Combining PCR and Next-Generation Sequencing (NGS) to explore the diversity of coccidia (*Eimeria* spp.) infecting turkeys globally

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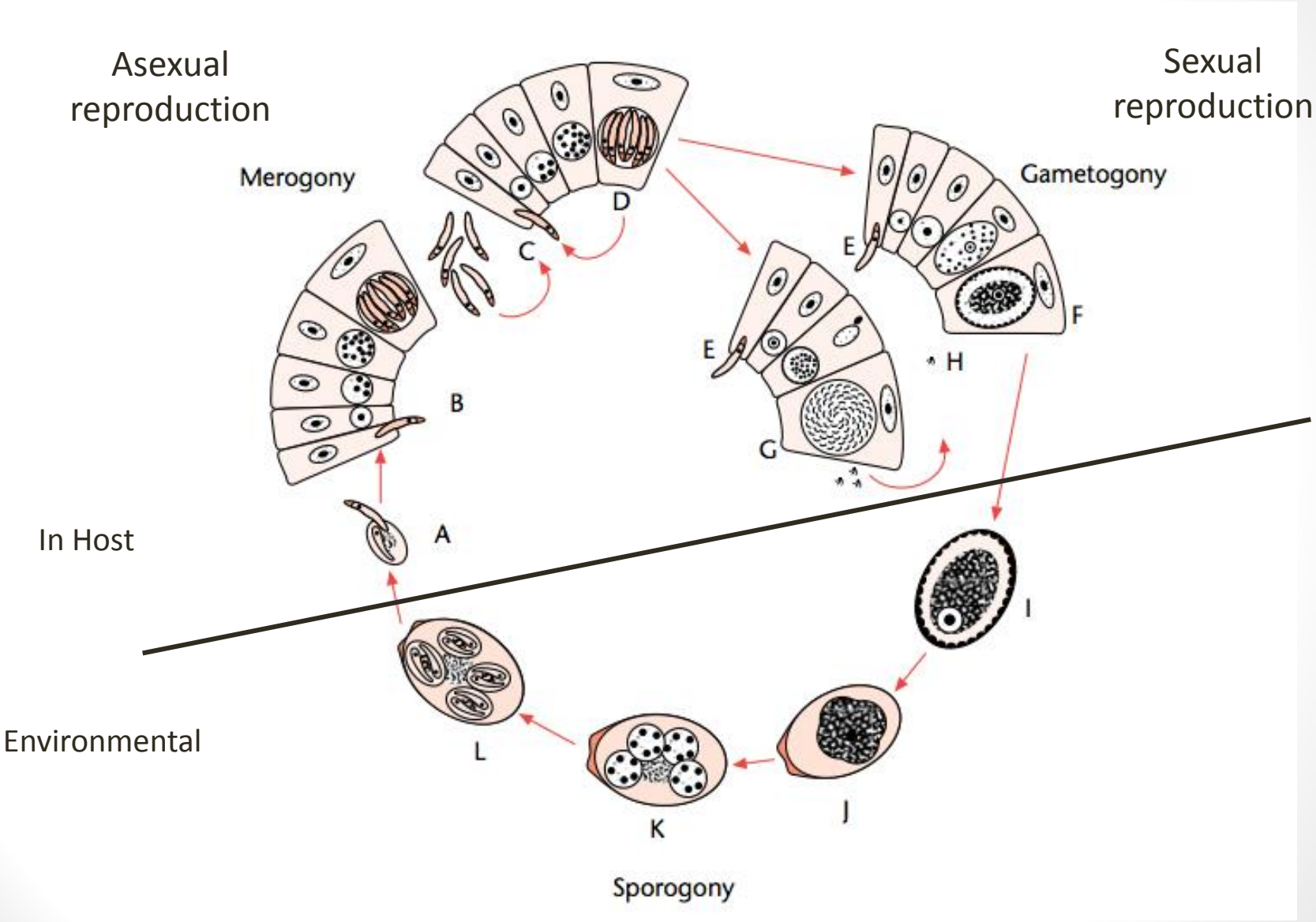
Pathobiology

