

CryptoNet: Molecular-based Tracking to Better Understand U.S. Cryptosporidiosis Transmission

Why track *Cryptosporidium* transmission in the U.S.?

Cryptosporidium causes a long-lasting diarrheal illness that can be life-threatening in immunocompromised people. It is spread from humans or animals, particularly cattle, via water, food, animal-to-person, or person-to-person. The parasite's extreme chlorine tolerance has facilitated it emerging as a leading waterborne pathogen; it is now the leading cause of outbreaks associated with swimming pools. An estimated 748,000 cryptosporidiosis case reports occur in the United States annually. Since 2004, annual reports of cryptosporidiosis have risen >3-fold in the U.S.¹.

Why is molecular tracking of *Cryptosporidium* needed?

As recently as the late 1990s, cryptosporidiosis in humans and other animals was thought to be caused by only one *Cryptosporidium* species, *C. parvum*. Molecular testing has since rewritten *Cryptosporidium*'s taxonomy. We now know that what we thought of as a single species is really a collection of at least 30 species, many with multiple subtypes causing varying severities of illness. **All forms are morphologically indistinguishable by traditional clinical lab tests.** Only a few of the species cause human infection. National molecular surveillance data in the United Kingdom has demonstrated that >95% of human *Cryptosporidium* infections are caused by multiple subtypes of *C. hominis* and *C. parvum*. *C. hominis* primarily exists in a human-to-human transmission cycle in more urban settings, while *C. parvum* is more rural transmission impacting both humans and ruminants (e.g., pre-weaned calves). Other rarer species can also infect humans. **Only molecular methods can distinguish these species, genotypes, and subtypes to elucidate the complex transmission of *Cryptosporidium* in the U.S.**

What is CryptoNet?

In response to the inability of traditional clinical diagnostics to distinguish *Cryptosporidium* species, genotypes, and subtypes and increased national reporting of cryptosporidiosis, CDC is developing CryptoNet, the first molecular tracking system for a parasitic infection. CryptoNet will be a multidisciplinary, molecular-based surveillance system built on the common BioNumerics platform successfully used by PulseNet and CaliciNet. CryptoNet planning is aimed at the efficient use of existing infrastructure to facilitate the systematic collection and molecular characterization of *Cryptosporidium* isolates to further understand cryptosporidiosis epidemiology.

Potential Public Health Impact

By facilitating real-time sharing of molecular epidemiology data among U.S. national, state, and local public health departments, CryptoNet will further elucidate the epidemiology of cryptosporidiosis and *Cryptosporidium* species, genotype, and subtype transmission by:

- Improving detection, investigation, and interpretation of waterborne, zoonotic, person-to-person, and foodborne cryptosporidiosis outbreak data
- Identifying geographic and temporal changes in the distribution of *Cryptosporidium*
- Increasing capacity to identify traditional and novel epidemiological links and risk factors, outbreak sources, and sources of contamination
- Identifying *Cryptosporidium* species, genotypes, and subtypes not previously known to infect humans

¹ Hospitalizations due to cryptosporidiosis cost >\$45 million annually; ambulatory visits cost ~\$270–760 each.



How Molecular Tracking and CryptoNet Can Help

Examples of how molecular typing has improved our epidemiologic understanding of cryptosporidiosis include:

- Oklahoma, 2007: Determined that two distinct outbreaks of cryptosporidiosis had occurred in adjacent counties during the same month.
- Colorado, Idaho, New Mexico, and Iowa, 2007: Identified a previously rare *C. hominis* subtype in 40 cases (70%) from all four states.
- New Mexico, 2007: Identified *Cryptosporidium* horse genotype in a pet shop employee with severe clinical symptoms.
- North Carolina, 2009: Identified zoonotic transmission of *C. parvum* that had occurred from pre-weaned calves to camp attendees and staff, an epidemiologic link that the retrospective cohort study failed to identify.

Technical Aspects

Cryptosporidium Genotyping Techniques. CryptoNet has been built for comparison of the 18S rRNA genes and 90 kDa glycoprotein gene (gp60) and has established a DNA sequence database using:

- A nested PCR-RFLP method on the 18S rRNA gene extracted from cryptosporidiosis diagnostic samples. The 18S rRNA gene has high copy numbers and the presence of conserved regions interspersed with highly polymorphic regions. The 800 bp nested PCR amplicon is used in RFLP reactions with different sets of restriction enzymes to differentiate *Cryptosporidium* species and genotypes.
- A nested PCR method on the gp60 gene extracted from clinical samples submitted for subtyping of *Cryptosporidium* species. GP60 is the most commonly used target for subtyping *C. parvum*, *C. hominis*, and *C. meleagridis*. Sequencing of the 800 bp nested PCR product identifies subtype family and subtype.

Laboratory Resource Requirements and Costs. Laboratories that regularly perform molecular analysis **will need little to no additional equipment or resources to complete the necessary methodologies for *Cryptosporidium* genotyping**. Specific equipment needs include a thermal cycler, microcentrifuge, electrophoresis system, water bath, PCR workstation, ABI 3130xl Genetic Analyzer or similar sequencing machine, and BioNumerics software. The estimated cost per sample beginning with DNA isolation and ending with DNA sequencing is approximately \$9.50². CDC will cover expenses to train and certify partnering laboratories across the U.S.

For More Information

If interested in becoming a partner laboratory and/or learning more about the advantages of using CryptoNet in your investigations, please email cryptonet@cdc.gov.

² Cost may vary depending on the brand of reagents and consumables, as well as contract pricing.