An immunomagnetic separation method for concentrating and increasing the recovery efficiency of *Cyclospora*

Summary

Risk assessment and mitigation require simpler and more efficient methods to concentrate *Cyclospora* oocysts. Their low concentration in food, soil, and water renders *Cyclospora* difficult to detect and characterize. Current detection methods rely on either microscopic examination or qPCR-based detection. Unfortunately, these methods are hampered by low concentrations and are sensitive to inhibitory contaminants, resulting in less than 30% DNA recovery even when using purified oocyst samples. To overcome these challenges, we are developing a rapid, specific, and efficient antibody-based method to separate and enrich *Cyclospora* oocysts and improve downstream DNA extraction methods. Following precedents established for other zoonotic enteric parasites, we intend widespread adoption of these methods to facilitate the identification and characterization of *Cyclospora* oocysts in a more accessible, effective, and cost-effective manner.

Objectives

1. Develop immunomagnetic separation (Cyclo-IMS) directly from environmental samples using magnetic beads linked with antibodies against a principal oocyst wall protein (COWP2 and/or TA4 antigen-like surface protein) of *C. cayetanensis*.
2. Evaluate the value of Cyclo-IMS to concentrate oocysts of *C. cayetanensis* from environmental samples.
3. Evaluate improvements to DNA isolation by coupling Cyclo-IMS to extraction methods, previously optimized on *Eimeria* oocyst surrogates, for *C. cayetanensis* (including freeze-thaw, bead beating, and osmotic shock to lyse oocysts).

Methods

To develop an immunomagnetic separation protocol (**Figure 1**), we will express the COWP protein, a key component of the *C. cayetanensis* oocyst wall, and TA4 antigen-like surface protein using codon-harmonized sequences cloned into *E. coli*. Expression will be verified via quantitative RT-PCR and protein will be purified using NiNTA chromatography. Two rabbits will be immunized with purified proteins to generate antibodies, and ELISA will be used to assess antibody titters. IgG will be purified, and its specificity will be evaluated using immunofluorescence on *Cyclospora* and *Eimeria* oocysts. High cross-reactivity, if found, will be addressed via preabsorption with *Eimeria* oocysts. Finally, we will crosslink polyclonal IgG to Dynabeads to create Cyclo-IMS for oocyst capture. We will assess bead-based capture efficiency by spiking *Cyclospora* oocysts in food and environmental water samples, and compare DNA extraction methods (UNEX, PowerSoil Pro, freeze-thaw, and osmotic shock) based on DNA yield and integrity.

Results to Date

We synthesized the *C. cayetanensis* COWP and TA4 antigen-like surface genes, both with and without codon-harmonization, using GenScript and expressing the proteins in *E. coli* after cloning them into the pTrcHisA vector. Recombinant plasmids were prepared through restriction digestion, gel purification, and sequence verification. *E. coli* BL21 was transformed for protein expression, induced with IPTG, and protein extracts were analyzed using SDS-PAGE, Coomassie staining, and immunoblotting (**Figure 2**). The proteins were purified via NiNTA affinity chromatography. For antibody production, rabbits were immunized with NiNTA-purified COWP and TA4 antigens emulsified in Freund's adjuvants. These methods will support the development of reagents targeting *C. cayetanensis* surface proteins for diagnosing and concentrating oocysts from food matrixes and environmental water samples.

Benefits to the Industry

In the United States, around 48 million people are affected by foodborne illnesses each year, costing the economy an estimated $7 billion annually. The economic burden of different pathogens varies, with *C. cayetanensis* costing $2.6 million in 2018. Therefore, it is crucial for produce growers and regulators to have reliable and sensitive tools that can detect the presence of foodborne pathogens, including *Cyclospora*. Our project aims to develop tools that efficiently concentrate and purify *Cyclospora* oocysts from environmental samples, substantially increasing DNA recovery efficiency. Importantly, the antibodies employed in this bead-based kit could further serve as the basis for a fluorescence-based detection assay, greatly aiding risk assessment and environmental sampling.

