CENTER for PRODUCE SAFETY

CPS 2020 RFP
FINAL PROJECT REPORT

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

Project Period
January 1, 2021 – December 31, 2022

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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.*

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CPS Campaign for Research

FINAL REPORT

Abstract

Cyclospora cayetanensis is the food- and waterborne coccidian parasite that causes the gastrointestinal disease cyclosporiasis. Recent domestic outbreaks, primarily traced back to contaminated fresh produce, have demonstrated a need for effective control measures. Frustrating efforts in combating cyclosporiasis outbreaks are the time- and labor-intensive methods required to identify the parasites and subsequently assay their viability (infectivity). To address this, we used a combination of robotics-assisted automation and an image detection algorithm based on machine learning and established a pipeline for chemical and physical inactivation assays for *Cyclospora* and surrogate organisms. However, sufficient numbers of viable *Cyclospora* required for this work were impossible to obtain, so we focused the majority of our inactivation work on the closely related and economically important chicken parasite, *Eimeria acervulina*. Although the availability of this parasite was hindered by recent outbreaks of avian influenza, we obtained and purified sufficient numbers of oocysts to assess several methods for chemical and physical inhibition of oocyst sporulation. Our multi-pronged approach examined the efficacy of several chemical phytochemicals of interest combed from the literature as well as exposure to UV light, a ^{60}Co gamma irradiation source, and ozonated water.

Background

Cyclospora cayetanensis is an emerging parasitic coccidian that causes the human gastrointestinal disease cyclosporiasis. To date, humans remain the only recognized host for *C. cayetanensis*. Symptoms of cyclosporiasis include severe diarrhea, dehydration and weight loss, and these symptoms can be especially severe in immune compromised patients (Sarfo et al., 2022). Previously associated with international travel to endemic regions, domestic outbreaks of cyclosporiasis have been reported in recent years in the United States. The first domestic outbreak in the United States occurred in 1990 and since 2000, nearly every year has seen an outbreak of cyclosporiasis, with the first instance of domestic transmission reported in 2018 (Almeria et al., 2019). These outbreaks are invariably linked to contaminated fresh produce such as lettuce, raspberries, and cilantro, thus highlighting the need for cost-effective and safe produce sterilization methods.

C. cayetanensis is a small (~8-10 uM) apicomplexan with a complex life cycle. The infective stage manifests as the sporulated oocyst. Sporulation occurs in the environment and requires from one to two weeks, during which the amorphous internal structures of the oocyst reorganize and form two visually distinct sporocysts. These football-shaped structures each contain two sporozoites, which are ultimately responsible for host cell invasion. The life cycle continues within host tissues upon infection via the fecal-oral route. The "cyclosporiasis season" in the United States generally spans May through early October, with peak season occurring between June and August and sporadic cases occurring through the winter months, presumably linked to international travel. In 2022, domestic outbreaks in the United States were traced to bagged salad mix from a specific producer, and meals served in a correctional facility in the southeastern USA.

To evaluate control efficacy, oocyst viability (as evidenced by sporulation) must be determined. This requires microscopic examination by a trained researcher. Even under this "gold standard," investigators often score samples of sporulated oocysts with wide margins of error across individuals. Therefore, an easy-to-use, objective method to score viability is needed. We sought

to establish a largely automated pipeline to inactivate *Cyclospora* and closely related surrogate parasites, and to objectively quantify viability. Inactivation and viability scoring of tens of thousands of oocysts can be accomplished with our workflow in mere days.

During this funding period, we have exploited the availability of a relatively low-cost robotic liquid handling system to screen a battery of compounds in a series of inactivation assays. In addition, we tested the effects of UV light, ozone, and gamma irradiation. In each assay, the final step is the collection of scanned images of treated parasites, which are then fed to an image detection model to identify and count sporulated and thus infectious oocysts. Since nearly all *Cyclospora* cases submitted through state labs and health departments arrive under fixative and are thus nonviable, we have validated the pipeline using the more readily available coccidian parasite of chickens, *Eimeria acervulina*.

Research Methods and Results

Objective 1. Validate strategies for inactivation of Cyclospora oocysts, including gamma radiation, UV, ozonation, and chlorine dioxide gas.

Eimeria purification

To maintain viability, *Eimeria acervulina* must immediately be purified from chicken feces upon arrival, otherwise sporulation efficiency rapidly diminishes in storage. Chicks were inoculated with *E. acervulina* according to protocols in the Jenkins lab at the U.S. Department of Agriculture according to Tucker et al. (2021). Immediately after collection, infected chicken feces were shipped overnight to the University of Tennessee. Upon receipt, the material was vigorously mixed with tap water at a ratio of approximately 1:5 and further mixed into suspension for 30 minutes using a magnetic stir bar. The resulting slurry was squeezed through cheesecloth placed in a funnel and collected in a large beaker. Aliquots were provisioned into 50-mL conical tubes and centrifuged at 2,000 rpm for 10 minutes. Pellets were resuspended in saturated sodium chloride (360 g/L), centrifuged at 2,000 rpm for 10 minutes, and allowed to sit for 10 minutes. The top fractions were collected, washed with water, and centrifuged at 2500 rpm for 15 minutes. Supernatant was discarded and this step was repeated twice more to remove residual salt. Finally, pellets were combined by resuspending in water. To induce sporulation for all experiments, oocysts were resuspended in 2.5% potassium dichromate and placed at 25˚C in the dark for 7–14 days.

