

Schrödinger's Oocyst

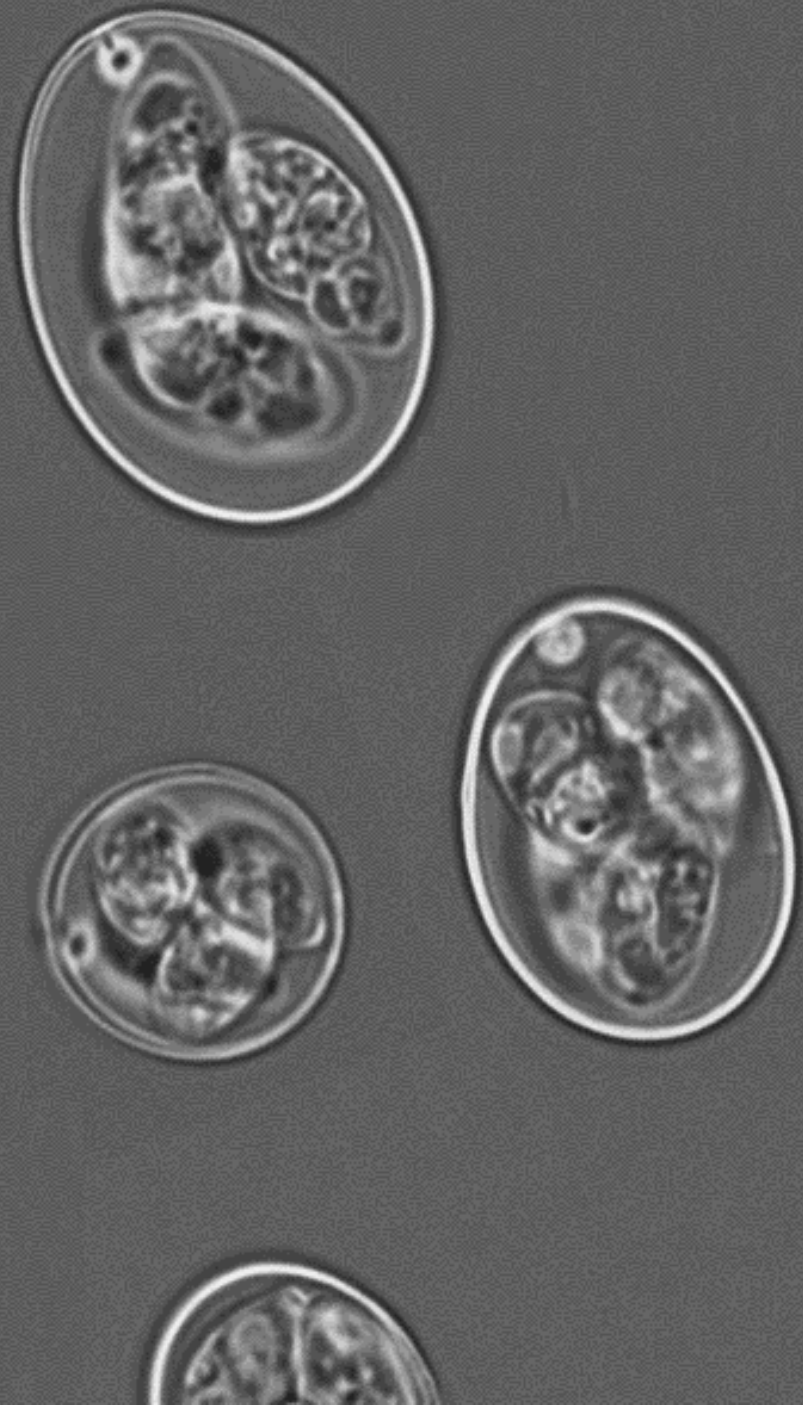
A molecular assay for determining the viability of *Eimeria* oocyst for the optimization of coccidiosis vaccines

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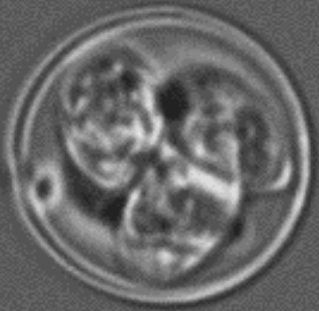
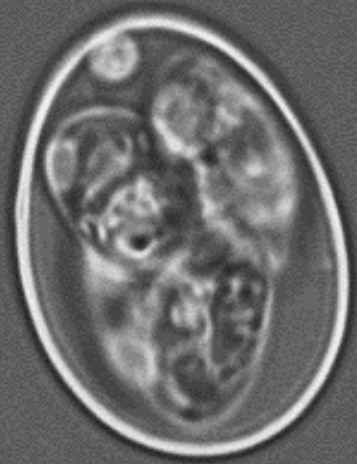
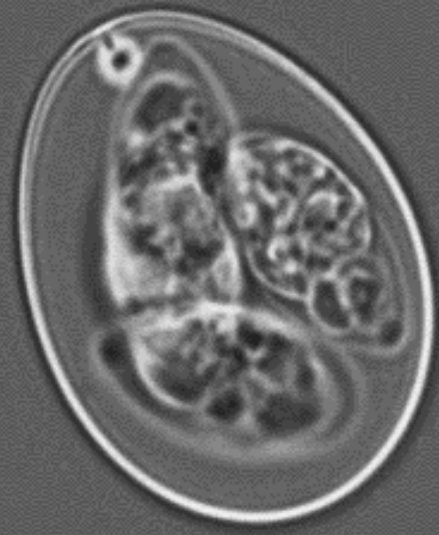
Collaborators & funding-bodies