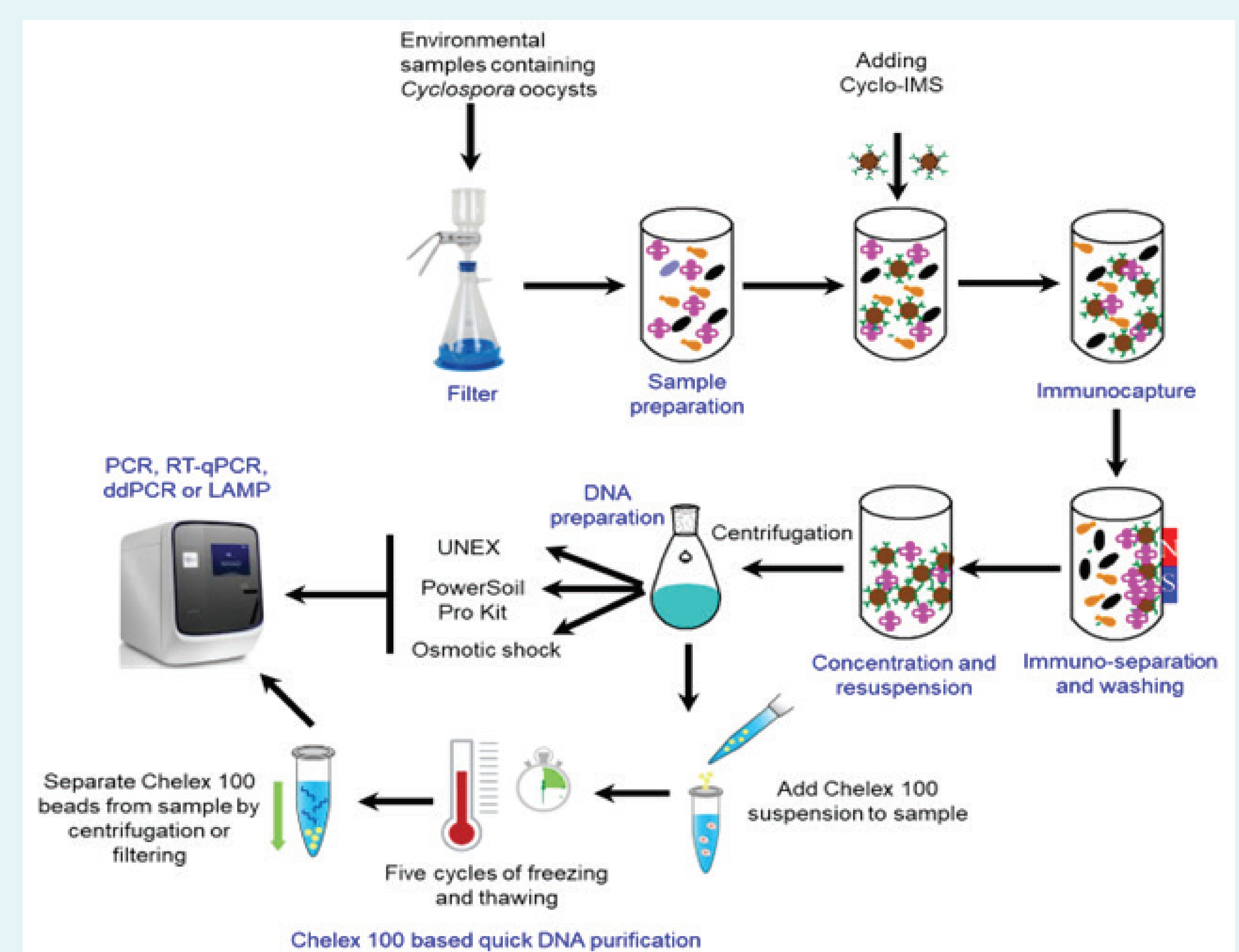

Figure 1:
Schematic representation of *Cyclospora* immunomagnetic separation and DNA purification method for improved sensitivity and reproducibility of *Cyclospora* detection system

