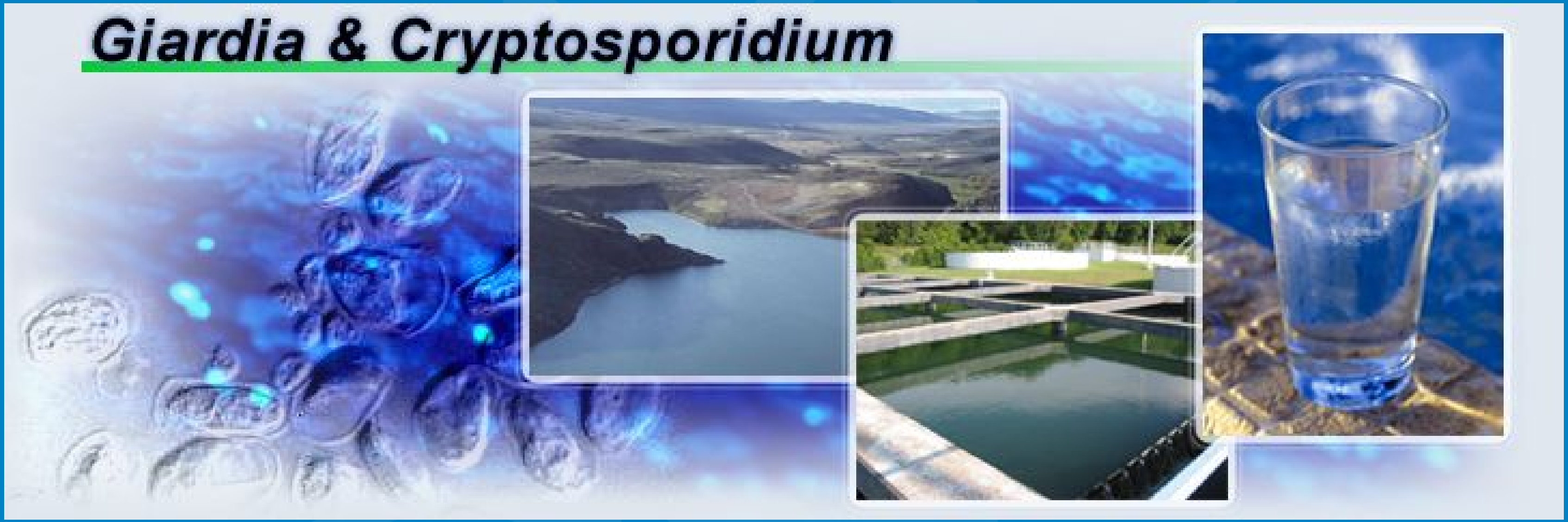
Molecular-Based Detection Systems for *Cryptosporidium* Oocysts



US EPA Office of Water

Eric N. Villegas, Ph.D.

STAR Grants Workshop on Innovative Approaches for Detecting Microorganisms and Cyanotoxins in Water

US EPA Region 3, Philadelphia, PA

May 20-21, 2009

Office of Research and Development
National Exposure Research Laboratory | Microbiological and Chemical Exposure Assessment Research Division | Biohazard Assessment Research Branch

Overview

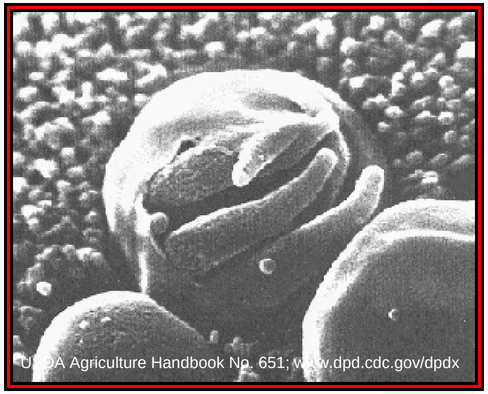
1. **Brief introduction to waterborne *Cryptosporidium***
   - **Biology and diversity of *Cryptosporidium* species**
   - **Current detection methodologies**