Ontario Genomics

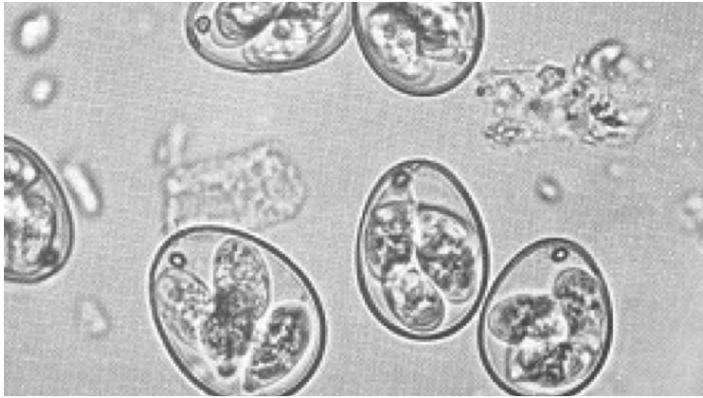


GenomeCanada



Eimeria species and coccidiosis

Eimeria species cause coccidiosis in poultry



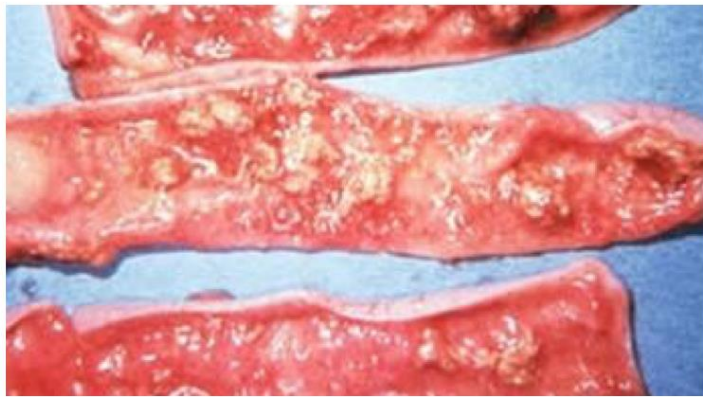
caused by *Eimeria* spp.