Outline

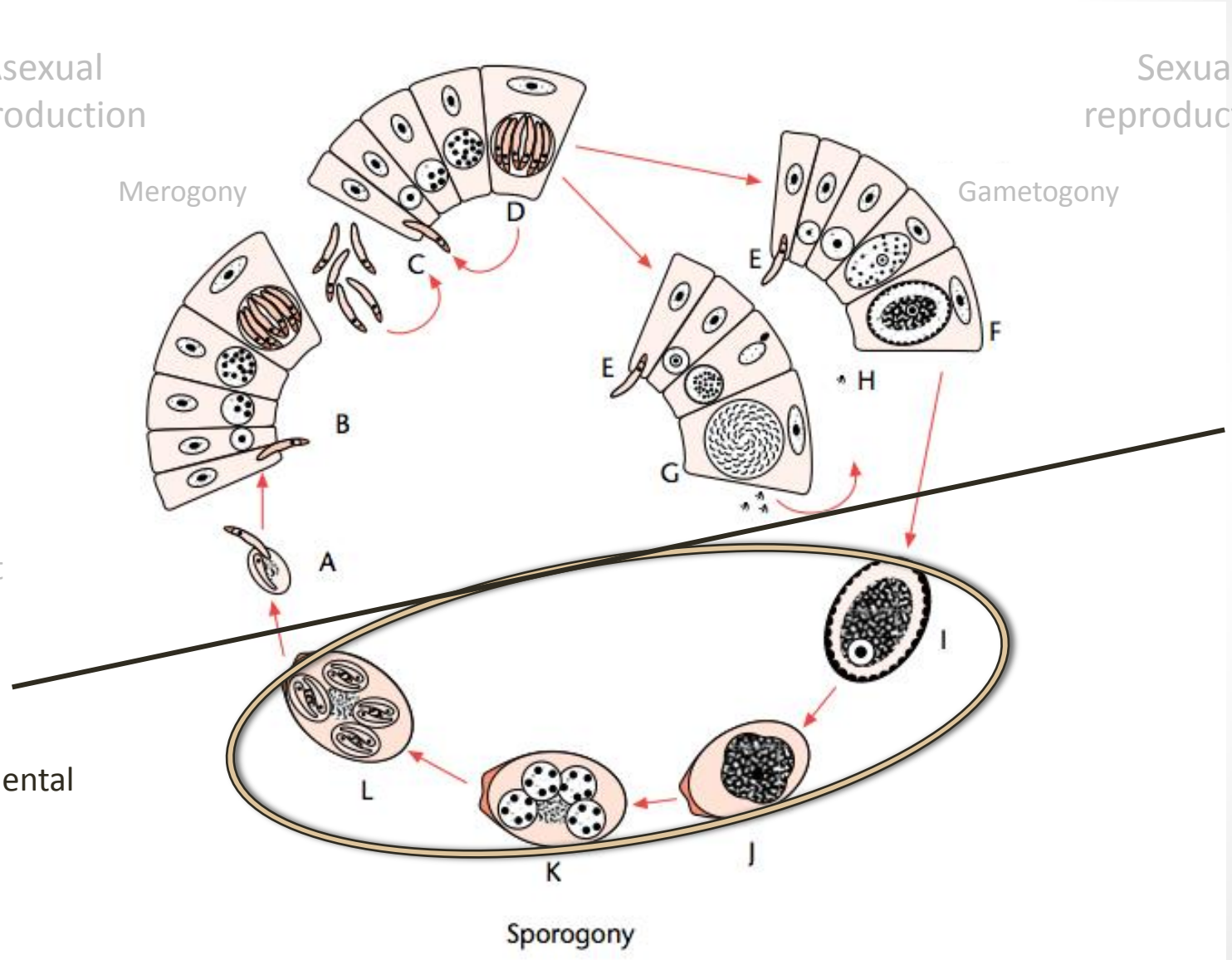
- Background
  - Life cycle
  - Species
  - Coccidiosis
- Objective
- Methods
- Future directions
- Summary

