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**Source Tracking Studies
in the
New York City Watershed**
(The ever changing Microbial Toolbox!)

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Outline

- Introduction to the NYC Water Supply
- Research Microbiology Unit mission
- Methods
- Case studies
 - Coliphage typing
 - Ribotyping
 - Genotyping
- Summary

NYC System Statistics

- 1,972 square-mile watershed
- 19 reservoirs, 3 controlled lakes
- 1.2 BGD, 9 million people
- Catskill and Delaware 90%
- Croton 10%
- Varying land uses

The NYC Water Supply



Research Microbiology Unit

- Mission:
 - To compliment routine microbiology operations by investigating unusual microbiological occurrences in the NYC watershed.
 - May include investigation of algal or bacterial blooms, bacterial source identification, protozoan analysis, and any contamination of the system through accidental or intentional actions.

Why?







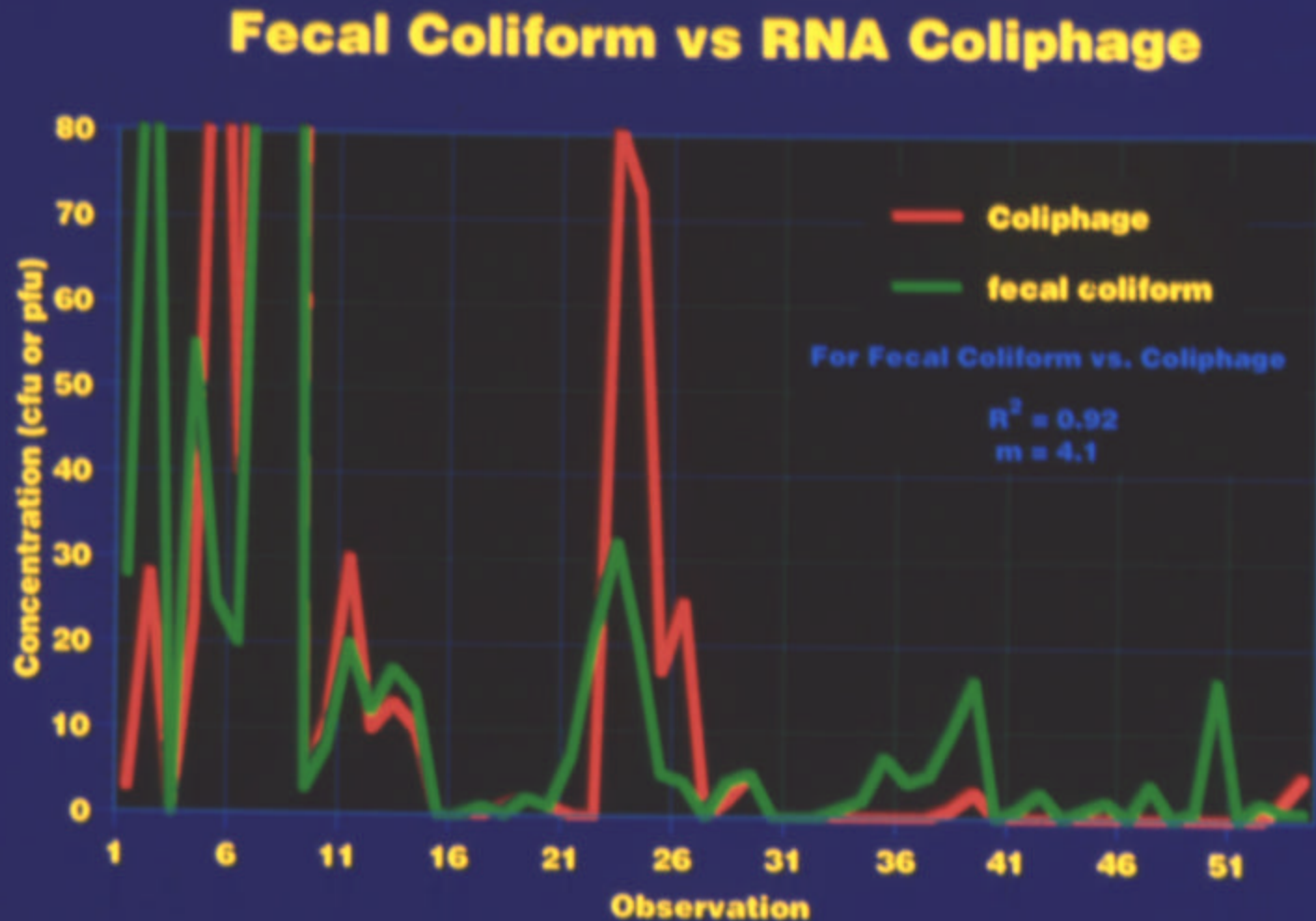
Methods

- Serotyping *E. coli*
- Electrophoretic typing *E. coli*
- Fecal Streptococcus/ Enterococcus
- *Clostridium perfringens*
- Coliphage typing
- Ribotyping *E. coli*
- *Cryptosporidium* genotyping