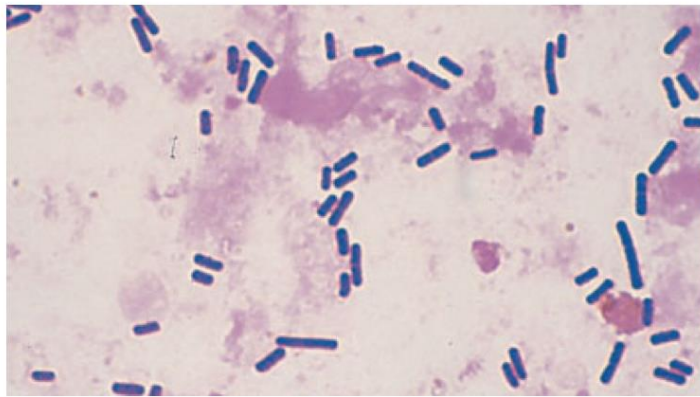
cosmopolitan disease



poor feed conversion efficiency



damage to intestinal epithelium



risk of 2° infection



major economic impact

The hypobiotic, infectious oocyst



4 sporocysts



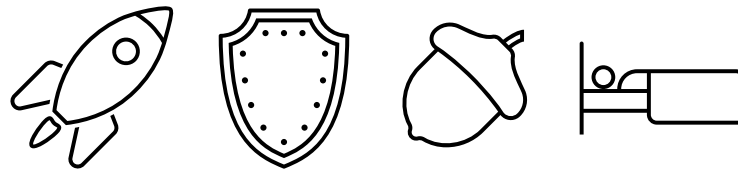
8 sporozoites



sporocyst walls



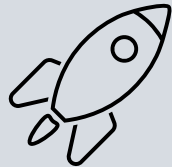
oocyst walls



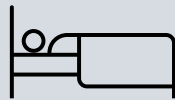
The hypobiotic, infectious oocyst



Protected from the environment by rugged oocyst & sporocyst walls



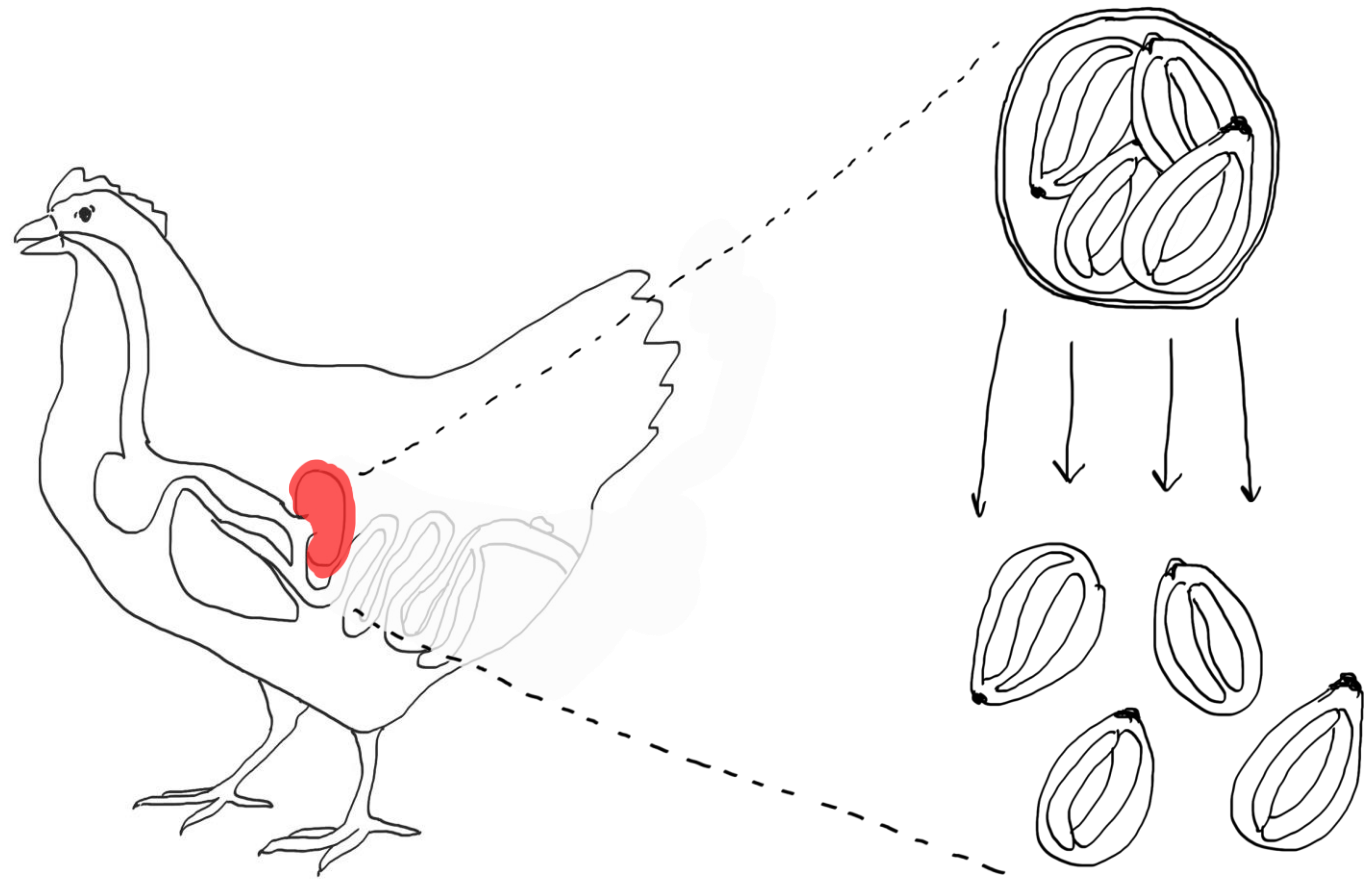
Can only access the resources enclosed within, cannot get rid of waste



