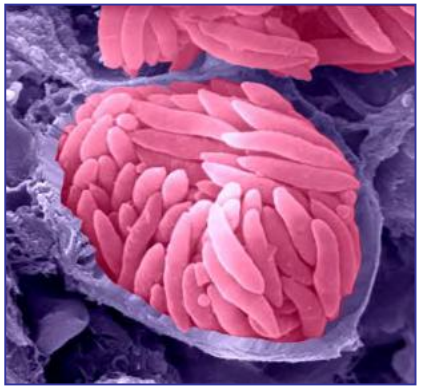
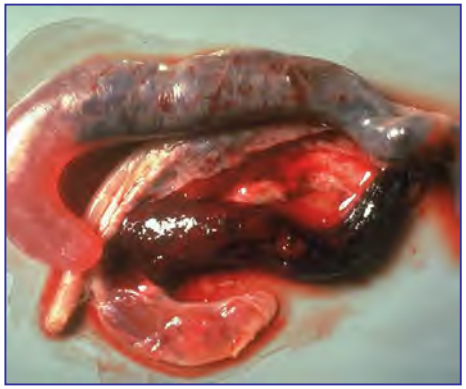
Cryptic *Eimeria*.
Are they coming? Do they matter?



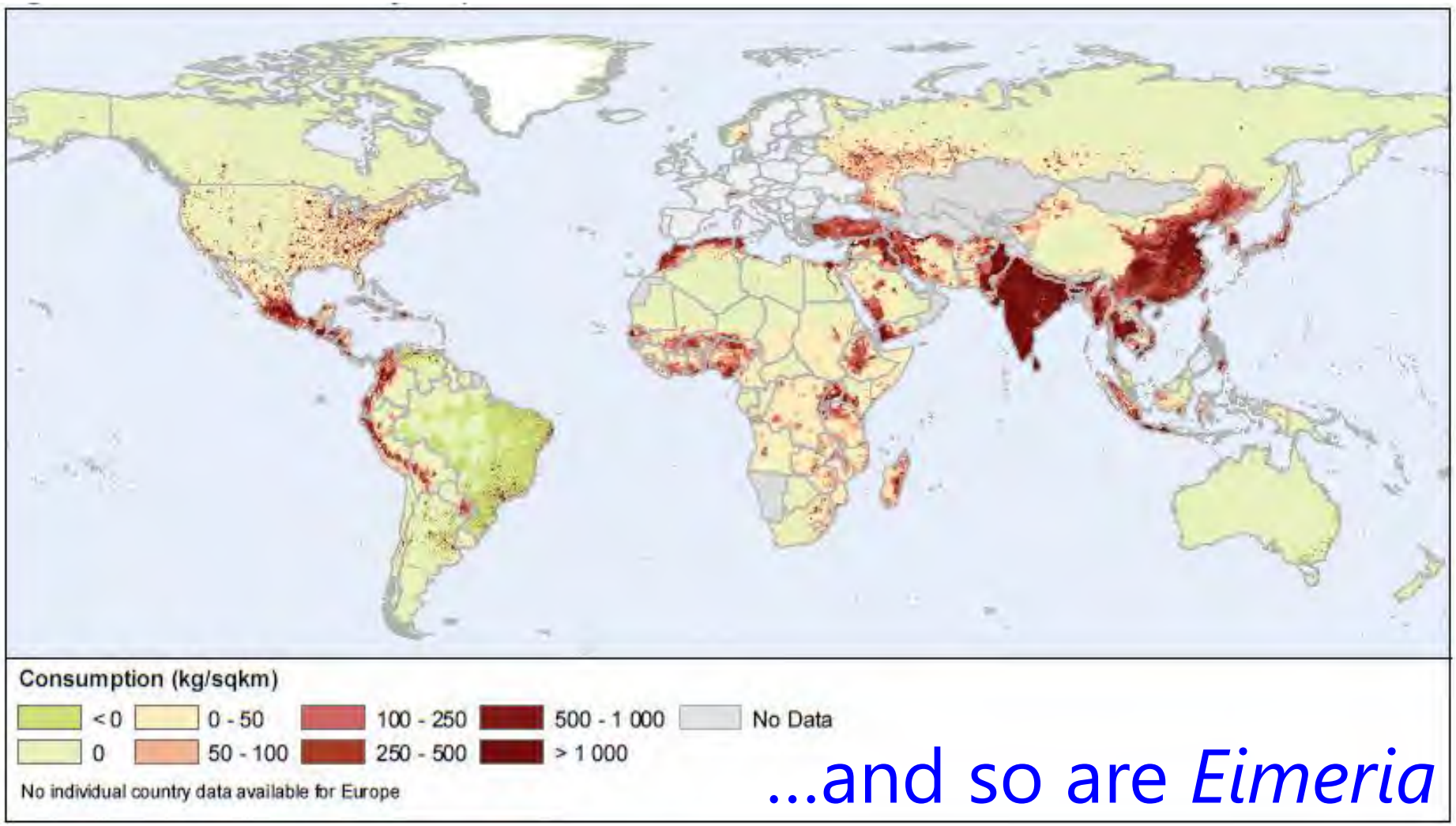


Damer Blake, Simon Spiro, Vlad Vrba, Isa Jatau, Matthew Nolan, Dong Xia, Greg Underwood, Fiona Tomley

Royal Veterinary College
University of London

Chicken production is booming…

- > 66 billion broilers produced in 2017 (FAO)



…and so are *Eimeria*

Eimeria species from the chicken

- Seven recognised species

ELSEVIER

Available online at www.sciencedirect.com
ScienceDirect
Veterinary Parasitology 152 (2008) 226–234

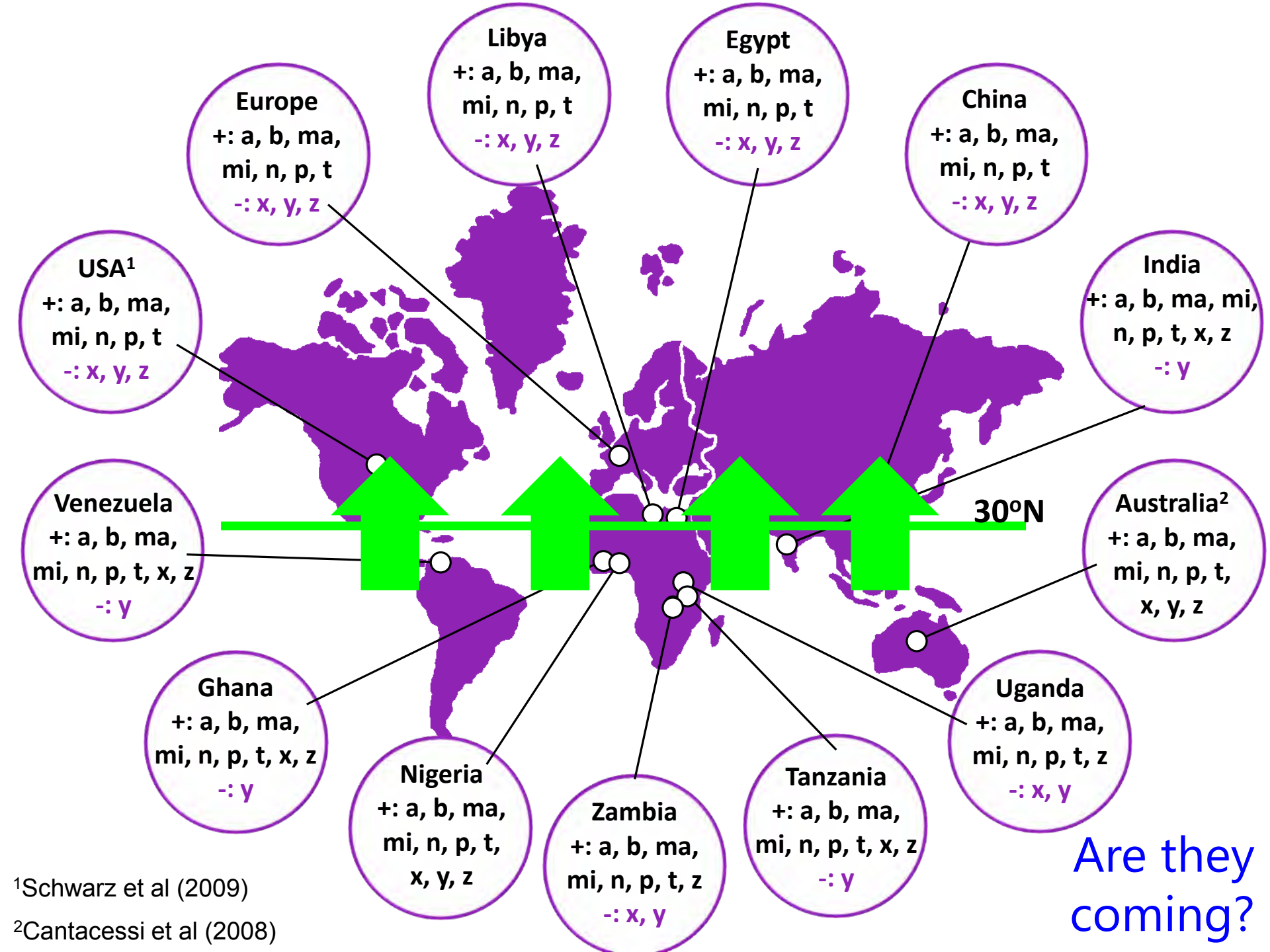
veterinary parasitology
www.elsevier.com/locate/vetpar

