RESEARCH IN PROCESS

Methods for Detection of Diverse Parasites on Packaged Salads Based on (Viable) (Oo)cysts

SUMMARY

Contamination of packaged salads with disease-causing microorganisms constitutes a significant health risk for produce consumers. Protozoan pathogens are targeted because they are implicated in produce-borne illness outbreaks, optimal methods for their detection in produce are lacking, and their prevalence in developed countries such as the U.S. is likely to rise due to increasing demands for imported vegetables as well as climate variability projections. In order to detect human pathogens on produce in a rapid, accurate, and affordable manner, we are developing a simple one-step procedure (multiplex polymerase chain reaction (PCR)) to simultaneously detect and differentiate *Cryptosporidium* spp., *Giardia* spp., *Toxoplasma gondii*, and *Cyclospora cayetanesis*. Additional molecular techniques, propidium monoazide quantitative PCR (PMA-qPCR) and reverse transcription (RT)-qPCR will be applied for further quantification of viable protozoan pathogens. Molecular method validation will be performed through laboratory spiking experiments in cooperation with our industrial cooperator.

OBJECTIVES

The purpose of this project is to address the key issue that challenges the produce and packaging industry – detection of human pathogens on produce in a rapid, accurate, and affordable manner. Specific objectives include:

1. Develop a simple one-step procedure for routine detection of the parasites *Cryptosporidium, Giardia, Cyclospora,* and *Toxoplasma* using qualitative multiplex-PCR to simultaneously detect and differentiate protozoan pathogens.
2. Develop and optimize PMA-qPCR and compare its performance with established RT-qPCR and microscopy methods for best detection of viable (oo)cysts.
3. Validate newly developed and optimized molecular methods through laboratory spiking experiments using leafy greens and determine optimized procedure and sampling plan emphasizing detection limits, potential inhibitors, speed and cost.

METHODS

Multiplex primer sets were designed to amplify all four protozoan targets simultaneously. Two methods will be compared for differentiating the parasites in a second step: 1) nested PCR reaction and 2) a restriction fragment length polymorphism (RFLP) assay.

Existing RT-qPCR and PMA-qPCR assays for target protozoan (oo)cysts were adapted. PMA-qPCR for *Toxoplasma* and *Cyclospora* will be developed to specifically detect live oocysts within a mixed population of live and dead (oo)cysts. The performance of PMA-qPCR assays will be compared with existing RT-qPCR or microscopy methods to evaluate the best quantitative methods for viable (oo)cysts.

Validation experiments will be performed by spiking known amounts of *Cryptosporidium* spp., *Giardia* spp., *Cyclospora cayetanensis*, and *Toxoplasma gondii* (oo)cysts on leafy greens. The produce will be stored in a zip-lock bag at 4°C. After 0, 1, and 4 days the produce will be 1) washed or 2) homogenized. Then the pathogen retention and recovery will be evaluated from the wash solutions and homogenized samples.

RESULTS TO DATE

All four target protozoan (oo)cysts were identified and enumerated using a combination of bright field and epifluorescent microscopy. Viability assays were established for *Cryptosporidium* and *Giardia* using enumeration of (oo)cysts via a combination of DFA (direct fluorescent antibody) staining and PI (propidium iodide) uptake. Standard operating procedures (SOPs) for multiplex PCR and viable qPCR were developed. Primer sets for multiplex PCR assays were designed to amplify a region of the 18S ribosomal RNA (rRNA) gene. Experiments are currently underway to test newly designed multiplex primers with nested PCR reactions for parasite differentiation. PMA-qPCR methods for *Cryptosporidium* and *Giardia* were adapted from existing methods based on up-to-date literature review. New PMA-qPCR assays for *Toxoplasma* and *Cyclospora* are currently being developed. Available RT-qPCR methods for *Cryptosporidium, Giardia,* and *Toxoplasma gondii* were adapted from existing methods.

BENEFITS TO THE INDUSTRY

We anticipate that the outcomes of this research will yield novel approaches to identify key protozoan pathogens in produce in a rapid, sensitive and specific way. Improved detection of specific pathogens by molecular means fills a big gap to improve monitoring strategies for pathogens, and further provides data for a comprehensive approach to predict risk – via quantitative microbial risk assessment – that characterizes the risk of contracting an illness due to parasites on produce. The beneficiaries are the relevant growers, harvesters and processors of the fresh produce. The ability to certify the absence of certain pathogens will guide more specific and effective risk management practices for the industry and provide new insights into optimization of processes. Our toolset is not limited to these produce types, and the total number of potential beneficiaries extends to growers, harvesters and processors of other specialty crops, especially produce industries that suffer common protozoa contamination and associated health risks to consumers.