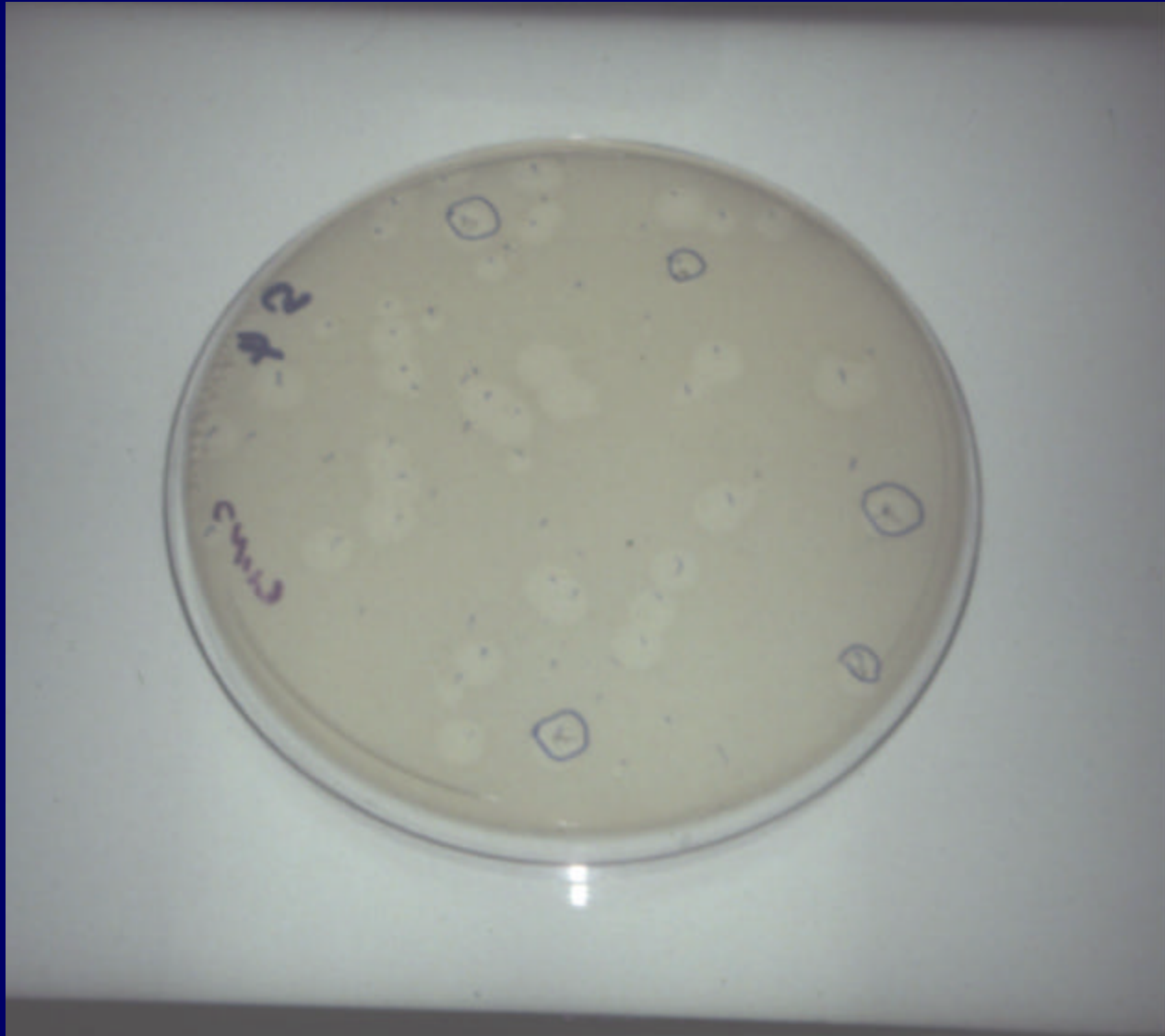
How do they work?

- F-specific RNA coliphage analysis
 - subtyping for group characterization
- Ribotyping *E. coli*
 - *comparing to a library of sources*
- Genotyping *Cryptosporidium* oocysts
 - Subspeciating and comparing genetic patterns

Correlation of fecal coliforms and F-specific RNA coliphages



Plaque growth

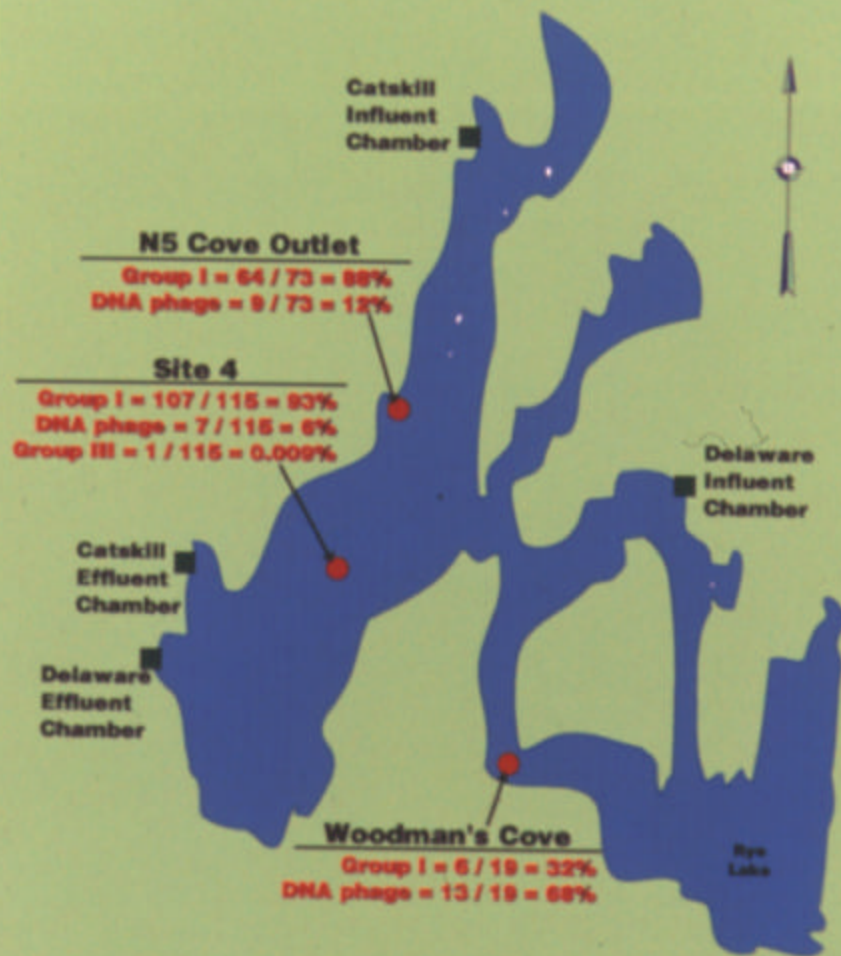


F-specific RNA Coliphage Groups

- **Group I** – almost exclusively non-human animals
- **Group II** – predominantly human source, (bovine, swine)
- **Group III** – predominantly human and swine sources
- **Group IV** – predominantly non-human sources

Coliphage Sampling Sites and Categorization of Results

Kensico Reservoir (1992 - 1994)



Coliphage Results Summary

Kensico Reservoir Study

Group	Site 4	N5 Cove	Woodman's Cove
I	107/115 (93%)	64/73 (88%)	6/19 (32%)
II	0	0	0
III	1/115 (0.9%)	0	0
IV	0	0	0

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Fecal Coliform Concentrations of Local Gulls and Geese