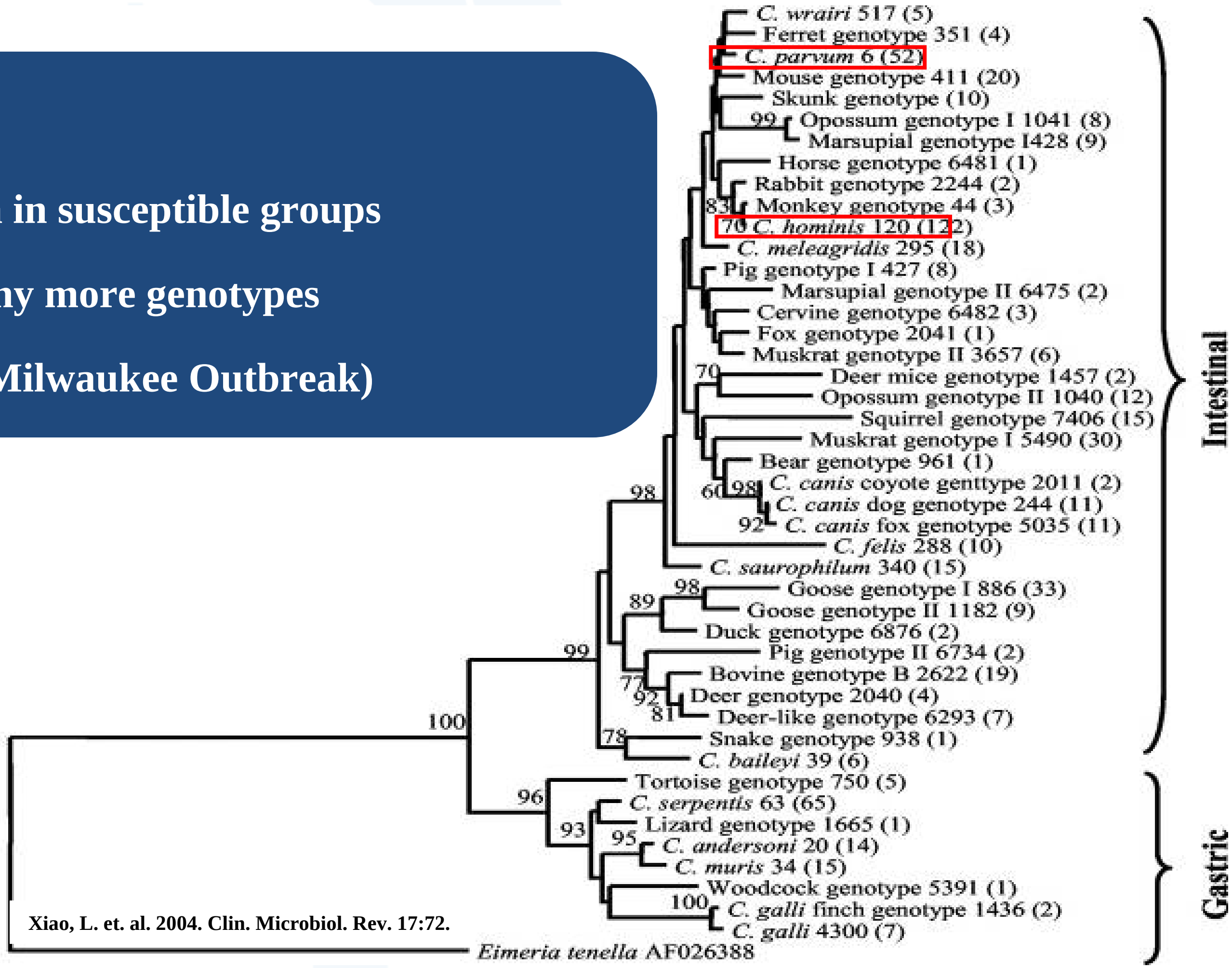
2. **US EPA-NERL's waterborne protozoan research program**
   - **Building a "Protozoan Detection Toolbox"**

3. **Perspectives on the future of the "Protozoan Detection Toolbox"**
   - **Future directions and considerations**

Cryptosporidium species

- **Enteric protozoan parasite**
- **Chronic diarrhea and death in susceptible groups**
- **At least 20 species, with many more genotypes**
- **Waterborne transmission (Milwaukee Outbreak)**


USDA Agriculture Handbook No. 651; www.dpd.cdc.gov/dpdx



Xiao, L. et. al. 2004. Clin. Microbiol. Rev. 17:72.