Life Cycle



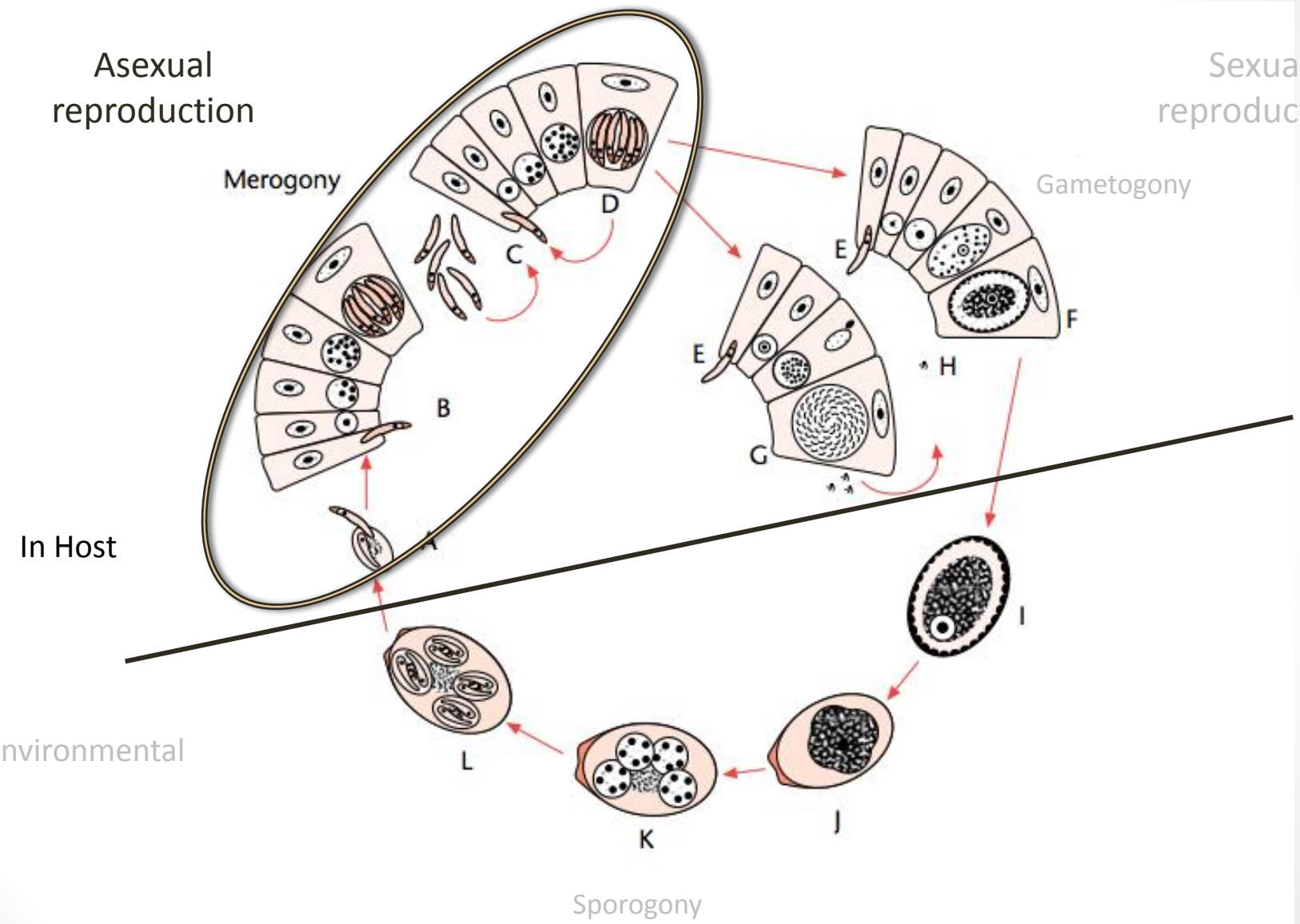
Barta, 2000

Life Cycle



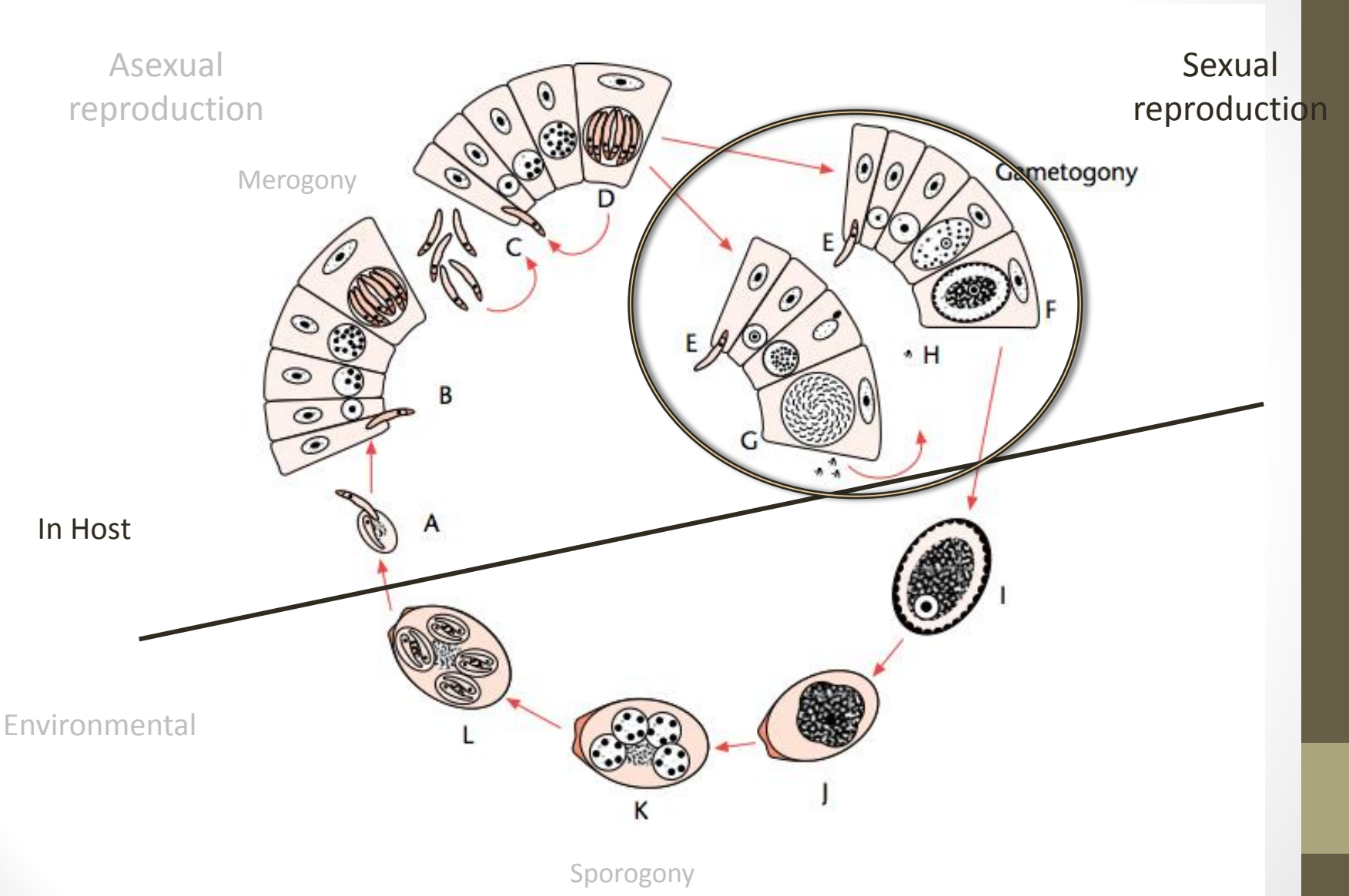
Barta, 2000

Life Cycle



Barta, 2000

Life Cycle



Barta, 2000

Species

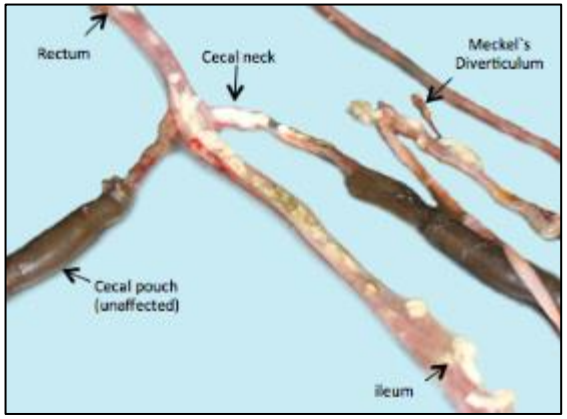
Pathogenic

- *E. meleagrimitis*
- *E. adenoeides*
- *E. gallopavonis*
- *E. dispersa*

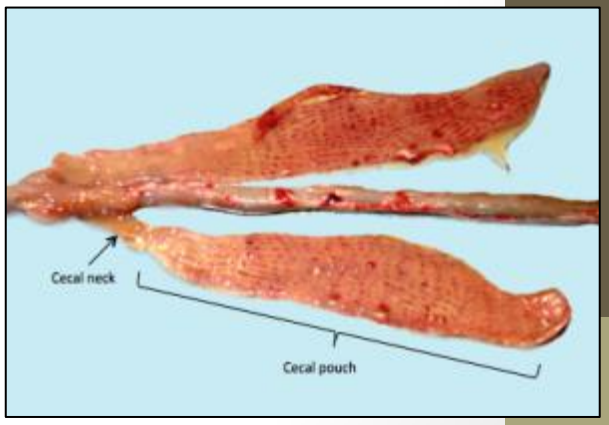
Relatively non-pathogenic

- *E. meleagridis*