Genetic characterization of three unique operational taxonomic units of *Eimeria* from chickens in Australia based on nuclear spacer ribosomal DNA☆

Cinzia Cantacessi[a], Shane Riddell[b], Genevieve M. Morris[a], Timothy Doran[b], Wayne G. Woods[a], Domenico Otranto[c], Robin B. Gasser[a,*]

- Genetic variants: X, Y and Z
  - Divergent strains?
  - Cryptic species?
  - …?

Internal transcribed spacer (ITS)



[1]Schwarz et al (2009)

[2]Cantacessi et al (2008)

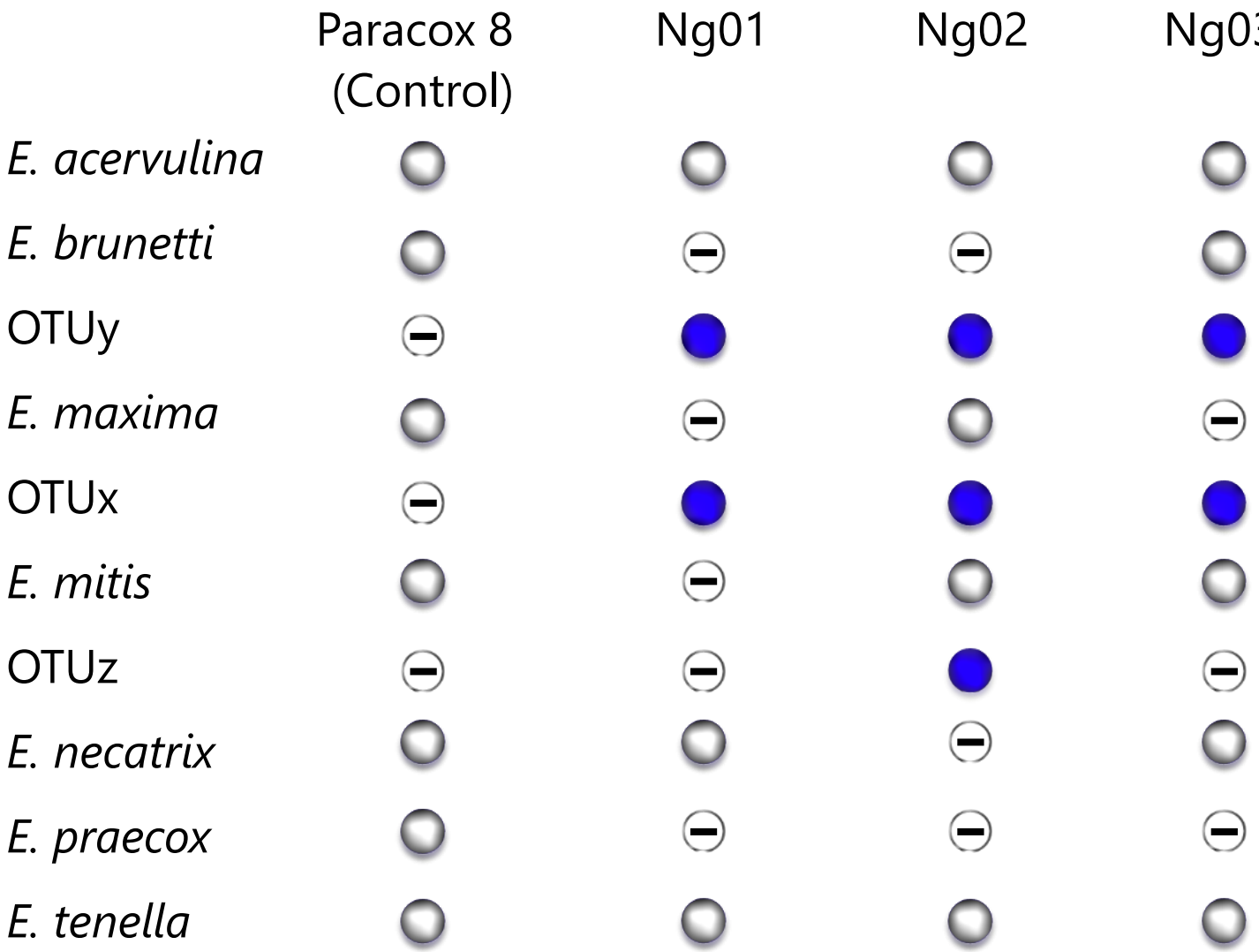
Does it matter?

- Drugs likely to be equally 'effective'
- Are live anticoccidial vaccines effective against OTUs?
- Passage OTU samples using Paracox® 8 vaccinated birds for selective enrichment

- Three Nigerian field populations
- Five to seven species/genotypes
- Triplicate vaccination: Paracox® 8 (x10)
- Experimental challenge