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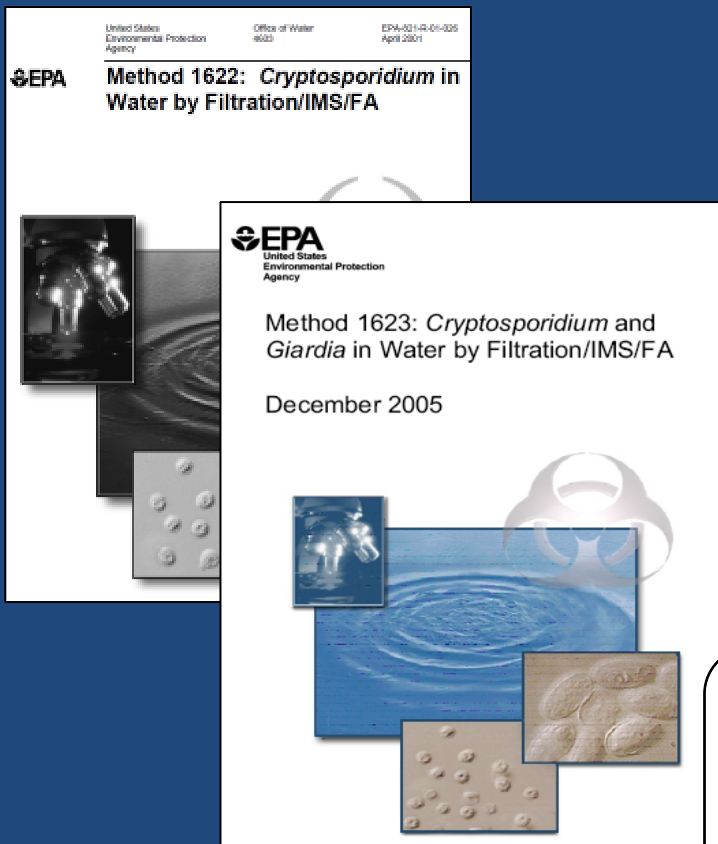
Cryptosporidium Species Infecting Humans and Selected Animals

Host	Major Species	Minor Species
Humans	*C. hominis and C. parvum* <u>(90% of all infections)</u>	*C. meleagridis, C. felis, C. canis, C. suis, cervine genotype*
Cat	*C. felis*	
Cattle	*C. parvum, C. bovis, C. andersoni, deer-like genotype*	*C. suis*
Chickens	*C. baileyi*	*C. meleagridis*
Deer	*C. parvum, deer genotype*	
Dog	*C. canis*	
Turkey	*C. meleagridis, C. baileyi*	
Pig	*C. suis*	*Pig genotype II*
Sheep	*Cervine genotype 1-3, bovine genotypes*	

Modified from Fayer and Xiao. 2008.

Method 1622/1623:
Detection of *Cryptosporidium* and *Giardia* "Now"


EPA

Method 1622: *Cryptosporidium* in Water by Filtration/IMS/FA

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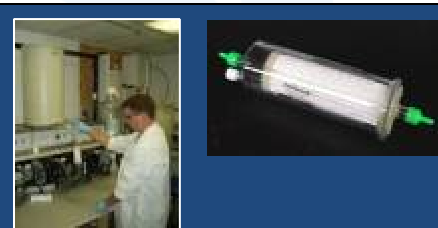
Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA

December 2005



Sample Collection Elution

Immunomagnetic Separation

Immunofluorescence Detection

Limitations:

- **Does not differentiate human infectious vs. animal forms**
- **No live vs. dead discrimination**

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Challenges for the 21st Century “Water Quality Tricorder”

Protozoan Detection Systems:

1. Fast and user friendly
2. Sensitive and quantitative
3. Species/genotype specific
4. Live vs. dead

United States Environmental Protection Agency

Research Focus Areas

HV, FiltaMax CFC → IMS/Acid → Microscopy

Method Now

- Improve filtration and concentration
- New technology to capture multiple pathogens

- New IMS reagents
  - antibodies
  - magnetic beads
- Improve recovery
  - Dissociation

- New reagents
  - Antibodies
- Cell Culture
- Molecular-based assays

Method of the Future

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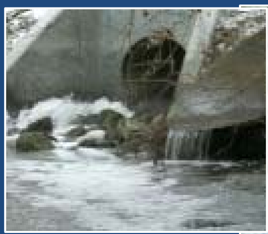
Question Driven Research