- *E. innocua*
- *E. subrotunda*

E. adenoeides

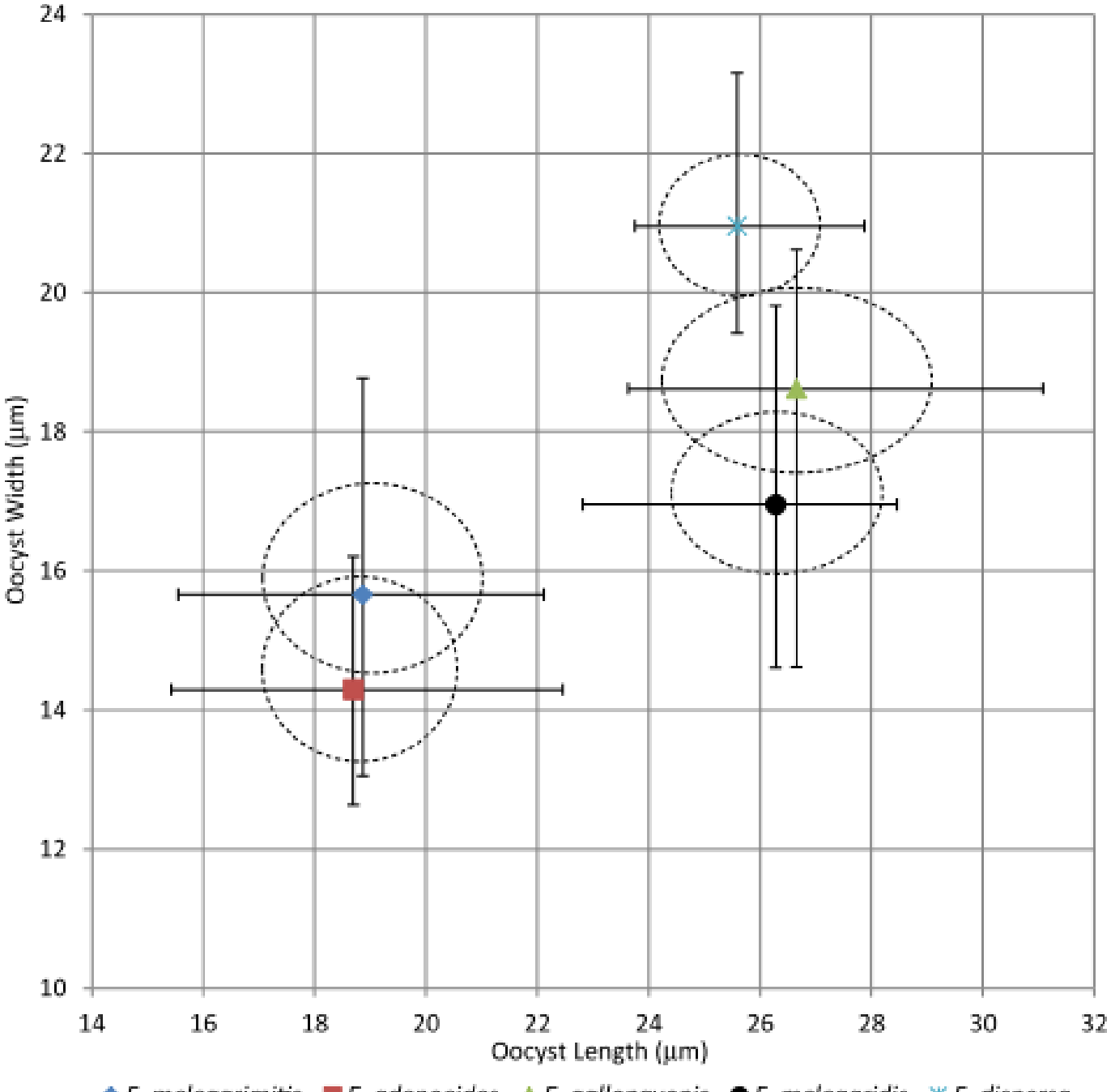


E. gallopavonis



E. meleagridis

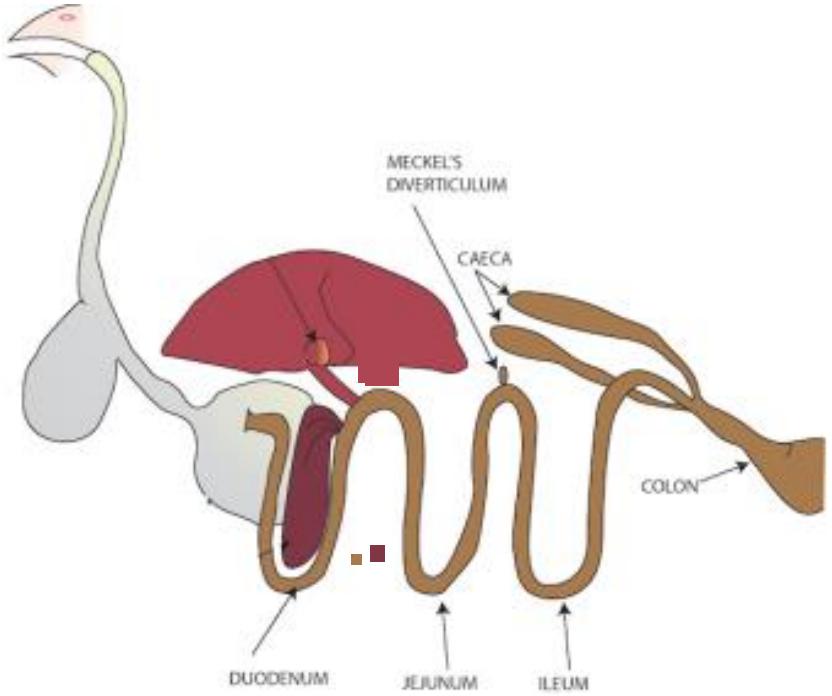
El Sherry, 2014; El-Sherry 2016



El-Sherry et al., 2015

Coccidiosis

- Enteric disease – parasite growth destroys enterocytes
- Malabsorptive diarrhea from infection with eimeriid parasites (genus: *Eimeria*)
- Severe infections, lesions, mortality



