Determination of physical and chemical mechanisms to prevent *Cyclospora* infection

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Summary

Cyclospora is a ubiquitous foodborne parasite that causes gastrointestinal illness in humans. Although it is difficult to trace *Cyclospora* infections to a single product, foodborne infections are primarily from the consumption of produce. While molecular techniques can be applied as a sensitive tool to identify *Cyclospora* oocysts, the molecular data on the viability/infectivity of oocysts is not available. Currently, the viability of oocyst can only be assessed by analysis of sporulation rates, which must be determined microscopically by a trained investigator. This inability to rapidly determine oocyst viability creates a significant bottleneck for testing new control measures. The primary goal of this work is to identify new control measures for inactivation of *Cyclospora* in agricultural water inputs and on the surface of produce.

Objectives

1. Validate strategies for inactivation of *Cyclospora* oocysts, including gamma radiation, UV, ozonation, and chlorine dioxide gas.
2. Develop an automated method for rapid determination of *Cyclospora* oocyst viability that would enable screening of antimicrobial libraries.
3. Utilize the automated method to identify novel antimicrobial compounds and effective delivery systems leading to inactivation of *Cyclospora*.

Methods

Inactivation methods of *Cyclospora* oocysts are being investigated by dosing oocysts with gamma radiation, UV, ozonation, chlorine dioxide gas, and a library of chemical compounds. The inactivation protocol consists of 1×10^4 oocysts/treatment in water with the same concentration of natural organic matter, at a starting pH of 7.0, and constant temperature of 25°C. Gamma radiation, UV, ozonation, and ClO2 treatments are applied manually. An automated liquid handler will be used for loading 96-well plates with oocysts and dosing with chemical compounds (**Figure 1**). Image acquisition is being automated with a motorized stage mounted on a confocal microscope with a 40X objective. A machine learning algorithm is being developed and evaluated with Matlab software to determine % sporulation from images acquired by the automated system.

Results to Date

Protocols have been established for UV-C, Far-UV, ozonation, and chlorine dioxide gas inactivation methods. A list of antimicrobial compounds has been identified for testing on an automated platform. Inactivation strategies are being tested on *Eimeria* oocysts until *Cyclospora* oocysts are available. To establish an automated method for image acquisition, *Eimeria* oocysts were imaged by various methods in the lab. Confocal images taken with a 40X objective and CMOS camera have suitable resolution for training machine learning algorithms. An algorithm was trained to identify sporulated and unsporulated oocysts (**Figure 2** and **Figure 3**, respectively). A script was written to evaluate the algorithm's performance (**Figure 4**). Thousands of images will be required to fully train the algorithm to identify sporulated oocysts at levels matching trained parasitologist.

Benefits to the Industry

The primary beneficiaries of this project will be the water treatment and produce industries. The benefits to near-term food safety solutions in the produce industry will be dependent on the strategy that yields the best inactivation. Ideally an approved food-safe chemical disinfectant would be successful at inactivation of *Cyclospora*, as identified in the high-throughput screen, which would speed the path forward for a near-term solution. A secondary beneficiary of the project will be other researchers studying inactivation of *Cyclospora* and related parasites. The automated image analysis system that will be developed will lower the barrier of entry for researchers, and increase the number of researchers focused on *Cyclospora* and other parasites important to the produce industry.

Figure 1. Robotic system housed at the Center for Agricultural Synthetic Biology. The system consists of a TECAN Evo 150 liquid handler and plate mover, 3 Inheco heating/cooling stations, two magnetic bead isolation stations, a plate-based centrifuge, a magnetic stirrer, and an imaging plate reader.

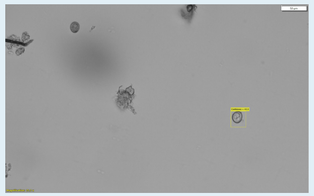

Figure 2. Detection of sporulated *Eimeria* oocysts. A machine learning algorithm was trained in Matlab to identify sporulated oocysts from 40X images. The algorithm detected the sporulated oocyst in this image with 40% confidence (yellow box).

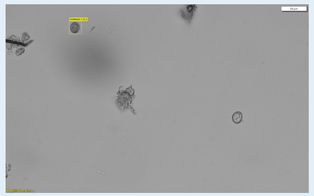

Figure 3. Detection of unsporulated *Eimeria* oocysts. The algorithm was also trained to identify unsporulated oocysts. The algorithm detected the unsporulated oocyst in this image with 23.1% confidence (yellow box).

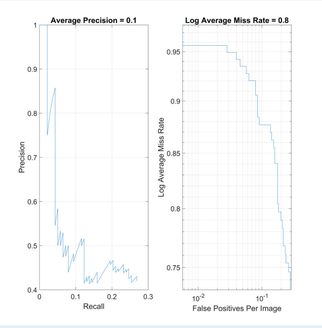

Figure 4. Evaluation of the unsporulated object detection algorithm using an independent data set. Average precision measures the accuracy of the object detector by plotting precision vs recall. Precision measures the percentage of predictions that are correct, and recall measures how good the algorithm is at finding all positives. The log average miss rate compares the number of false positives to the number of false negatives per image. To improve algorithm performance, further training on thousands of images is required.