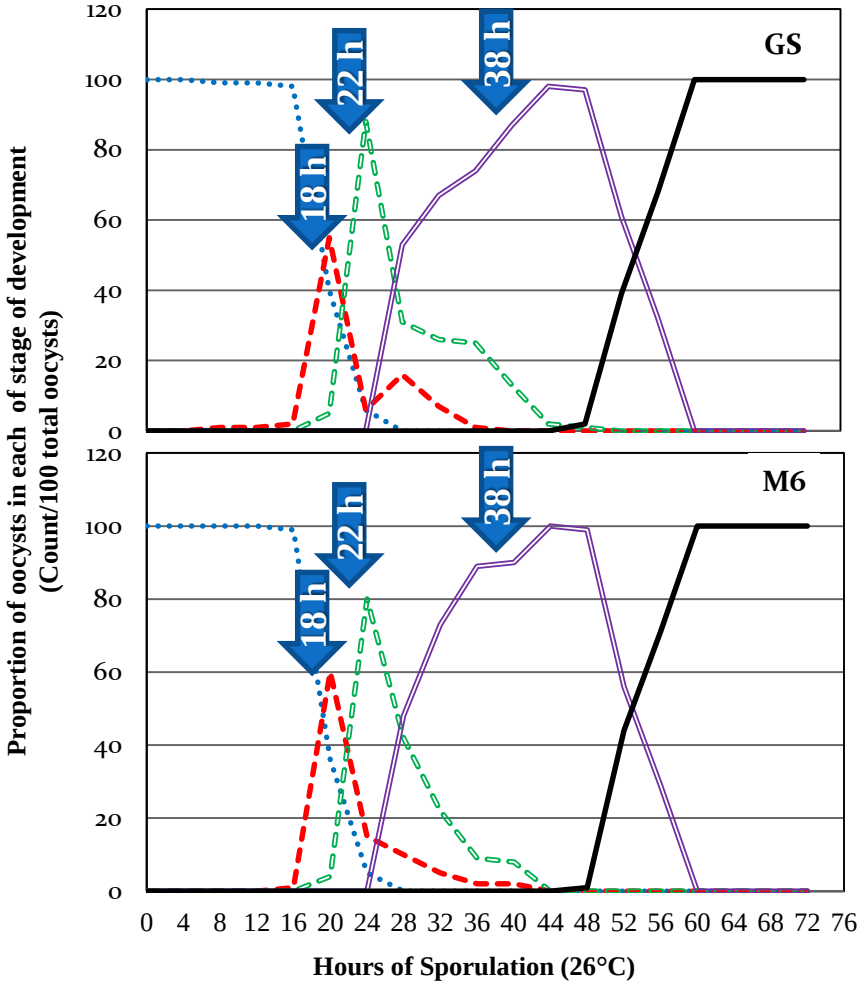


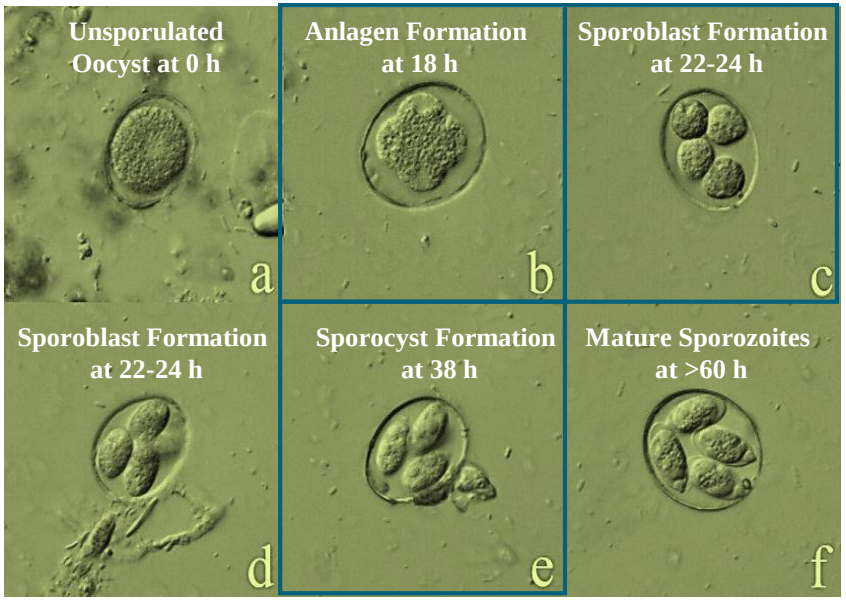
Eimeria species – Life Cycle
Exogenous Development - Sporulation



Stages of Sporulation – *Eimeria* species

Results:





Unsporulated oocysts
Sporoblast Anlagen
Sporoblasts without Sporocyst Walls
Sporocysts without Mature Sporozoites
Sporocysts with Mature Sporozoites

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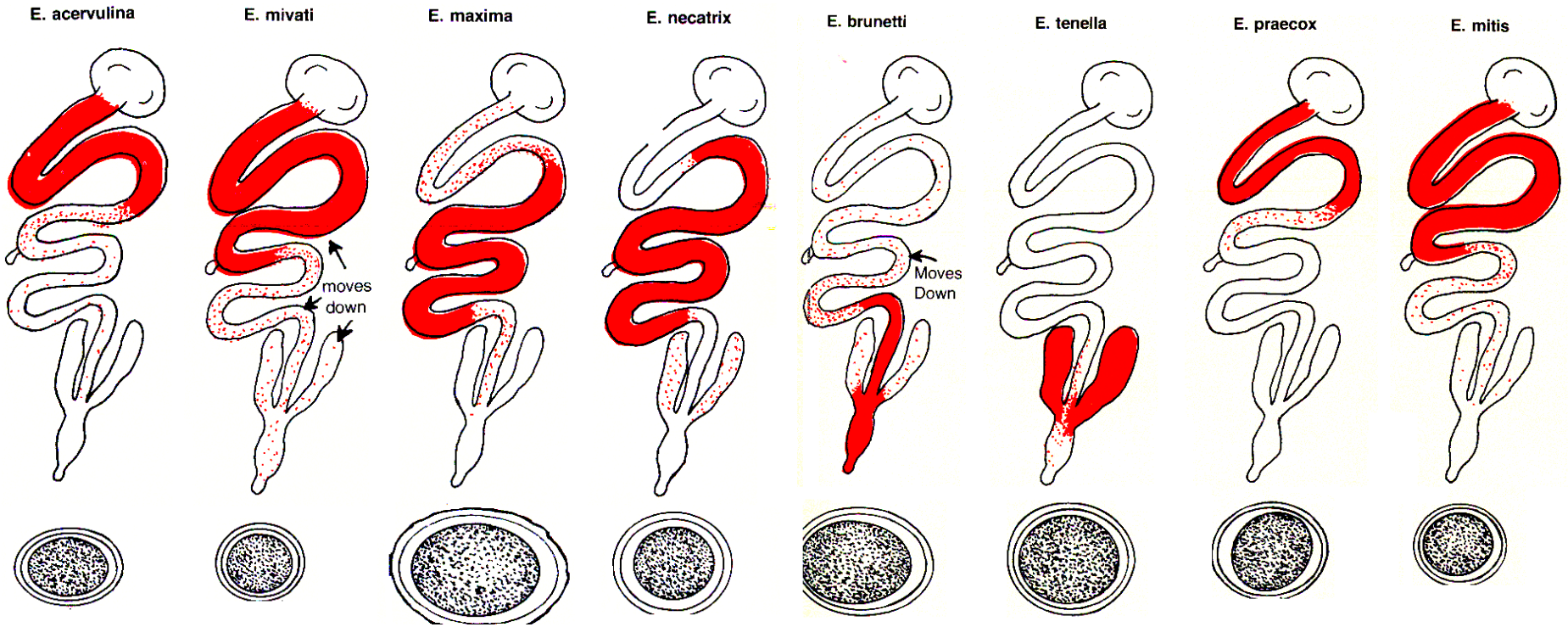
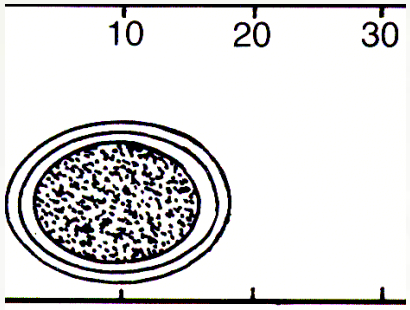
Not just one disease
Eimeria species in chickens



Reid and Long, 1979

Eimeria species in Chickens

Eimeria acervulina



AV = 18.3 x 14.6

17.7-20.2

13.7-16.3

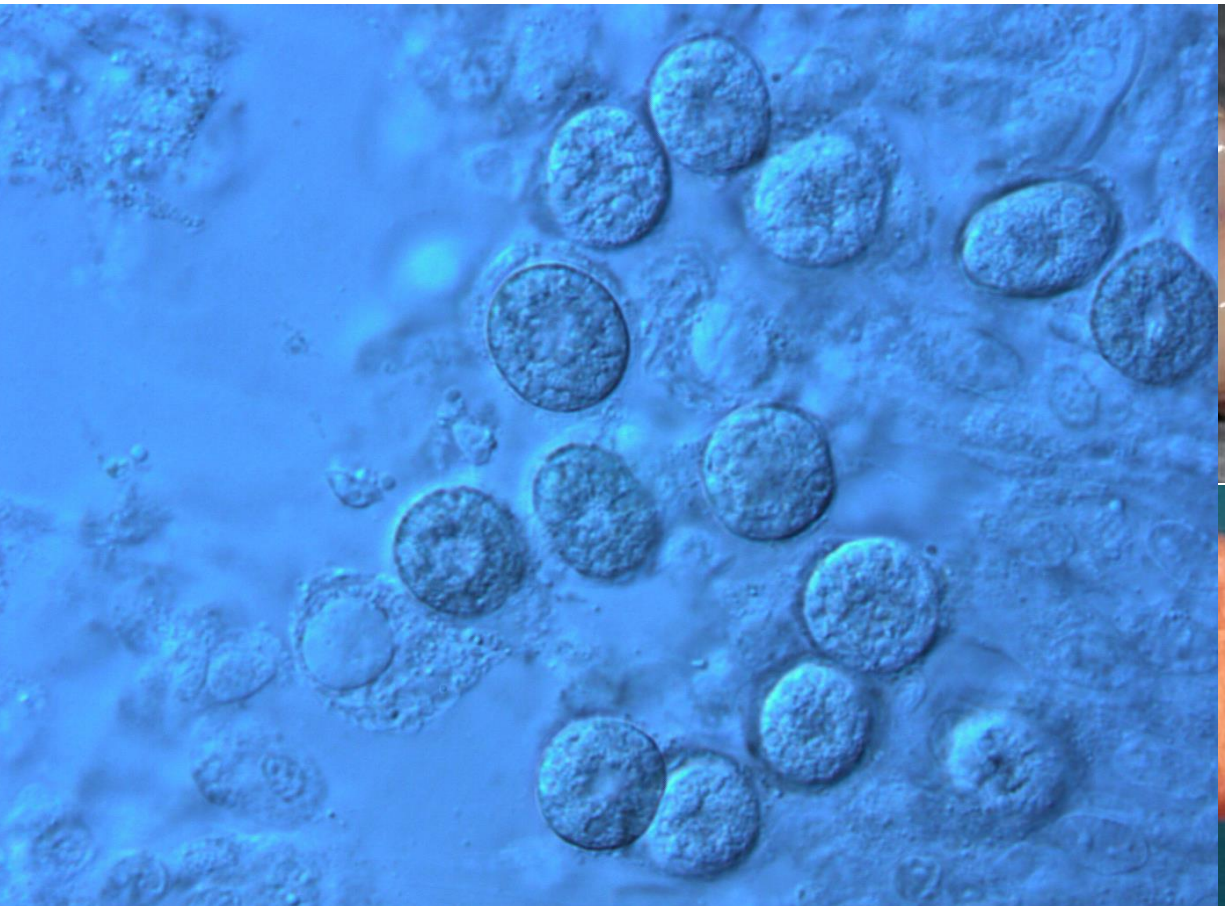
Pathogenic;
Prolific

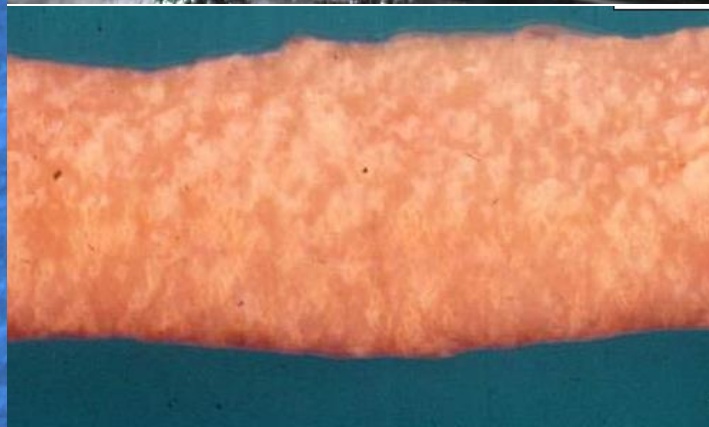
Merck Veterinary Manual and Reid and Long (1979)

