

Report for 2001NY1841B: Epidemiologic Risk Analysis of Cryptosporidium parvum in Watershed: the Role of Genetic Variation Among Isolates

- Other Publications:
 - Lindergard, G; DV Nydam, SE Wade; SL Schaff; HO Mohammed, Detection of human infective Cryptosporidium isolates by multiplex PCR in fecal and soil samples, J Vet Diag Invest 2002, (Conditional acceptance).
 - Nydam, DV; GL Lindergard; SE Wade; SL Schaff and HO Mohammed, 2002 Prevalence of Cryptosporidium parvum genotypes H and C in isolates collected from cattle within the area of New York State that comprises the New York City Watershed, Epidemiology and Infection, (Submitted).

Report Follows:

Problem and Research Objectives:

The specific objectives of the proposed studies will focus on:

- i) Expand our panel of polymorphic markers for identification of a wider range of genotype of *Cryptosporidium spp.* isolates.
- ii) Using combination of deterministic and probabilistic approaches we will develop scenario path models describing the transmission of infectious *C. parvum* from the environment to the stream edge. These models will take into consideration the infectious nature of the isolates, the management practices that promote and or inhibit this rate of environmental contamination and spread on the farm. The models will be based on data collected previously and methods advanced in aim 1.
- iii) Design cost-effective strategies to control and manage the potential risk associated with the presence of these organisms on CAFOs. These strategies will be based on the findings in aims 2 and 3 and allow for input from stakeholders.

Methodology:

A PCR based protocol targeting multiple loci is expected to identify differences between genotypes of isolates from many different potential hosts. DNA is being isolated directly from whole fecal samples by a method modified after Zhu et al. (7), which uses glass beads to break the oocyst wall or by a freeze-thaw method designed in our laboratory.

Samples that have tested positive for *Cryptosporidium* oocysts at this stage will be further analyzed with a nested multiplex PCR method to verify its genotype (3,4).

In these proposed studies, in addition to expanding our markers to include additional species of *Cryptosporidium*, we will reexamine the 478 samples that were confirmed by the flotation method. The reexamination will be by PCR only. By doing so, we will be able to see whether all the samples had only one genotype or were contaminated with different species of *Cryptosporidium*. Furthermore, we will examine an equal number of randomly selected samples from the pool of negatives.

Principal Findings and Significance:

The funding was used to address aim 1. We carried out studies to expand our markers to include additional species of *Cryptosporidium* and examine fecal samples that were collected from cattle in New York City watershed. These samples were confirmed by the floatation and enzyme-linked immunosorbent assay (ELISA) methods to have *C. parvum*. Furthermore, an equal number of randomly selected samples from the pool of negatives were examined using the PCR method. The purpose of examining the negative samples was to confirm that we have not miss-classified animals as the result of employing a more sensitive technique, the PCR.

DNA was isolated directly from whole fecal samples using the glassbeads method to break the oocysts' wall. A nested PCR was performed for a portion of the *Cryptosporidium* SSU rRNA gene with first set of primers SSU-1 and SSU-2 followed by nested primers SSU-3 and SSU-4. In an earlier study we found that the SSU approach is more sensitive than TRAP-C gene for detection of the presence of *Cryptosporidium sp.* in fecal samples.

A restriction enzyme, *Vsp I*, that specifically cuts the human *C. parvum* type was used at this stage to differentiate it from the bovine type. Similarly, a 331 bp fragment of the TRAP-C2 gene

was amplified exclusively from *Cryptosporidium* DNA by primary primers TRAP-1, TRAP-2 and secondary primers TRAP-3. Again, both the human and the bovine *C. parvum* type were identified by digestion with specific endonucleases; *BsuRI* digests the human type only while *Eco9II* specifically cuts the bovine type.

A nested multiplex PCR approach was adapted for the simultaneous detection of four human infective genotypes of the protozoan parasite *Cryptosporidium*. Specific PCR primers were designed for the heat shock protein 70 gene of three types of *C. parvum*, human type, bovine type and dog type and for *C. felis*. These four genotypes have all been found in human fecal samples. The primers amplified DNA fragments of specific sizes each representing a unique genotype. The limit of detection of the method was found to vary between 10 and 100 oocysts per 1 ml fecal material. The results of these studies are published in the manuscript by Lindergard et al., 2002.

Fecal samples were collected from 437 calves at risk for *Cryptosporidium*. This sample represents a subset of positive and negative samples that have been previously examined by concentration/flotation microscopy and oocysts were detected in 214 of them. The DNA from whole feces from all samples was extracted. This was used to amplify fragments of the SSU rRNA and the thrombospondin-related adhesion protein C-2 (TRAP C-2) genes by nested polymerase chain reaction (PCR). Amplicons were generated for 200 of the samples. The SSU rRNA fragments were subjected to restriction digestion by the enzyme *VspI* and the TRAP C-2 fragments were digested by the restriction enzyme *Eco9II* to distinguish between the H genotype (genotype 1) and the C genotype (genotype 2) of *C. parvum*. None of the 200 samples tested by this 2 locus nested PCR-restriction fragment length polymorphism (RFLP) approach had the H genotype. We are 95% confident that the cattle in the NYCW did not harbor the H genotype. Focusing solely on methods to decrease or eliminate watershed contamination with oocysts by cattle will likely miss the important human sources of many urban drinking water outbreaks that are caused by the H genotype.