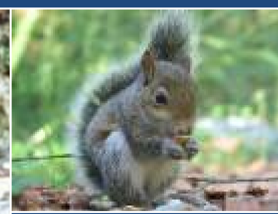
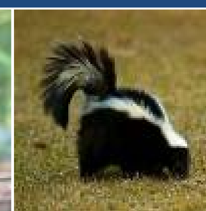
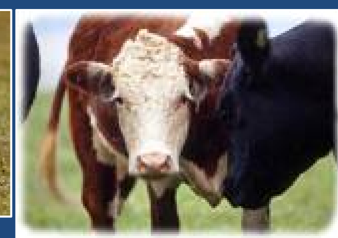
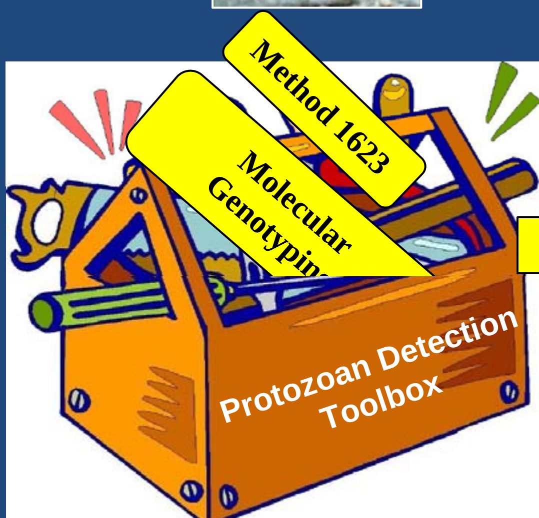
1. What are the total levels of *Cryptosporidium* in the watershed?
2. How complex is the *Cryptosporidium* species diversity in the watershed?
3. What are the total levels of pathogenic *Cryptosporidium* in the watershed?
4. Are the *Cryptosporidium* oocysts in the watershed viable/infectious?
5. Other questions…

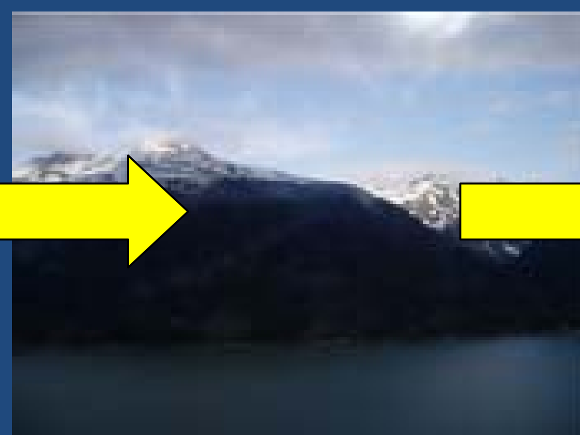
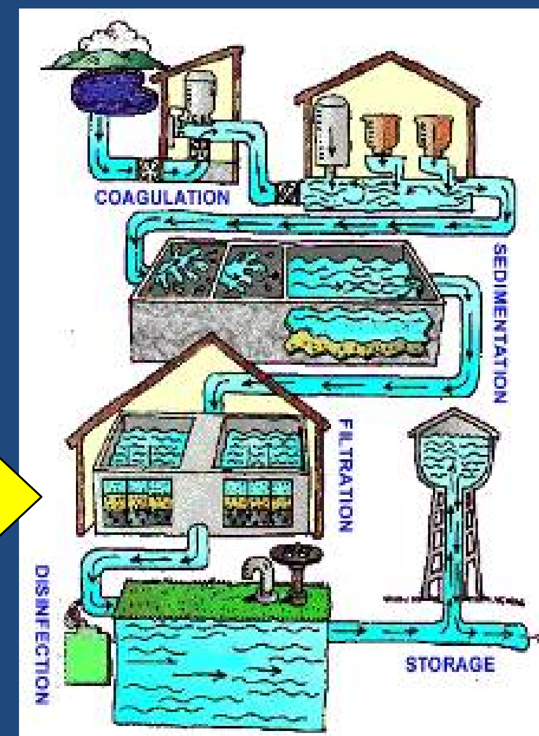
Tools for Source Tracking, Species Identification, and Genotyping

Sources of Contamination

Humans **Wild Animals** **Cattle/Livestock**



Tracking Sources of Contamination in a Watershed

Applied and Environmental Microbiology, Nov. 2008, p. 6495–6504 Vol. 74, No. 21
0099-2240/08/$08.00+0 doi:10.1128/AEM.01345-08