Eimeria species in Chickens

Eimeria acervulina

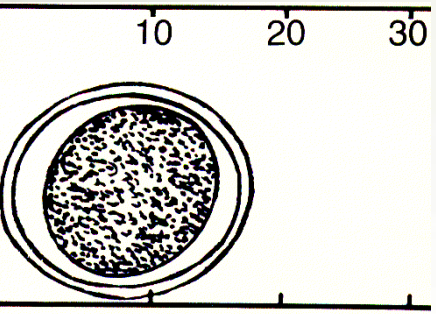
Pathogenic; Prolific; Ubiquitous





Eimeria species in Chickens

Eimeria praecox

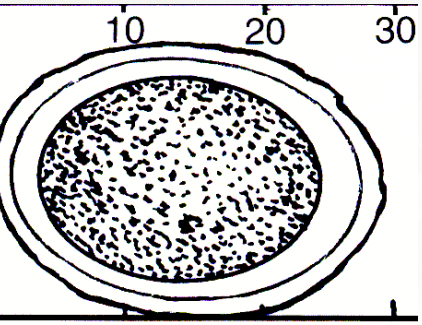


Under-recognized,
Mildly pathogenic

Merck Veterinary Manual and Reid and Long (19

Eimeria species in Chickens

Eimeria maxima



30.5 x 20.7

21.5-42.5

16.5-29.8

Immunogenic
Pathogenic
Promotes NE

Merck Veterinary Manual and Reid and Long (1979)

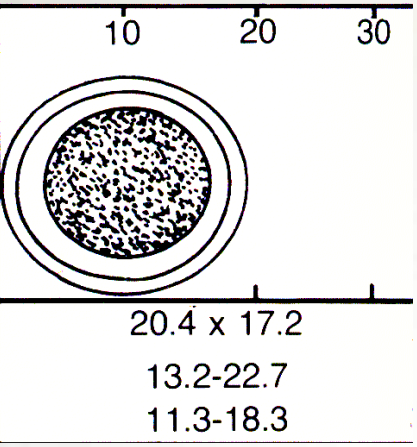
Eimeria species in Chickens

Eimeria maxima

Immunogenic; Pathogenic; Promotes NE (excess mucus/inflammation)

Eimeria species in Chickens

Eimeria necatrix



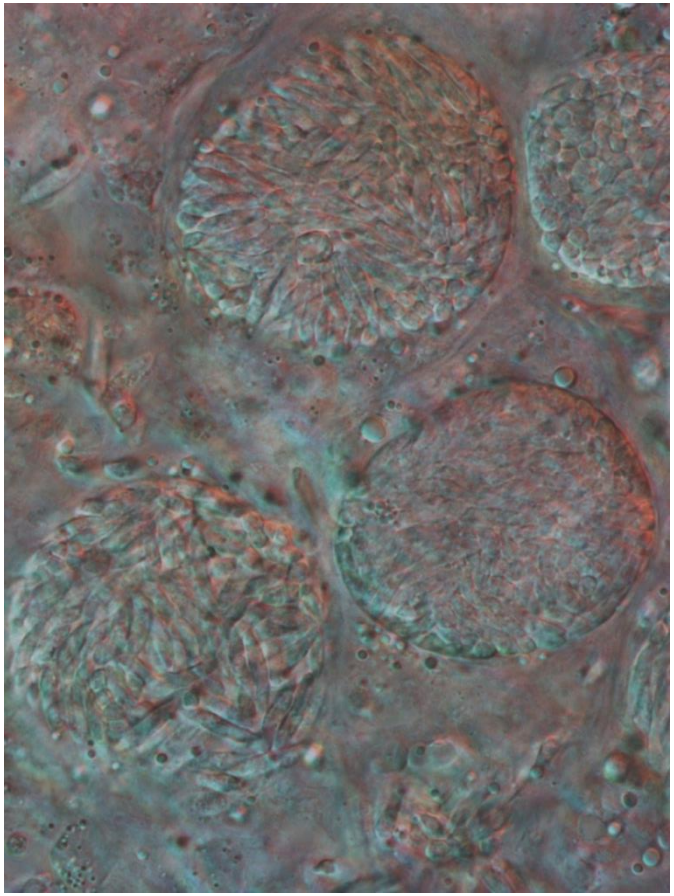
Very pathogenic
Haemorrhage
Low productivity

Merck Veterinary Manual and Reid and [illegible]

Eimeria species in Chickens

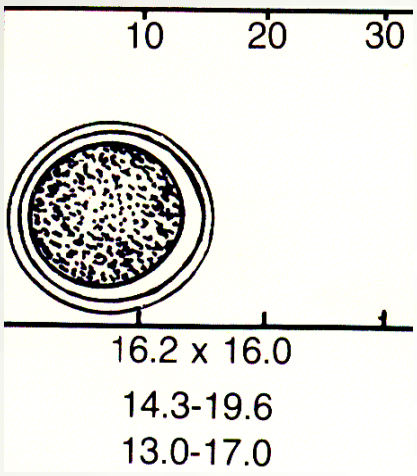
Eimeria necatrix

Very pathogenic; Haemorrhage; Low productivity



Eimeria species in Chickens

Eimeria mitis

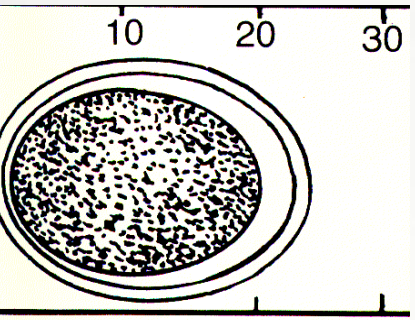


Less common,
Mildly pathogenic

Merck Veterinary Manual and Reid and Long

Eimeria species in Chickens

Eimeria brunetti



24.6 x 18.8

20.7-30.3

18.1-24.2

Less common

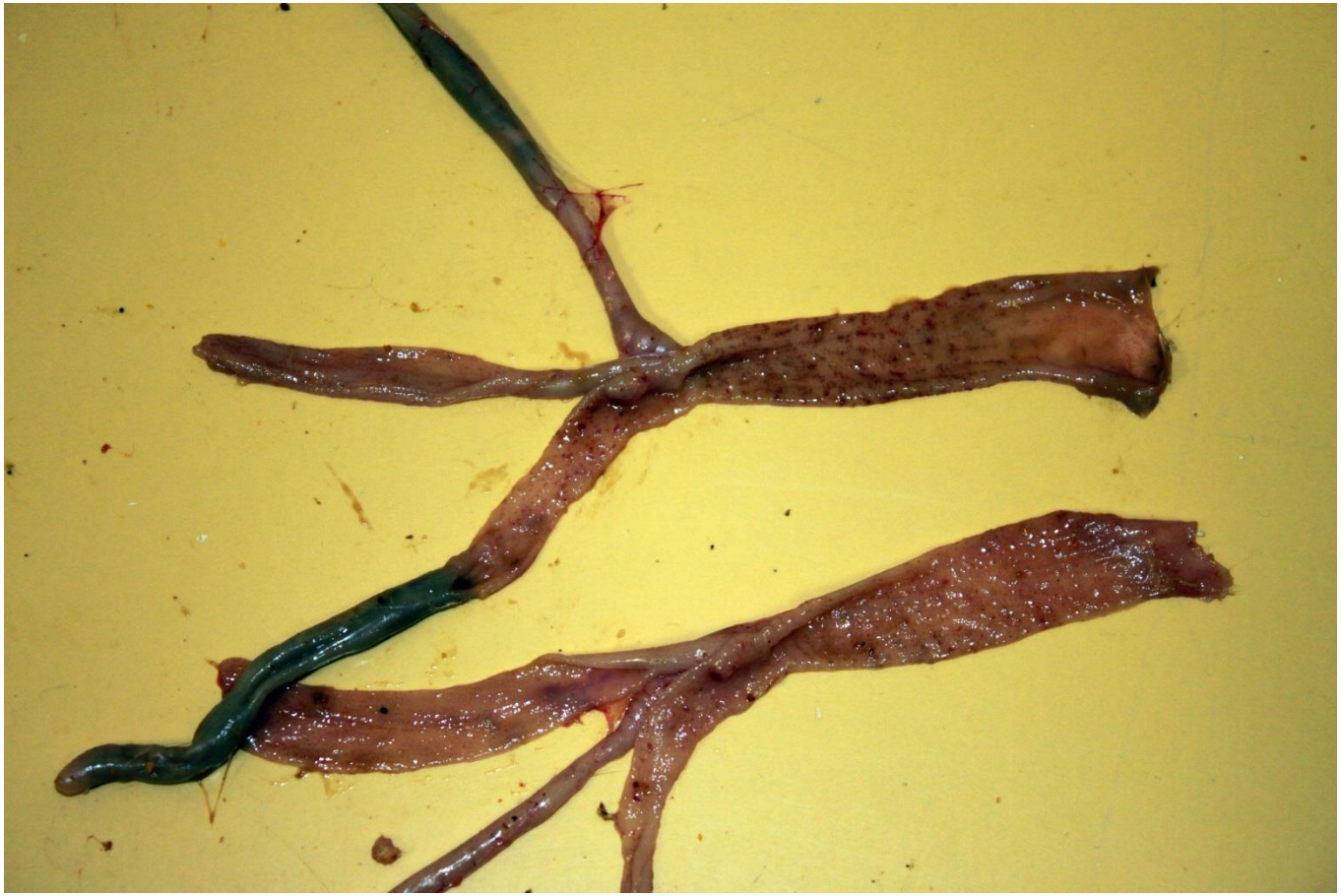
Mildly pathogenic

Merck Veterinary Manual and Reid and Long (1979)

Eimeria species in Chickens

Eimeria brunetti

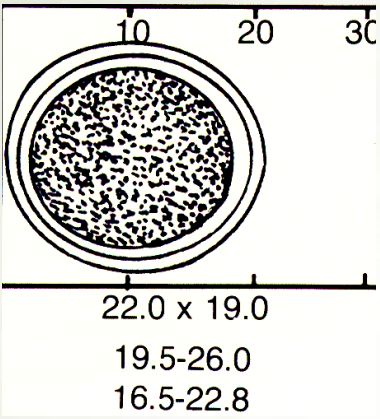
Less common; Mildly pathogenic; Marked Dehydration; Modest Lesions



http://images.objectivepathology.com/zoom.aspx?cols=10&zoomtarget=/UoGuelph/OVC/PathoBiology/Parasitology/PB-C84

Eimeria species in Chickens

Eimeria tenella



HIGHLY Pathogenic
Haemorrhage

Merck Veterinary Manual and Reid and [illegible] (1979)