Challenge dose

Escape

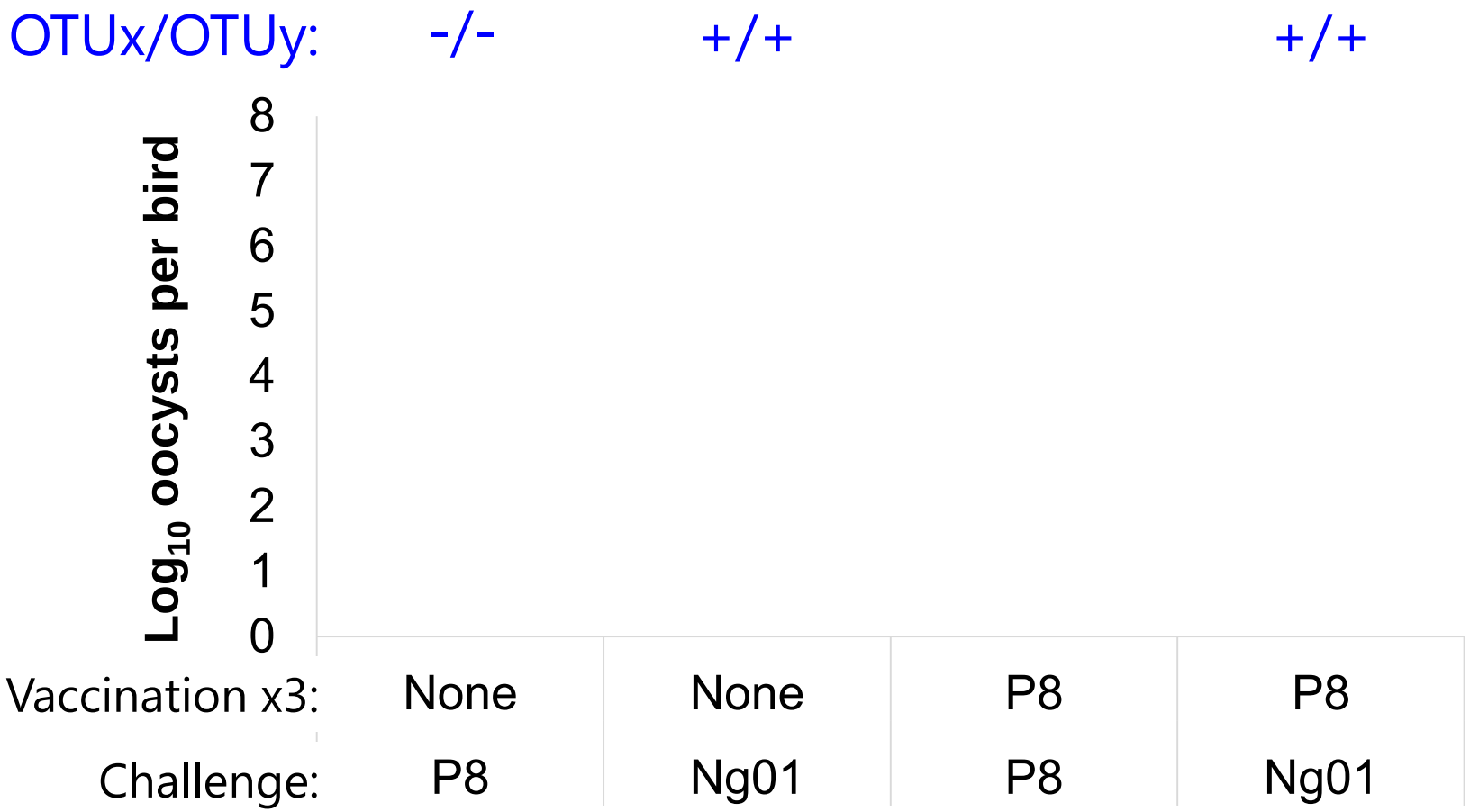


: Parasite present

: Parasite failed to produce oocysts

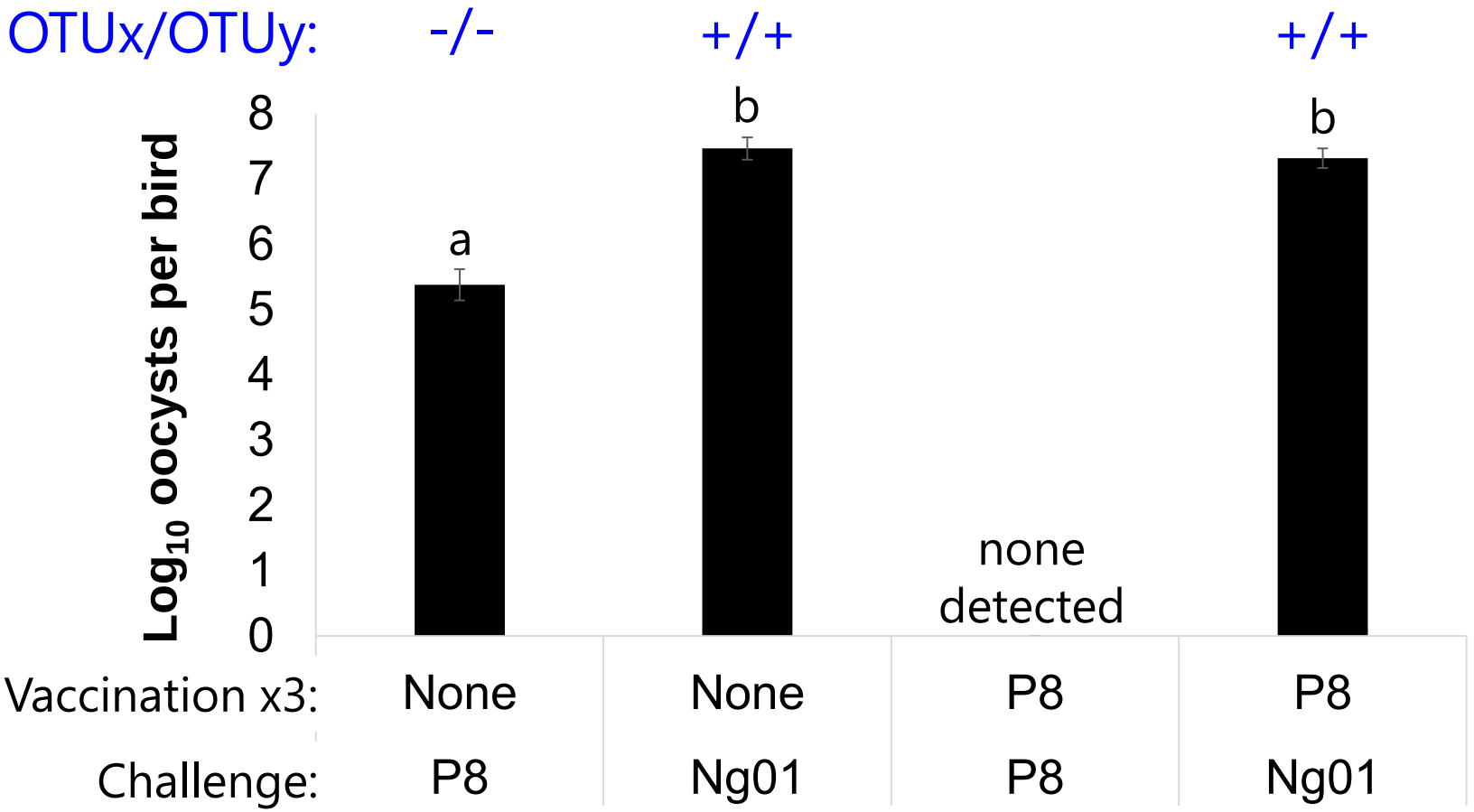
OTU genotype vaccine escape

- Passage OTUx/OTUy sample using Paracox® 8 (un)vaccinated birds

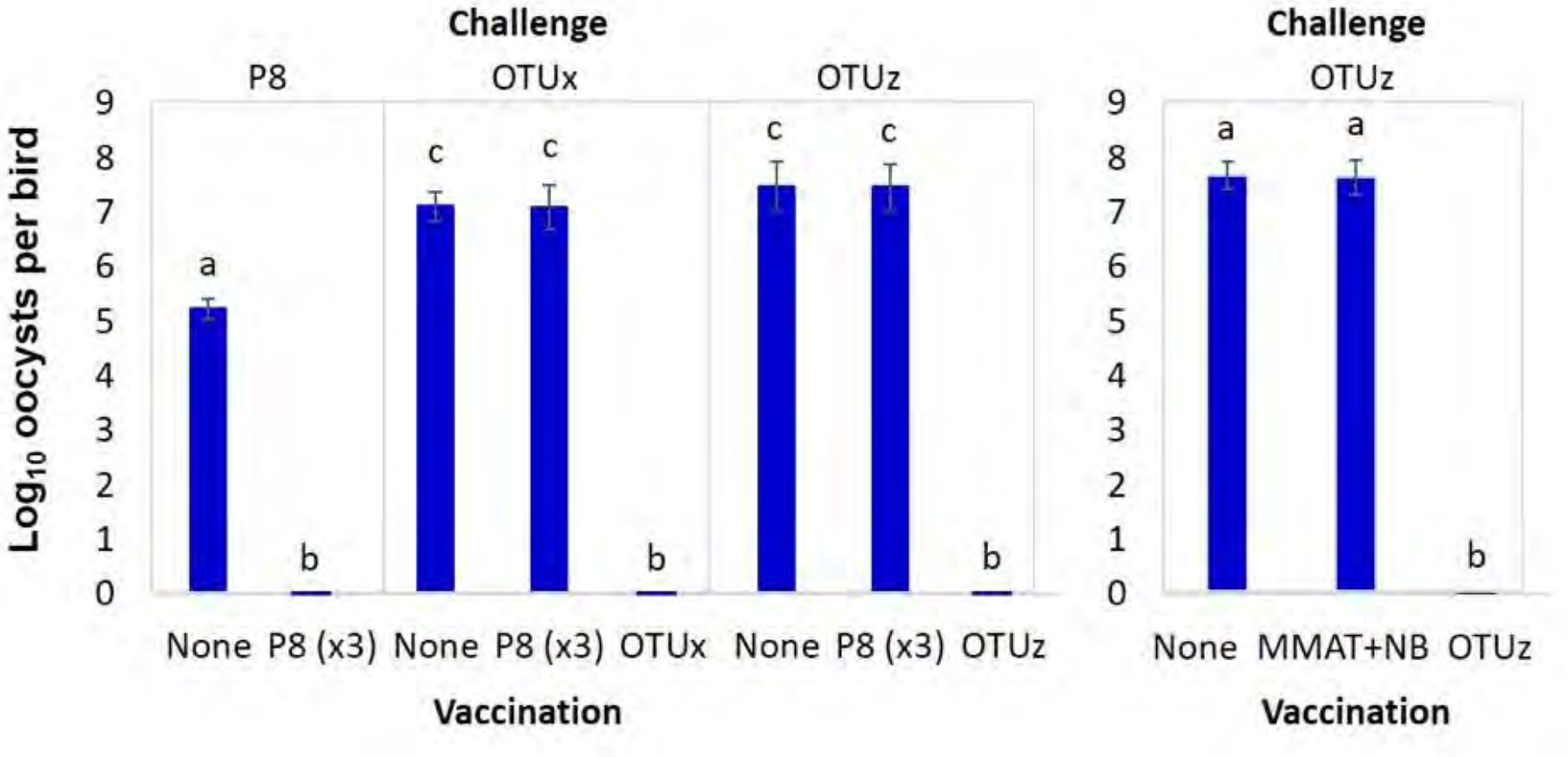


OTU genotype vaccine escape

- Passage OTUx/OTUy sample using Paracox® 8 (un)vaccinated birds

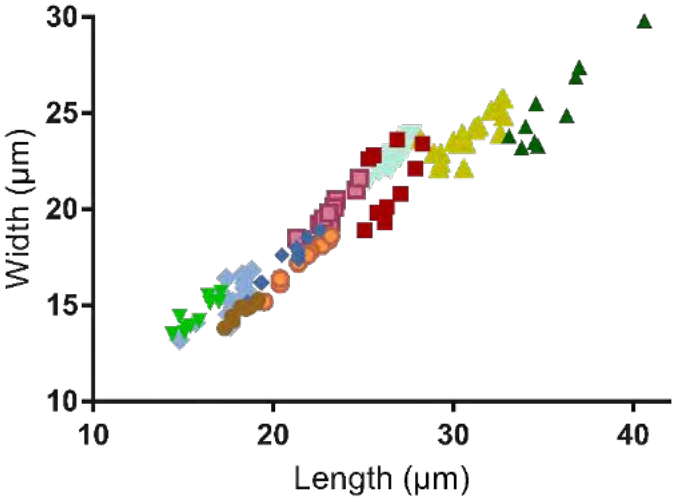


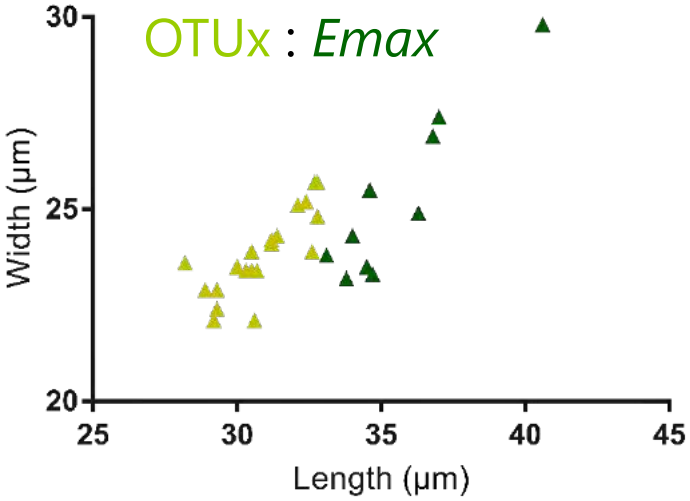
OTU genotype vaccine escape

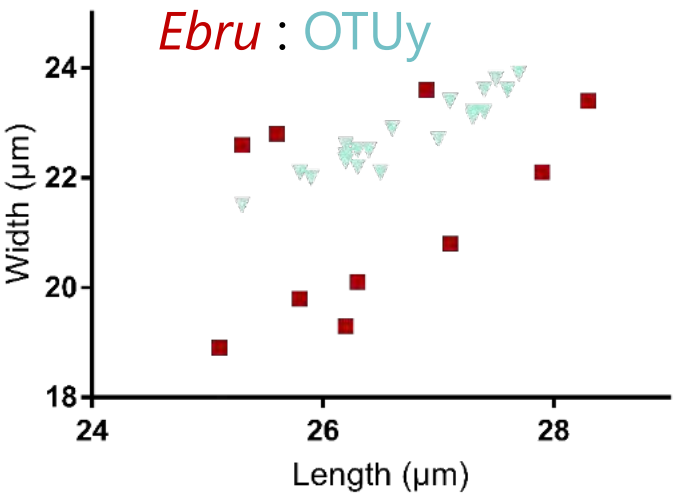


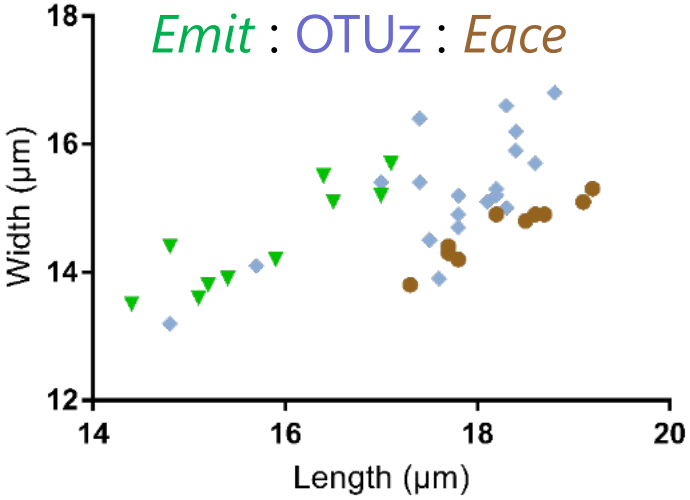
- Are live anticoccidial vaccines effective against OTUs? NO.

OTUs: strain or species? Oocysts…



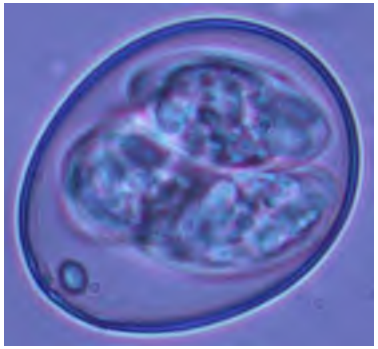






OTUs: strain or species?

Genotype:	OTUx
Source(s) analysed:	Nigeria, Australia
Closest relative (ITS):	*E. maxima*
Oocyst morphology:	Length: 30.5 µm Width: 23.4 µm L/W ratio: 1.30
Prepatent period:	130 hours
Fecundity:	Low
Site of replication:	Duodenum, extending into the jejunum



OTUs: strain or species?

Genotype: OTUy

Source(s) analysed: Nigeria

Closest relative (ITS): *E. brunetti*

Oocyst morphology: Length: 26.8 µm
Width: 22.9 µm
L/W ratio: 1.17

Prepatent period:

Fecundity: Low

Site of replication:

OTUs: strain or species?