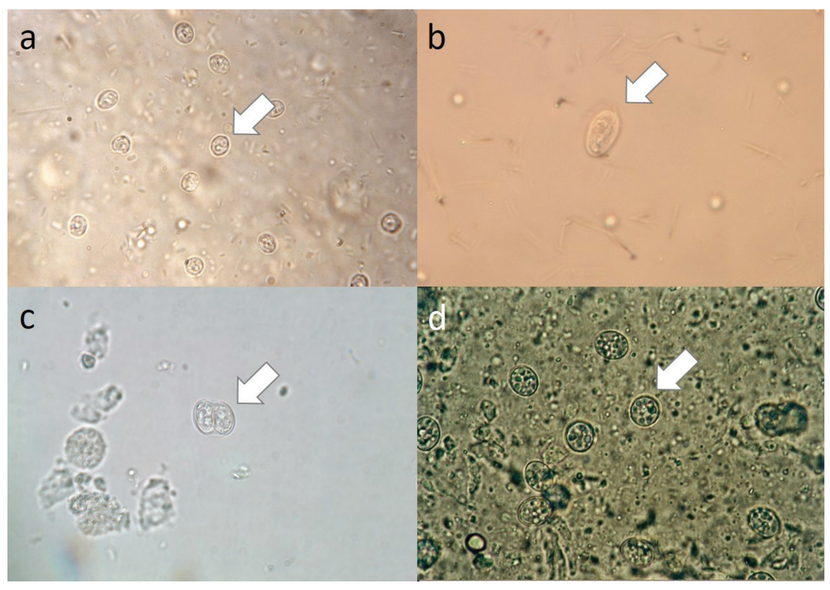

Figure 1. Microscopic images of (a) *Cryptosporidium parvum*, (b) *Giardia lamblia*, (c) *Toxoplasma gondii*, and (d) *Cyclospora* spp. [credit: Public Domain Image (a), Dr. Karen Shapiro (b and c), and Rataj et al. 2011 (d)].

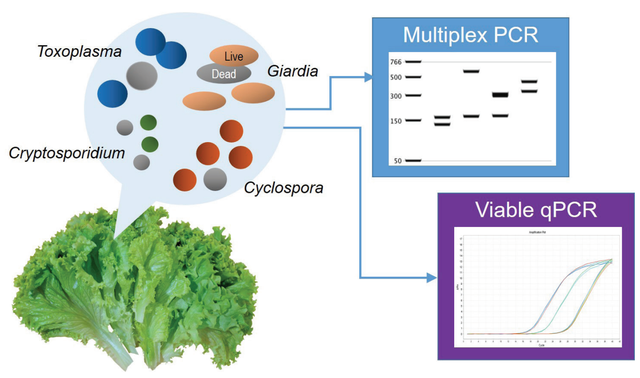

Figure 2. A schematic diagram of our project

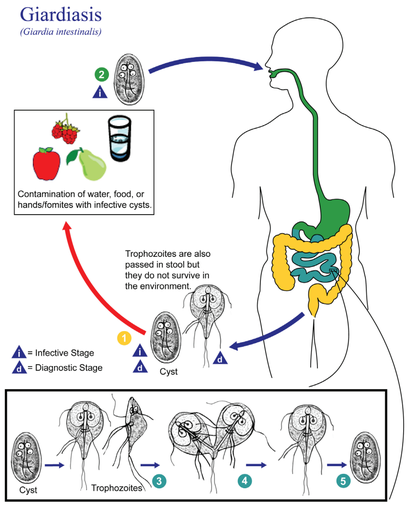

Figure 3. Life cycle of *Giardia* (credit: CDC Webpage)

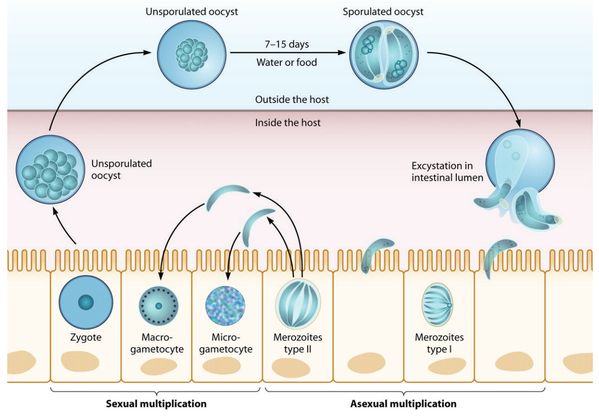

Figure 4. Life cycle of *Cyclospora* (credit: Ortega et al. 2010)

CONTACT

Stefan Wuertz
University of California, Davis
swuertz@ucdavis.edu

AUTHORS

Stefan Wuertz, Veronica Rajal, Karen Shapiro, Minji Kim, Beatriz Aguilar

ACKNOWLEDGEMENTS

Patricia Conrad, Andrea Packham, Lezlie Rueda, Brittany Leung, Samantha Duran

LENGTH OF FUNDING

January 1, 2016 – March 31, 2017

CPS CENTER *for* PRODUCE SAFETY