A viability assay for *Cyclospora* and its surrogates *Eimeria*

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Summary

To mitigate human health risks posed by *Cyclospora*, produce growers and regulators require tools for assessing not only their presence but also their viability. Comparative microscopic analyses of senescence oocysts identified the presence of autofluorescent granular structures only in dead oocysts of *Eimeria*. Based on the presence or absence of the autofluorescent granular structure, we developed fluorescence-activated cell sorting (FACS) and an AI-based model to distinguish live from dead parasites. RNAseq of senescence oocysts also showed great promise in developing biomarkers to differentiate live from dead parasites. Hence, we are conducting viability assessments using FACS, an AI-based model, and biomarkers identified from RNAseq data. Success would afford the industry and regulators a sensitive and specific assay to diagnose parasite contamination and test the presence of viable protozoan pathogens.

Objectives

1. Adapt and validate the sensitive biomarkers for risk assessments.
2. Develop quantitative viability assays for *Cyclospora* using *Eimeria* as a surrogate.

Methods

- To conduct the viability assessment of *Eimeria*, we utilized senescence oocysts of 0 to 30-month-old oocysts stored at 4°C. We evaluated the morphological changes of the 0 and 30-month-old *E. acervuline* oocysts representing live and dead parasites respectively using light, DIC, phase contrast, and confocal imaging.
- We adopted FACS to differentiate live from dead oocysts using a violet laser (405 nm) with a 525/50 nm filter (AmCyan) and blue laser (488 nm) with a 530/30 filter (FITC, fluorescein isothiocyanate).
- We conducted chicken infection with 1,000 FACS-sorted live (without granular structure) and dead (with granular structure) oocysts.
- The AI model was developed with 200 images each of live, dead, and unsporulated oocysts using YOLOv7.
- We conducted RNAseq with senescence oocysts.

Results to Date

We used *Eimeria* (as a surrogate for *Cyclospora*) to examine morphological differences between live and dead oocysts. Microscopic examinations identified the presence of granular structures in dead oocysts, which are autofluorescent under UV exposure, and autofluorescent intensity is much higher in dead oocysts than in live oocysts (**Figure 1**). By utilizing the autofluorescent intensity, we sorted the live from dead parasites using FACS with specific gates (**Figure 1**). Finally, an *in vivo* infection assay using chicks demonstrated the significantly low infectivity of the FACS-sorted dead oocysts compared to live. We developed an AI model that differentiates live from dead parasites based on the presence of granular structures (**Figure 2**). Our RNAseq experiments with senescence oocysts identified a cluster of genes that are differentially expressed in live and dead parasites (**Figure 3**).

Benefits to the Industry

A globalized food trade, extensive production, and complex supply chains broaden the reach and stakes of microbiological food safety outbreaks. The CDC estimates approximately 48 million people in the United States are sickened by a foodborne illness, annually. The estimated cost of food safety incidents for the U.S. economy is around $7 billion per year. Thus, produce growers and regulators urgently need mitigation tools capable of assessing not only the presence of foodborne pathogens, but also their viability. If successful, our project will provide the produce industry and its regulators with the means to quantify viable parasites contaminating fresh produce. This will aid risk assessment and mitigation, enhancing the safety of fresh produce and the reputation of the fresh produce industry.

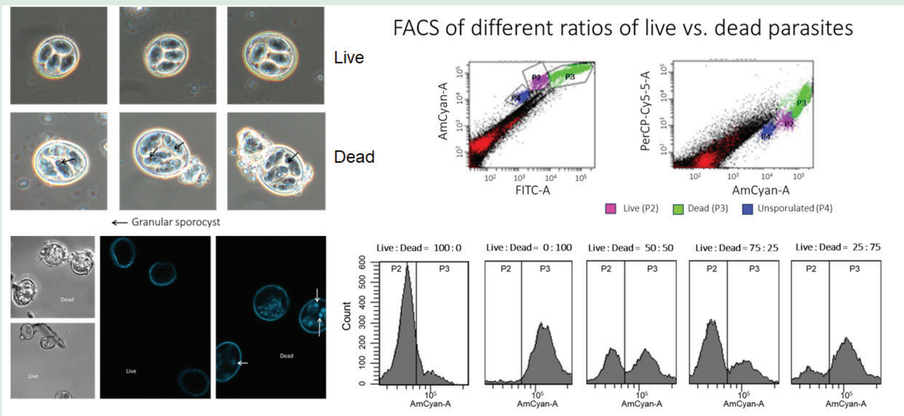

Figure 1. Developing a viability assay using high-resolution microscope and FACS.