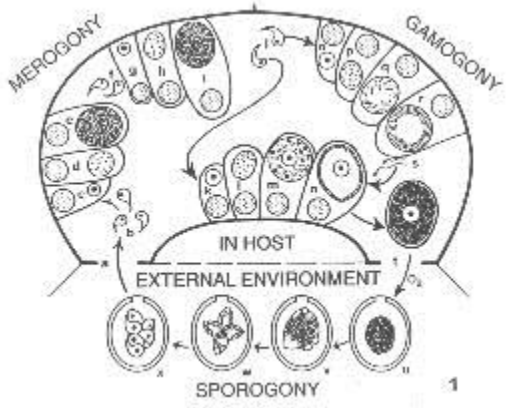
nttp://www.poultryhub.org/physiology/body-systems/digestive-system/

Float oocysts and sporulate

Morphometrics

Oocyst shape and size

Lifecycle



Location and number of lifecycle stages

Attempt to identify species

Current challenges with identifying species

- Using only morphological and biological features can be **misleading**.
- Morphometric data regarding *Eimeria* spp. in turkeys reported to **vary** among different strains of a single species (Long et al. 1977; and Poplstein and Vrba 2011).
- All of the original strains of these parasites used to describe species have been lost

Float oocysts and sporulate

Morphometrics

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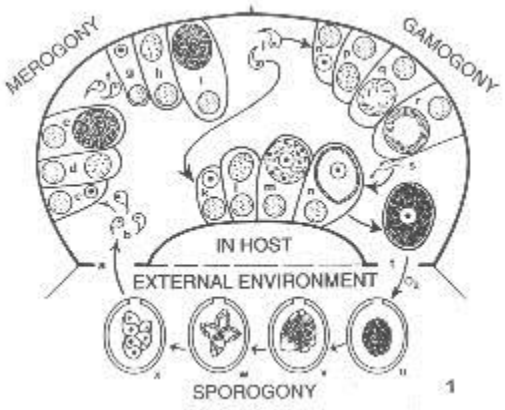
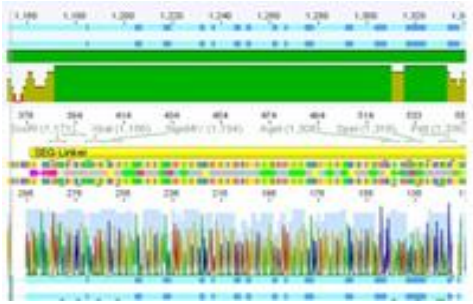


Location and number of lifecycle stages

DNA extraction

PCR , sequencing and analyses for **COI and 18S rDNA**



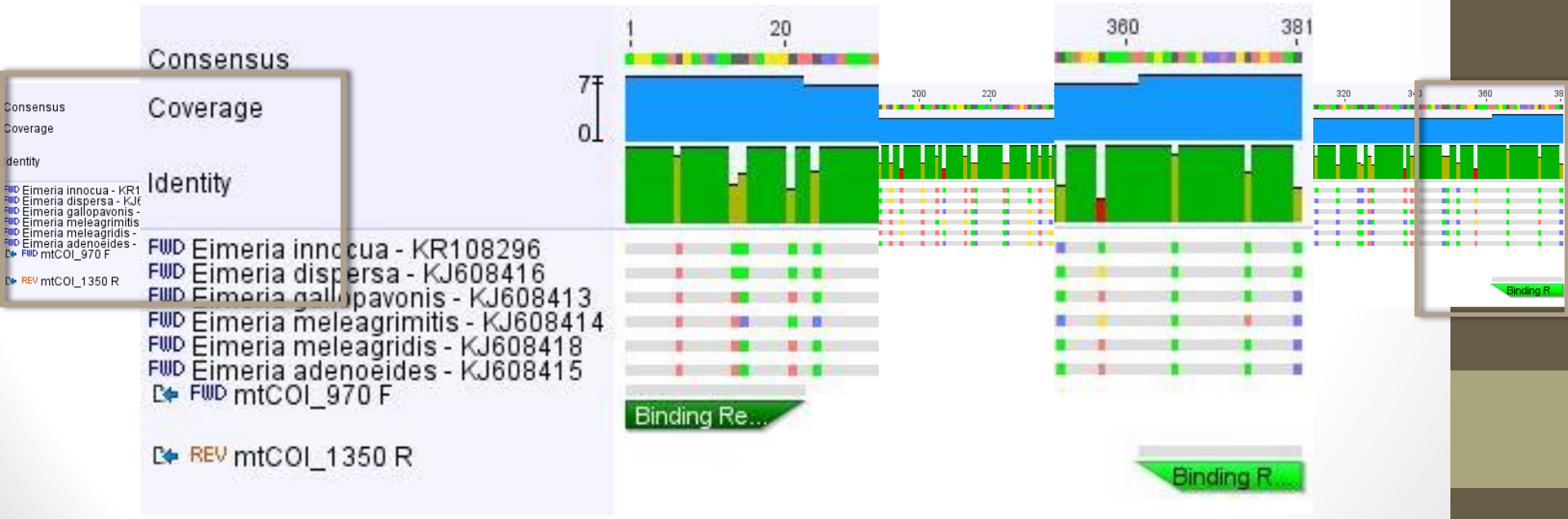
Attempt to identify species

Objective

Optimize genotyping methods targeting the mtCOI locus - *Eimeria* species in mixed samples