Genotype: OTUz

Source(s) analysed: Nigeria, Australia

Closest relative (ITS): ? *(E. mitis)*

Oocyst morphology: Length: 17.6 µm
Width: 15.3 µm
L/W ratio: 1.15

Prepatent period: 135 hours

Fecundity: Moderate

Site of replication: Duodenum, extending into the jejunum

(ileum)

OTUs: strain or species?

Species/OTU	Oocyst L x W µm (L/W ratio)	Pre-patent period	Fecundity	Pathogenicity	Location
E. acervulina	18.3 x 14.7 (1.25)	89	+++++	++	SI (upper)
E. brunetti	26.5 x 21.3 (1.25)	120	++	++++	SI (lower), rectum
E. maxima	35.5 x 25.3 (1.41)	120	++	+++	SI (mid)
E. mitis	15.8 x 14.5 (1.09)	91	++++	++	SI (lower)
E. necatrix	20.8 x 17.3 (1.20)	138	++	+++++	SI (mid), caeca*
E. praecox	21.7 x 17.4 (1.25)	84	++++	+	SI (upper)
E. tenella	23.1 x 19.8 (1.17)	132	+++	++++	Caeca
OTUx	30.8 x 23.8 (1.29)	130	+++	+++	SI (upper)
OTUy	26.7 x 22.8 (1.17)	tbc	tbc	tbc	tbc
OTUz	17.7 x 15.2 (1.17)	135	+++	++	SI (upper-mid)

Are OTU *Eimeria* pathogenic?

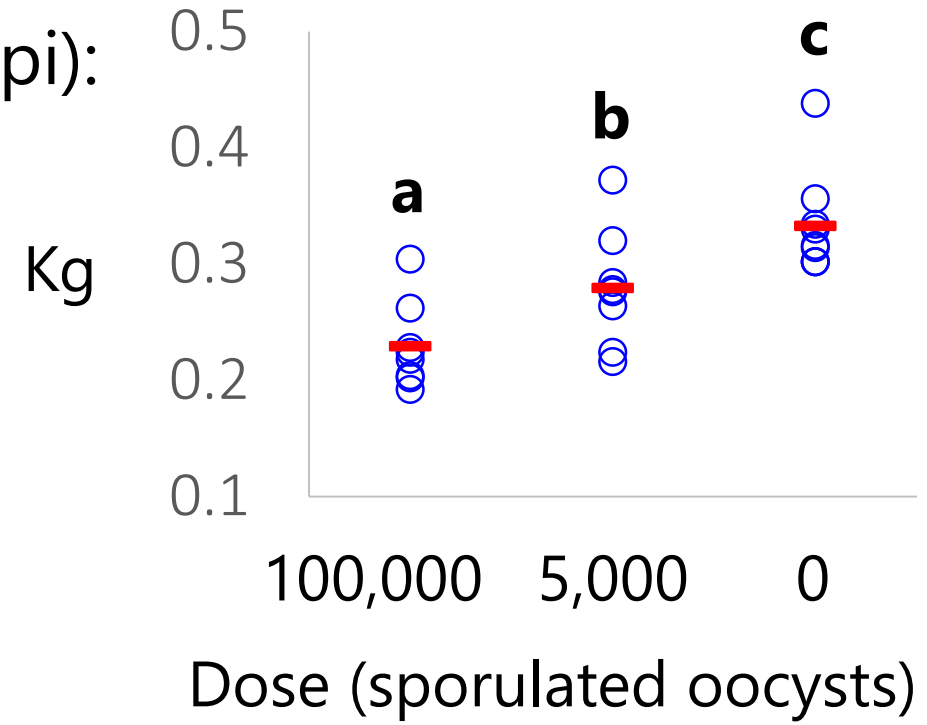
Genotype: OTUz

Source(s) analysed: Australia

BWG (days -3 to +10 pi):

[Ross 308, 21 days old]

Mortality: None



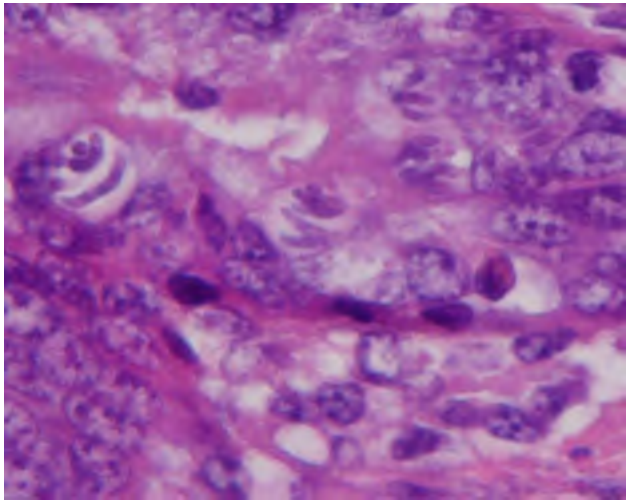
Watery mucus (days +5 to +7 pi). No discrete lesions

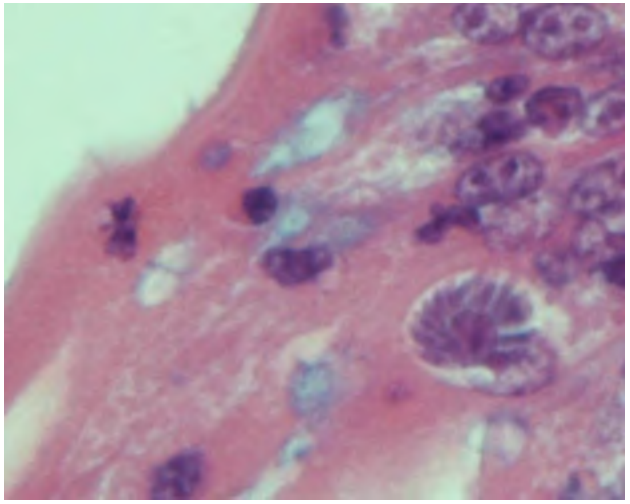
Are OTU *Eimeria* pathogenic?

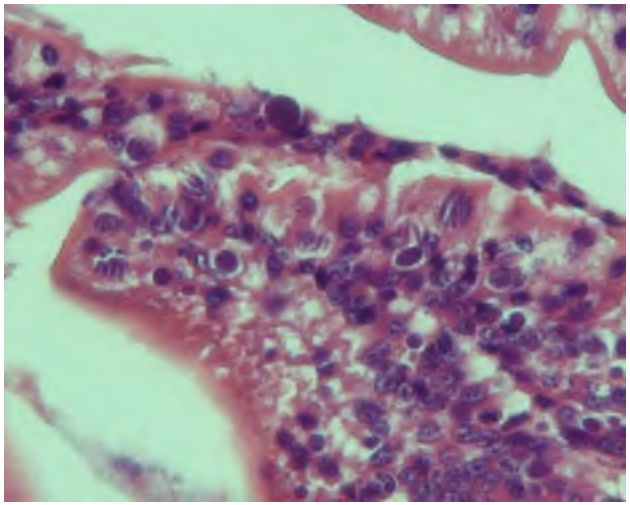
Schizonts detected from ~50 hpi

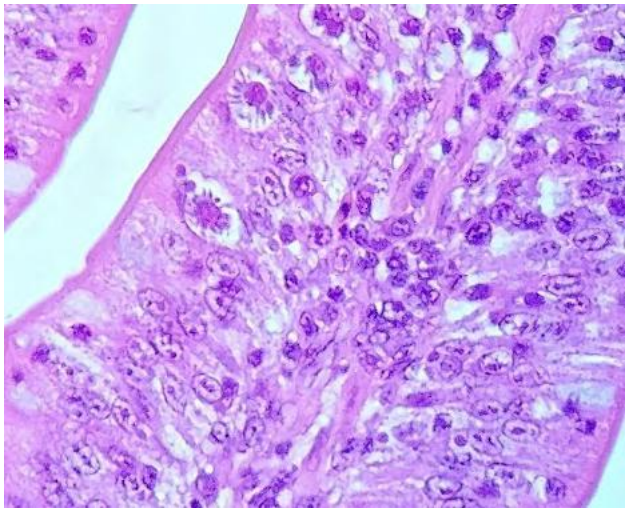
Late generation schizonts (between 96 and 168 hpi)

