Project Title: Attachment and transport mechanism of *Cryptosporidium parvum* oocysts in subsurface environments: a multi-scale study

Project Type: Research

Focus Categories: water quality, groundwater, agriculture

Research Category: Groundwater Flow and Transport, Water Quality, Biological Sciences, Engineering

Keywords: pathogen transport, groundwater contamination, manure

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Congressional District of the university where the work is to be conducted: 15

1. Research Objective

Pathogens including *Cryptosporidium parvum* oocysts found in surface runoff are one of the leading causes of impaired river and estuary water. Knowledge on the fate and transport of *C. parvum* oocysts in agricultural runoff is currently lacking and is urgently needed to protect water supplies for many parts of the state. The results of this project will provide a scientific basis for water resources and environmental ***sustainability***.

This project uses a ***multi-scale approach*** to identify *chemical and physical factors* that influence attachment and mobility of *C. parvum* oocysts. A comprehensive understanding of these factors will be used to develop a model to predict the fate and transport of oocysts in the subsurface environment. The ***objectives*** of this project are: (1) to investigate the role of oocyst wall macromolecules in the deposition and transport of *C. parvum* oocysts by systematically modifying the oocyst wall; (2) to determine the attachment mechanisms of *C. parvum* oocysts on inorganic (i.e. quartz) and organic (i.e. coated with natural organic matter) soil surfaces on a microscopic scale; and (3) to determine the transport of *C. parvum* oocysts in the subsurface environment in micromodel setups. The experimental approach ranges from a ***microscopic to a macroscopic scale***. A novel microscopic technique consisting of a radial stagnation point flow (RSPF) cell combined with a microscope will be used to monitor attachment and detachment kinetics of oocysts under well-defined flow conditions in real time. Deposition and detachment experiments will be conducted with systematically varied solution conditions to determine the mechanisms of oocyst interaction with representative soil surfaces. Pore scale transport of oocysts will be studied using a precisely fabricated micromodel.

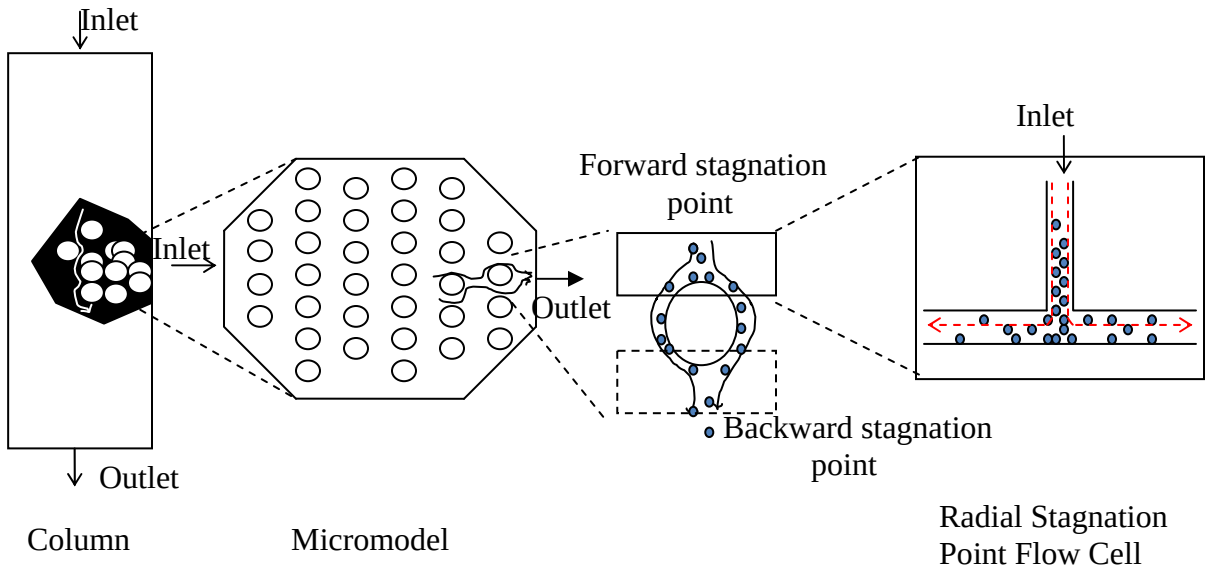

Figure 1 Radial Stagnation Point Flow Cell and Micromodel used in this study

2. Methodology.

Task 1 Characterize *C. parvum* oocyst wall properties

1) Purification of *C. parvum* oocysts. *C. parvum* oocysts (viable, 4-5μm in diameter) were purified from the feces of male Holstein calves (IACUC protocol # 04070). The purified oocysts

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were centrifuged and washed with Tris-ethylenediamine-tetraacetic acid (Tris-EDTA: 50 mM Tris, 10 mM EDTA) and stored at 4 °C in a solution of 50% Hanks' balanced salt solution (HBSS, GIBCO, Grand Island, New York) and 50% antibiotic-antimycotic solution (0.6% penicillin, 1% streptomycin, 0.0025% amphotericin, and 0.85% NaCl in sterile water).

2) Modification of *C. parvum* oocyst wall. We treated *C. parvum* oocysts with various digestive enzymes, including proteinase K (a broad-spectrum serine protease) and mixed glycosidases (capable of removing carbohydrate residues from proteins). Deposition kinetics of untreated and treated oocysts on quartz surface were also determined to study the effects of oocyst surface macromolecules on oocyst deposition.