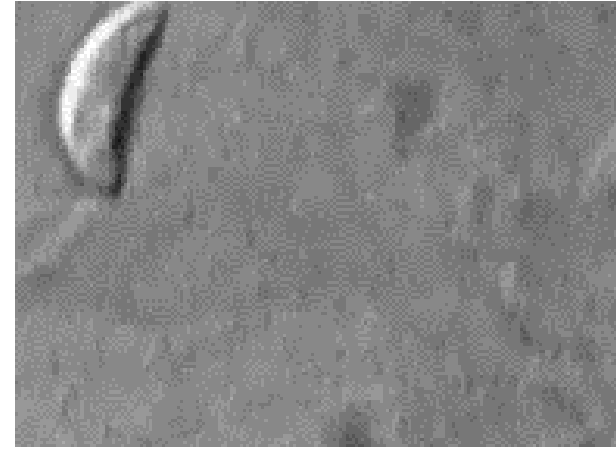
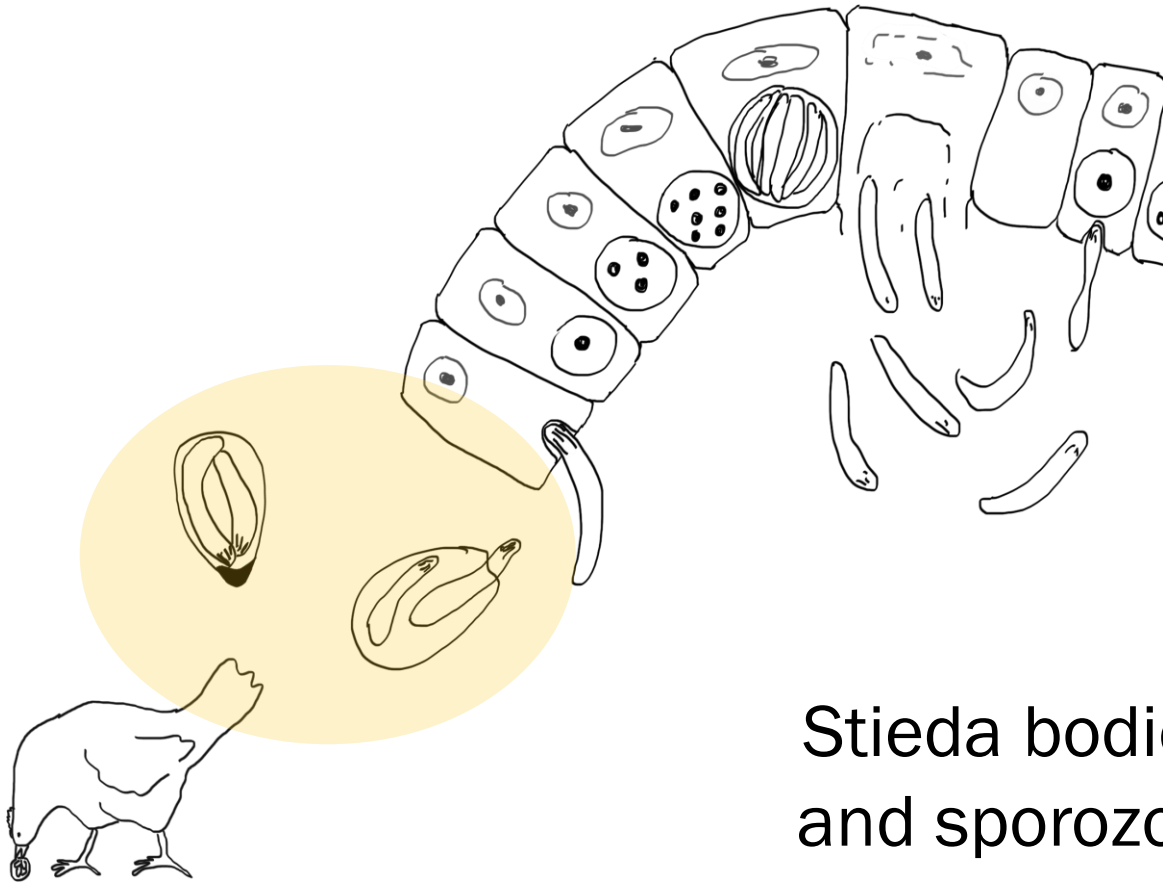
Dormancy ensures maintenance of viability for as long as possible