UV inactivation assays

Rather than using a continuous flow device, UV-C testing was accomplished using a custom-built chamber (**Figure 1**). An advantage of this approach is obviating the need to reconcentrate oocysts for sporulation and scoring. On opposing walls in our chamber, 1/2-inch diameter holes were drilled to position the light source at various distances corresponding to a series of UV-C irradiation dosages. Treatment distances, and thus UV intensity, were determined using an International Light Technologies IL1700 radiometer in a dark room, calibrated to 222, 254, and 282 nm. Oocysts were treated for 1, 3, and 5 minutes at the desired UV-C dose using a Viqua VH2000 UV bulb (model 602805). For far UV testing (222 and 282 nm), a Sterilray™ Microbe Buster™ apparatus was used, with the ballast housing positioned at appropriate distances from the sample. Following treatment, oocysts were mixed 1:1 with 2.5% potassium dichromate and incubated in the dark at 25°C for 7–14 days. Oocysts were imaged at 60x under immersion oil using confocal microscopy and manually scored for sporulation.

UV-C inactivated *E. acervulina* in a dose-dependent manner (**Figure 2**). The initial sporulation rate of purified E. *acervulina* was calculated as ~11.9%. All plates were imaged at 14 days

post-treatment and sporulation rates were scored. A significant difference in 1- versus 5-minute treatments was observed for each dose from 30-200 mJ/cm^2 ($p<0.001$). At 0 mJ/cm^2 dose, a significant difference between 1- and 5-minute treatments was noted ($p=0.043$, unpaired t-test) that indicates intrinsic variability in sporulation within the sample, since this dose received no UV-C light. At the two lowest doses tested, 30 and 40 mJ/cm^2, time under irradiation rather than irradiation intensity alone had a more substantial impact on sporulation, congruent with the notion that lower doses of UV require longer exposure times to inactivate the oocysts. At the time of this report, the MicrobeBuster™ has had to be reconfigured to ensure the proper function of a toroid assembly, and testing is currently underway.

We have noticed that, while the trends remain similar across individuals, the total numbers of sporulation counts can differ depending on the investigator. Therefore, three independent counts on the same images are required to ensure the accuracy of the scoring, supporting the need to refine our automated image detection method. Further, UV inactivation has been scored specifically as a function of sporulation relative to total oocyst counts, per field of view (multiple fields of view; n>500 oocysts/treatment). UV-C appears inconsequential to oocyst physical integrity.

Ozone inactivation assay

Ozone was generated using pure oxygen passed through an A2Z Ozone Inc. portable ozone generator and bubbled into a graduated cylinder containing 500 mL sterile water. After 50 seconds, the ozone concentration was measured at 2.5 ppm according to spectrophotometry (Hach DR1900) using AccuVac ampules containing indigo trisulfonate. Twelve mL of ozonated water was added to 3 mL of *Eimeria* (concentration: $7.5*10^6$ oocysts/mL) and left at room temperature for 10 minutes. The oocyst suspension was pulled through a 0.22-µm filter into a 50-mL syringe, enabling oocyst capture in the filter and separating organic matter from the treated water. The syringe plunger was removed and the filtered water was measured for residual ozone concentration. After 10 minutes the residual ozone concentration was measured at 0.2 ppm. Sterile water was then passed back through the syringe to release the oocysts from the filter membrane and facilitate collection. Residual ozone was quenched using 6% (w/v) sodium thiosulfate, which was also added to the negative control samples. *Eimeria* oocysts released from the filter membranes were resuspended in water, after which they were reconcentrated by passing the suspensions through silica columns (Epoch Bioscience) over a vacuum. Concentrated oocyst pellets were then collected in 2.5% potassium dichromate and allowed to sporulate in the dark at 25°C as above. A viable batch of oocysts arrived in mid-January and the first ozone treatment was completed on 1-27-23. Pending the results of this initial test, we will tailor ozone dosage to determine the optimal dose, followed by modifying variables such as organic matter content and pH (increasing pH alone leads to a reduction in sporulation efficiency).

Cobalt-60 irradiation

We used the ^{60}Co source at the Leach Nuclear Science Center at Auburn University (**Figure 3**) to assay the effects of gamma rays on oocyst viability. Briefly, purified *E. acervulina* were aliquoted into 15-mL conical tubes and placed at the appropriate distances from the gamma ray source such that doses of 0, 0.25, 0.5, 0.75, 1.0, and 2.5 kGy would be administered simultaneously. The 0 kGy control sample was placed outside of the chamber to ensure no gamma ray exposure. The first batch of *E. acervulina* tested showed that they were nonviable upon receipt. This set of experiments will therefore be repeated using the latest oocyst shipment from USDA.