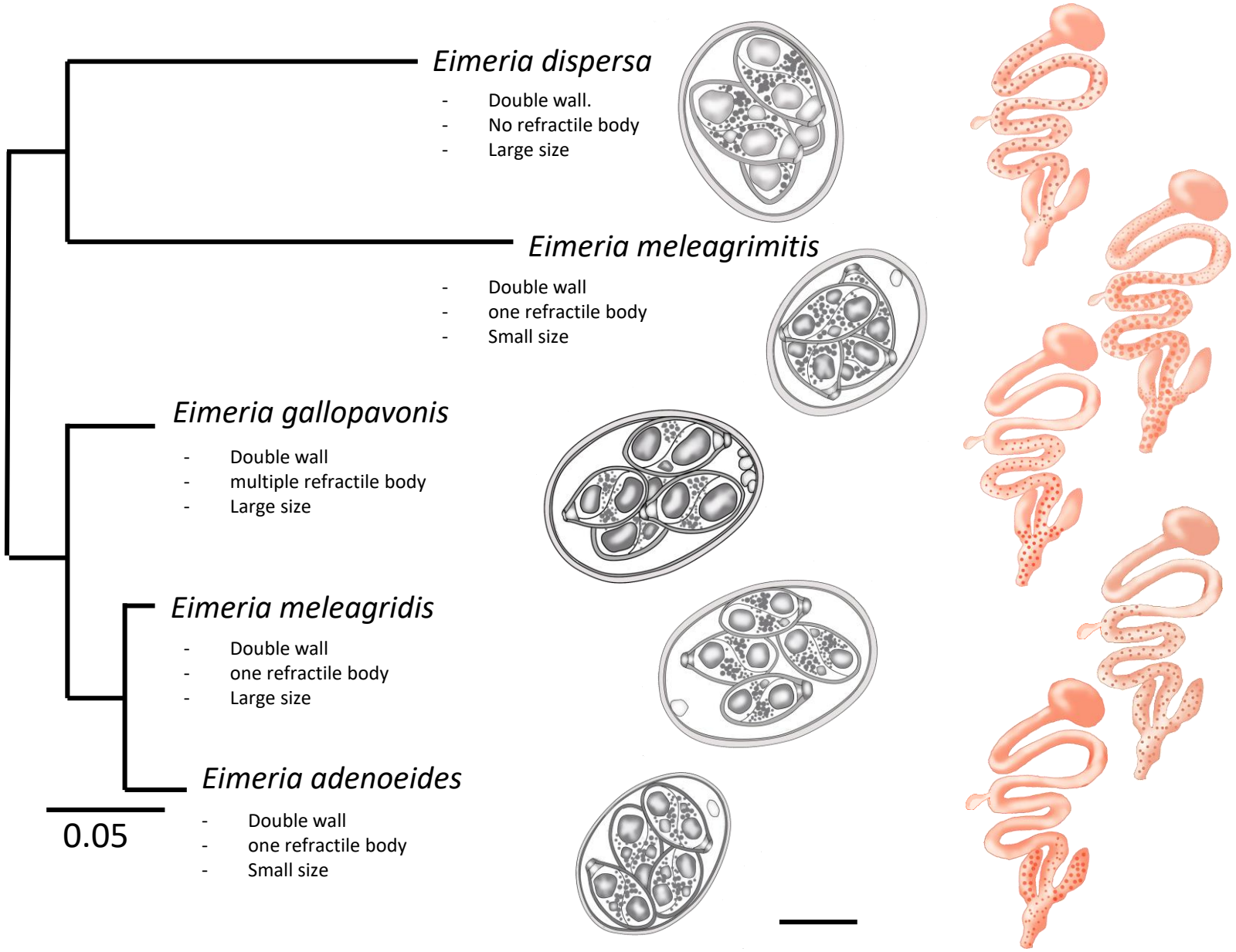
Eimeria species in Chickens

Eimeria tenella

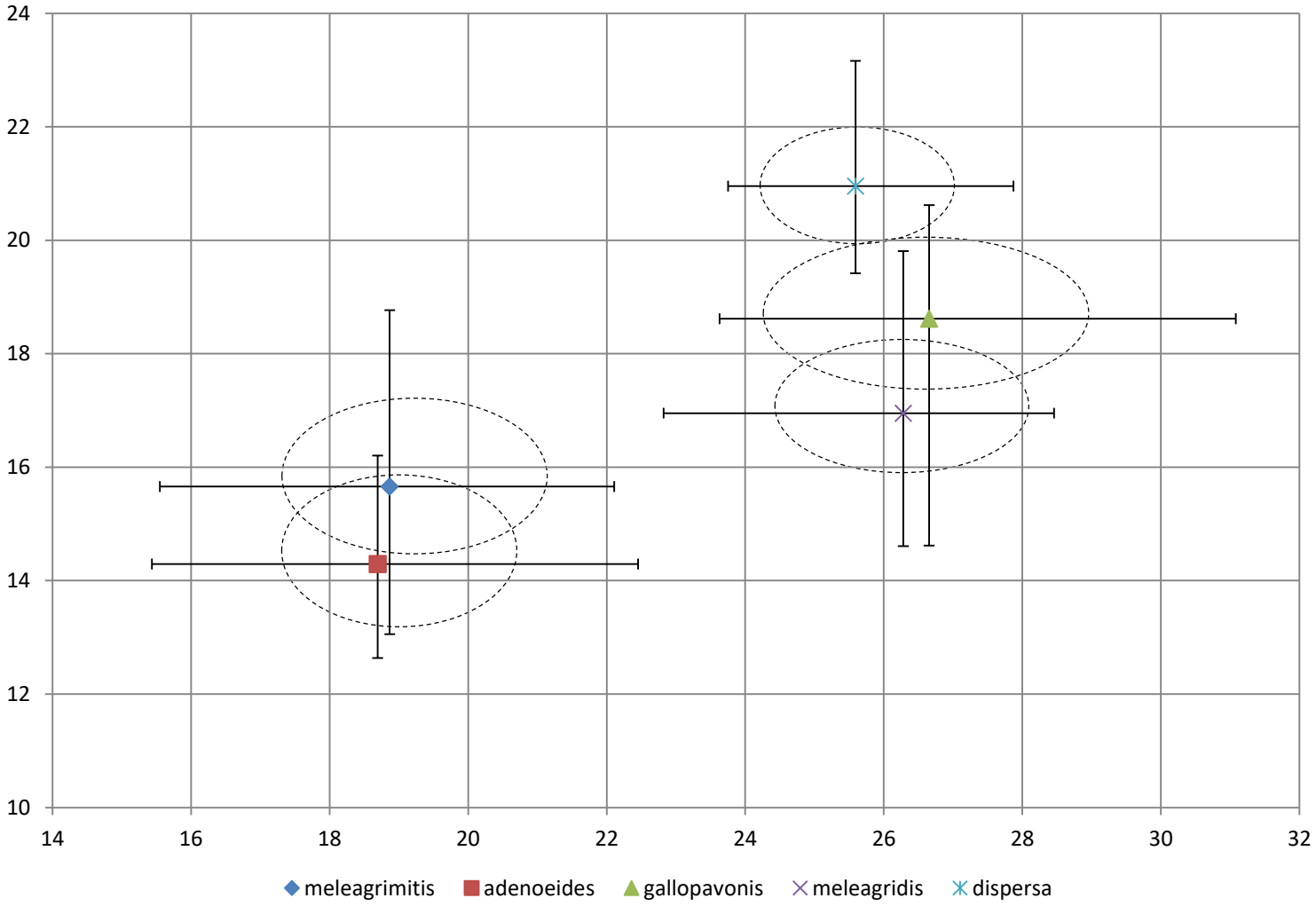
HIGHLY Pathogenic; Haemorrhage

http://images.objectivepathology.com/zoom.aspx?cols=10&zoomtarget=/UoGuelph/OVC/PathoBiology/Parasitology/PB-C61J

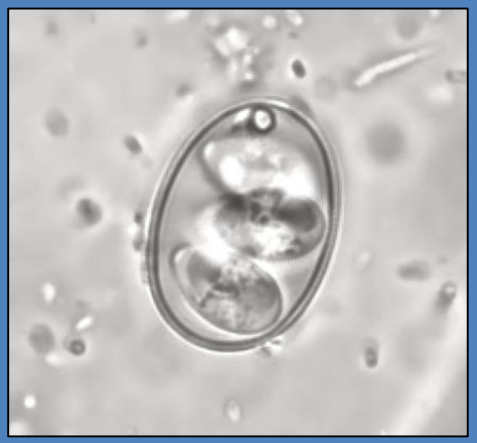
Eimeria species in Turkeys



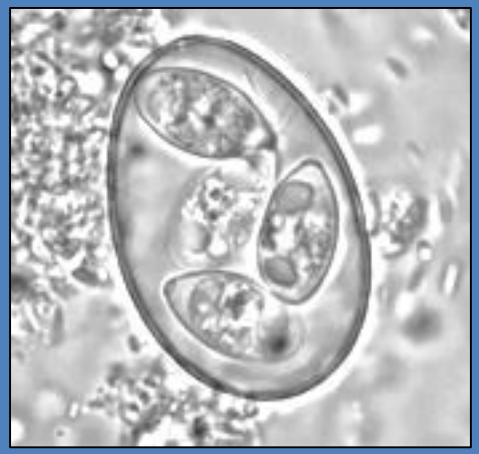
Eimeria species in Turkeys



Eimeria adenoeides and *E. gallopavonis*



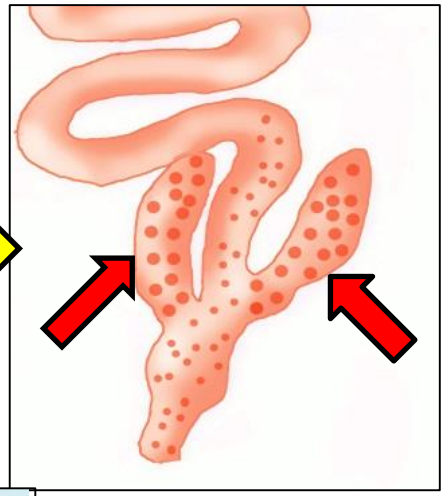
18.8× 14.2µm

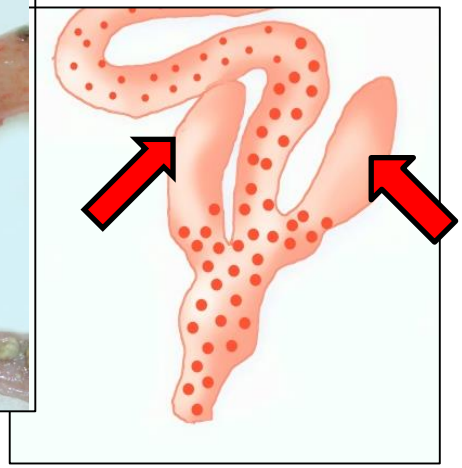


28.8× 17µm

Mainly in cecal pouch

Mainly in cecal neck





Topics

- coccidiosis as an “artificial” disease (a by-product of poultry domestication)
- the coccidian life cycle
- the *Eimeria* species in chickens and turkeys
- coccidiosis in the gut
- coccidiosis in a flock

Coccidiosis – Gross lesions

- Johnson and Reid, 1970 for descriptions
- host variation to infections (age/breed/type)
- highly dose associated
- ‘clean’ lesion scores in naïve birds BUT immunopathology/inflammation can confuse lesion scores in cocci-exposed birds.

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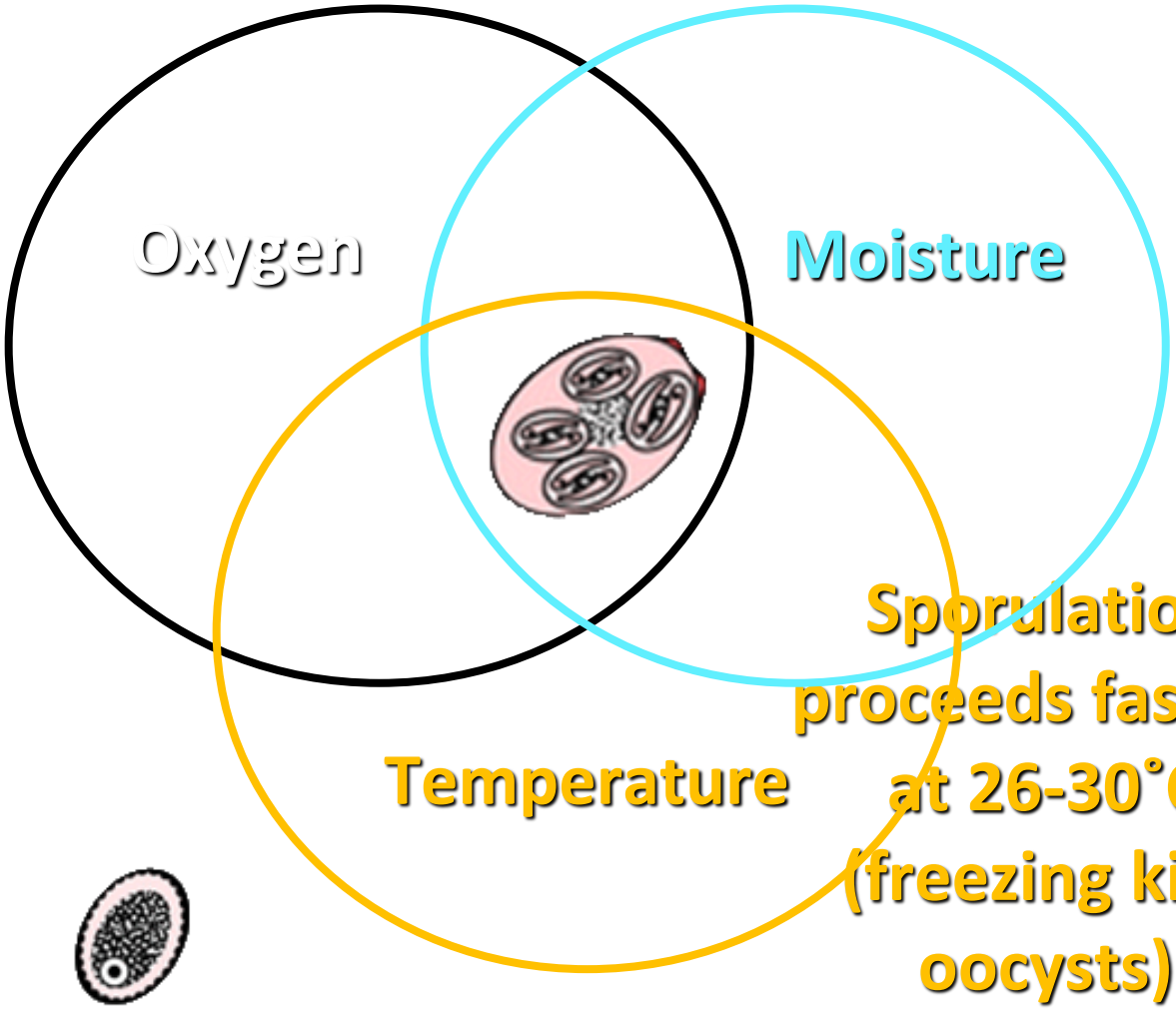
Coccidiosis in the Barn

Coccidia in the Barn - Oocyst Development and Survival

Oocysts are susceptible to dessication - >16% litter moisture needed (25% optimal) >30% reduces sporulation



Sporulation proceeds fastest at 26-30˚C (freezing kills oocysts)

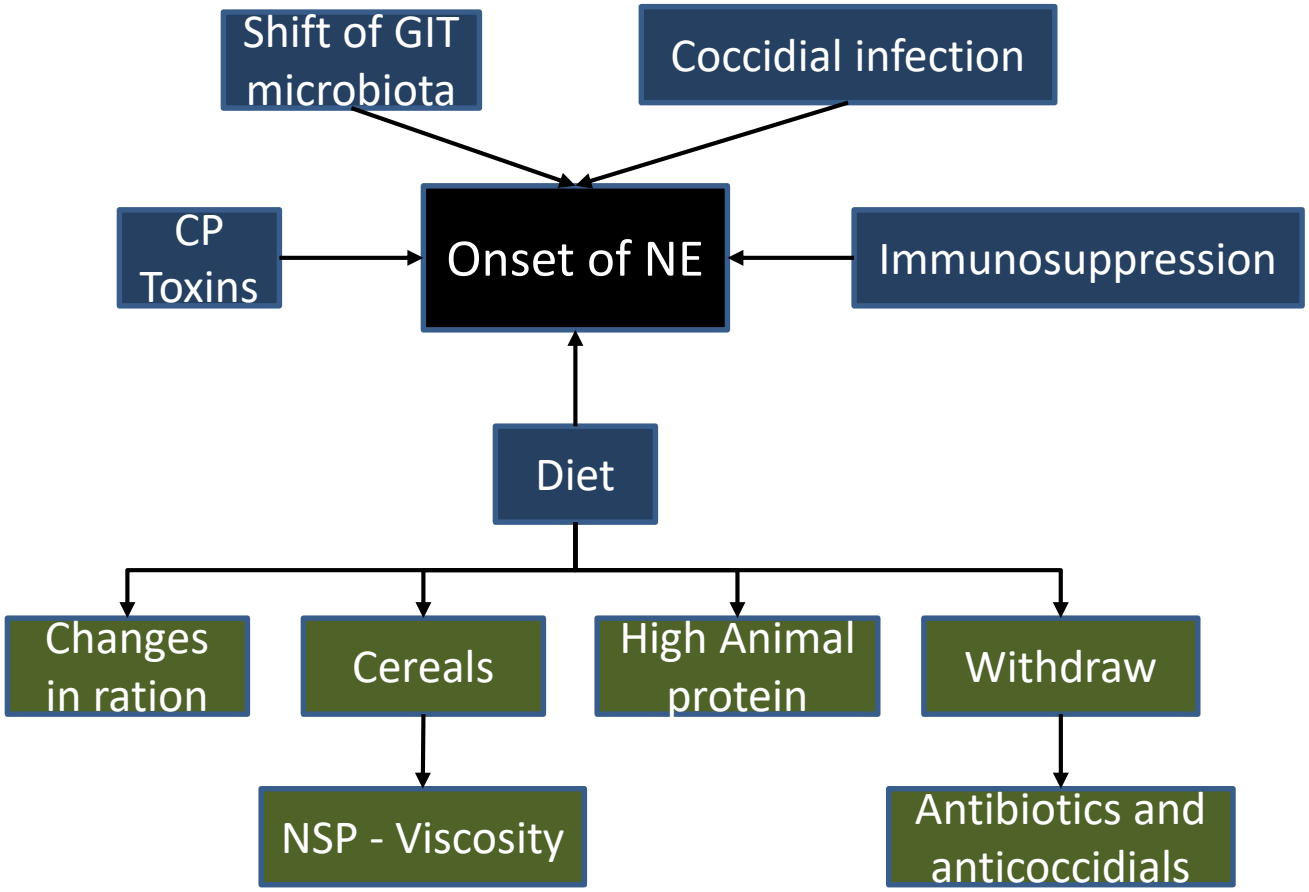
Sporulation is an aerobic p ess – access t oxygen