Initiation of infection

Grinding in the gizzard breaks open oocysts to release sporocysts

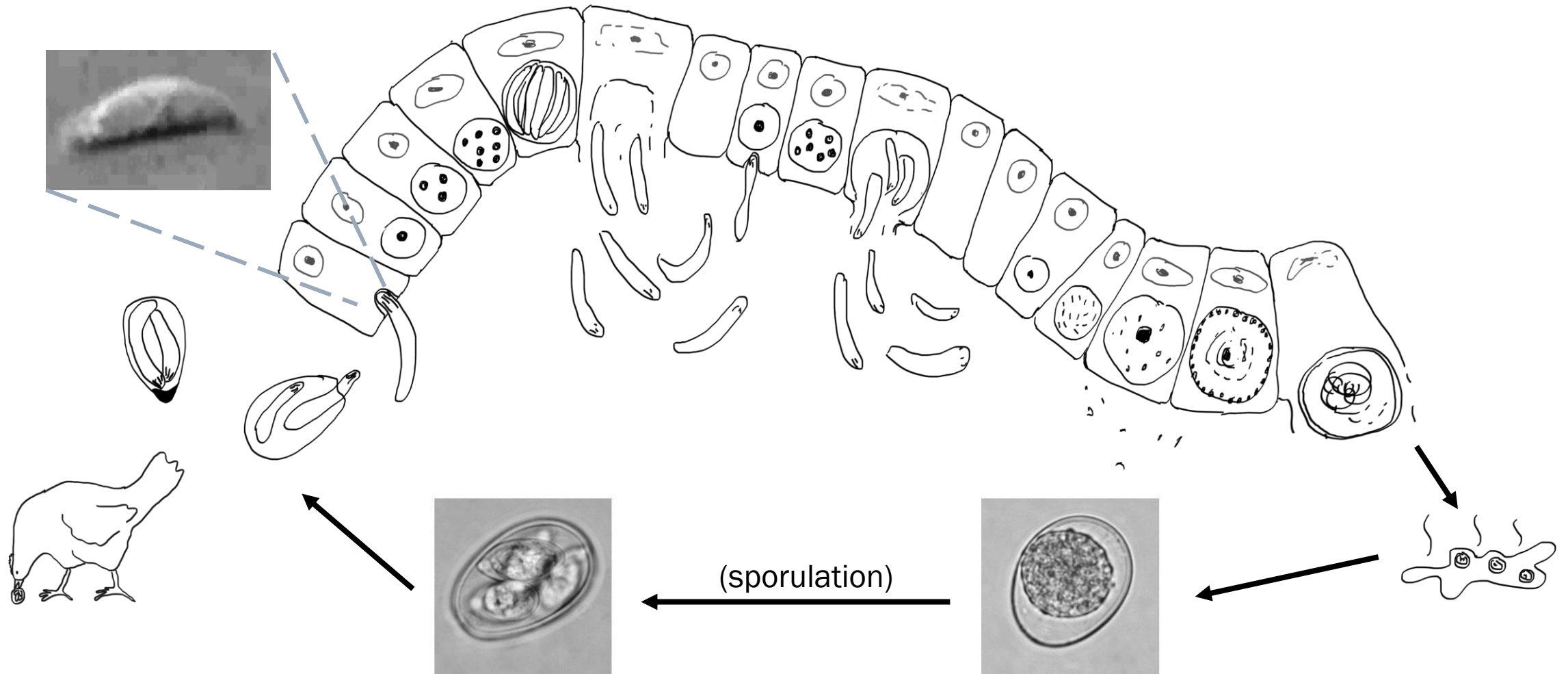


Initiation of infection



Stieda bodies are enzymatically dissolved and sporozoites actively exit the sporocyst

Lifecycle overview

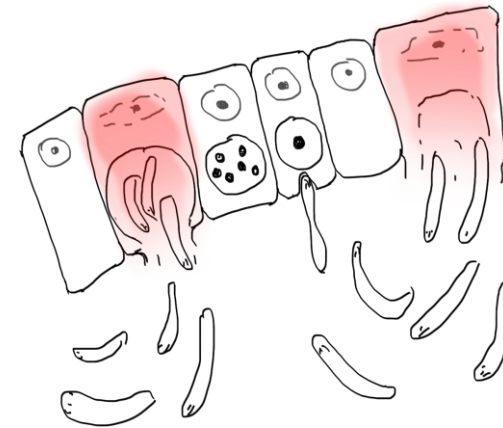
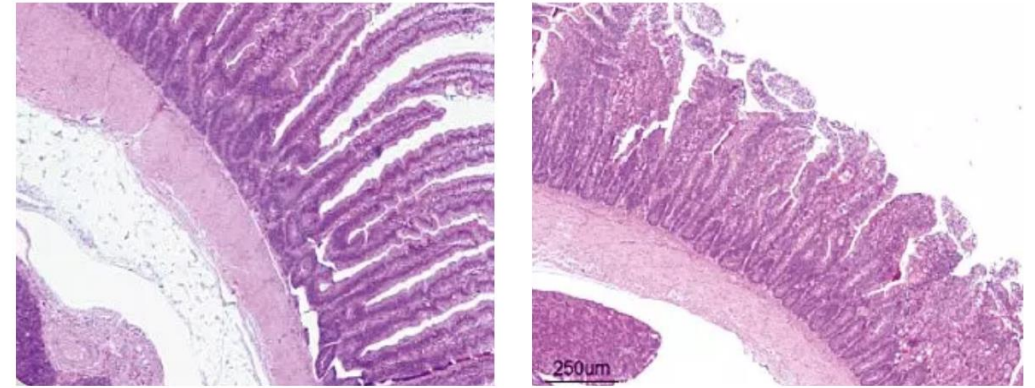