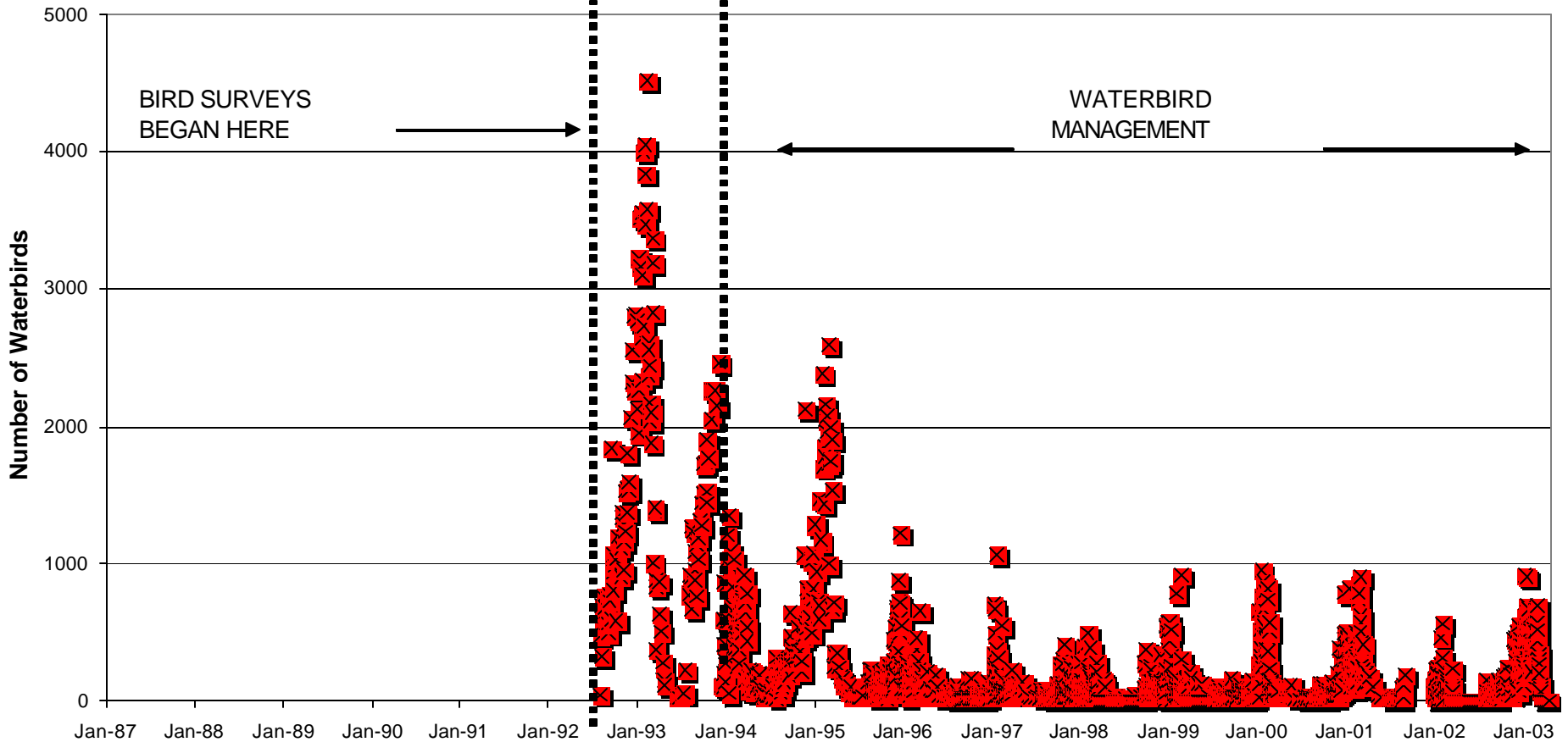
- 249 Ring-Billed Gulls
 - Gull feces = 3.68×10^8 FC per gram
 - Gull feces weight = 0.48g
- 236 Canada Geese
 - Goose feces = 1.53×10^4 FC per gram
 - Goose feces weight = 8.35g

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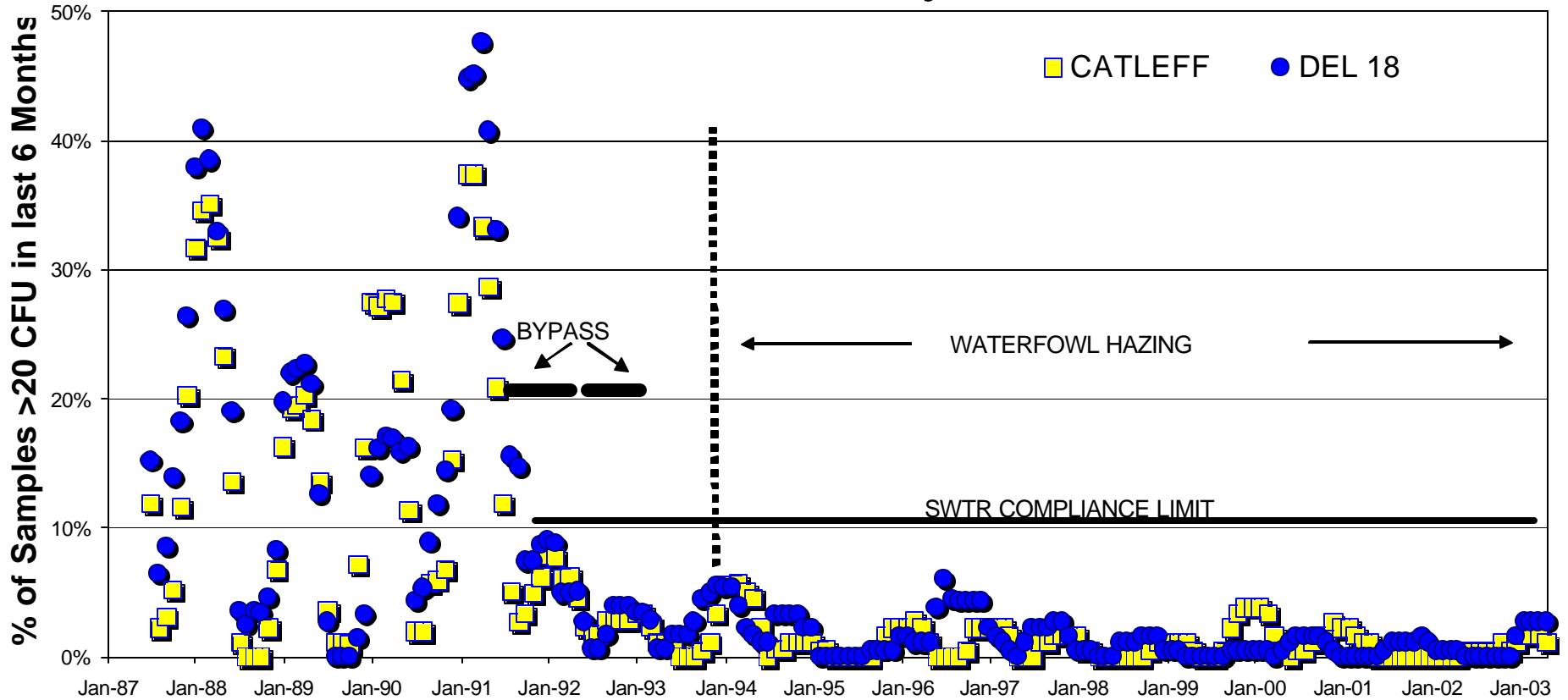


'I'll put a diaper on a sea gull,
but I won't change it.'