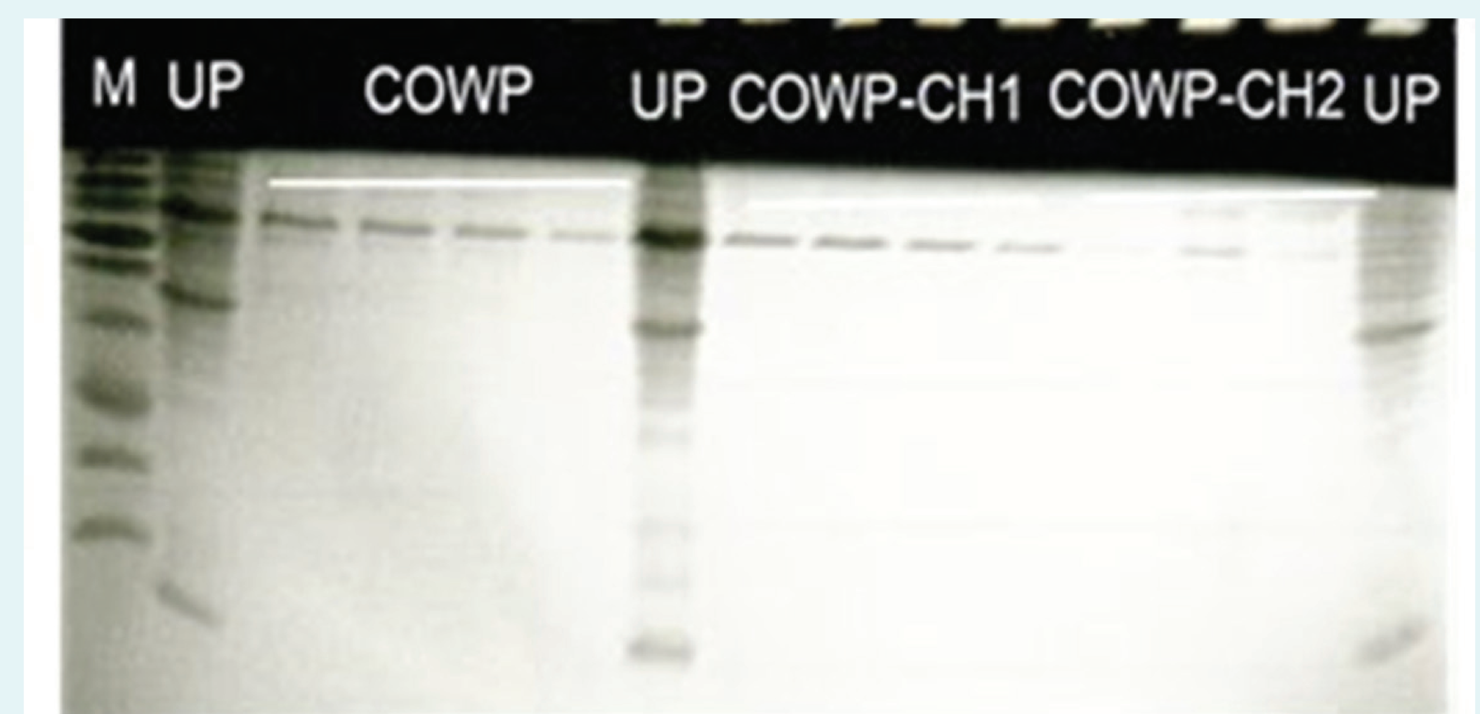

Figure 2:
Expression profile of COWP with or without codon-harmonization (COWP-CH1 and CH2) after expressing in *E. coli*

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