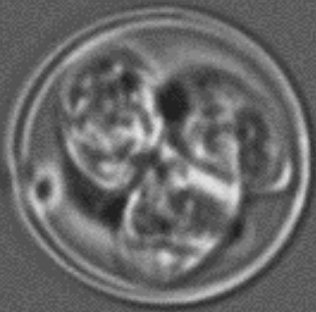
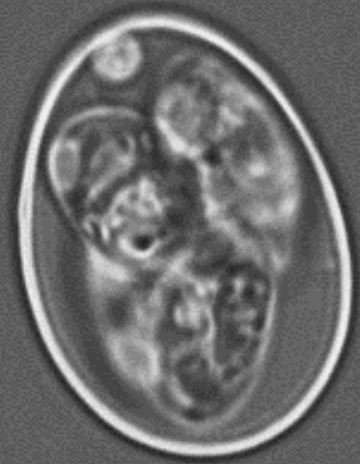
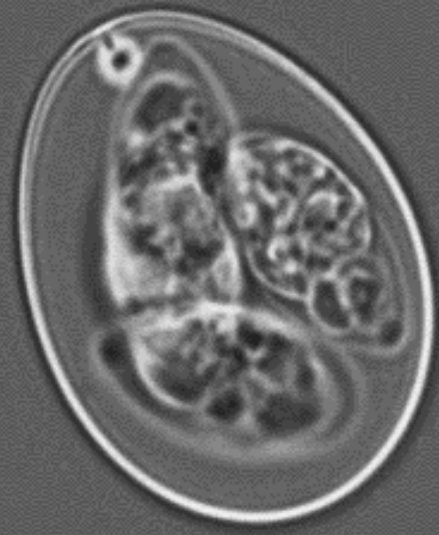
Endogenous development

Merogonic parasite development causes enteritis

Number of round of merogony are a genetically determined feature of each *Eimeria* spp.

- Infection is self-limiting
- Severity directly proportional to # of infective parasites ingested





Live vaccines for
coccidiosis control
& the importance
of viability

Anticoccidials allowed industry intensification, but...

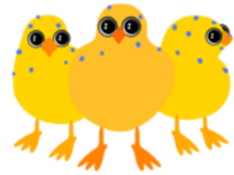
- Ongoing expense
- Demand for RWA products
- Mandatory withdrawal periods
- **Widespread resistance**



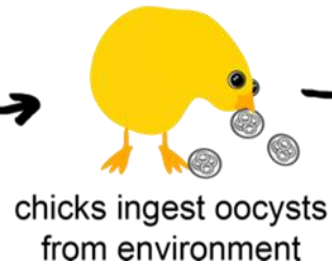
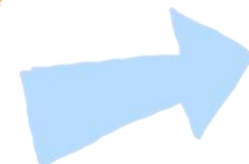
**Immunological control of coccidiosis:
An effective and sustainable alternative!**