Cryptosporidium Source Tracking in the Potomac River Watershed[∇]

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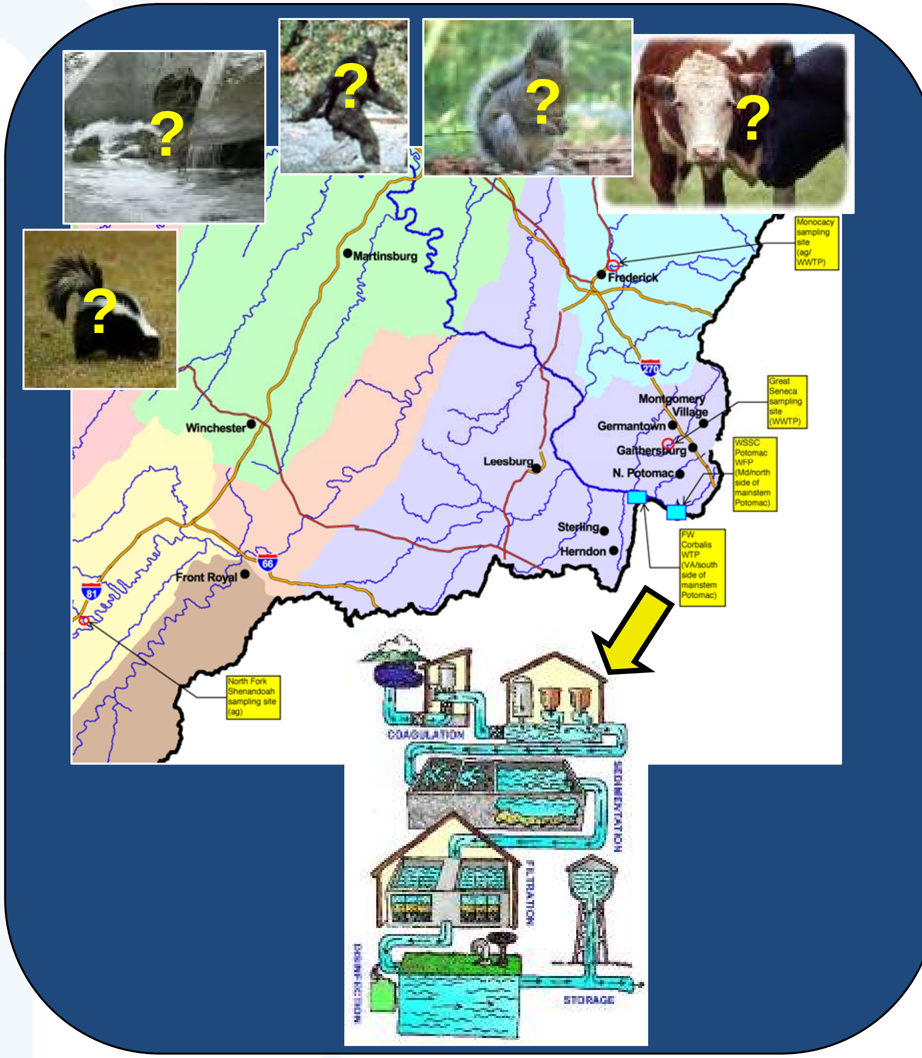
Goals

- **Identify types of *Cryptosporidium* oocysts present**
- **Use PCR-RFLP and Method 1623**
- **Identify potential sources of *Cryptosporidium* oocysts in the Potomac River**

Potential Sources:

Storm water runoffs
Wastewater treatment discharges
Wild animals
Agricultural/animal operations



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Methodology

Collection of 20-L water samples (93 samples)

Filtration of two 10-L samples

Method 1623

- One filter to an LT2 certified laboratory
- Immunomagnetic separation of oocysts
- Microscopy

Other branch:

- One filter to CDC laboratory
- Immunomagnetic separation of oocysts
- PCR, DNA sequencing




Species and Genotypes Found

TABLE 5. *Cryptosporidium* genotypes found in water samples in the Potomac watershed