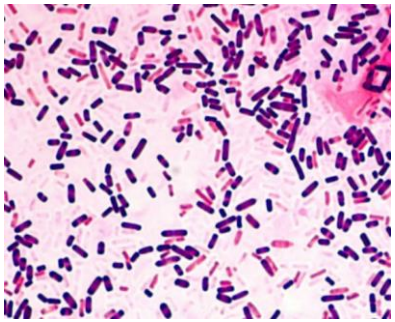
Sporogonic development in environment is essential for "cycling"

Summary

- coccidiosis as an “artificial” disease (a by-product of poultry domestication)
- reproductive potential of life cycle is fixed
- multiple species makes it a disease complex
- lesions occur both microscopically and macroscopically – immune response can exacerbate
- control is highly dependent on controlling oocyst numbers at appropriate times

Necrotic enteritis (NE)





Adapted from Shojadoost et al., 2012

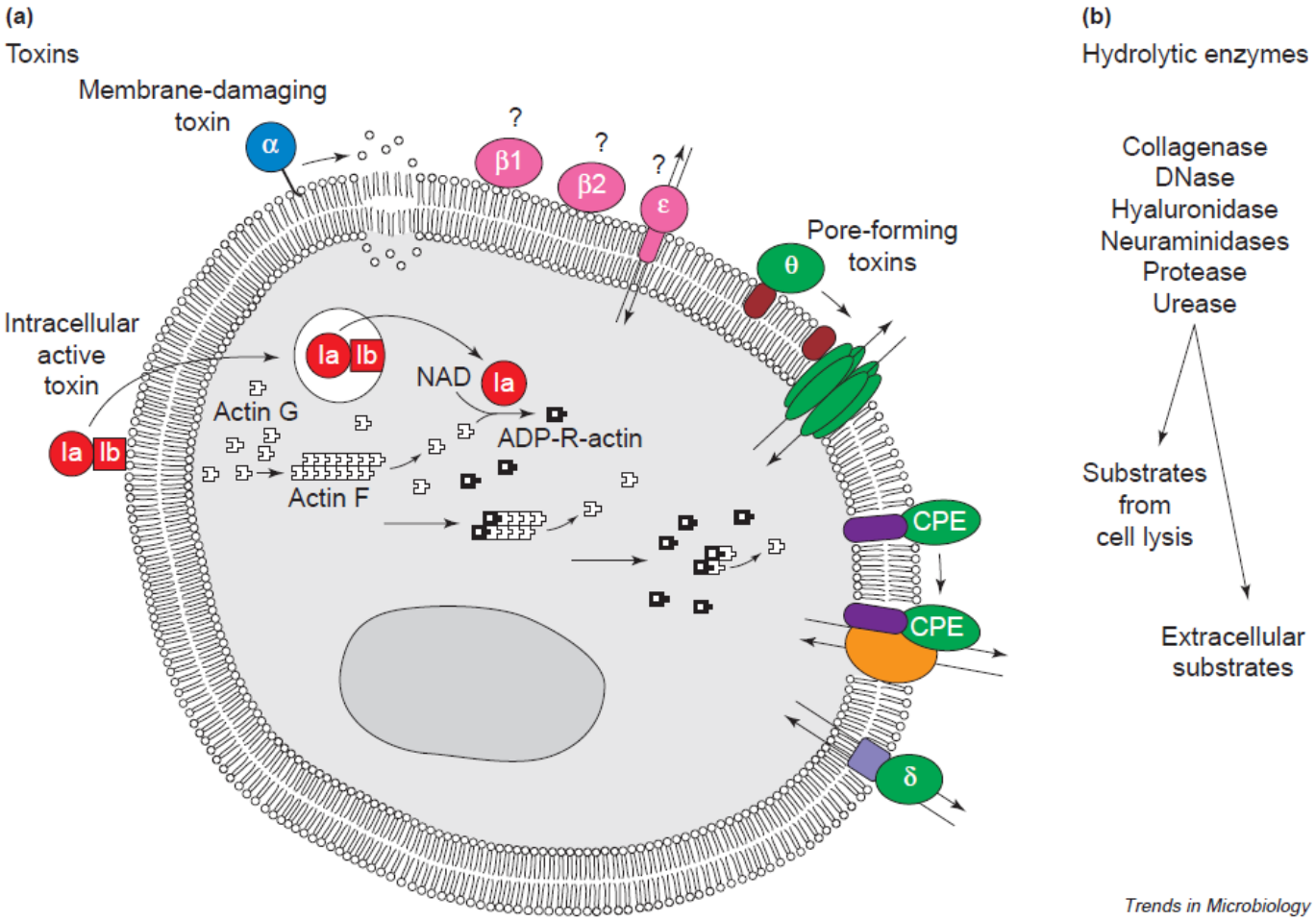
Classification system of *C. perfringens* based on the production of six major toxins

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., 2018. Anaerobe. doi.org/10.1016/j.anaerobe.2018.04.011

Schematic representation of the targets and modes of action of some *C. perfringens* toxins



Petit et al., 1999. Trends. Microbiol. 7:104-110

Predisposing factors:

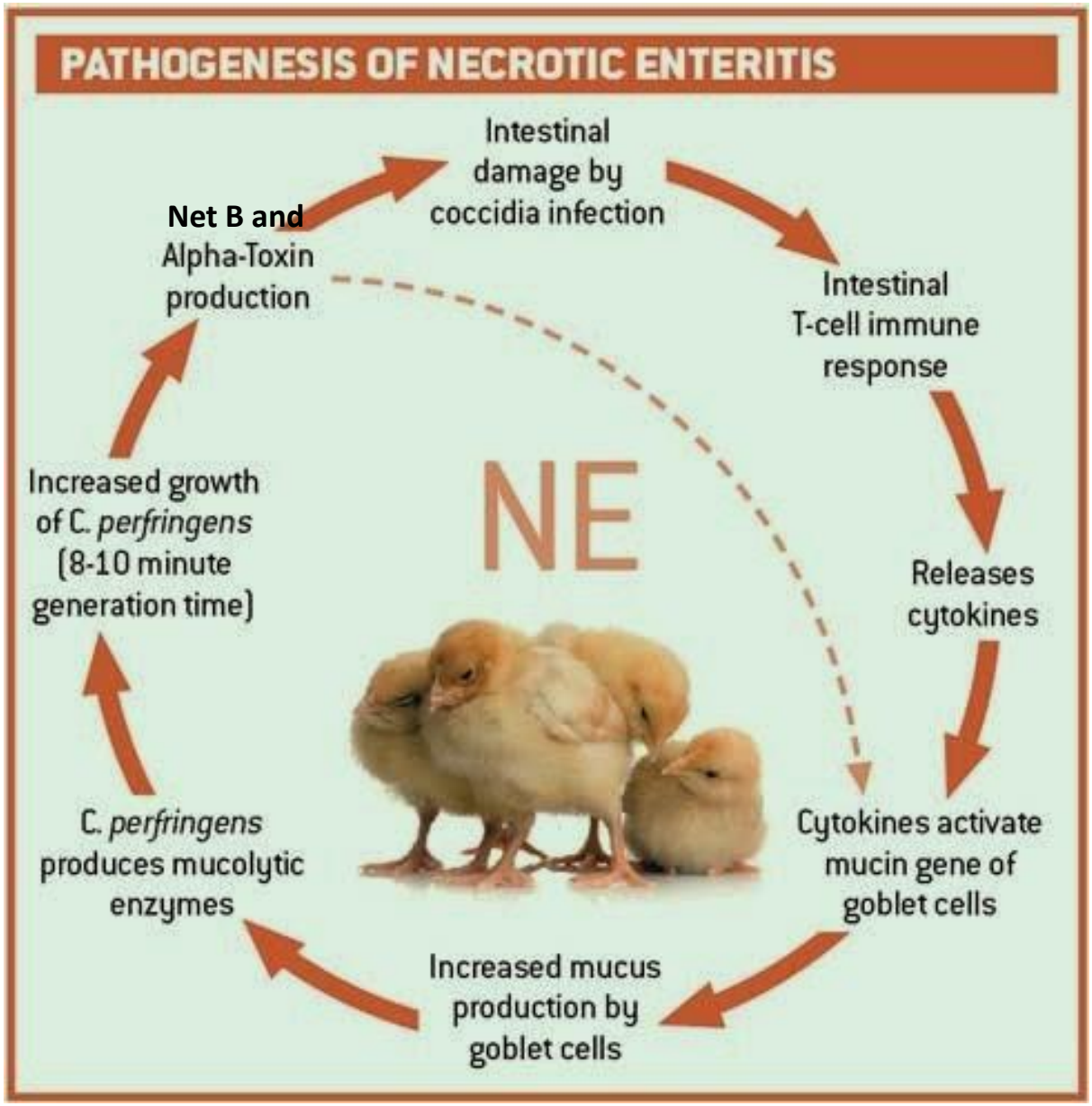
Non-starch polysaccharides

Animal protein (Fish meal)

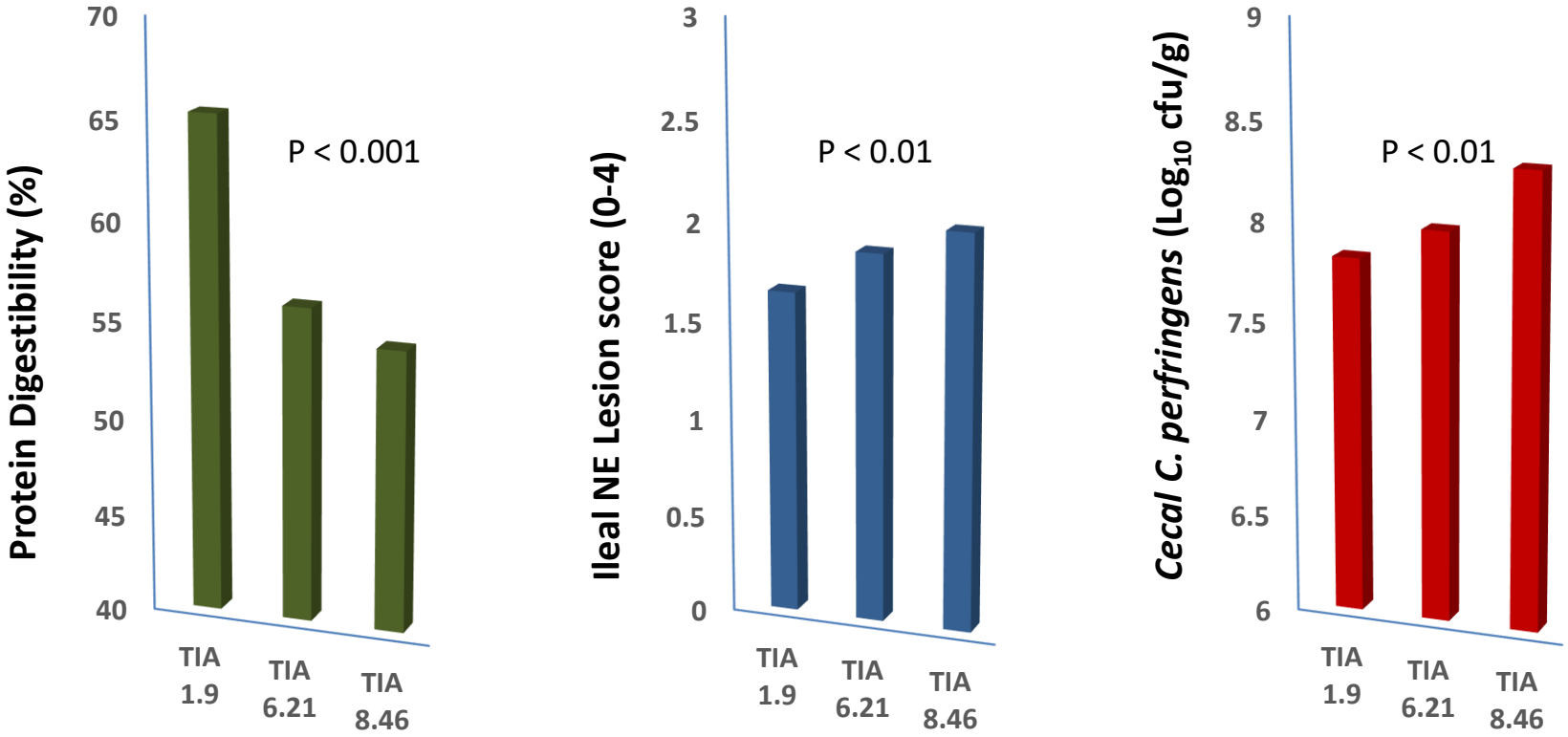
Coccidiosis

Immunosuppression and stress

<u>Appropriate intestinal environment</u>



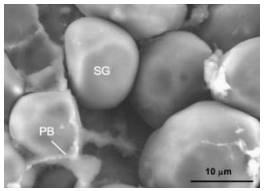
Effect of TIA on Necrotic enteritis presentation



