Water and Pathogen Testing During Waterborne Disease Outbreak Investigations

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Division of Foodborne, Waterborne, and Environmental Diseases

Water testing is an investigative tool

- **Water testing can be an effective component of epidemiological investigations**
  - Confirming water source associated with an outbreak
  - Identifying source of fecal contamination
  - Identifying system deficiencies contributing to an outbreak
  - Adding confidence to the conclusions of an epidemiological investigation

Testing strategy depends on the implicated water

CDC Capabilities for Collaborative Investigations

- Sampling
- Sample processing
- Rapid pathogen testing via real-time PCR
- General water quality testing (e.g., *E coli*)
- Molecular epidemiology testing
  - PFGE (from culture isolates)
  - Sequencing (pathogen identification, subtyping)

Sampling

- **Grab samples**
  - 100 - 300 mL → water quality testing, microbial indicators
  - 1 - 4 L → swimming pool backflush samples
- **Large-volume filtration**
  - Pre-2004, used microfilter cartridges for viruses and parasites
  - Currently, use ultrafiltration
    - Hollow-fiber ultrafilters (dialysis filters)
    - Dead-end ultrafiltration
    - Performed in field, ultrafilters shipped to lab

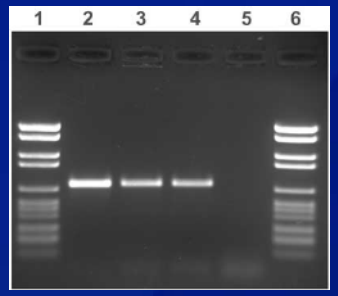
Ultrafilter

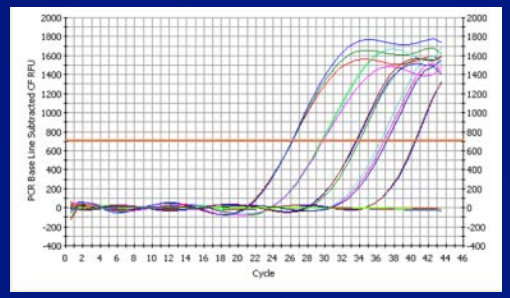
Local preparedness facilitates rapid response

- **Water Sampling Kits developed by states**
  - Peristaltic pump
  - Chlorine test kit
  - Coolers and supplies
    - Ultrafiltration (filters, tubing, connectors)
    - Grab sample bottles
    - Sodium thiosulfate
    - Gloves, scissors, ice packs, ziploc bags, markers, data shee

Rapid screening using real-time PCR

- Faster turnaround times vs. conventional PCR

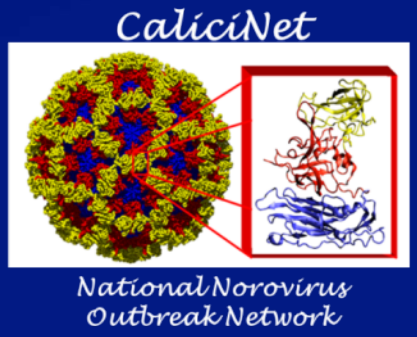


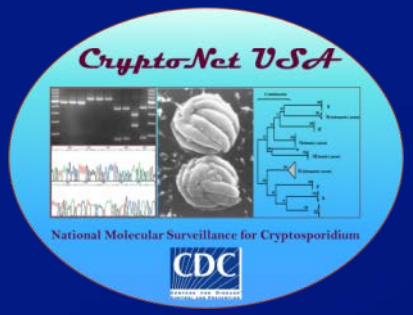


- Potentially enables quantitation ("qPCR")
- Bacteria → culture first, then PCR
- Viruses → direct analysis by RT-qPCR
- Parasites → direct analysis by qPCR
  - For *Cryptosporidium* and *Giardia*, can also perform fluorescence microscopy for confirmation, quantitation

Supporting molecular epidemiology

- Bacteria → match PFGE patterns (PulseNet)
- Viruses → match nucleic acid sequences (CaliciNet)
- Parasites → compare sequences, subtypes (CryptoNet)





Water testing: supporting public health response

- Milwaukee (1993)
  - Human "*C. parvum*" genotype (later named *C. hominis*) identified in patient stools in 1998 and in Milwaukee wastewater in 2000, confirming that waste source was primarily from sewage system
  - Spurred improvements in filtration and disinfection (ozonation); also Milwaukee's water treatment plant intake pipe was extended 4,200 ft further into Lake Michigan
- Oklahoma (2008)
  - Norovirus GI.4 identified in stool samples and a water sample from the well that served a condominium complex.
  - The complex was eventually connected to the public water supply
- Alamosa, CO (2008)
  - *Salmonella* Typhimurium detected throughout non-disinfected public water supply system
  - System shut down until new chlorination plant brought online
- Louisiana (2011): Water testing confirmed presence of *Naegleria fowleri* in tap water used by cases for nasal rinsing

Working together to investigate WBDOs

- Support for outbreak investigations
- Training (e.g., sampling)
- Technology transfer (e.g., molecular testing)
- Surveillance: CryptoNet

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- State and local health departments

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NOTE: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Questions?