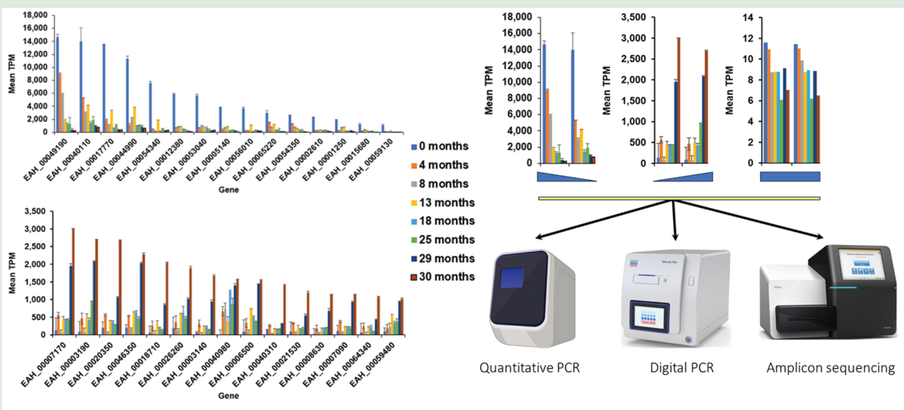

Figure 3. Developing biomarkers to distinguish live from dead oocysts using RNAseq profile of senescence oocysts.

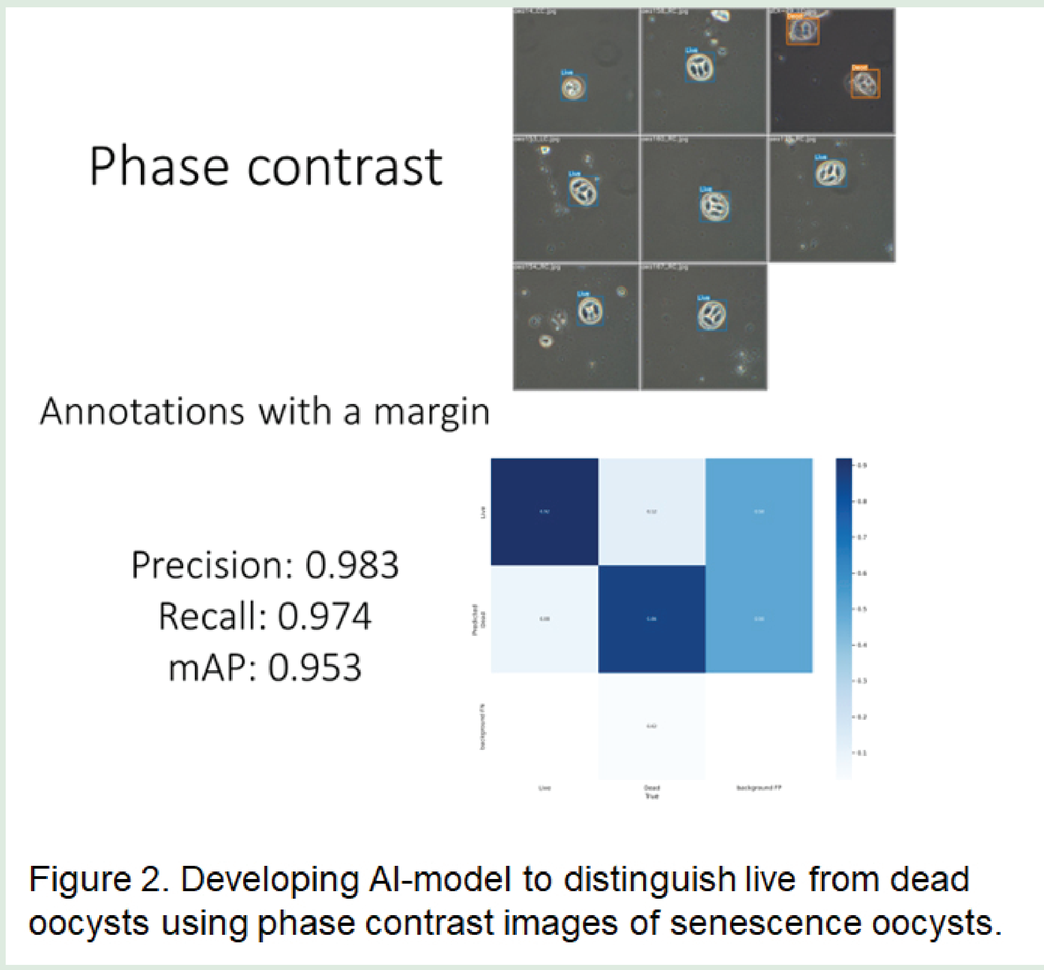

Figure 2. Developing AI-model to distinguish live from dead oocysts using phase contrast images of senescence oocysts.