TIA: Trypsin Inhibitor Activity mg/g

Palliyeguru et al., 2011. Br. Poult. Sci. 52:359-367

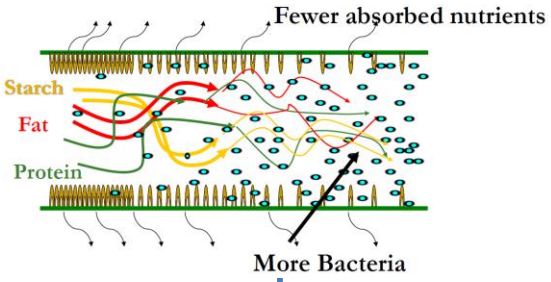
Nutrient utilization and alternative grains: Non-starch polysaccharides (NSP)



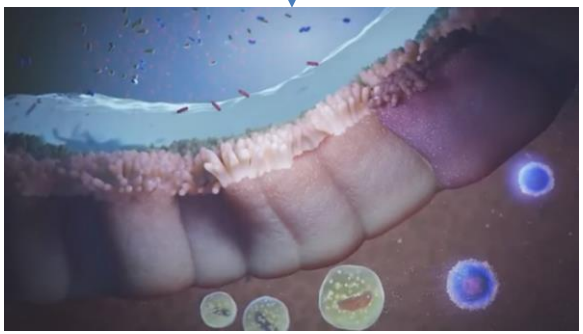
Wong et al., 2009

Ingredient	NSP g/kg DM
Corn	97
Wheat	119
Rye	152
Barley	186
Oats	232

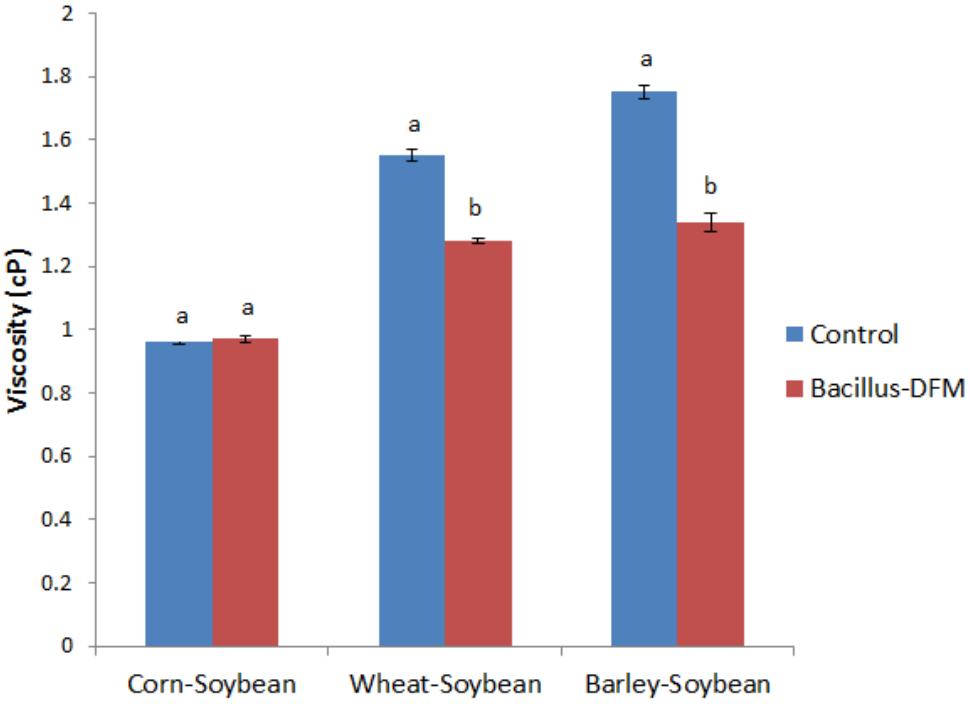
Knudsen (1997)

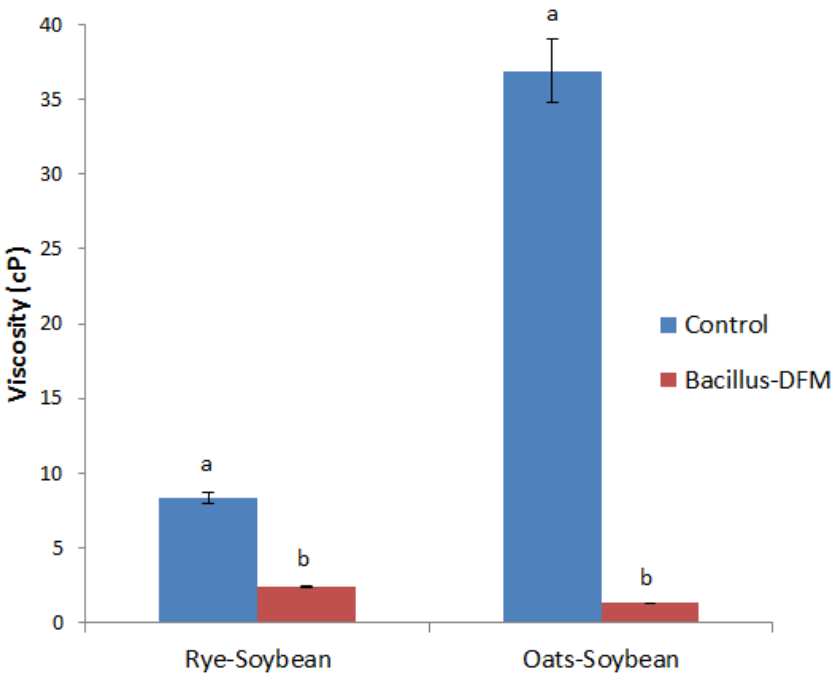


Bedford (2002)



Evaluation of *in vitro* viscosity of different diets with or without inclusion of a *Bacillus*-DFM candidate



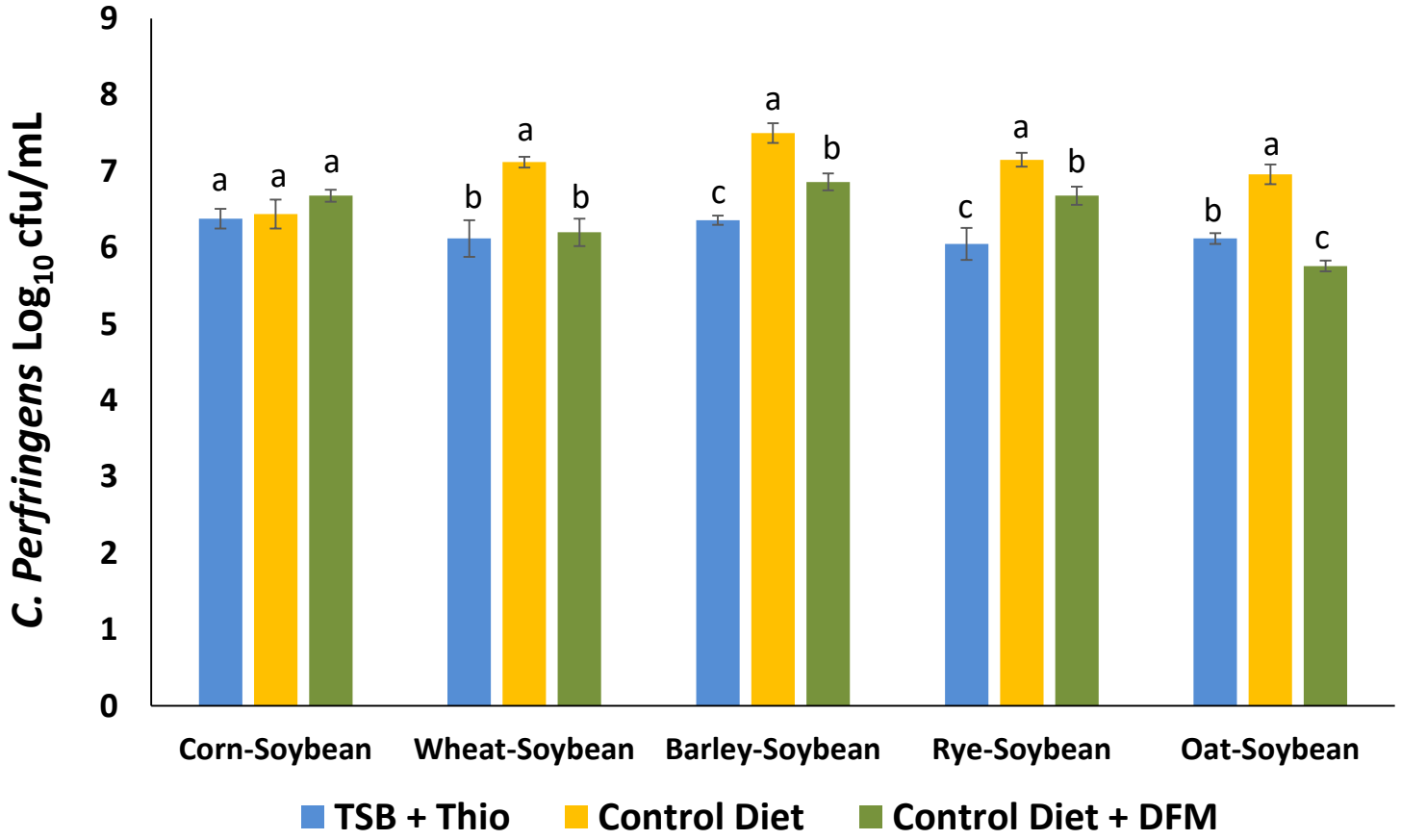


[a-b] ($P<0.05$).

[*] Viscosity was measured after 3 h and 15 min of *in vitro* digestion at 40°C, the data reported is the mean of 5 replicates per diet per treatment.

Latorre et al., 2015. Front. Vet. Sci. 2:25

In vitro Proliferation of *C. perfringens* in different digested diets with or without inclusion of a *Bacillus*-DFM candidate



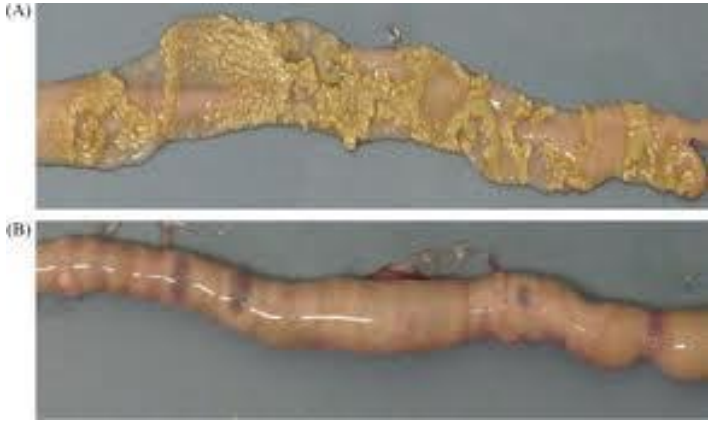
[a-c] ($P < 0.05$).

[*] Supernatant from each diet was used as part of the broth for *C. perfringens* growth. Inoculum used 10^5 cfu of *C. perfringens* and 10^8 spores/g of *Bacillus*-DFM candidate

Latorre et al., 2015. Front. Vet. Sci. 2:25

Necrotic Enteritis – Clinical Signs

Necrotic Enteritis - Lesions



Gross lesions typical of experimentally induced necrotic enteritis. **(A)** Typical mucosal lesion following experimental inoculation, with pseudomembrane forming. **(B)** Typical necrotic lesions viewed from the serosal surface

Cooper and Songer 2010

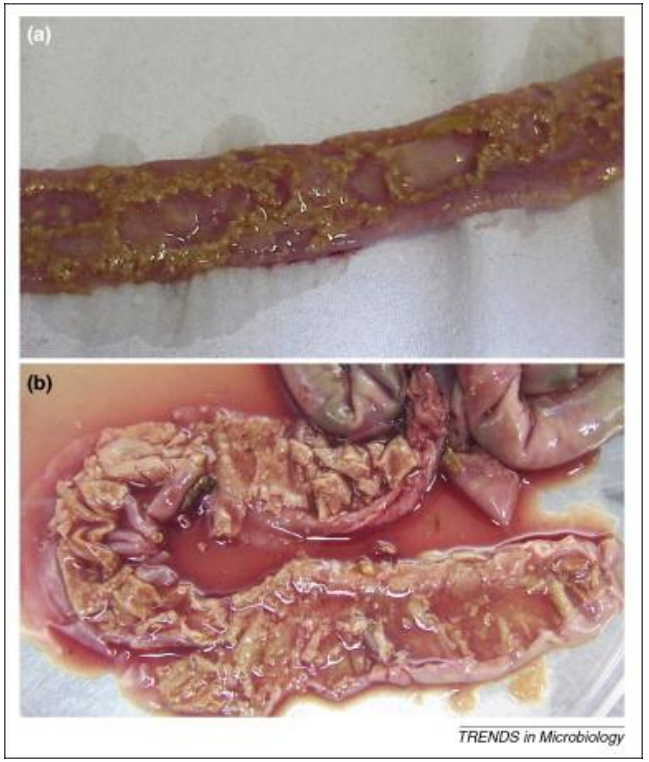


Figure above - Typical gut lesions in severe broiler necrotic enteritis. **(a)** These consist of patches of necrosis throughout the gastrointestinal tract and, in extreme cases, **(b)** extensive necrosis of the mucosal surface