Species or genotype	Major known host(s)	Minor known host(s)	No. of samples positive	No. of detections[a]	Detection site(s)
C. andersoni	Cattle	Sheep, humans (?)	41	167 (151 type A, 14 type B, and 2 type C sequences)	All except Great Seneca Creek[b]
C. felis	Cats	Cattle, humans	2	3	Great Seneca Creek
C. meleagridis	Birds	Humans, dogs, deer mice, brown rats	1	1	Great Seneca Creek
C. serpentis	Snakes, lizards		1	1	Potomac WFP
Deer mouse genotype III (W1)	Deer mice	Squirrels	3	5	Great Seneca Creek, Potomac WFP, Corbalis WTP
Deer mouse genotype IV (W3)	Deer mice		1	1	Great Seneca Creek
Cervine genotype (W4)	Sheep, zoo and wild ruminants, squirrels, chipmunks, woodchucks	Deer mice, beavers, raccoons, lemurs, humans	3	5	Great Seneca Creek
Muskrat genotype I (W7)	Muskrats, voles		3	4	Corbalis WTP, North Fork Shenandoah River, Monocacy River
Snake genotype (W11)	Snakes		1	1	Potomac WFP
W12			1	1	Great Seneca Creek
Skunk genotype (W13)	Skunks	Raccoons, otters, opossums, squirrels, humans	4	5	Great Seneca Creek, Potomac WFP, Corbalis WTP
Vole genotype (W15)	Voles		1	1	North Fork Shenandoah River
Tortoise genotype	Tortoises		1	1	Great Seneca Creek
C. bovis-like genotype			1	1	Potomac WFP
Mouse genotype II-like	Mice		1	3	North Fork Shenandoah River

[a] Total number of positive samples for five PCR replicates of all samples.
[b] Detected in one PCR replicate of one storm flow water sample from the Great Seneca Creek.

Yang, et.al. 2008. Applied and Environmental Microbiology

United States Environmental Protection Agency

Summary and Impact:

Summary

- **A cattle specific species (*C. andersoni)* was the predominant oocyst detected tested**
- **Pathogenic *C. hominis* and *C. parvum* were not detected in all 93 samples analyzed**
- **Only minor species/genotypes infecting humans were detected (10 samples)**
- **Molecular-based detection technique used in this project proves to be sensitive to detect and genotype oocysts in source waters**

Impact

- **Helped Utilities and Region 3 understand that oocysts in the surrounding county's source water are predominantly non-pathogenic**
- **Utilities are setting out to work with the agricultural community by encouraging and implementing better management practices (BMPs) in the local cattle/dairy industry**

What Lies Ahead for the Waterborne *Cryptosporidium* Research Program?

Multiple Pathogen Detection Systems

Quantitative PCR-Based Detection of *Cryptosporidium spp.*

- Many species and genotypes found in source water
- Most quantitative PCR published have varying degrees of specificities
- Development of multiplex qPCR assays

species	All *Cryptosporidium* spp.	*C. parvum* specific	*C. hominis* specific
C. parvum	+	+	-
C. hominis	+	-	+
C. muris	+	-	-
*C. meleagridis**	+	-	-
*C. felis**	+	-	-
*C. canis**	+	-	-
T. gondii	-	-	-

* Purified genomic DNA from CDC

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Molecular Detection Technologies: A Perspective

1. Molecular-based detection of *Cryptosporidium* is in its infancy

2. A better understanding of the differences between zoonotic and human-specific *Cryptosporidium/Giardia* is possible

3. Advances in the "Protozoan Detection Toolbox" will improve our understanding of these parasites and their relationship to public health

Using the Protozoan Detection Toolbox To Address our Questions

1. What are the total levels of *Cryptosporidium/Giardia* in the watershed?
2. What are the total levels of pathogenic *Cryptosporidium/Giardia* in the watershed?
3. How complex is the *Cryptosporidium/Giardia* species diversity in the watershed?
4. Are the *Cryptosporidium/Giardia* oocysts in the watershed viable/infectious?
5. Other questions…

Method 1623

PCR-RFLP, qPCR

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Interstate Commission for the Potomac River Basin
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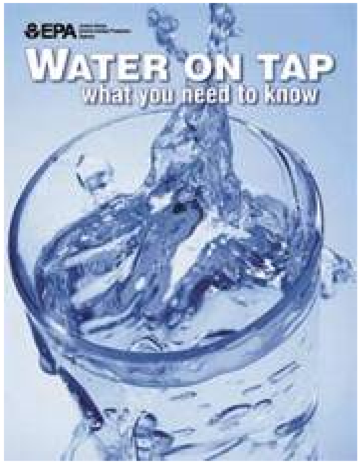
Questions?



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