Methods: primer design

- PCR: test universal primers that target conserved region in mtCOI in the isolates of the 6 species of *Eimeria* that are available

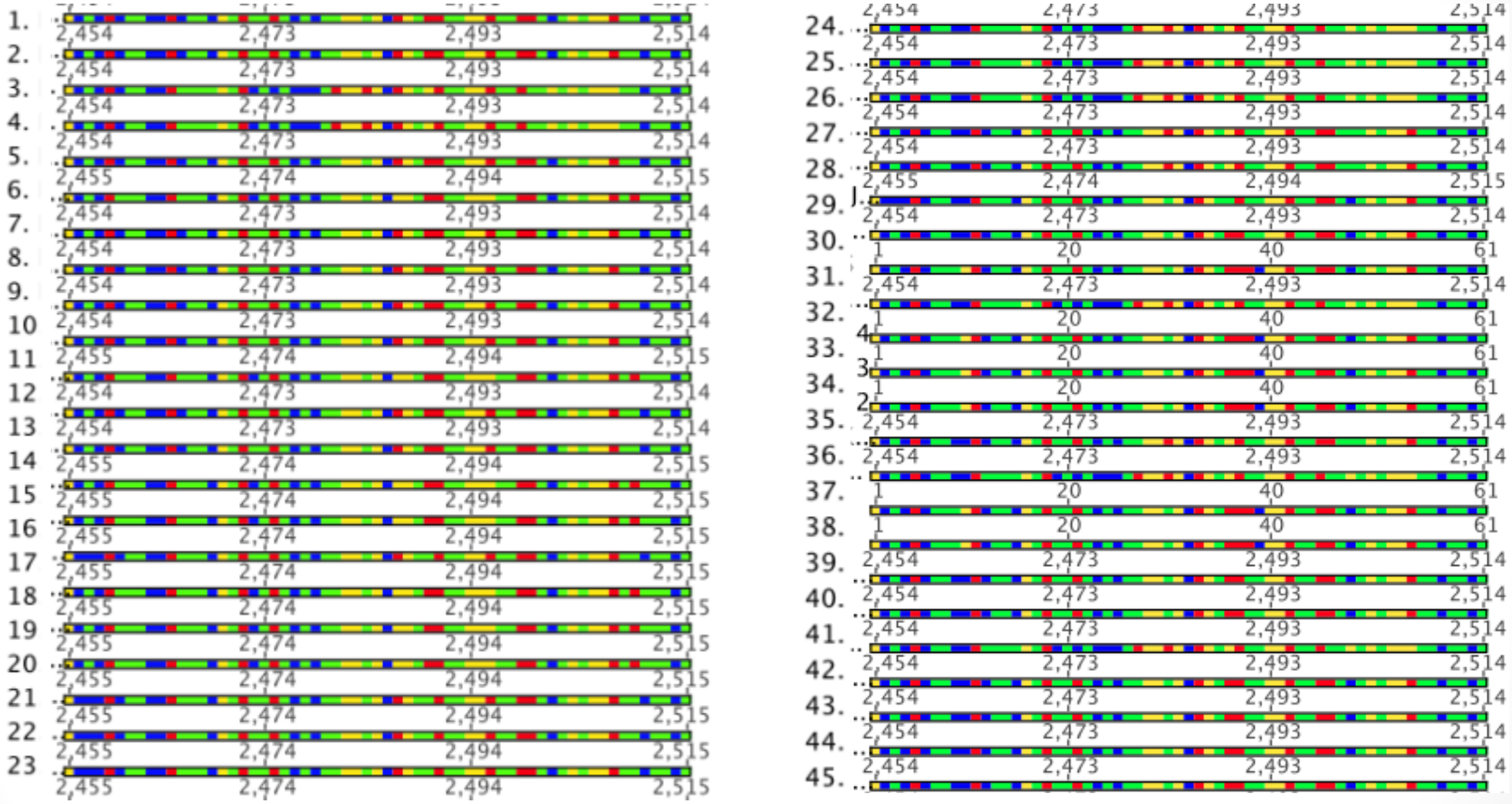


Methods: international sampling

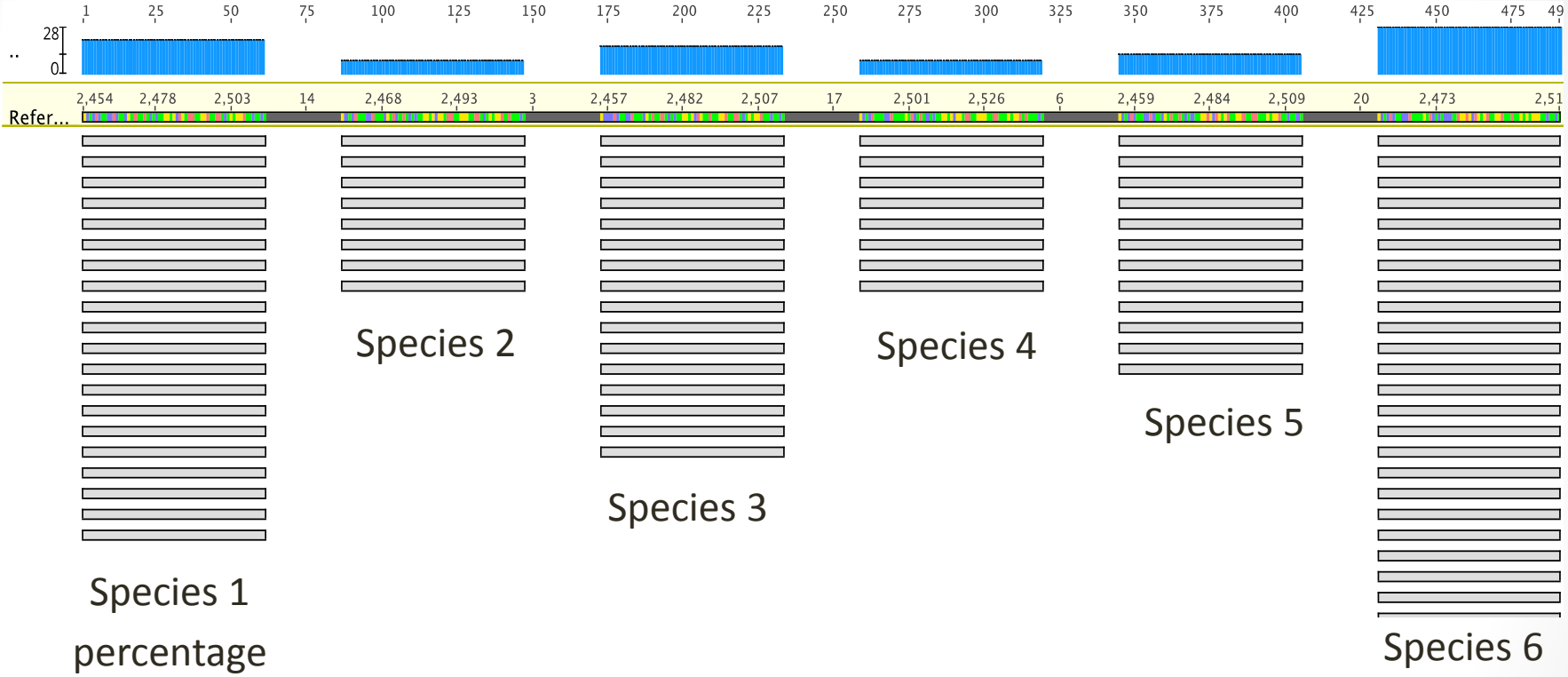
- Obtain litter/fecal samples from commercial turkeys internationally
- Isolate and (if possible) sporulate oocysts prior to DNA extraction
- Extracted DNA will be PCR amplified and sequenced through Illumina MiSeq
  (NGS on ≤378 PCR +ve samples)

```
@SRR038845.3 HWI-EAS038:6:1:0:1938 length=36
CAACGAGTTCACACCTTGGCCGACAGGCCCGGGTAA
+SRR038845.3 HWI-EAS038:6:1:0:1938 length=36
BA@7>B=>:>>7@7@>>9=BAA?;>52;>:9=8.=A
@SRR038845.41 HWI-EAS038:6:1:0:1474 length=36
CCAATGATTTTTTTCCGTGTTTCAGAATACGGTTAA
+SRR038845.41 HWI-EAS038:6:1:0:1474 length=36
BCCBA@BB@BBBBAB@B9B@=BABA@A:@693:@B=
@SRR038845.53 HWI-EAS038:6:1:1:360 length=36
GTTCAAAAAGAACTAAATTGTGTCAATAGAAAACTC
+SRR038845.53 HWI-EAS038:6:1:1:360 length=36
BBCBBBBBB@@BAB?BBBBCBC>BBBAA8>BBBAA@
```

Raw NGS sequence output



Example NGS Sequence Alignment