Kensico Reservoir Waterbirds Waterfowl Program Data



Kensico Reservoir Keypoints Fecal Coliform Bacteria Kensico Laboratory Data



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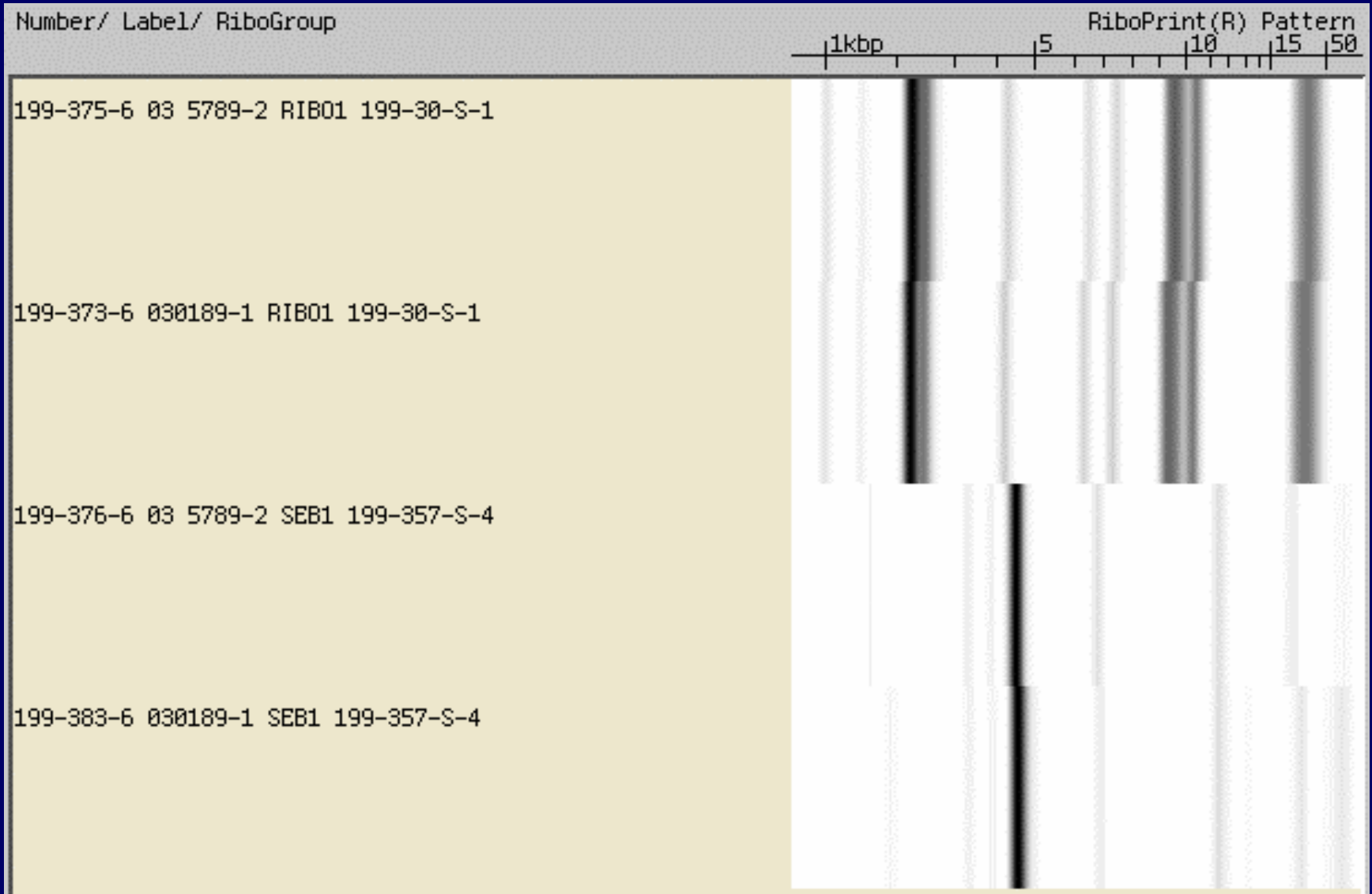


'Chasing geese all day. On the county payroll.
Pinch me, Al!'

Ribotyping

- Creating a library of *E. coli* sources:
 - Are all libraries created equally?
 - Is there geographic diversity among species?
 - What about transient and resident strains?
 - What about the consumption cycle?
 - Does *E. coli* have what it takes?
 - Promising for the future...

E. coli from Goose Feces and an Effluent Chamber Using EcoR1 and HIND III



Objectives of the *Cryptosporidium* Genotyping Study

- ❑ To capture oocysts from storm samples and recover them in the laboratory for molecular analysis
- ❑ To identify the sources of *Cryptosporidium* in two sub-basins thorough the use of molecular techniques
- ❑ To assess the human-infective potential of *Cryptosporidium* spp. found in storm water in two watersheds (Ashokan and Malcolm Brooks) of NYC DEP

Importance of Molecular Tools in the Study of *Cryptosporidium* in Water

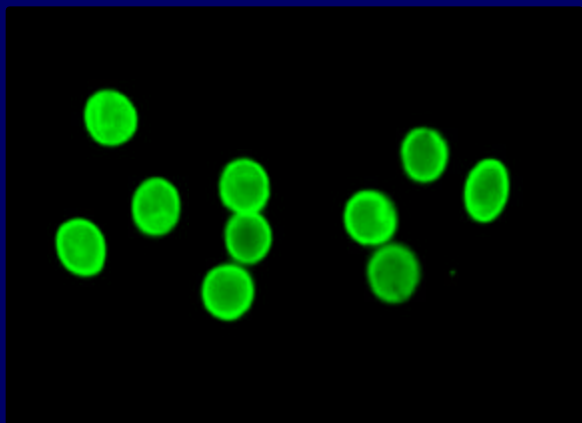
□ Useful in the assessment of human infection potential of *Cryptosporidium* parasites found in water

- Only a few *Cryptosporidium* spp. are human pathogens
- Among human-pathogenic *Cryptosporidium* spp., the public health significance of different species is different