Objective 2. Develop an automated method for rapid determination of Cyclospora oocyst viability that would enable screening of antimicrobial libraries.

Cyclospora imaging and viability scoring pipeline

Region-based Convolutional Neural Network (RCNN) modeling is a widely used deep-learning method for image processing and was used to conduct target cell recognition. A well-tuned and trained model can serve as a classifier that distinguishes sporulated oocysts from other regions (including nonsporulated oocysts and background areas) in scanned images. With the model, it is possible to identify the location and dimensional size of sporulated oocysts.

To train the model, purified *Cyclospora* oocysts were imaged at 60x under oil on an Olympus Fv1000 confocal microscope equipped with the cellSens software package (Olympus). Several thousand oocyst images were saved for subsequent training of our image detection algorithm. To generate the training set, images were imported into the Image Labeler application in Matlab (Mathworks) and region of interest (ROI) boxes were drawn around unequivocally sporulated oocysts. To qualify for inclusion, two clearly defined sporocysts had to be observed within the oocyst wall. Sporulating or partially sporulated oocysts and sporulated oocysts whose periphery was partially obscured by the image boundary were not designated as ROIs. **Figure 4** shows an example of a fully annotated frame, where green boxes indicate ROIs.

Our image detection model currently performs with an ~80% success rate, meaning that compared to a trained researcher, the model predicts sporulation correctly 80% of the time. We continue to generate and annotate newly acquired images to fine-tune image detection accuracy. Since initiating this work, we have partnered with investigators well versed in machine learning applications, expediting our progress in increasing algorithm performance. Many thousands of images have been scanned but only ~500 have been fully annotated with the Image Labeler for AI training. In the future it will be useful to develop and train a similar algorithm for *Eimeria acervulina* sporulation detection, given how difficult it has been to obtain viable *Cyclospora*.

Objective 3. Utilize the automated method to identify novel antimicrobial compounds and effective delivery systems leading to inactivation of Cyclospora.

Phytochemical inactivation assays

Various compounds showing promise for oocyst inactivation were pulled from the literature. To date, these include quercetin, luteolin, and cucurmin (each in shellac-encapsulated and free forms), thymol, caffeic acid, ferulic acid, carvacrol, catechin and epicatechin, resveratrol, apigenin, and ellagic acid. 100% DMSO and 0.1N NaOH were used as positive controls after showing efficacy while testing our workflow, and water was used as a negative control. Purified oocysts were mixed at a ratio of 1:1 with each compound in a 96-well plate and incubated in the dark at 25°C for 48 hours. Experiments were performed in triplicate. Dispensation, mixing, and aspiration of compounds and/or water was accomplished using the Opentrons OT-2 liquid handling robot running OS 4.7 (Opentrons, Brooklyn, NY). After 48 hours, the compounds were replaced with distilled water. Two subsequent washes with distilled water were performed, after which the water was removed and replaced with 2.5% potassium dichromate. Oocysts were then incubated in the dark at 25°C for at least 7 days, after which they were imaged and manually scored.

No phytocompound tested to date showed any appreciable effect on preventing sporulation. Some representative data for three compounds is given in **Figure 5**. However, we did validate our workflow with the positive and negative control treatments. Therefore, we have built the capacity to screen additional compounds in future collaborations.

LAMP isothermal PCR

While performing this work for CPS we also engaged in the molecular genotyping and surveillance of *C. cayetanensis* under the guidance of the Centers for Disease Control and Prevention (CDC) and the Tennessee Department of Health (TDH). As a corollary to this effort, we tried to establish a rapid, sensitive, and field-efficient diagnostic test for *C. cayetanensis* detection. LAMP isothermal PCR uses a series of internal and loop primers (targeting molecules) to bind DNA through complimentary base pairing, which then facilitate loop-mediated replication at a constant temperature. Therefore, this method requires only a heat source such as a water bath or incubator to activate the reaction, versus conventional or real-time PCR, which each require expensive, specialized equipment.

Each *C. cayetanensis* oocyst harbors three unique genomes: nuclear, mitochondrial, and apicoplast (a plastid organelle). Initial LAMP primers targeting the nuclear gene *TufA* and two apicoplast targets (**Table 1**) were designed using the New England Biolabs (NEB) LAMP primer design tool (http://lamp.neb.com/) and used to amplify *C. cayetanensis* DNA using the WarmStart Colorimetric LAMP assay kit. We chose nuclear and apicoplast targets after observing off-target amplification using mitochondrial primers. DNA isolated from a purified *C. cayetanensis* culture or the purified oocysts themselves were used as the reaction template. Incubation at 65°C for 30 minutes was performed in a water bath and any resulting color changes, based on the inclusion of phenol red pH indicator, were used to establish a positive vs. negative reaction (example, **Figure 6**).