Villi, not crypts







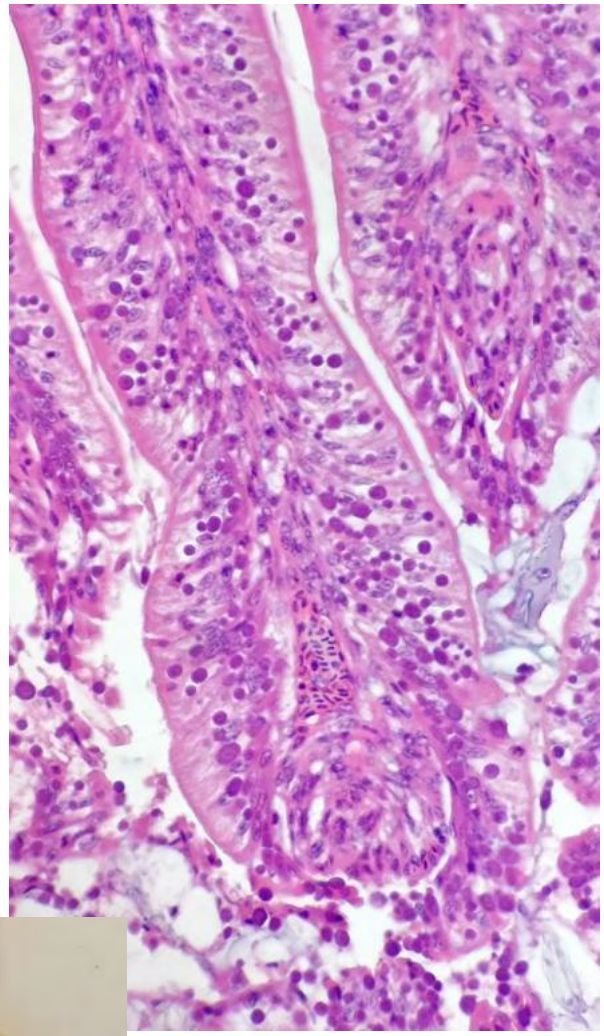


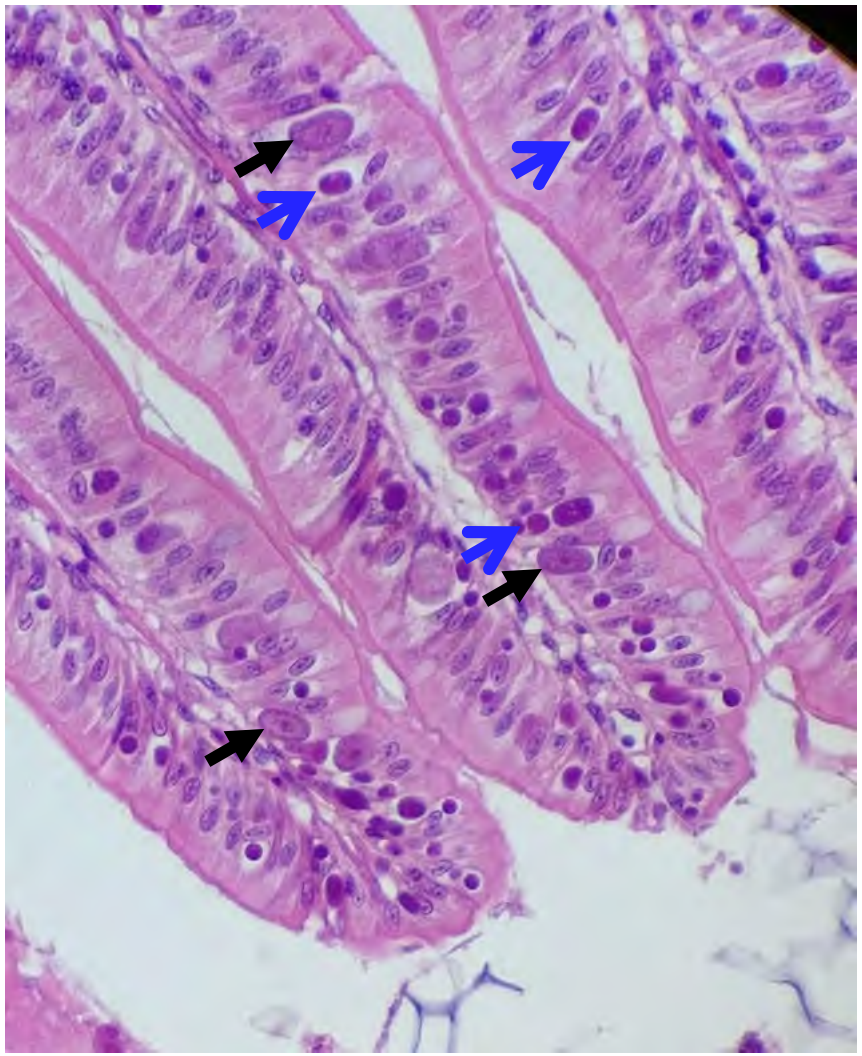
Are OTU *Eimeria* pathogenic?

Gametes
from 120 hpi

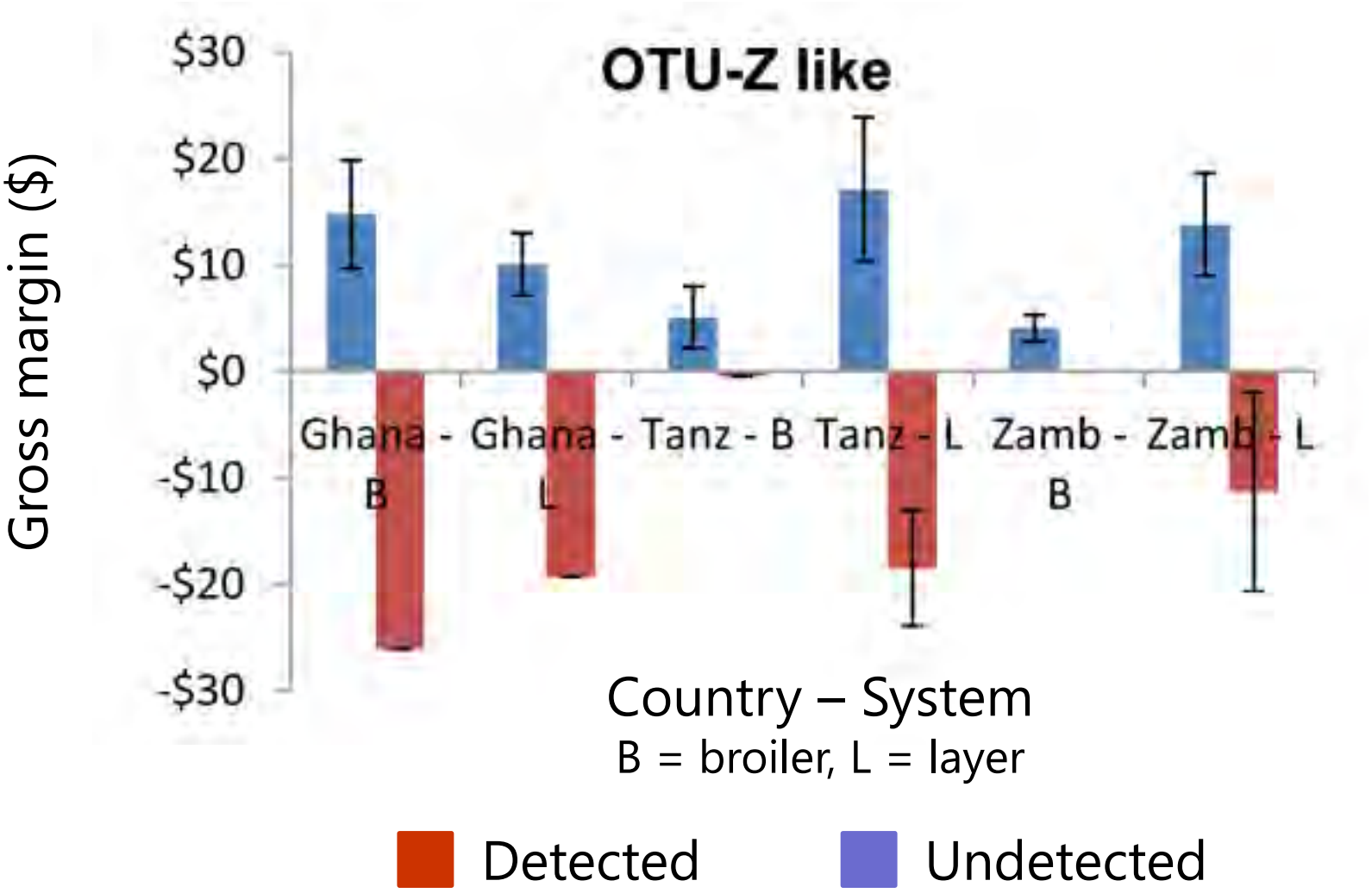
(144 hrs pi)