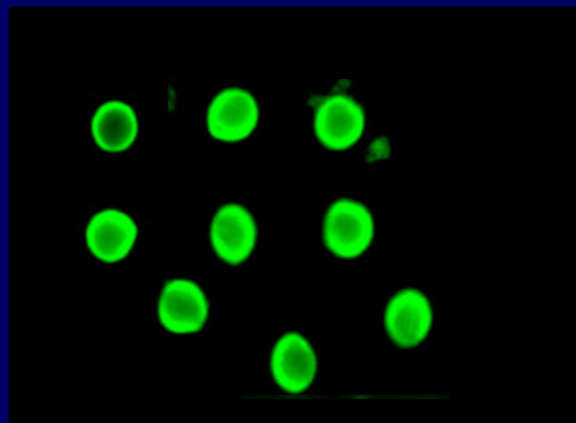
□ Helpful to the identification of contamination sources

- Most *Cryptosporidium* spp. preferentially infect specific hosts

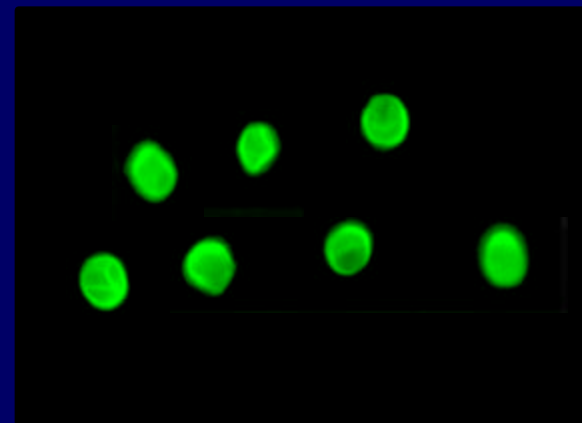
**Similarity in Immunofluorescence of Oocysts from
Human-pathogenic and Non-human-pathogenic *Cryptosporidium* spp.**