Initial results show promise for this method as a rapid presence/absence test for *Cyclospora*, with the primary advantage being minimal equipment required. However, the specificity of the reaction needs to be improved. We will continue to test further iterations of this method, ultimately testing spiked produce to determine the sensitivity and specificity, and to find optimal primer sets. Sensitivity of some genotyping primer pairs approaches 5 oocysts/mL, so we expect to achieve similar sensitivity using a refined iteration of our LAMP method.

Outcomes and Accomplishments

1. We established and validated a largely automated pipeline for oocyst treatment and viability scoring, using an affordable, open-source robotics platform in conjunction with machine learning to assess oocyst viability. The cost-effective Opentrons OT-2 robotics platform is open source and both the protocol and hardware definition files can be distributed.
2. We have defined a minimal UV-C dosage regimen (distance and time) required to prevent sporulation in *E. acervulina* oocysts. This basic study will allow us to conduct further analyses on systems designed for field implementation. Further, we can use this method to treat any number of organisms to determine best practices for UV sterilization. Since we tested only purified oocysts, further work needs to address more pragmatic concerns, such penetrating the convoluted surface areas of various fruits and vegetables and determining the best method to dose a given area in a production facility.
3. We developed a protocol for treating *C. cayetanensis* and surrogate organisms with ozonated water. Initial testing was recently completed, and the first batch of treated organisms was ready for annotation and scoring in early February 2023.
4. Capitalizing on our funding to perform molecular diagnostics with *C. cayetanensis*, we designed and tested the first iteration of a functional, field-friendly molecular diagnostic tool for *Cyclospora* detection using LAMP isothermal PCR. Once optimized, this tool could serve as an easy-to-use and rapid test for *Cyclospora* in produce washes or macerated materials.

Summary of Findings and Recommendations

During this funding period, we identified at least two methods for oocyst inactivation, with several experiments still underway due to sporadic and late availability of sufficient quantities of oocysts. First, UV-C irradiation was successfully employed to inhibit sporulation across a series of doses and time points. Since we noted a significant difference in the efficacy of UV-C irradiation at nearly every dose tested between the 1- and 3-minute time points, we have applied treatments with finer temporal resolution to oocysts derived from the same USDA batch of *Eimeria*. By further refining the dosage curve, we will define a minimal UV exposure time required to sufficiently inhibit oocyst development toward minimizing energy inputs in field applications.

UV-C treatment for sterilization of fresh produce has demonstrated significant benefits, including cosmetic improvement in button mushrooms with the prevention of browning (Wang et al., 2022), maintenance of post-harvest flavor profile in pepino fruits (Zhao et al., 2021), and accumulation of human health-promoting compounds in tomato fruit (Yan, et al., 2020). Thus, the benefits of UV-C inactivation methods are manifold. Upon completion of 222 and 282-nm testing, we expect to maintain the benefits of UV treatment at 254 nm while significantly lowering health risks associated with harmful wavelengths (Schuch & Menck, 2010; Zaffina et al., 2012).

In addition to UV light, we tested a battery of phytocompounds for their ability to disrupt oocyst development. While most compounds tested to date show little promise, we did develop and validate a largely automated pipeline to assay the disinfectant properties of antimicrobial compounds. While testing various methods to establish a positive control treatment, it was noted that pH modification using 0.1N NaOH was an effective measure in blocking development through sporulation. Interestingly, reducing pH using 1N HCl had no effect, showing that mildly alkalizing, but not acidifying, *Eimeria acervulina* suspensions can reduce viability. It would be valuable to determine the minimal concentration of NaOH or other alkalizing agents sufficient to prevent foodborne transmission of infective oocysts. One avenue for further exploration could be modifying pH in produce washes prior to further inactivation using UV light. Along these lines, synergism between or among inactivation methods used in this work could lead to new self-contained systems for field implementation.

Infectivity assays can be used to determine the viability of some coccidians in the endogenous phase, or host tissue penetration (Felici et al., 2021). However, since no method for culturing *Cyclospora cayetanensis* has yet been developed, evaluating the success of inactivation assays currently relies on microscopic examination of treated oocysts. We noticed first-hand that this method is subject to variation depending on the relative experience of individual researchers. To obviate complications involving human error in scoring oocysts, we developed an automated image detection system capable of accurately predicting viability based on sporulated state. Algorithm training sets are still being analyzed to increase the success in sporulation detection, with current a detection accuracy of ~80% on training sets. Accompanied by a largely automated and standardized screening assay, we hope to deliver tools that will alleviate the burden of manual counting and reduce the subjectivity inherent to viability screening. By making this algorithm publicly available, we will bolster the capacity for rapid screening of test compounds across research and production facilities.