Immerseel et al. 2009

Necrotic Enteritis – Lesion Score system

Figure 1: Score = 1

Figure 2: Score = 2

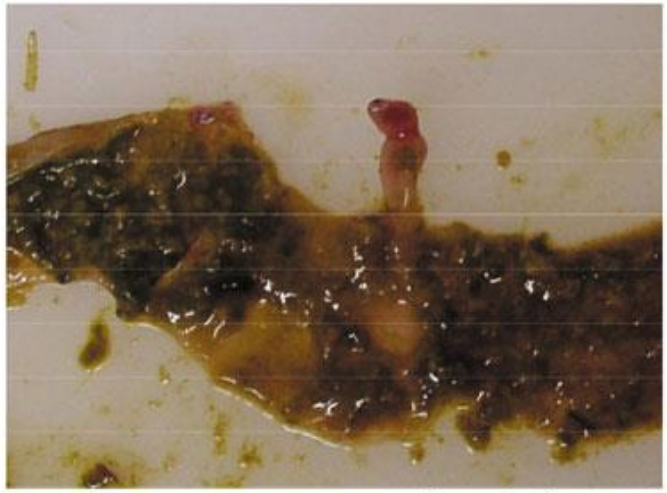


Figure 3: Score = 3

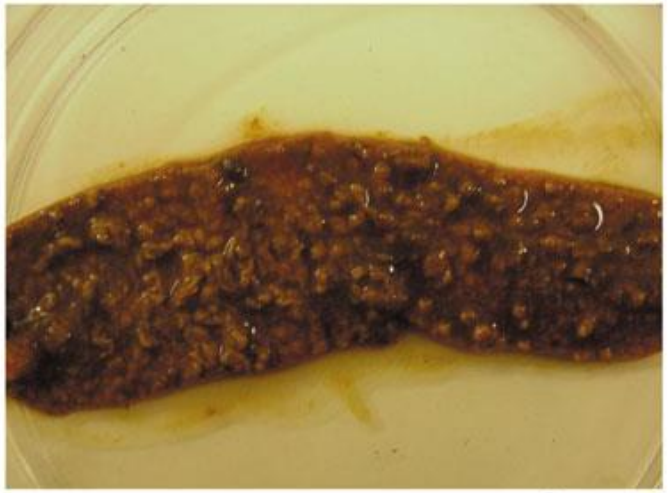


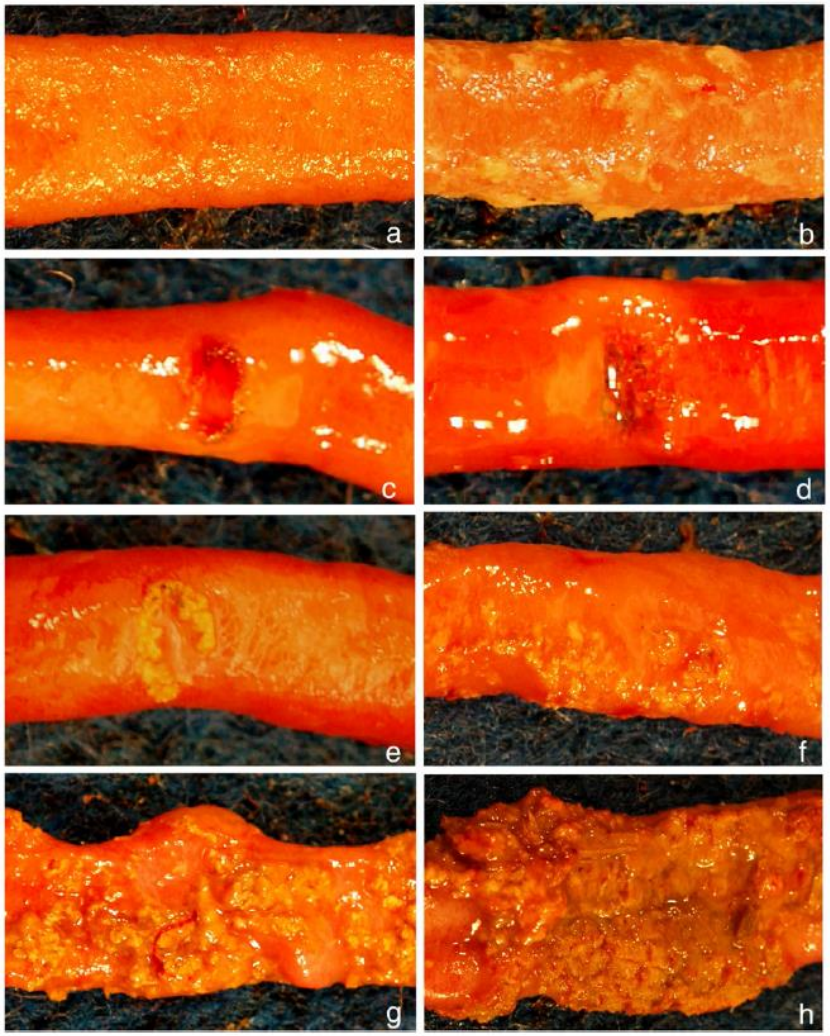
Figure 4: Score = 4

Poultryhub.org

Prescott et al., 1978. Can. Vet. J 19:181-183

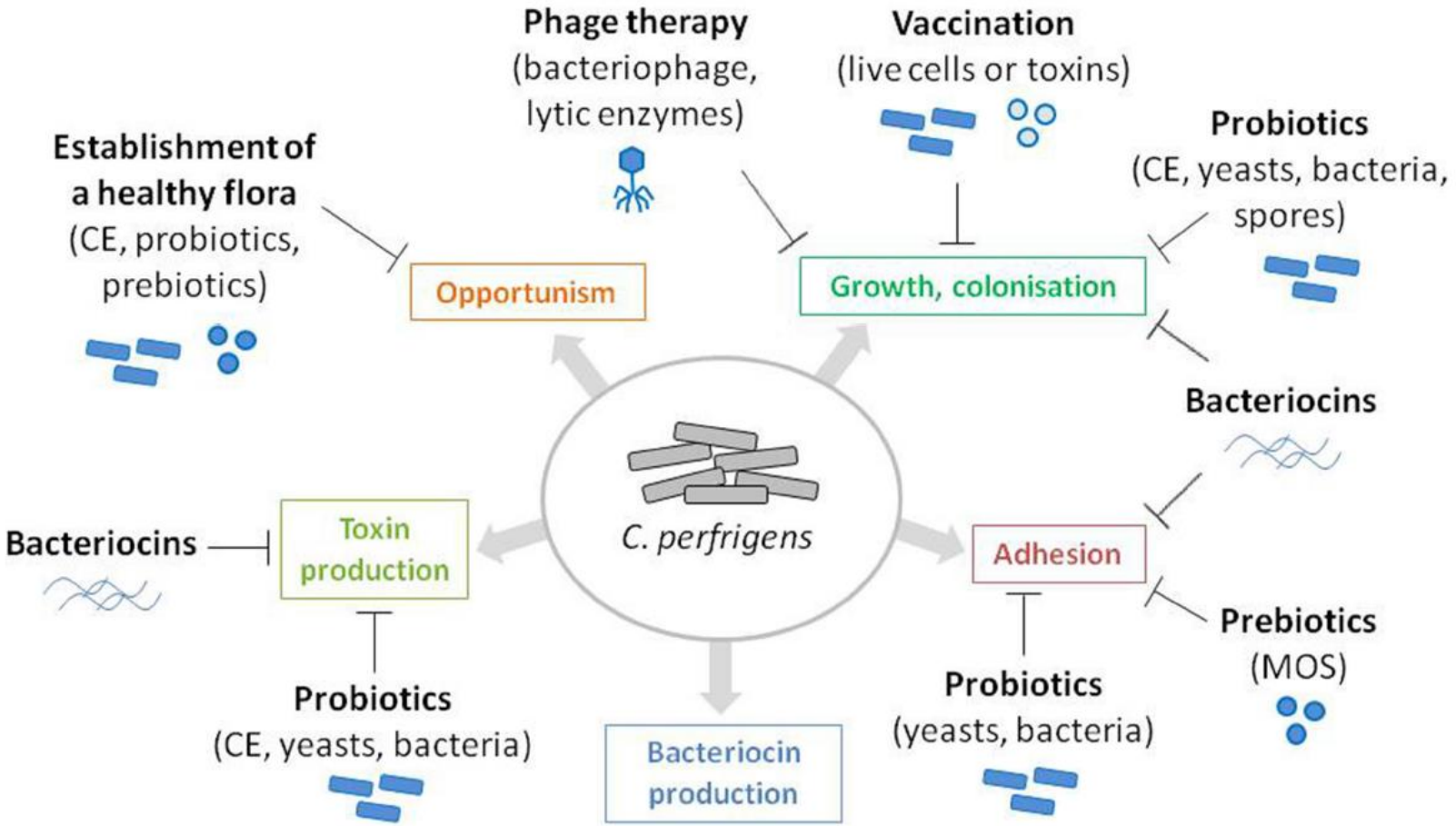
Necrotic enteritis – Lesion Score System

Different Scoring Systems



Shojadoost et al., 2012. BMC Vet Res 43:74

C. Perfringens virulence factors and potential targets for NE prevention



Caly et al., 2011. Front. Microbiol. 6:1336

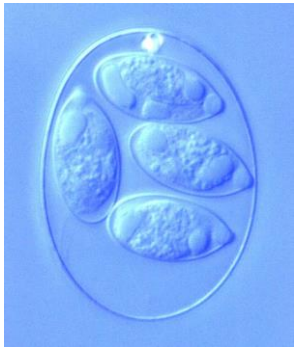
Objectives

1. Evaluate the possible role of:

- Neonatal *Salmonella* Typhimurium infection as a predisposing factor for NE

- *Eimeria maxima* strain (M6 or Guelph) on development and severity of NE

2. Evaluation of different NE models that could be used to determine the effect of different AGP alternative candidates



http://www.merckvetmanual.com/

AVIAN DISEASES 55:319–323, 2011

Research Note—

The Role of an Early *Salmonella* Typhimurium Infection as a Predisposing Factor for Necrotic Enteritis in a Laboratory Challenge Model

S. Shivaramaiah,[A] R. E. Wolfenden,[A] J. R. Barta,[B] M. J. Morgan,[A] A. D. Wolfenden,[A] B. M. Hargis,[A] and G. Téllez[AC]

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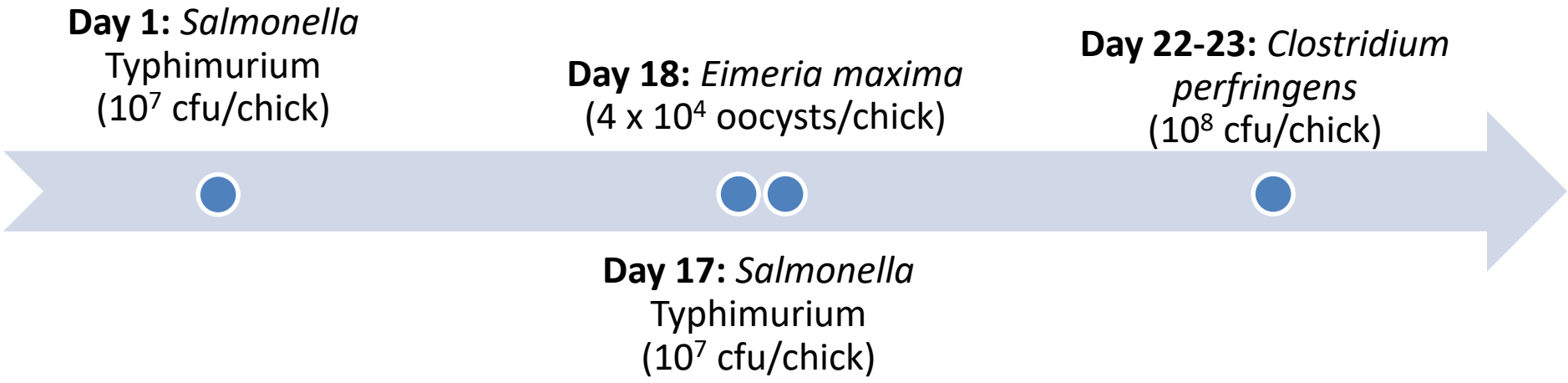
Received 23 November 2010; Accepted and published ahead of print 26 February 2011

SUMMARY. Necrotic enteritis (NE) caused by *Clostridium perfringens* (CP) in poultry is an important bacterial disease in terms of economic implications. The disease is multifactorial and is invariably associated with predisposing factors. In the present experiments, we investigated the potential predisposing role of neonatal *Salmonella* Typhimurium (ST) infection for NE-associated mortality in a laboratory challenge model. In two experiments, day-of-hatch chicks were randomly assigned to four groups: Group 1, nonchallenged control; Group 2, chickens received *Eimeria maxima* (EM) and CP; Group 3, chickens received EM and CP and were also challenged with ST at day 1 of age; Group 4, chickens received EM and CP and were also challenged with ST at day 17 of age. Challenged groups received an oral dose of EM at 18 days of age and CP (10^8 colony-forming units/chick) at 22–23 days of age. When compared to EM and CP, chicks challenged with ST (day 1) had increased NE-associated mortality and CP-associated lesion scores ($P < 0.05$) in both experiments. Furthermore, body weight and body weight gain were lower ($P < 0.05$) in chicks infected with ST (day 1) in the first experiment, even though no differences ($P > 0.05$) were observed in weight gain in the second experiment. Chicks challenged with ST (day 17) were similar to the EM and CP group in all of the above-mentioned parameters, indicating that a paratyphoid infection in younger chicks remarkably alters the susceptibility to secondary bacterial infections. Based on this work, the authors suggest that an ST infection early in the age of a chick may be important for altering susceptibility to NE, an observation that may be useful from the perspective of experimental reproduction of this disease and, perhaps, as an economically important reason to address the problem of paratyphoid *Salmonella* infections in young chicks.