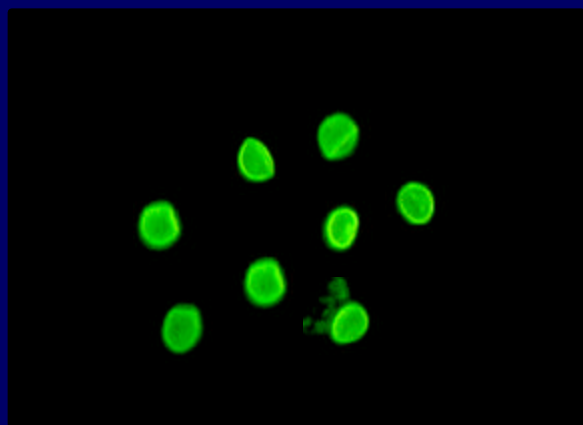
C. hominis



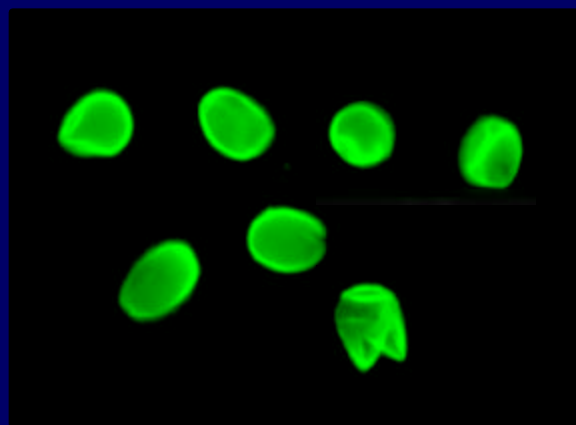
C. parvum bovine



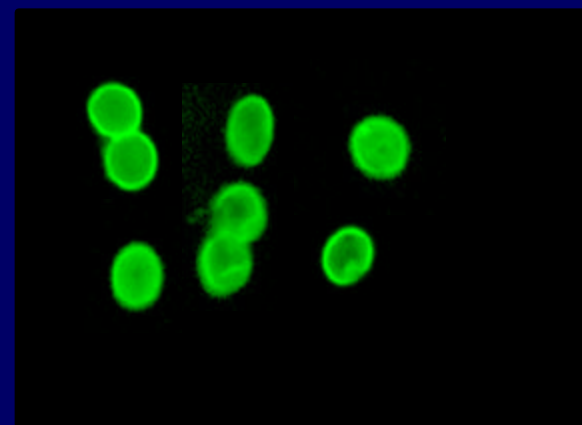
C. parvum mouse



C. saurophilum



C. muris



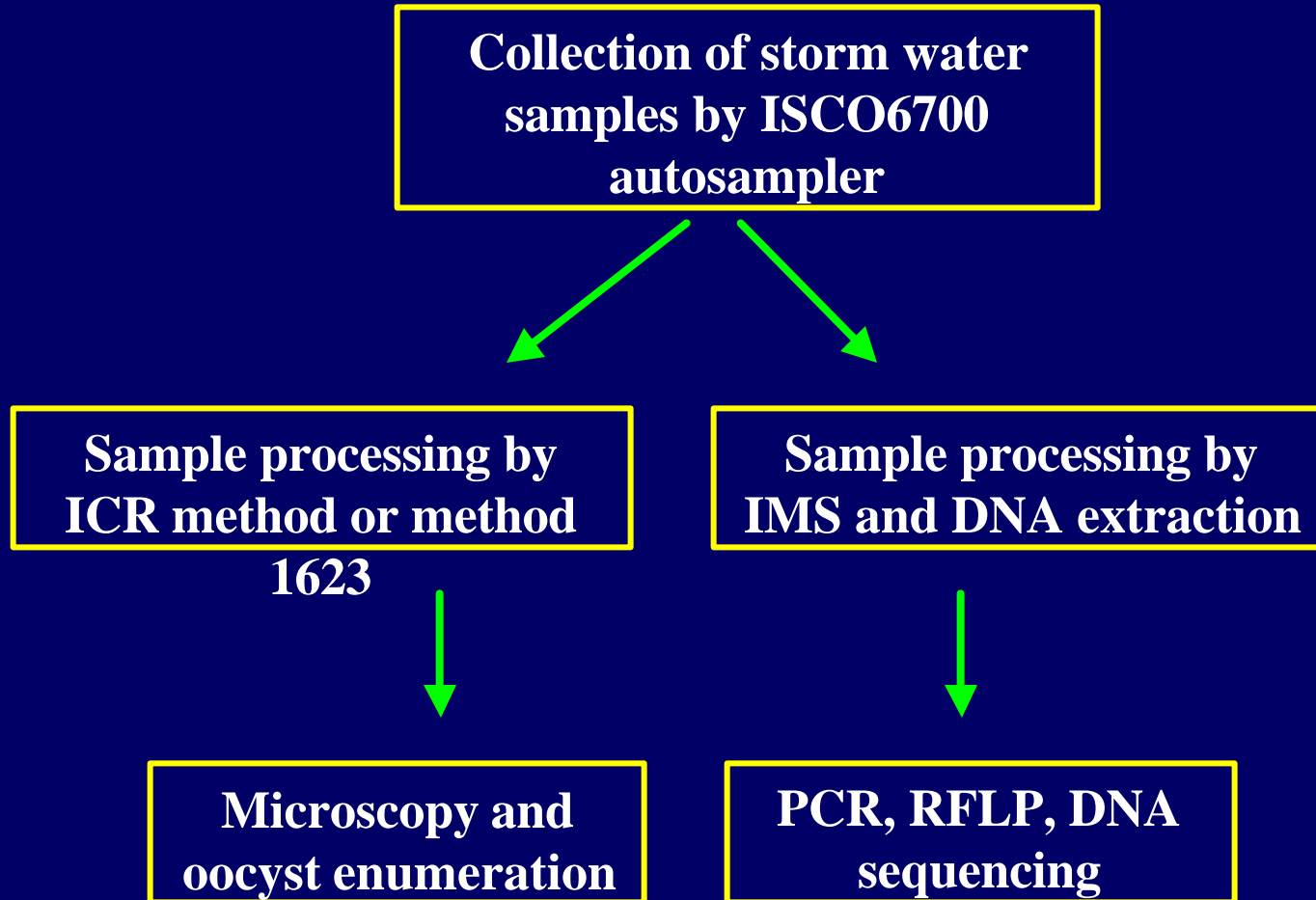
C. baileyi

Human-pathogenic *Cryptosporidium* spp.

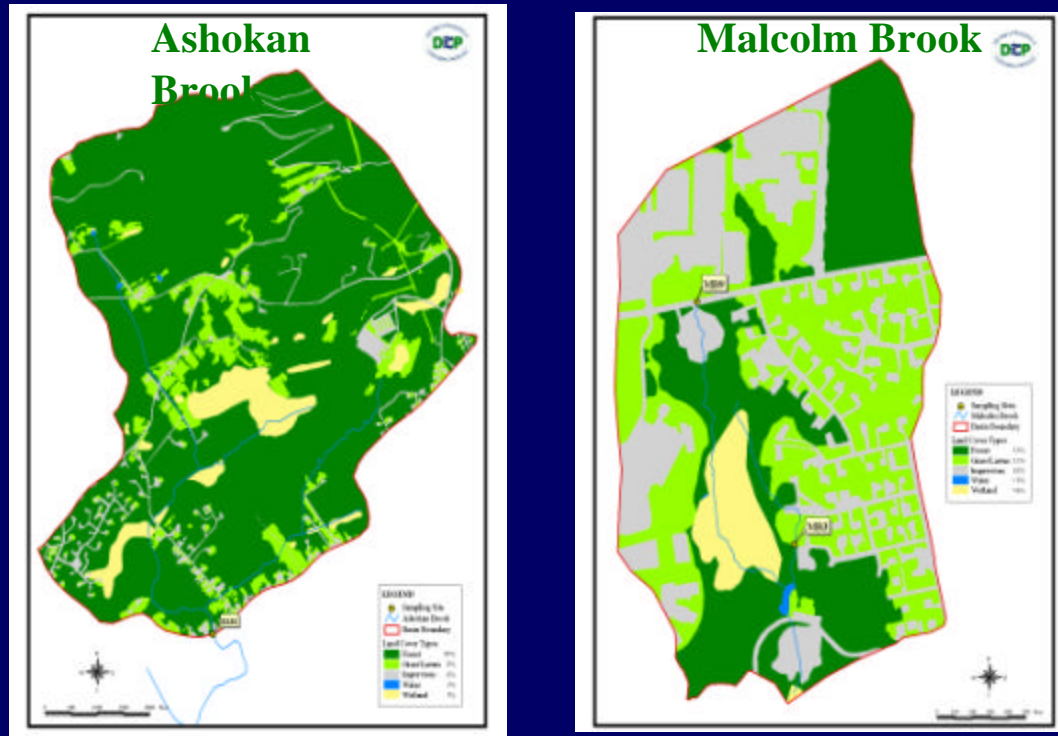
- *C. hominis*
- *C. parvum*
- *C. meleagridis*
- *C. felis*
- *C. canis*

- *C. muris*
- *Cryptosporidium* cervine genotype
- *C. suis*

Methodology

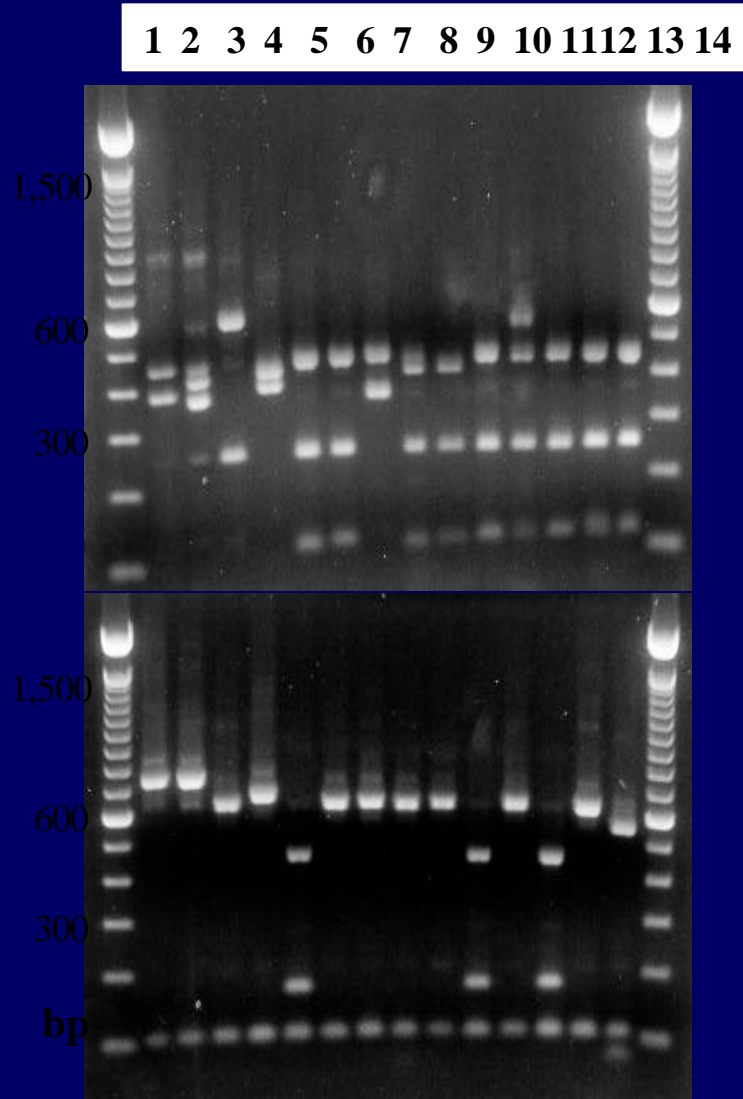


Differences in the Ecology between Ashokan and Malcolm Brooks, NYCDEP



	Ashokan	Malcolm
Forest	78%	31%
Grass/lawn	8%	31%
Impervious	6%	32%
Water	1%	<1%
Wetland	7%	<6%