3) Characterization of *C. parvum* oocyst wall macromolecules composition and conformation. The peptides released by proteinase K and carbohydrates hydrolyzed by mixed glycosidases were respectively analyzed with liquid chromatography/nano-electrospray ionization tandem mass spectrometry (LC-MS/MS) and phenol-sulfuric acid assay to determine the composition of *C. parvum* oocyst wall surface macromolecules. Surface potential and polarity of the untreated and proteinases treated *C. parvum* oocysts revealed information about the conformation of oocyst wall surface macromolecules.

Task 2 Determine the attachment mechanisms of *C. parvum* oocysts on inorganic and organic surfaces at the microscopic level

A radial stagnation point flow (RSPF) cell was used to determine the attachment efficiency of untreated and proteinase K treated *C. parvum* oocysts on quartz surfaces in the presence of monovalent cations. In addition, the deposition of untreated oosysts on quartz or natural organic matter in the presence of divalent cations was studied in RSPF cell. As seen in Figure 1, RSPF is used to mimic the forward stagnation point of irregular soil grains. With RSPF, it is possible to control the hydrodynamic conditions and conduct real time observation of *C. parvum* oocyst deposition on inorganic and organic surfaces under a microscope.

Task 3 Simulate the transport of *C. parvum* oocysts in the subsurface environment with micromodel and column setup

The micromodel (surface material: SiO_2), as shown in Figure 1, was designed to conduct direct and real time observation of *C. parvum* oocysts traveling along the granular particles. The collectors were etched onto a Si wafer and then the surface was oxidized to form SiO_2. electrolyte solutions containing oocysts were pumped into the micromodel and directly observed under microscope.

3. Principal Findings and Significance.

Each task of the proposed research provided knowledge on deposition and transport of pathogens in the natural environment.

1) For task 1, we characterized the composition and conformation of *Cryptosporidium parvum* oocyst wall surface macromolecules and studied their effect on interactions between *C. parvum* oocyst and quartz surface. The results illustrated that *C. parvum* oocyst wall is covered by a fluffy layer of glycoprotein.
2) For task 2, we studied the deposition of *C. parvum* oocysts on quartz and natural organic matter surface in the presence of divalent cations and deposition kinetics of untreated and

proteinase K treated *C. parvum* oocysts on quartz surface in the presence of monovalent cations. The results indicated that the fluffy layer on *C. parvum* oocysts wall leads to weaker van der Waals interaction and stronger steric repulsion. This fluffy layer makes oocysts more mobile in the subsurface environment. In addition, carboxyl groups of the fluffy layer on *C. parvum* oocysts wall and natural organic matter surface leads to specific interaction of Ca^{2+} with carboxyl groups and enhanced deposition of oocysts on SRNOM surfaces and decreases the mobility of oocysts in the subsurface environment.

3) A microscopic method for direct and real time observation of oocyst transport and distribution in a micromodel that simulates porous media is being developed.

4. Notable Achievements.

1) For task 1, we, for the first time, reported contact angles measured for oocyts and based on these data estimated the Hamaker constant between oocysts and quartz surface. The Hamaker constant is essential to calculate van der Waals interaction between those two surfaces.

2) For task 2, we found that proteinase K treated *C. parvum* oocysts significantly decreased compared to that of untreated oocysts. This observation indicated that the fluffy layer on *C. parvum* oocysts wall leads to weaker van der Waals interaction and stronger steric repulsion. Inductive coupled plasma (ICP) was employed to measure the free divalent cation concentration in solutions containing oocysts. ICP data showed more Ca^{2+} bound to oocyst surface than Mg^{2+}. Moreover, proteinase K treatment of oocysts led to a significant decrease in deposition rate due to less binding of Ca^{2+} to the surface of the treated oocysts as shown by the ICP data. The deposition and ICP results suggested that inner-sphere complexation of Ca^{2+} with carboxylate groups on both SRNOM and oocyst surfaces enhanced deposition of oocysts on a SRNOM surface.

3) For task 3, as of May 2010, we are developing a microscopic method to directly measure single-collector attachment efficiency of *C. parvum* oocysts.

5. Students Supported with Funding.

Ms. Yuanyuan Liu, Department of Civil and Environmental Engineering, Engineering School, University of Illinois at Urbana-Champaign. She is a PhD candidate and is expected to graduate in 2012.

6. Publications and Presentations.

Janjaroen, D.; **Liu, Y.**; Kuhlenschmidt, M. S.; Kuhlenschmidt, T. B.; Nguyen, T. H. Role of Divalent Cations on Deposition of *Cryptosporidium parvum* Oocysts on Natural Organic Matter Surfaces. *Environmental Science & Technology* 2010, in press, DOI: 10.1021/es9038566.

Liu, Y.; Kuhlenschmidt, M. S.; Kuhlenschmidt, T. B.; Nguyen, T. H. Characterization of *Cryptosporidium parvum* Oocyst Wall Macromolecules and Adhesion Kinetics of Oocysts on Quartz Surface. *Biomacromolecules* 2010, Submitted.

Liu, Y.; Kuhlenschmidt, M. S.; Kuhlenschmidt, T. B.; Nguyen, T. H. "Direct measurement of single-collector attachment efficiency of Cryptosporidium parvum oocysts: Method development", *239th ACS National Meeting & Exposition*, Mar. 2010

Liu, Y.; Kuhlenschmidt, M. S.; Kuhlenschmidt, T. B.; Yau P. M.; Nguyen T. H. "Role of *C. parvum* Oocysts Wall Macromolecules on Deposition Kinetics of Oocysts on Quartz Surface" *The Association of Environmental Engineering and Science Professors (AEESP)*, July, 2009