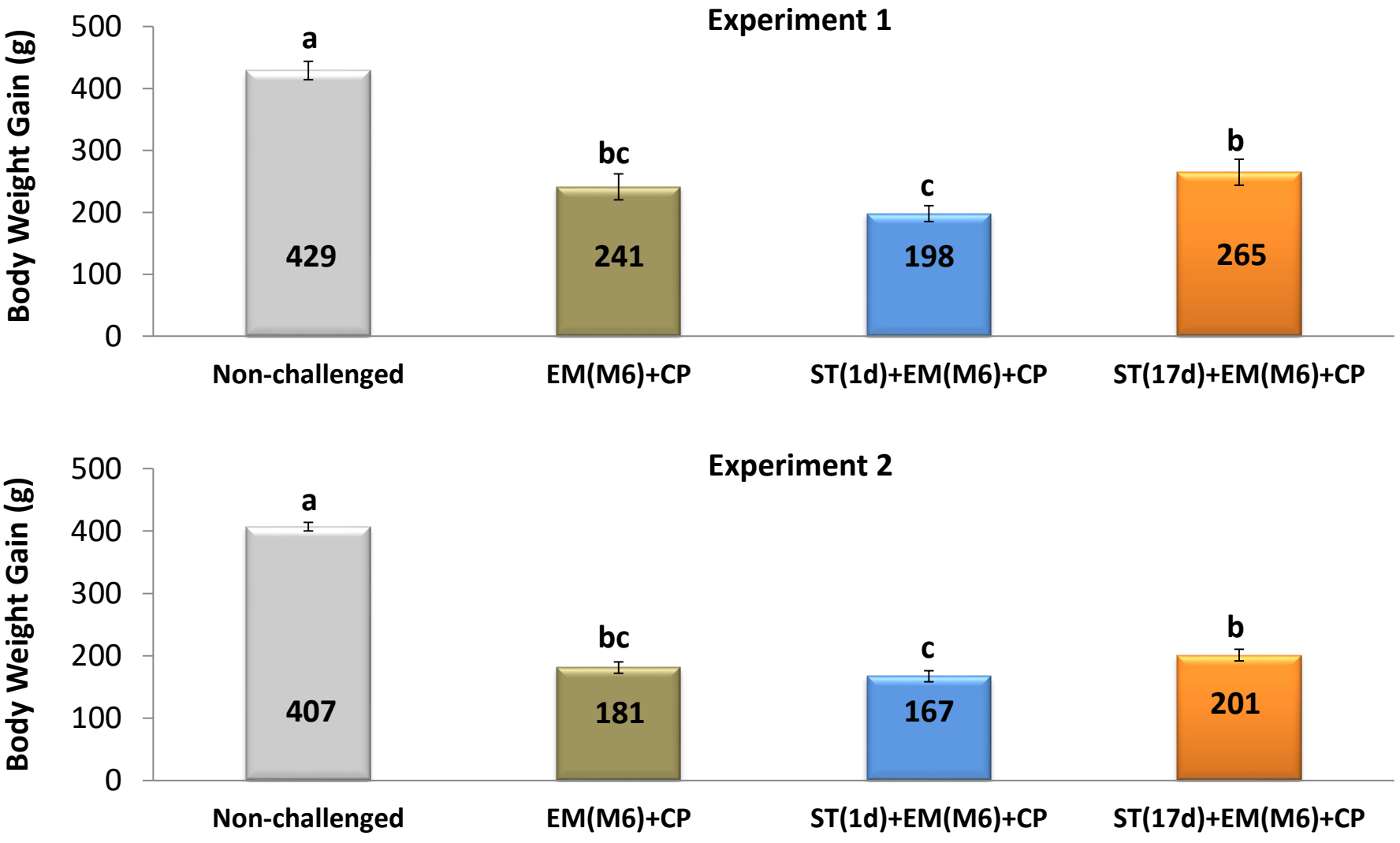
Experimental groups and challenge protocol (Experiments 1 and 2**)

Treatment*	*S.* Typhimurium	*E. Maxima*	*C. perfringens*
Non-challenged	-	-	-
EM + CP	-	+	+
ST (d1) + EM + CP	+ (day 1)	+	+
ST (d17) + EM+ CP	+ (day 17)	+	+

*ST: *Salmonella* Typhimurium, EM: *Eimeria maxima* (**M6**), CP: *Clostridium perfringens*
__Exp 1:__ 25 chickens/treatment
__Exp 2:__ 4 replicates of 25 chickens/treatment

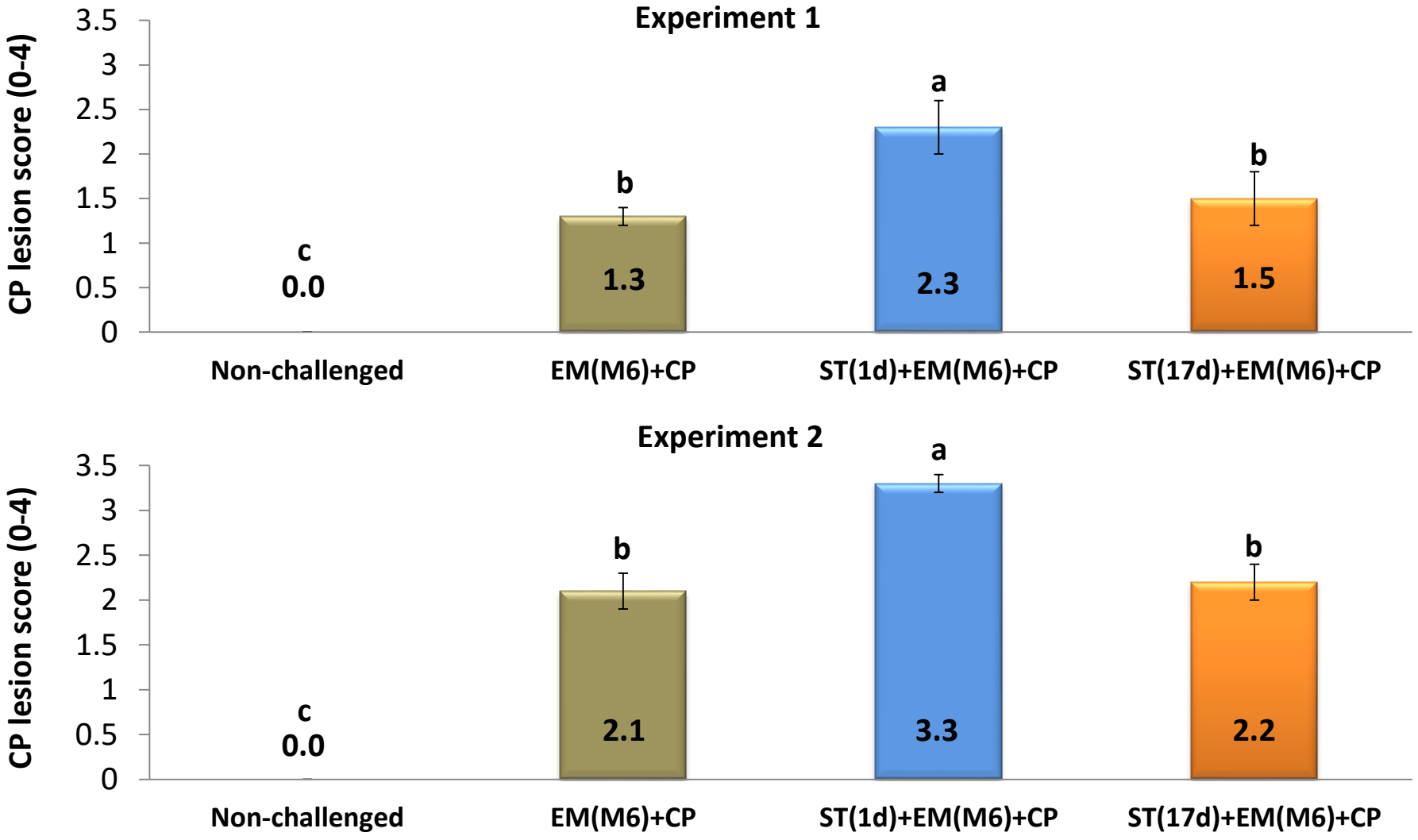


Effect of *Salmonella* Typhimurium infection on body weight gain in a necrotic enteritis model (18-25d-of age)



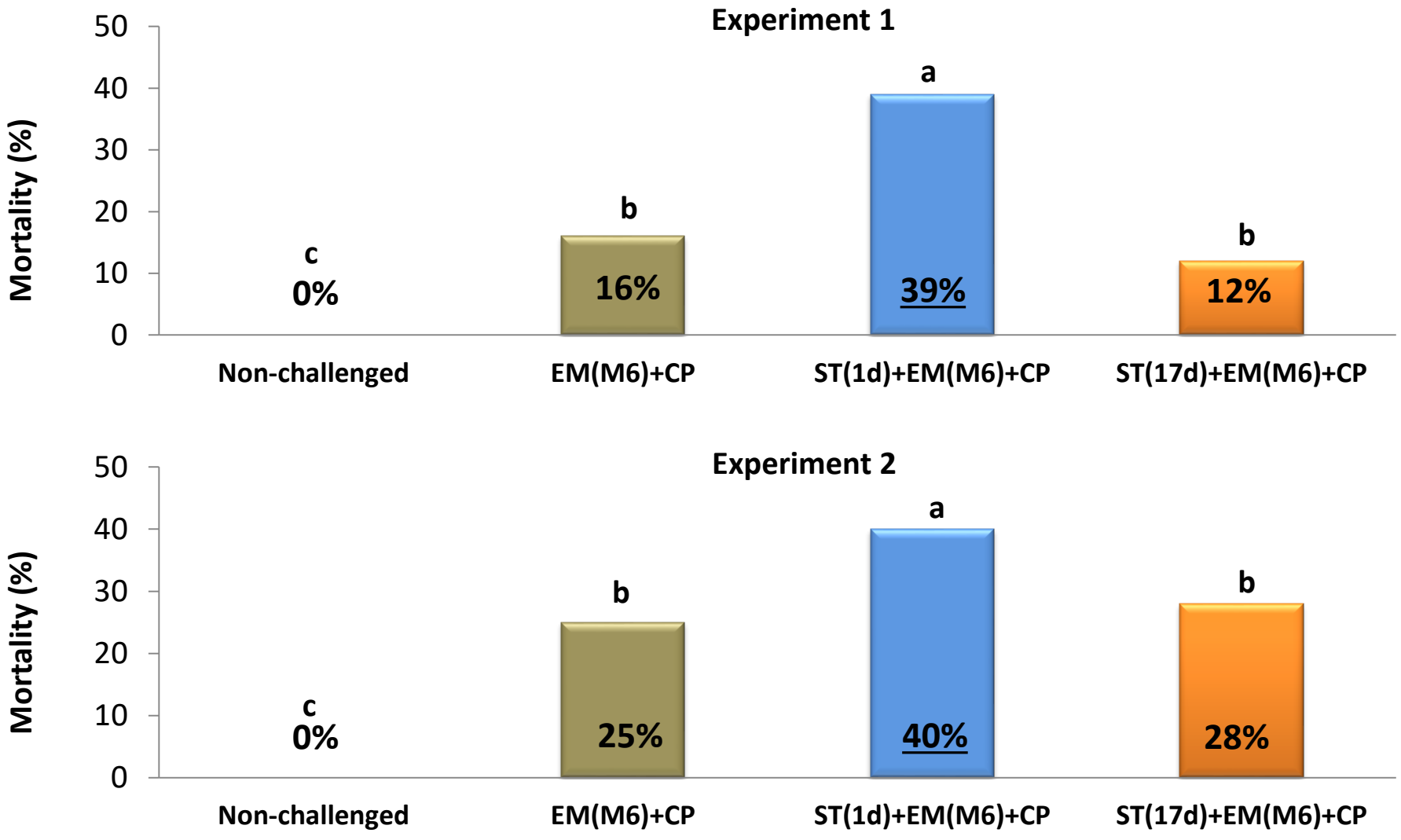
$P < 0.05$

Effect of *Salmonella* Typhimurium infection on *Clostridium perfringens* associated lesions in a necrotic enteritis model (25d-of age)



P < 0.05

Effect of *Salmonella* Typhimurium infection on total percent mortality in a necrotic enteritis model (25d-of age)



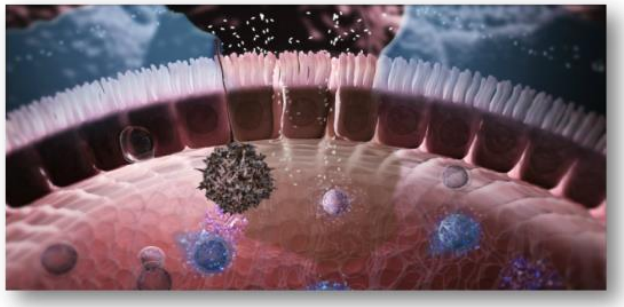
P < 0.05

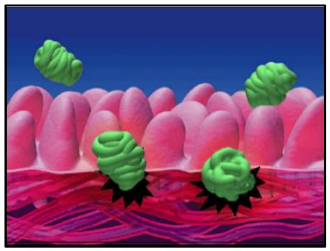
Serum FITC-d levels

Marker
Fluorescein isothiocyanate dextran (FITC-d)
3,000-5,000 Da; Green fluorescent dye

FITC-d oral gavage (8.34mg/kg)
1 h before taking blood samples

Gut leakage





Serum FITC-d levels

Serum FITC-d level:
Excitation wavelength of 485nm and emission wavelength of 528nm