Differentiation of *Cryptosporidium* Species and Genotypes by SSU rRNA-based PCR-RFLP



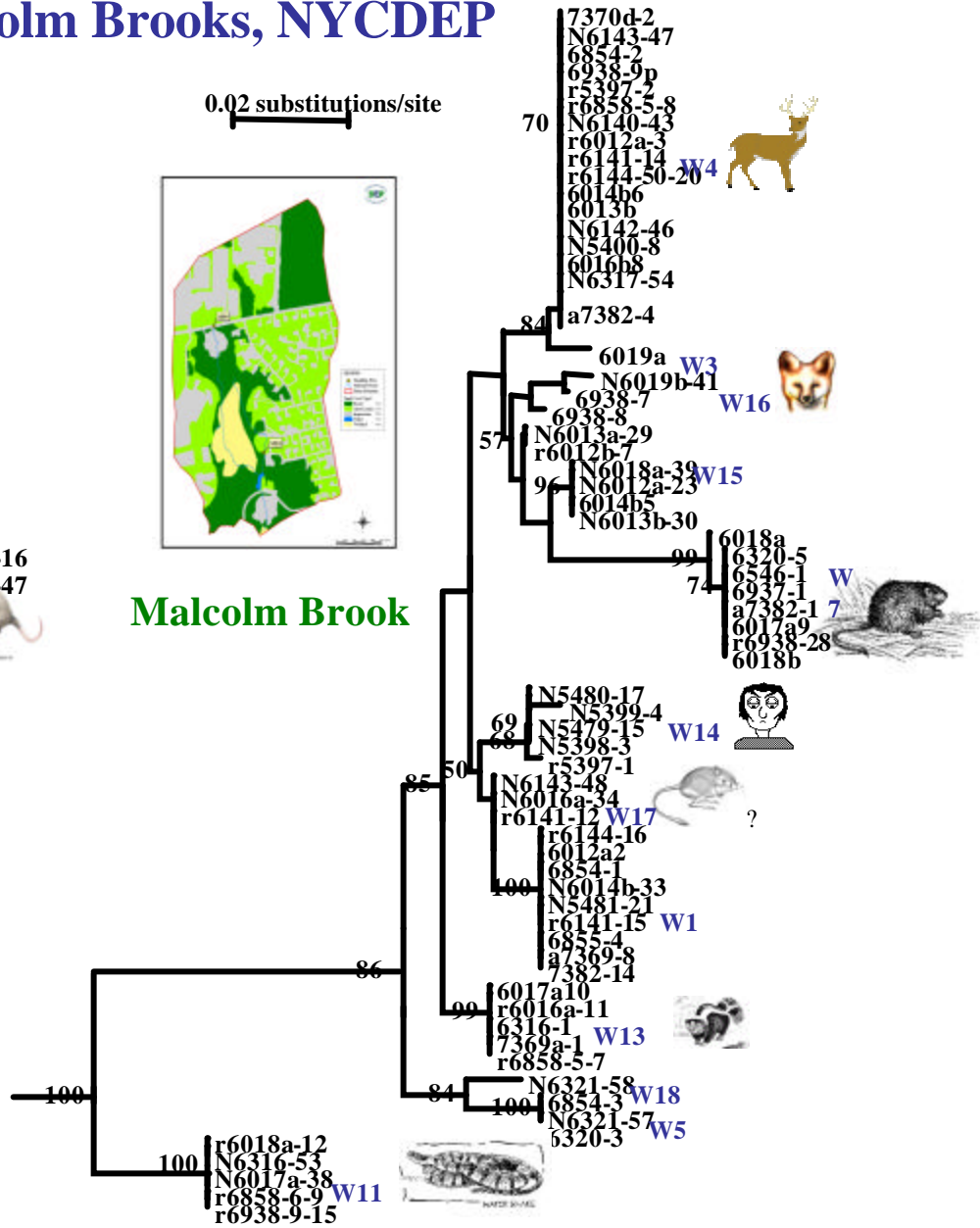
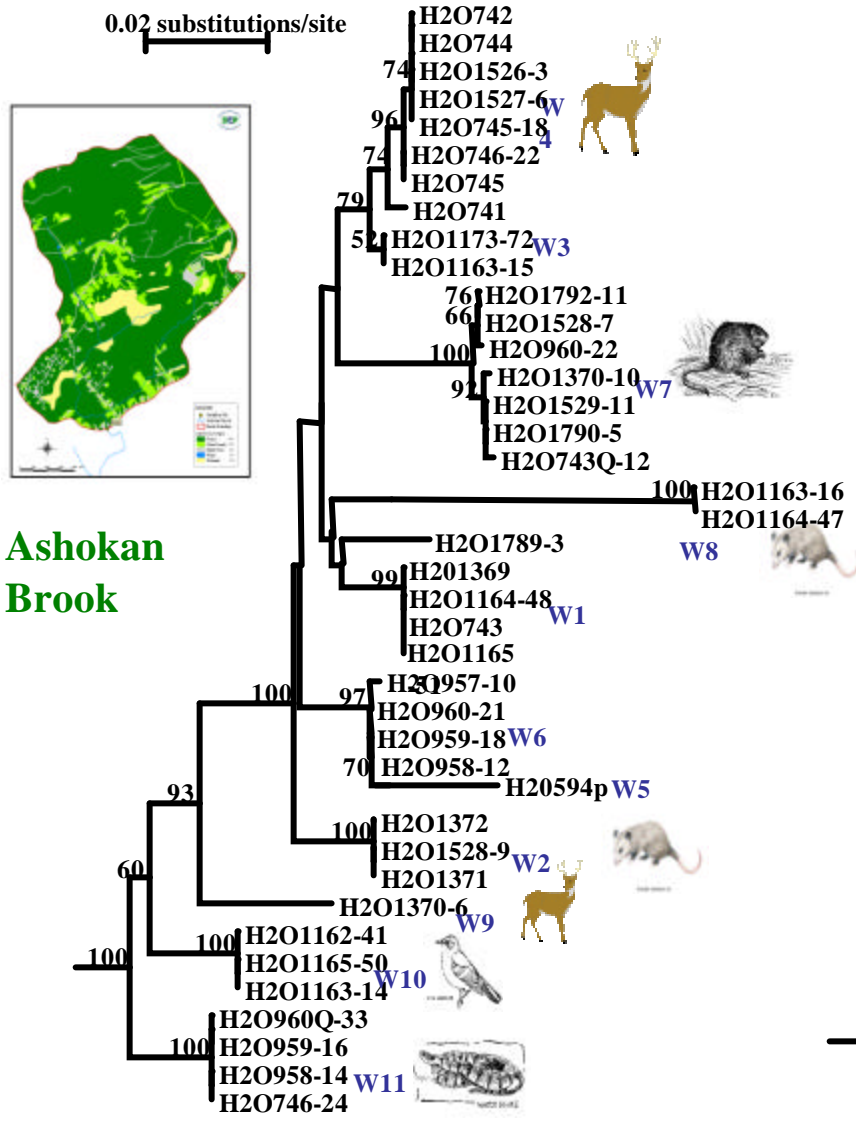
1. *C. muris*
2. *C. serpentis*
3. *C. baileyi*
4. *C. felis*
5. *C. meleagridis*
6. *C. wrairi*
7. pig genotype I
8. *C. canis*
9. *C. saurophilum*
10. ferret genotype
11. marsupial genotype I
12. mouse genotype
13. *C. parvum*
14. *C. hominis*