By mid-February 2023, data from the initial ozone screening and gamma ray testing will be available for analysis, potentially increasing the number of validated methods to break the life cycles of food- and waterborne coccidians. Ozonated water was shown to reduce the incidence

of *Giardia* contamination in drinking water (Kondo et al., 2020) and has proven effective to inactivate *Cryptosporidium parvum* (Morrison et al., 2022). Further, ozone exposure has demonstrated benefits in produce such as strawberries (Piechowiak et al., 2022). However, as exposure time increases, consequences begin to arise, such as a reduction in vitamin content or the development of unwanted cosmetic traits (reviewed in Sachadyn-Król & Agriopoulo, 2020). Thus, our current method relies on decontamination in a matter of minutes.

The above highlights some significant findings from the current work. As previously stated, a major hindrance to completing experiments has been the availability of test organisms. A coordination between CPS and local health departments in which *Cyclospora* specimens are received, or with the FDA, could help to promote awareness of the need for viable test organisms to combat the spread of cyclosporiasis.

References cited

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APPENDICES

Publications and Presentations

Presentations:

1. Determination of Physical and Chemical Mechanisms to Prevent *Cyclospora* Infection. CPS Research Symposium, La Jolla, California, June 21-22, 2022. [poster]

Manuscripts in preparation:

1. Myers, A., Baumann, A., Kazerooni, N.K., Wise, A.M., Lenaghan, S.C. Determination of optimal dosages for UV-C and far UV irradiation of *Eimeria acervulina* oocysts.
2. Baumann, A., Sears, R., Morgan, B., Lenaghan, S.C. Kinetics and mechanism of Cobalt-60 gamma ray inactivation of the chicken parasite *Eimeria acervulina.*
3. Baumann, A., Lenaghan, S.C. A rapid isothermal PCR diagnostic tool for detection of *Cyclospora cayetanensis* oocysts on fresh produce.
4. Yang, R., Baumann, A., Myers, A., Kazerooni, N.K., Lenaghan, S.C. Machine learning facilitates automated viability determination for the coccidian *Cyclospora cayetanensis*.

Budget Summary

The total amount awarded ($395,464) was sufficient to perform the necessary experiments and support the required personnel. Our *Cyclospora*/*Eimeria* work was additionally funded by TDH, so the entire amount awarded by CPS was not exhausted. For instance, consumables required to run the Opentrons robotics platform were split across these funding sources. As a result, some money was returned to CPS.

Figures 1–6 and Table 1 (see below)