Kuttappan et al. 2015
Vicuña et al. 2015

http://www.nature.com/ni/multimedia/mucosal/animation/index.html

Bacterial translocation

Right half of liver → Weighed and Homogenized → 1:4 wt/vol dilutions - Saline

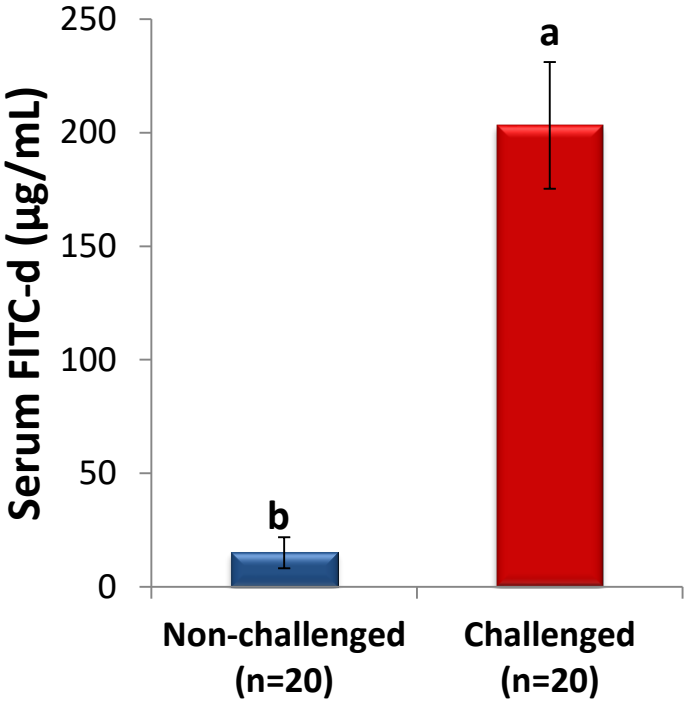
↓

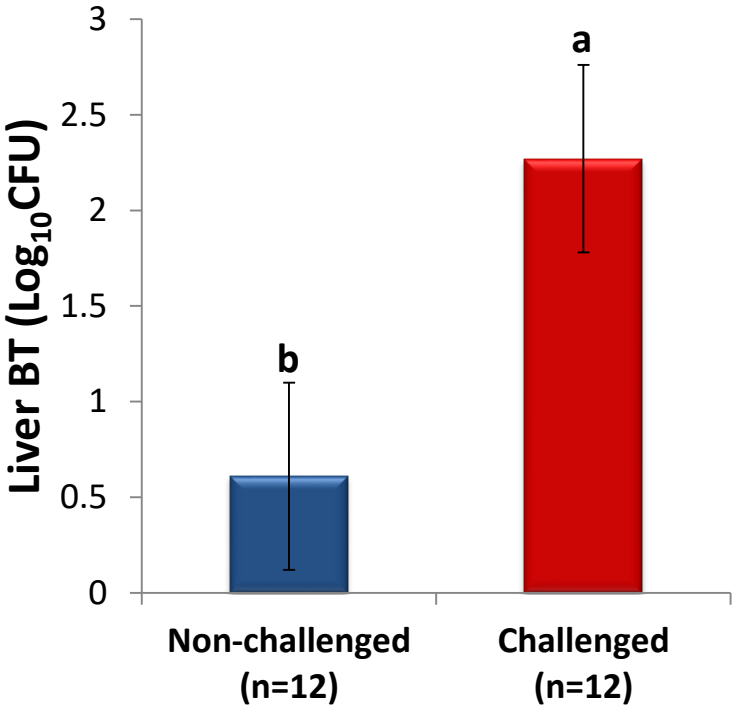
Tenfold serial dilutions – sterile 96 well plate

←

Tryptic soy agar with Thioglycollate plate

Serum FITC-d level and liver bacterial translocation of broiler chickens in a challenged necrotic enteritis model in Experiment 3 (25d-of age)

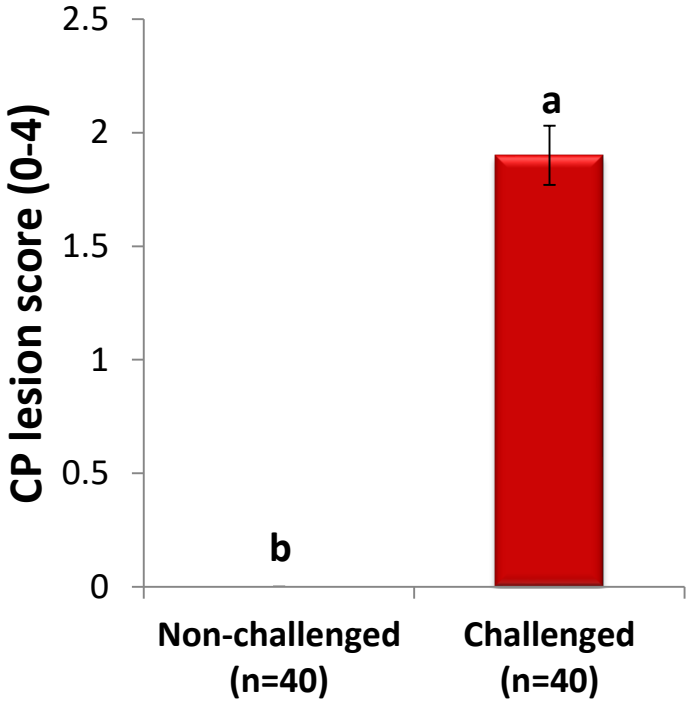


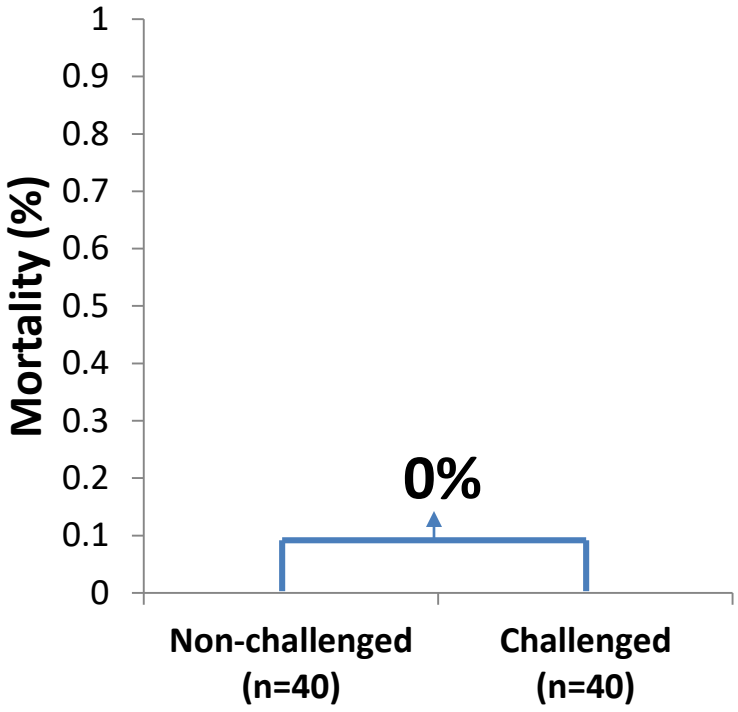


Challenged: ST(1)+EM(**Guelph**)+CP

P < 0.05

Clostridium perfringens lesion score and total percent mortality of 25d-old broiler chickens in a necrotic enteritis model in Experiment 3





Challenged: ST(1)+EM(**Guelph**)+CP

$P < 0.05$

On going research

frontiers in Veterinary Science | Veterinary Infectious Diseases

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The Animal Microbiome in Health and Disease

ORIGINAL RESEARCH ARTICLE Provisionally accepted The full-text will be published soon. Notify me

Front. Vet. Sci. | doi: 10.3389/fvets.2018.00199

Evaluation of the epithelial barrier function and ileal microbiome in an established necrotic enteritis challenge model in broiler chickens

Juan D. Latorre[1, 2], Bishnu Adhikari[1, 2], Si Hong Park[2], Kyle D. Teague[1, 2], Lucas E. Graham[1, 2], Brittany D. Mahaffey[1, 2], Mikayla F. Baxter[1, 2], Xochitl Hernandez[3], Young Min Kwon[1, 2], Steven C. Ricke[2], Lisa Bielke[4], Billy M. Hargis[1, 2] and Guillermo Tellez[1, 2*]

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[3]Department of Avian Medicine, Universidad Nacional Autónoma de México, Mexico
[4]Department of Animal Science, The Ohio State University, United States

Conclusion

- *S*. Typhimurium = Predisposition to NE – Gut damage, immunosuppression, $$$.
- *Eimeria maxima* strain may have a critical role on development and severity of NE
- In the urgent search for AGP alternatives, some candidates may be more likely to promote recovery of the enteric epithelium whereas others may be more protective for the inflammation-induced shock and high acute mortality associated with the more virulent challenge.
- Consideration of appropriate models for different candidate AGP alternatives may be important in future studies.

Histomoniasis

- Synonyms: Blackhead disease and infectious enterohepatitis;
- Parasitic (protozoal) disease;
- **Etiology:** protozoa *Histomonas meleagridis*

- **Characteristics:**
  - highly transmissible within a flock;
  - incubation period 7-12 days;
  - Intermediate host: *Heterakis gallinarum.*

Figure 1. Histomonads in light microscopy.

Figure 2. Histomonad in light microscopy.

Lesions

Figure 2. A normal ceca (A) and a ceca of a turkey experimentally infected with *H. meleagridis* (B).

Caseous core - “cheesy core”

Lesions

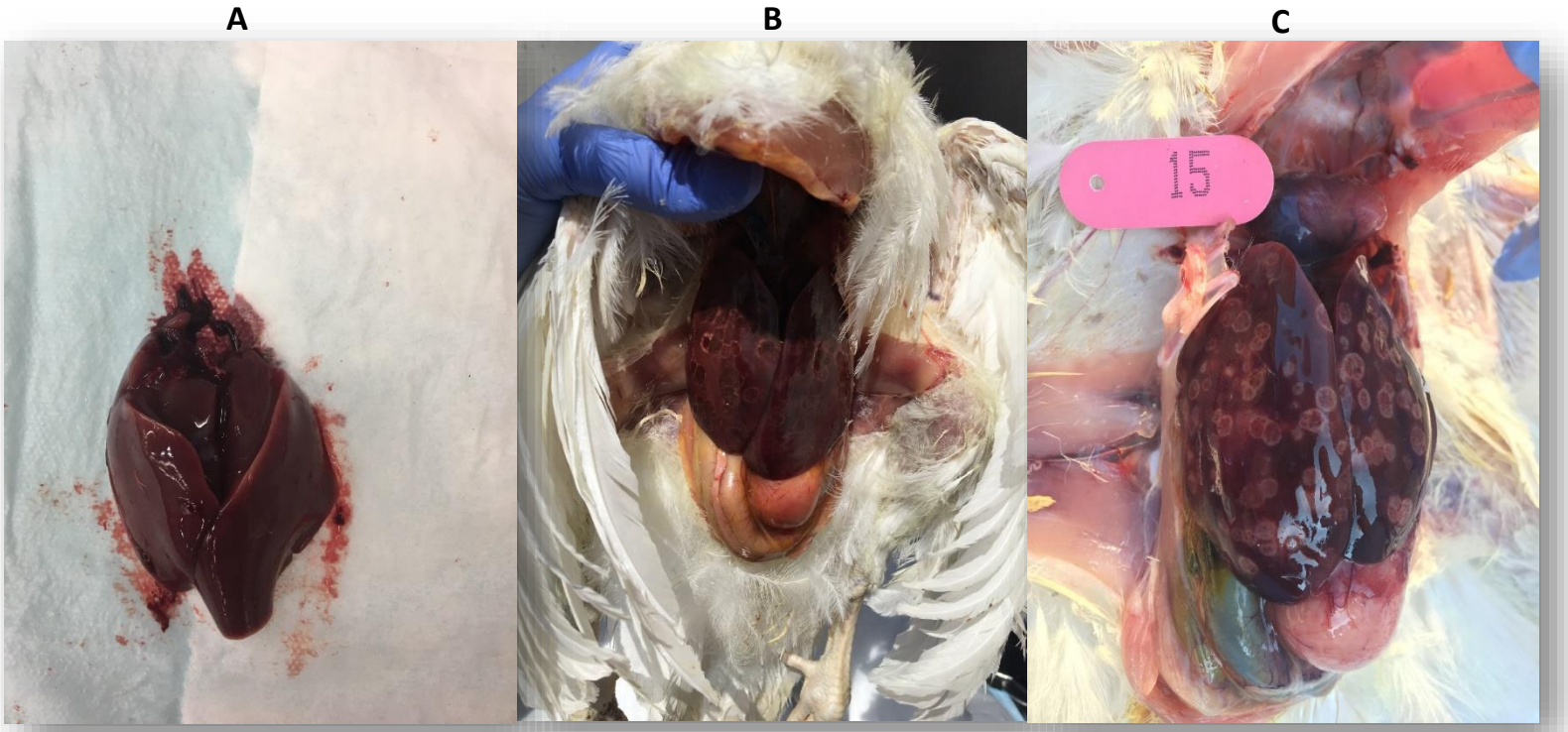

Figure 3. Normal turkey liver (A); Livers of turkeys experimentally infected with *H. meleagridis* (B and C).

Lesions

Figure 4. Ceca and liver of experimentally infected turkeys (C) and chickens (D). From Sulejmanovic, Liebhart and Hess (2013). *In vitro* attenuated *Histomonas meleagridis* does not revert to virulence, following serial *in vivo* passages in turkeys or chickens. *Vaccine 31*, 5443-5450.

Transmission

- Intermediate host
  - Ingestion of the cecal worm (*H. gallinarum*)
  - Earthworm may carry the *H. gallinarum*
- Bird to bird or fresh droppings (cloacal drinking)

- **Treatment?**
  - No chemotherapeutic substances available

- **Prophylaxis?**
  - Vaccines
    - no commercial option available.
    - Autogenous vaccine – modified live – growing in abnormal conditions (attenuation)
  - Biosecurity measures.
    - Avoid contact between turkeys and chickens;
    - C and D.
  - Control of the cecal worm especially in chicken flocks.

Thank you