Upper panel: *SspI* digestion

Lower panel: *VspI* digestion

Based on Xiao et al., 1999a, 1999b

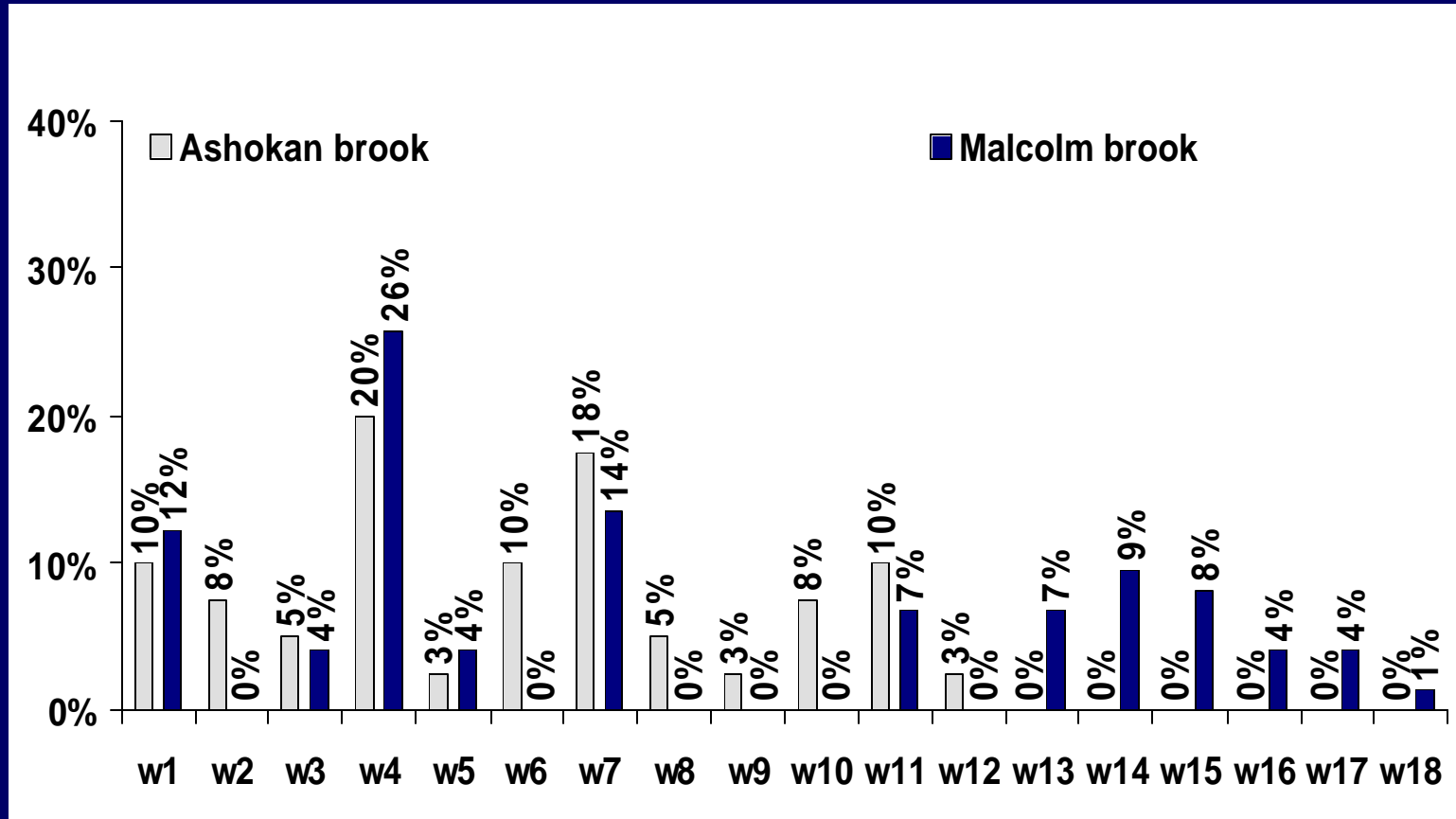
Sources of *Cryptosporidium* Contamination in Storm Water from Ashokan and Malcolm Brooks, NYCDEP



Number of Genotypes per Storm Water Sample from the Malcolm and Ashokan Brooks

No. of genotypes by sample	No of samples (%)	
	Ashokan Brook	Malcolm Brook
0	2 (6.9)	8 (20.0)
1	16 (55.2)	12 (30.0)
2	9 (31.0)	10 (25.0)
3	2 (6.9)	8 (20.0)
4	0	1 (2.5)
5	0	1 (2.5)

Distribution of *Cryptosporidium* genotypes in storm water from Ashokan and Malcolm Brooks in New York



Summary

- There is no single source tracking analysis that points to the “smoking gun”
- All source tracking strongly suggests predominantly non-human influence in NYC watershed so far
- Need more work on sources for RT, and positive controls for human influences
- Any Questions?