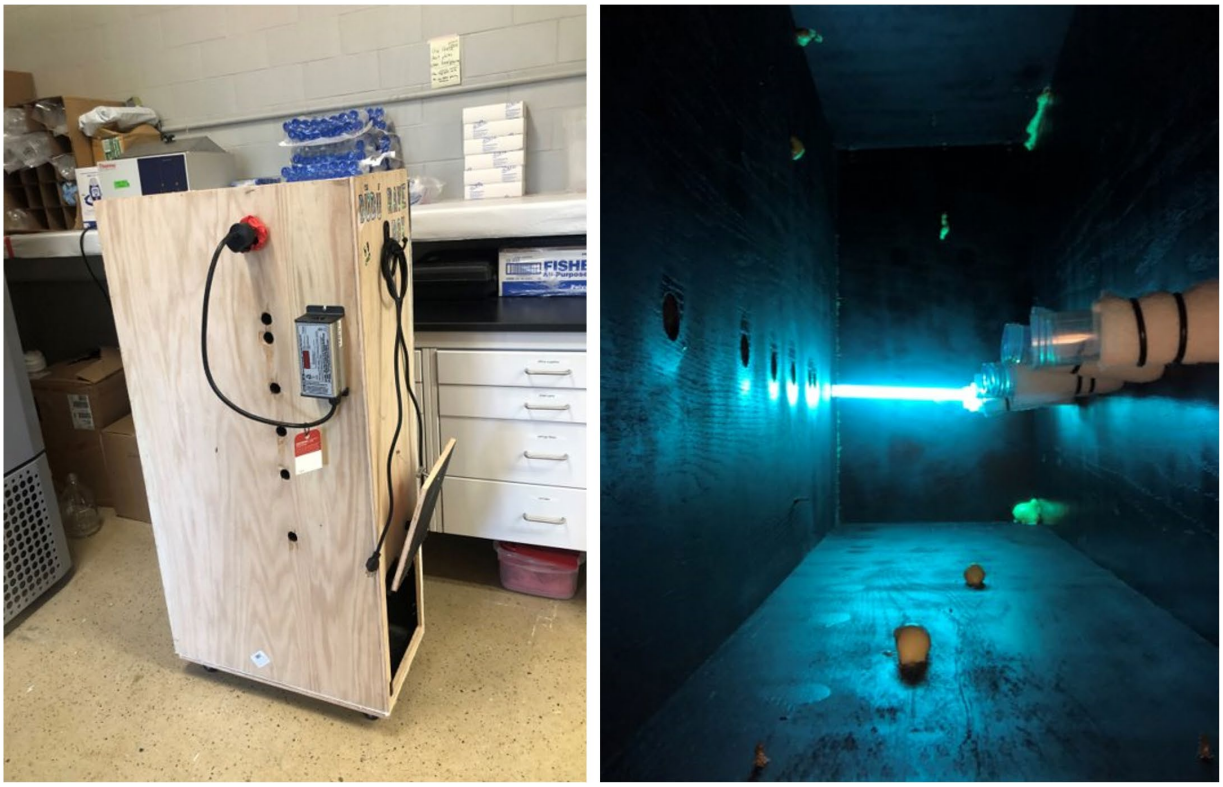

Figure 1: UV treatment chamber with Viqua UV-C bulb assembly attached; samples are loaded into the chamber and the door is sealed (left). Right: 254nm UV bulb positioned at slot 7, corresponding to the 30mJ/cm^2 dose.

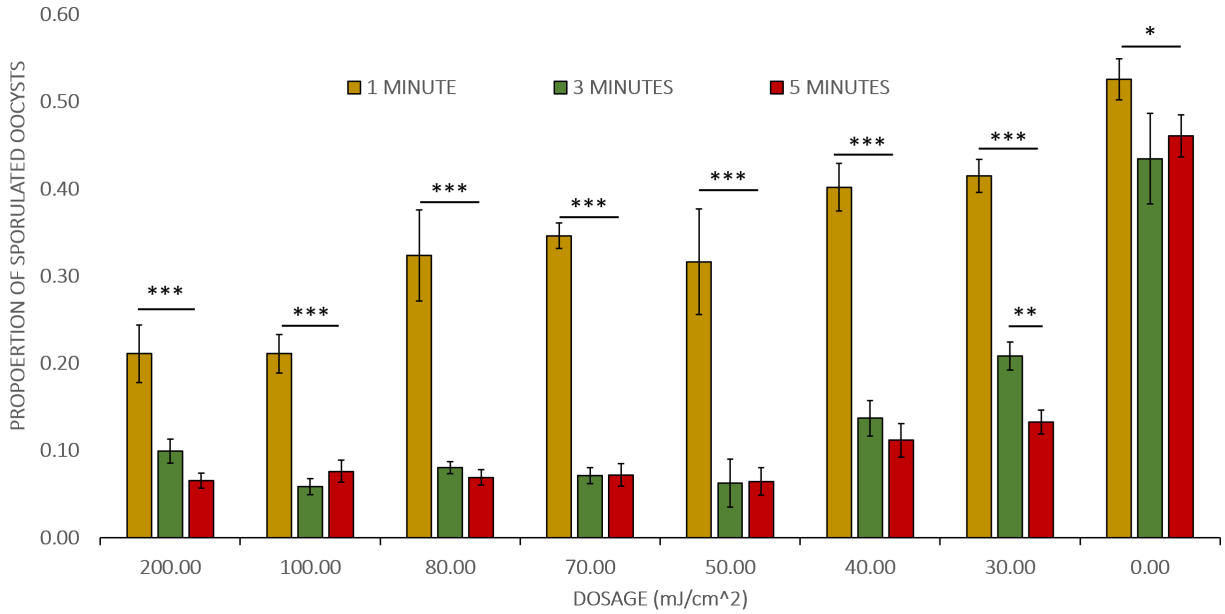

Figure 2: Effects on *Eimeria acervulina* sporulation efficiency of exposure to UV-C irradiation at 1, 3, and 5 minutes. Sporulation is scored as the proportion of oocysts sporulated versus the total per field of view (FOV). Five counts to 100 oocysts across 10 to 20 FOVs were performed for each treatment (dosage and time).

Figure 3: *Eimeria acervulina* oocysts suspensions placed at the appropriate calculated distances from a ^{60}Co gamma ray source housed in the Leach Nuclear Science Center at Auburn University.