Correlates with the most severe pathology



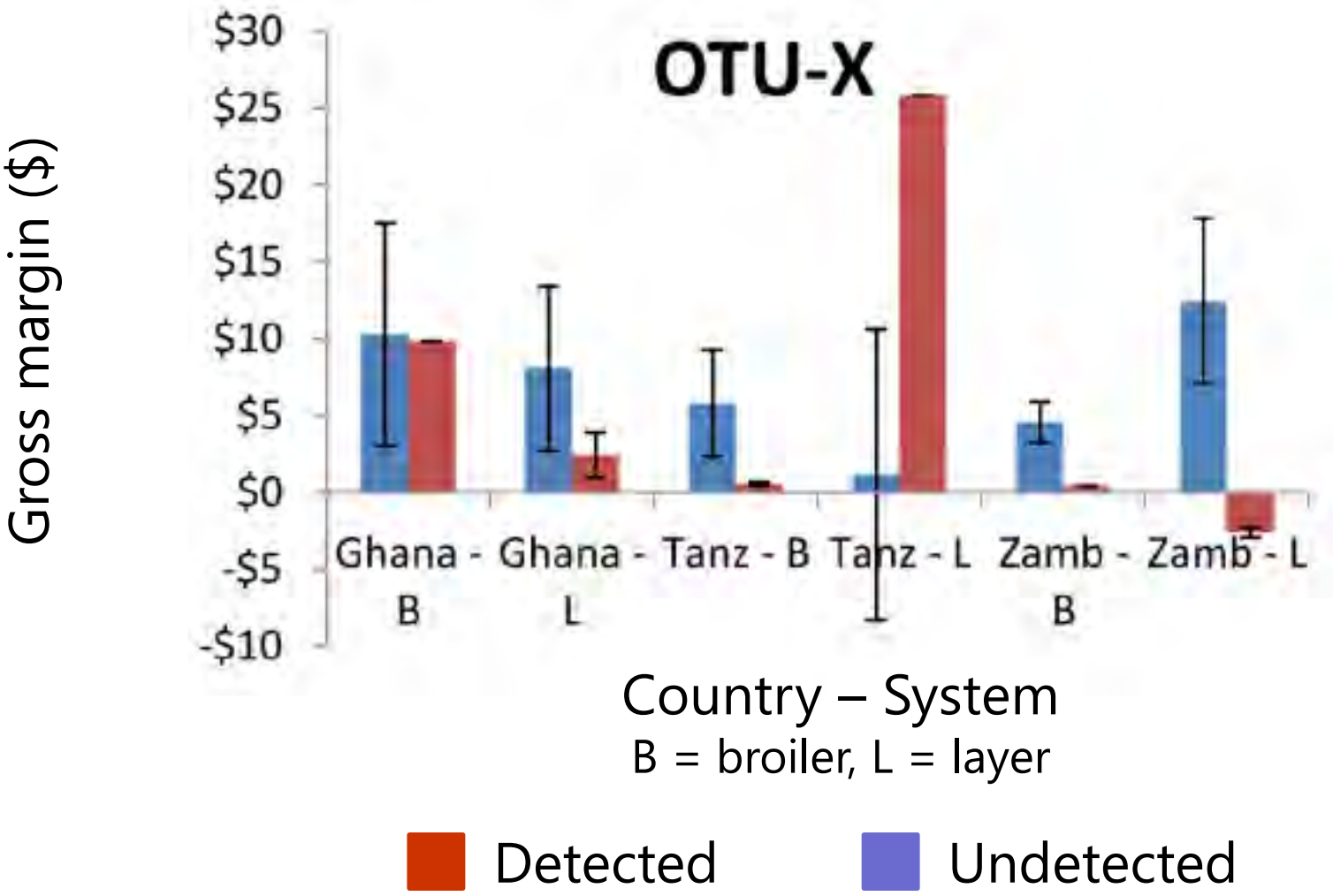


Are OTU *Eimeria* pathogenic - $?



Fornace et al (2013) PLoS One 8: e84254

Are OTU *Eimeria* pathogenic - $?



Fornace et al (2013) PLoS One 8: e84254

Eimeria genomes

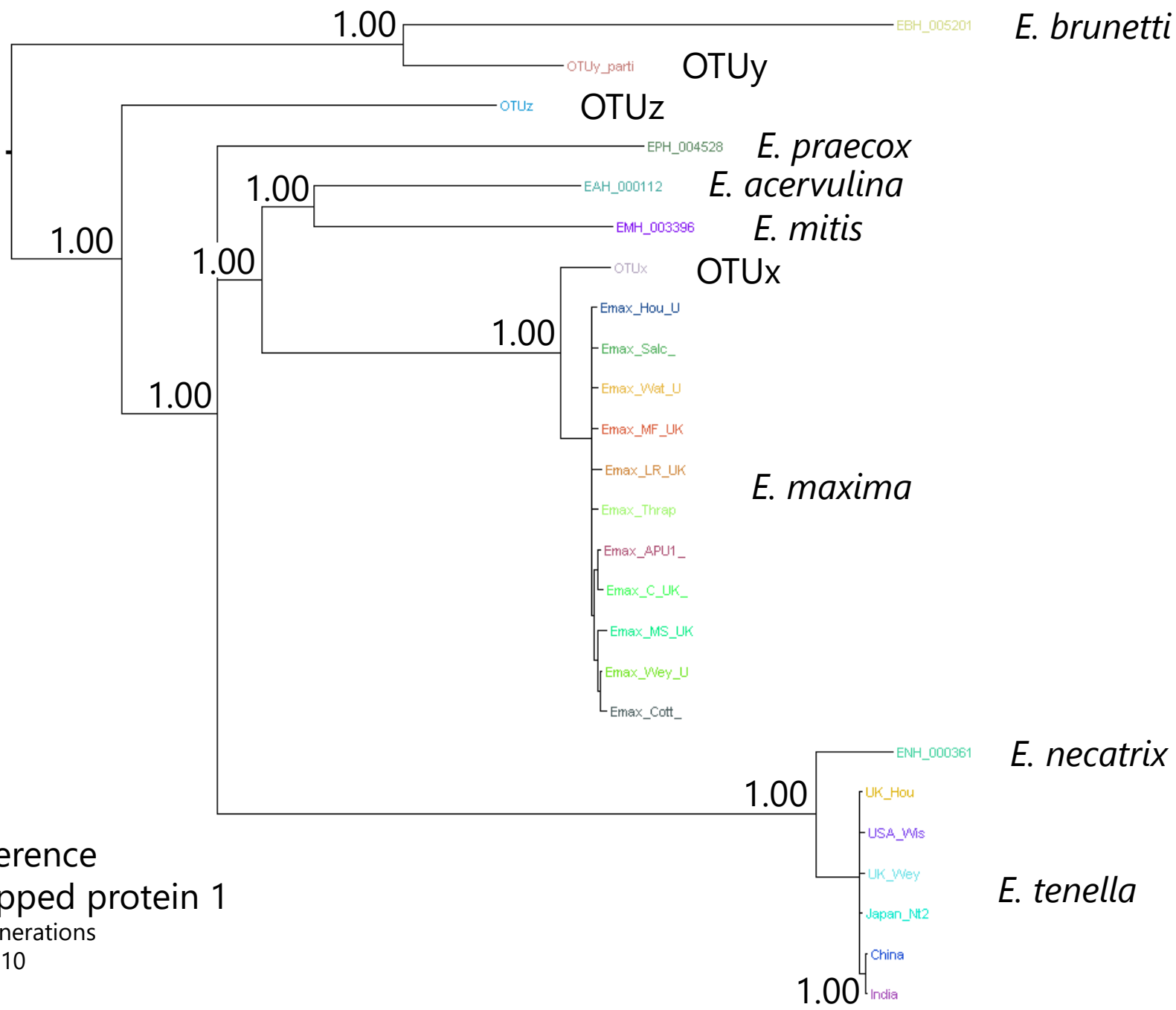
Species/OTU	Reference origin	Assembly size (Mbp)	Scaffolds	% complete vs T. gondii	Additional strains available
E. acervulina	UK	45.8 (61.1*)	3,415 (627*)	98.6	
E. brunetti	UK	66.9	5,317	na	
E. maxima	UK	46.0	3,564	93.3	UK
E. mitis	UK	72.2	15,978	na	
E. necatrix	UK	55.0	3,707	95.2	
E. praecox	UK	60.1	12,929	na	
E. tenella	UK	51.9	4,664	98.1	Japan, Nigeria, US
OTUx	Aus	42.9	16,071	na	
OTUy	Aus	58.0	40,800	na	
OTUz	Aus	50.6	27,925	na	