```
                1                  10                  20                  30                  40                  50                  60 61
1. Eimeria unknown ...GCTCACTTTGACTTCGTATTATCTCTTGGTGCAGTAAACGGATTAATCTGTGGATTCTTCT
2. Eimeria unknown ...GCTCACTTTGACTTCGTATTATCTCTTGGTGCAGTAAACGGATTAATCTGTGGATTCTTCT
3. Eimeria unknown ...GCTCACTTTGACTTCGTATTATCTCTTGGTGCAGTAAACGGATTAATCTGTGGATTCTTCT
4. Eimeria unknown    GCTCACTTTGACTTCGTATTATCTCTTGGTGCAGTAAACGGATTAATCTGTGGATTCTTCT
5. Eimeria unknown ...GCTCACTTTGACTTCGTATTATCTCTTGGTGCAGTAAACGGATTAATCTGTGGATTCTTCT
6. Eimeria unknown ...GCTCACTTTGACTTCGTATTATCTCTTGGTGCAGTAAACGGATTAATCTGTGGATTCTTCT
```

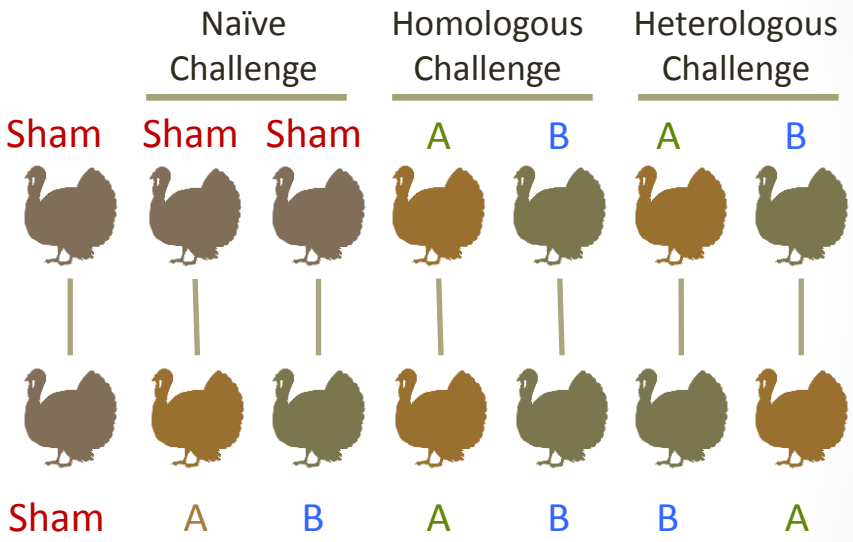
Future Directions

- This project will allow us to explore the global biodiversity of mixed infections using a PCR/NGS approach
- May identify minor (infrequent) species and other previously unidentified species in samples with multiple parasites
- Cross-immunity studies will be performed to establish if protection against all identified *Eimeria* spp. infecting turkeys is generated