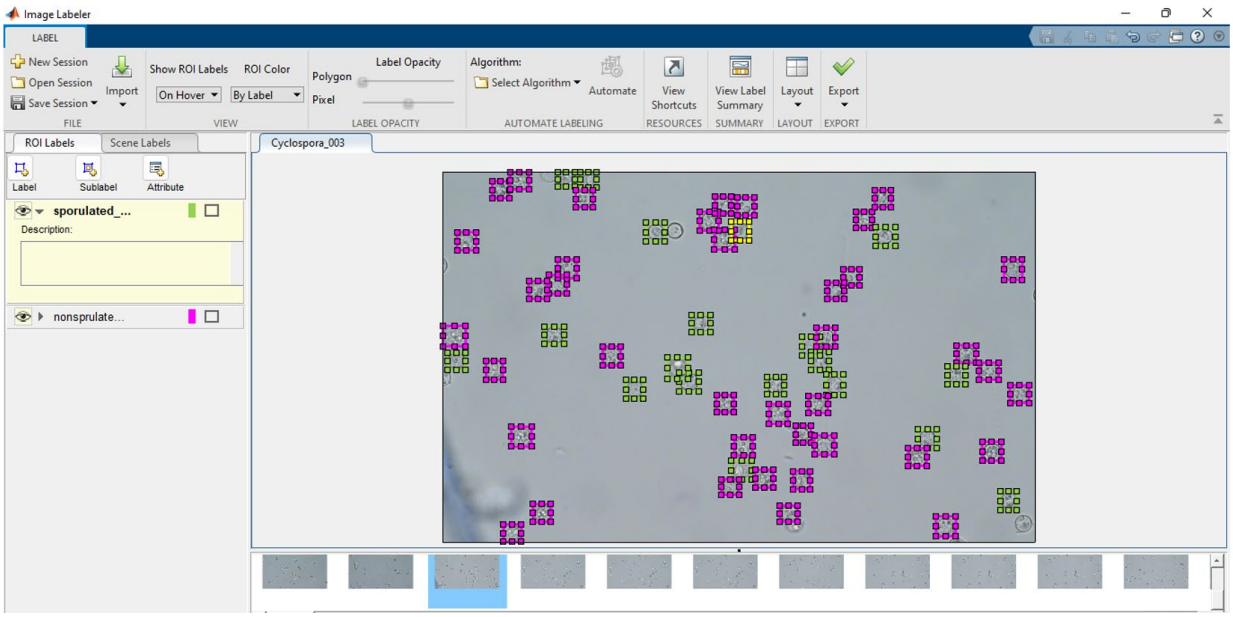

Figure 4: Example of an annotation panel using the Image Labeler application in Matlab. Sporulated oocysts are shown as labeled with a green region of interest (ROI) box. In this image, nonsporulated oocysts are shown as labeled with magenta ROIs.

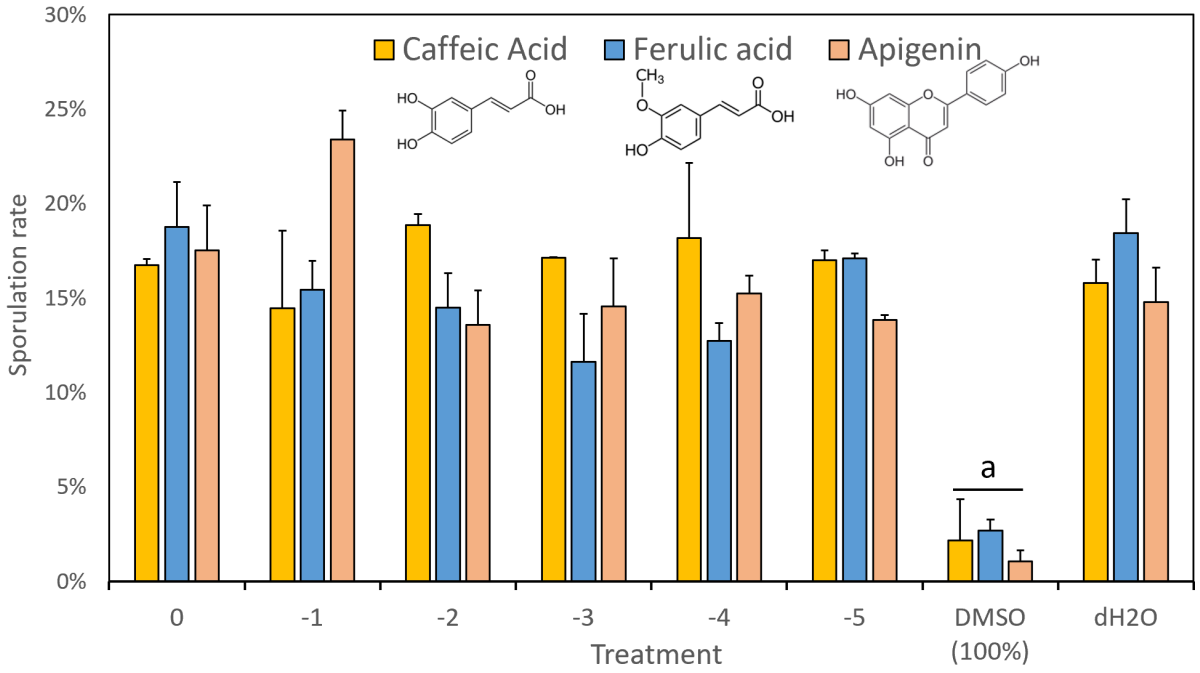

Figure 5: Representative data from a chemical inactivation screen using caffeic acid, ferulic acid, and apigenin to inhibit *E. acervulina* sporulation. 100% DMSO was found during our screening to serve as an effective positive control and in this case, was the only treatment to significantly inhibit sporulation relative to the negative control (water).