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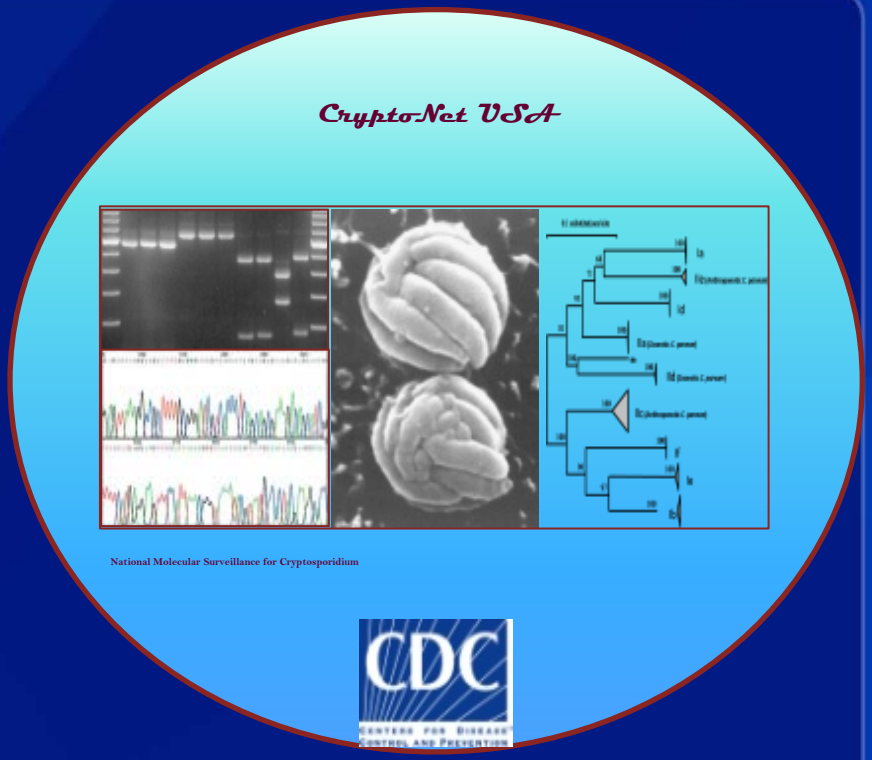

PATHOGEN TESTING

How useful is it?

OUTBREAK INVESTIGATIONS USING GENOTYPING AND SUBTYPING

North Carolina Outbreak-2009

- Youth Summer camp
  - livestock, recreational water venues, well-supplied drinking water, meals from a central kitchen and garden that provided >50% of produce for meals
- 46 lab-confirmed and probable cases of cryptosporidiosis
- Analysis of data from retrospective cohort study was used to ID risk factors and implement control measures
  - Ham from a sandwich bar significantly associated with illness

Collier et al., MMWR 2011

NC outbreak 2009-Sampling

- **Stool samples**
  - Animals: calves, goats, kid, and piglet
  - Human cases: 12 confirmed by PCR and genotyping
- **Environmental samples**
  - Water sources
    - All negative
  - Composite soil samples from the garden
    - *Cryptosporidium* spp. detected but typing could not be performed

NC outbreak 2009-Molecular Epidemiology

	Cryptosporidium parvum	
	#	Subtype
Human	8	IIaA17G2R1 (8)
Calf	3	IIaA17G2R1(2) IIaA15G2R1
Goat kid	1	IIaA17G2R1 (1)
Piglet	1	IIaA17G2R1 (1)

Usefulness of molecular epidemiology in NC outbreak

- Traditional epidemiologic methods identified eating ham from a sandwich bar as the main risk factor
- Genotyping revealed identical genotypes between human cases and livestock on the property
- Ham may have been a vehicle for transmission, but source of infection was likely the livestock
- Enabled accurate directives to prevent new infections
  - Previously a diluted chlorine spray was used for "washing" after handling animals
  - New prevention efforts included running water, soap, and appropriate hand-washing techniques

Value of Cryptosporidium Molecular Epidemiology

- Collaborate to develop, test, and implement standardized lab methods for Crypto investigations
- Build a network for tracking *Cryptosporidium* strains in humans, animals, and environmental samples
- Share this info in real time with partners to identify outbreaks and source infections
- Analyze trends in *Cryptosporidium* transmission

Public Health Impacts of a *Cryptosporidium* genotype tracking system

- Increase laboratory data sharing among national, state, and local public health laboratories
- Promote improved laboratory-based tracking of cryptosporidiosis in the United States
- Improved efficiency in detection and investigation of foodborne, waterborne, person-to-person, and zoonotic outbreaks of cryptosporidiosis
- Improve understanding of *Cryptosporidium* transmission in the United States