Future Directions: Cross-immunity

Method

- Trickle infections
  - Generate sterile immunity
- Homologous and heterologous challenges
- Comparing homologous and heterologous challenges:
  - Protection against lesion formation (high dose challenge)
  - Oocyst shedding (low dose challenge)



Future Directions: Portability

- Portable DNA sequencing
  - MinION
  - SmidgION

https://nanoporetech.com/products/minion

https://nanoporetech.com/products/smidgion

Summary

- Using oocyst morphometrics or biological features of oocysts is misleading and therefore not reliable for *Eimeria* spp. identification
- Universal primers targeting a conserved mtCOI region in all species being tested for ability to more accurately speciate parasites [in mixed infections]
- In the near future, cross immunity studies will be performed to determine if cross species protection is possible

Acknowledgements

Advisory Committee

- Dr. John Barta
- Dr. Nicole Nemeth
- Dr. Leonardo Susta

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- Kobus Van- Heerden

Scholarships

- OVC Scholarship

Parasitology Lab (Room 4818)

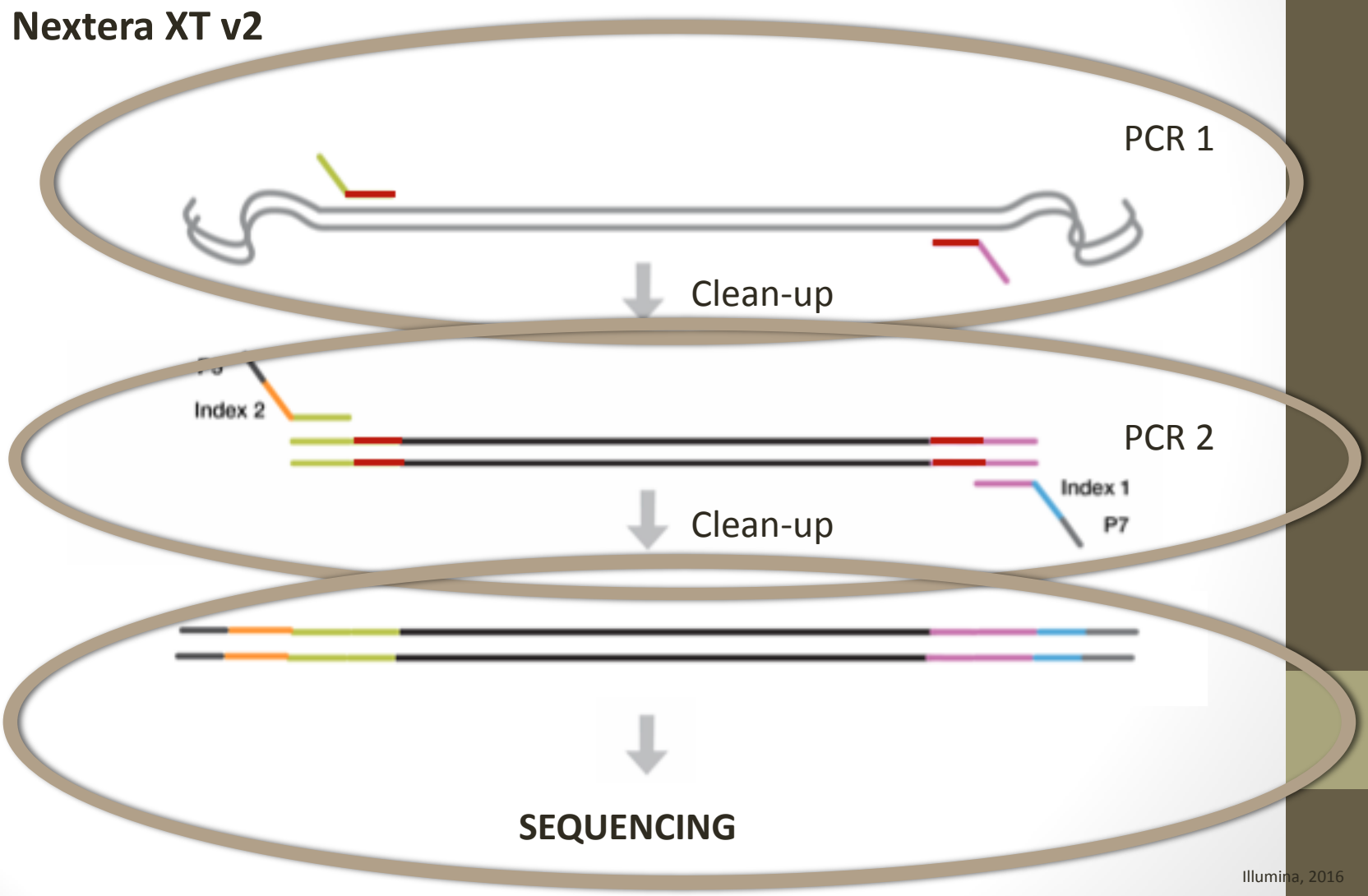
- Julia Whale
- Julie Cobean
- Perryn Kruth
- Ryan Snyder
- Alex Leveille
- Evelin Rejman

Questions?

Limitations

- Availability of samples
  - Countries
  - Quantity
- Variability in medication vs. vaccination of the turkeys
- Time of sample collection
  - Varying periods of shedding

Methods: library preparation



Illumina, 2016