Live vaccines for coccidiosis control

chicks are given low doses of viable oocysts



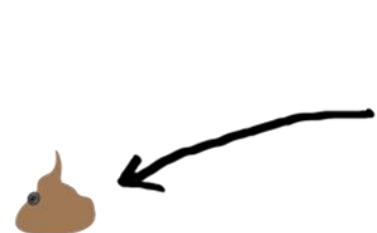
subclinical infection is established



chicks ingest oocysts from environment



second round subclinical infection

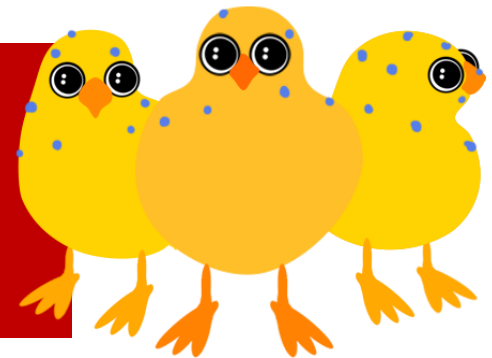


Vaccine oocysts must be viable

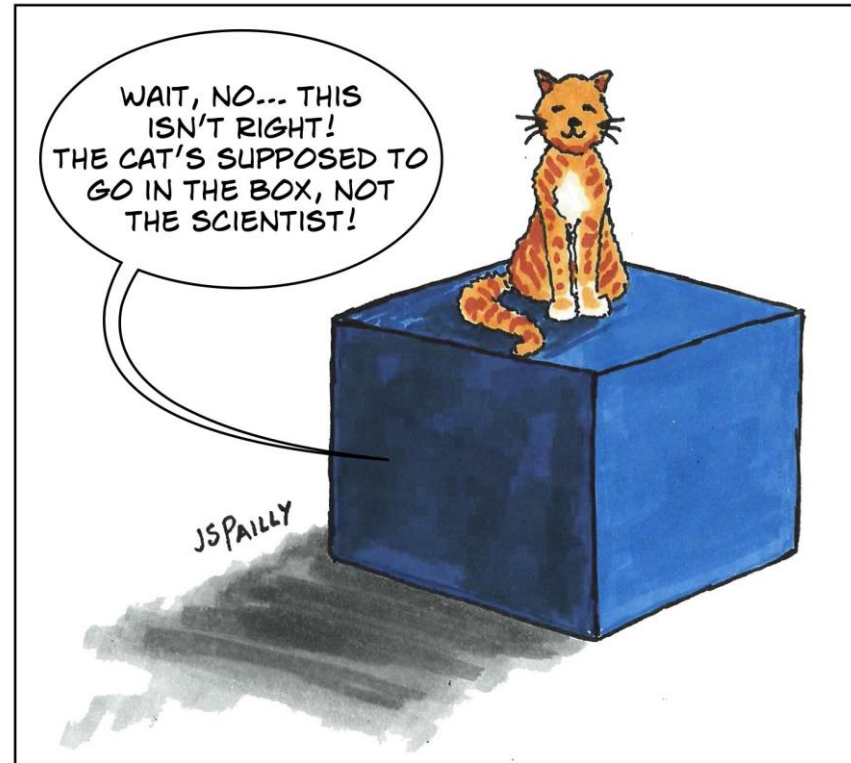
Dose too high = disease; Too low = insufficient immune stimulation

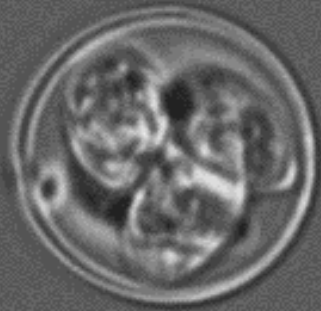
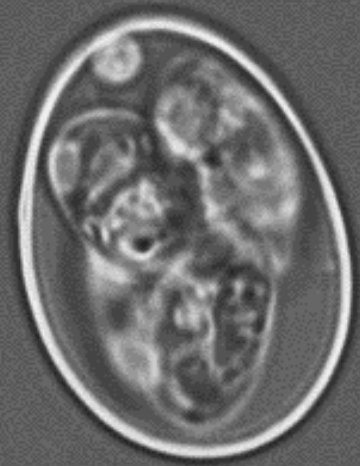
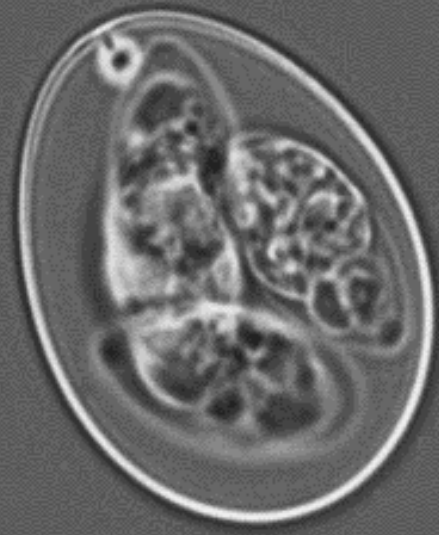
- No morphological differences between live and dead oocysts
- Longevity affected by numerous factors
- Current viability testing strategy: Infection trials

Optimal dosage cannot be determined
without knowledge of oocyst viability



Is it alive, or is it dead?





A new way:
molecular determination
of oocyst viability

Gene expression



Gene:

- What could happen (words, sentences that make up instructions)

Gene expression:

- What is happening (reading the gene and following the instructions)
- Involves production of **mRNA**

Protein:

- What has happened (the end-product of gene expression)

Gene expression



Robust, stable,
long-lived



Short lived!
Abundance reflects
recent biological
activity (aka aliveness)



Robust, stable,
long-lived

Abundance of mRNAs....



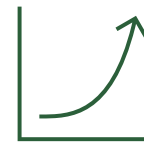
- Abundance of specific mRNAs changes in response to environmental/developmental queues
- Reflects current (\pm) biological activity
- Can be measured with accuracy & specificity (via qPCR)