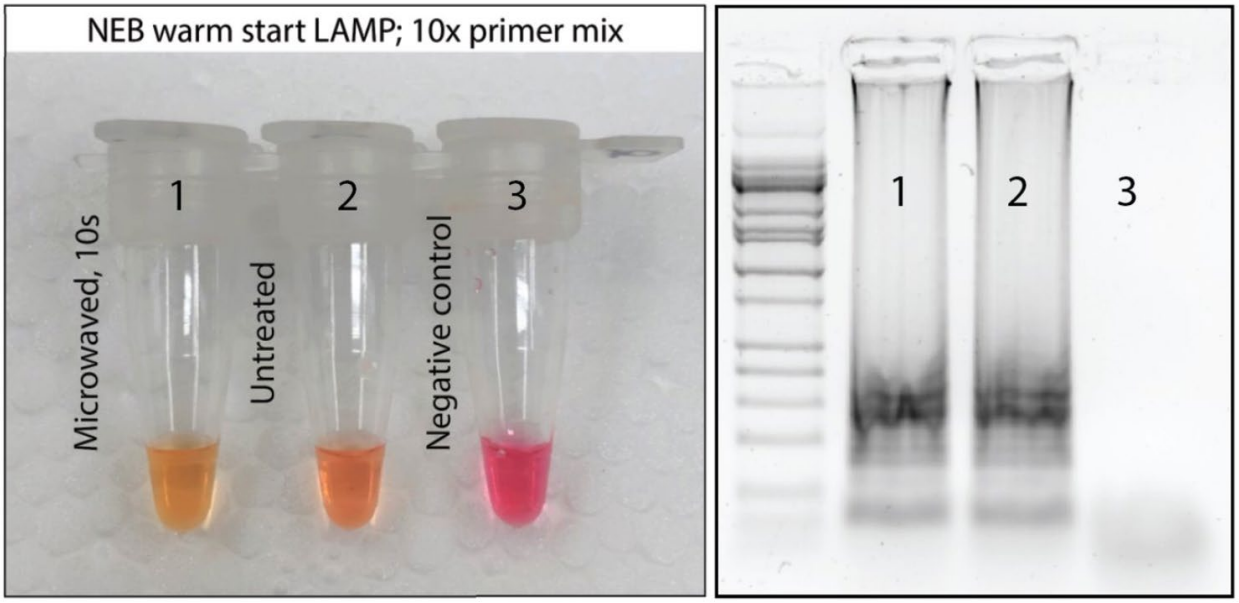

Figure 6: Left: Example of pure *C. cayetanensis* oocysts used as input for LAMP isothermal PCR. Microwaved (1; this method is known to rupture oocyst walls in coccidia) or untreated (2) oocysts are sufficient to render a color change, indicating a positive reaction. Right: Gel electrophoresis shows evidence of the positive and negative LAMP reactions from the corresponding tubes at left.

Table 1: Primers used for three separate LAMP isothermal PCR reactions targeting the nuclear gene TufA, and two apicoplast targets (AP1 and AP2).

Primer name	Sequence
TufA_LAMP_F3	TTATTCAGAAATTGACTCTGCT
TufA_LAMP_B3	AAATGTTCACGAGTTTGAGG
TufA_LAMP_FIP	ACCTGGACAATCAATATGAGCAAAAGGGGAATTACAATTAATACTTCTCA
TufA_LAMP_BIP	TGATTACAGGTGCCGCACAAATAGGTCCGTCTGTAGCA
TufA_LAMP_F2	GGGGAATTACAATTAATACTTCTCA
TufA_LAMP_F1c	ACCTGGACAATCAATATGAGCAAAA
TufA_LAMP_B2	ATAGGTCCGTCTGTAGCA
TufA_LAMP_B1c	TGATTACAGGTGCCGCACAA
AP1_LAMP_F3	GAAGAAAAAGCTAGGGGAATT
AP1_LAMPB3	AAATGTTCACGAGTTTGAGG
AP1_LAMPFIP	GCATGACCTGGACAATCAATATGA CTTCTCATGTAGAATATGAAACTTC
AP1_LAMPBIP	TGATTACAGGTGCCGCACAA CATAGGTCCGTCTGTAGC
AP1_LAMPF2	CTTCTCATGTAGAATATGAAACTTC
AP1_LAMPF1c	GCATGACCTGGACAATCAATATGA
AP1_LAMPB2	CATAGGTCCGTCTGTAGC
AP1_LAMPB1c	TGATTACAGGTGCCGCACAA
AP2_LAMPF3	GATGGCTACTGTAATTGGAAT
AP2_LAMPB3	GGTTTATATCCTTCAAAAAATGGTG
AP2_LAMPFIP	ATCCCACGTCTTACTTCTTCTTTTT AAAAAACATTAACTATAGCAGAGGC
AP2_LAMPBIP	AGCTCTCCCCTCAAGTATTTTAAC TACCTCCTTCTGACGCAG
AP2_LAMPF2	AAAAAACATTAACTATAGCAGAGGC
AP2_LAMPF1c	ATCCCACGTCTTACTTCTTCTTTTT
AP2_LAMPB2	TACCTCCTTCTGACGCAG
AP2_LAMPB1c	AGCTCTCCCCTCAAGTATTTTAAC