*PacBio sequencing. All other sequences generated using Illumina

Australian OTU genome sequencing

OTUx assembly:	~42.9 Mb ~24x coverage 16,071 contigs	10,309 gene models
OTUy assembly:	~58.0 Mb ~14x coverage 40,800 contigs	12,777 gene models
OTUz assembly:	~50.6 Mb ~27x coverage 27,925 contigs	11,705 gene models

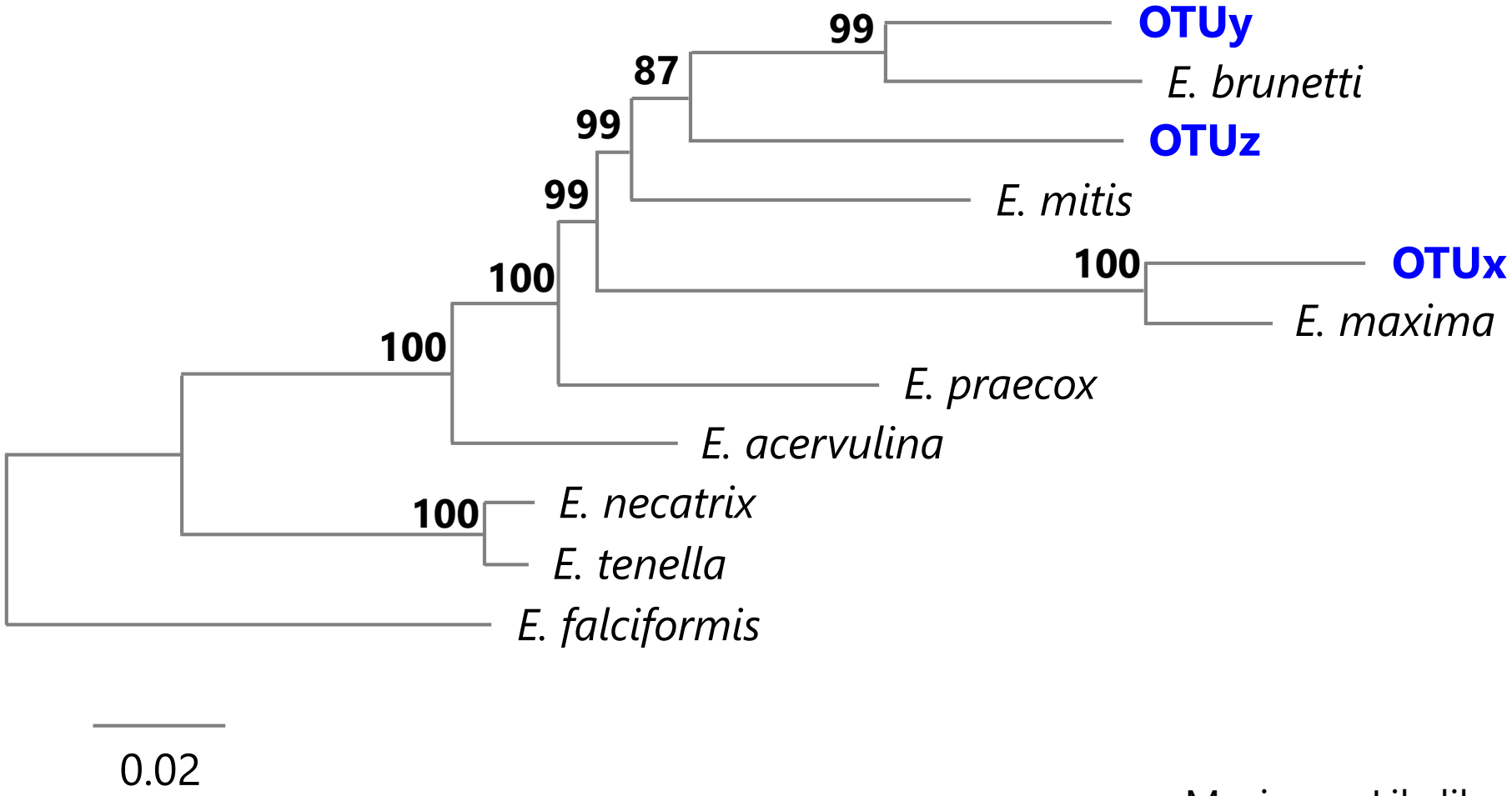
β-tubulin
18S rDNA
Cytochrome oxidase I
Actin depolymerising factor
Mitochondrial



Bayesian inference
Immune mapped protein 1
4 runs, 500,000 generations
Sample frequency 10
Burn in 25%

0.1 Expected Substitutions per Site

Consensus phylogeny



Maximum Likelihood
GTR+G
56 orthologues, concatenated
1,000 iterations

Summary

OTUs

✗

✓

Distinct

- Geographically
- (Morphologically)
- Antigenically
- Genetically

- New species names?

Acknowledgements

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Newcastle University

- **Abdalgader Moftah**

Nigeria

Ahmadu Bello University

- **Isa Danladi Jatau**

BBSRC bioscience for the future

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GCRF

Global Challenges Research Fund

UK Research and Innovation

GCRF

One Health Poultry Hub

Population growth is driving global demand for safe, low-cost poultry meat and eggs but rapid intensification creates conditions for diseases. This Hub explores how to meet rising demand while minimising the risk to public health. It will study how intensification increases risk of infectious disease and antimicrobial resistance, why certain processes and behaviours are risky and establish when and where to test interventions for disease control. Working with strong networks of local, regional and global stakeholders, the evidence generated will be put to immediate use.

"Sri Lanka is nearly self-sufficient with poultry products but needs to improve product quality to comply with international food safety standards. Our Hub will provide a golden opportunity for us to share knowledge and technical know-how among collaborative countries while providing the benefit of capacity building to improve quality standards assuring food safety for consumers."

Dr Ruwani Kalupahana, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Sri Lanka

KEY FACTS

£18.1M

11 partner countries

27 partner organisations

Lead organisation: Royal Veterinary College

Principal Investigator: Professor Fiona Tomley

Geographical focus: South-Central Asia and South-East Asia

Contact details: Professor Fiona Tomley
ftomleyervc.ac.uk