Assay concept

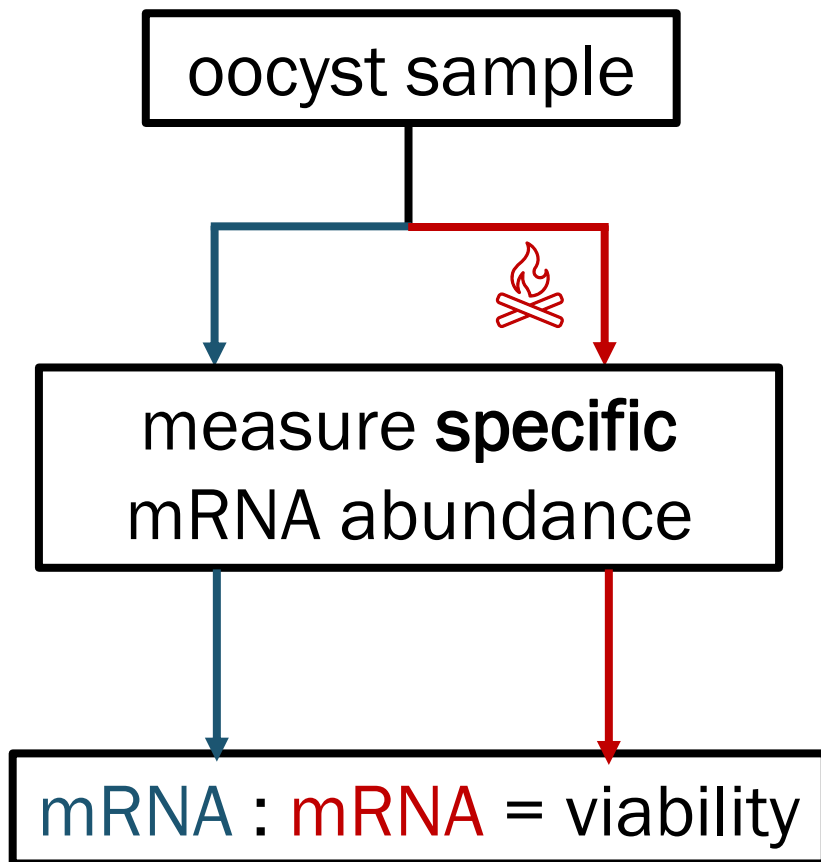


Stimulate parasites with heat (simulate host environment) = big shift in gene expression

Magnitude of shift in gene expression
 \propto oocyst viability



Assay development



- ✓ Low numbers of oocysts required
- ✓ ~5 hours to perform (~1 person hr)
- ✓ Control material generated
- ✓ High reproducibility, consistency of
 - RNA recovery efficiency
 - control abundance
 - technical repeat consistency
 - target quant

Assay development

mRNA : mRNA = viability

... ongoing

- mRNA targets in warmed samples showed increases of abundance from 250 to 9,000% in high viability samples = **High dynamic range**
 - Recent total infectivity loss = near-total loss of response to heating
 - Quality of fecundity data reflecting oocyst infectivity not yet sufficient to provide “gold-standard” support for assay accuracy
- ★ Larger scale live-infection trials will be required

Future directions

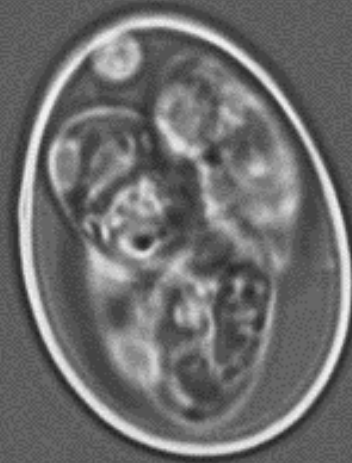
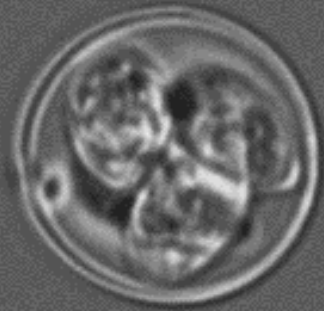
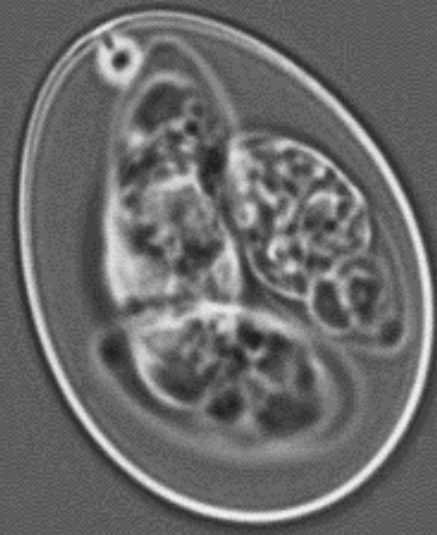
- Further validation via longitudinal trials
- Multiplexing for ability to detect viability of each *Eimeria* species in mixed-species samples
- New technologies (ddPCR, card-based PCR) to improve accessibility
- Application of assay



Recap

- Infection by *Eimeria* spp. is **highly immunogenic**
- Controlled administration of viable oocysts = effective vaccination
- **...but viability must be known!**
- No rapid and accurate way to determine viability currently available
- Concept for assay based on measuring products of **evidence of gene expression**
- Preliminary viability assay shows strong performance
- Further live-infection trials required
- Multiple future applications of the assay





Thank you for having me!

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