Factors Related to the Occurrence and Distribution of Select Bacterial and Protozoan Pathogens in Pennsylvania Streams

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Background

- What do indicators indicate?
- New studies dealing with fecal indicator bacteria (FIB) relation to pathogens
  - (Streams) Season, land use, discharge, turbidity, chemistry
  - (Lakes) Wave height, wind direction, storm drain flow
- Few studies have the same level of chemical and hydrologic data available from the WQN

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Shiga toxin-producing *E. coli* (STEC)

- **STEC are a main cause of intestinal disease in humans**
  - **Over 200 STEC types identified with various combinations of virulence genes**
- **STEC have been associated with outbreaks and death**
  - **Food and Water**
- ***E. coli* O157:H7 is most frequently isolated type in North America**
  - **Only STEC type that is “easy” to identify**
  - **Small infectious dose (fewer than 10 cells)**
  - **Typically most virulent combination of genes**
- **Microbial Source Tracking (MST) application**

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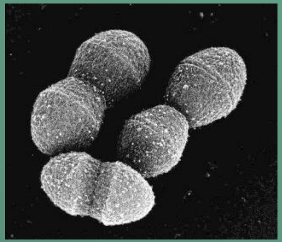
Pathogenic Enterococci

- Pathogenic *Enterococcus*
  - Not a common intestinal pathogen (i.e. does not typically cause diarrhea)
  - However, enters body via oral/fecal transmission
  - Enterococcus strains with the *esp* gene
    - Bacteremia
    - Endocarditis
    - Wound infection
    - Urinary Tract Infection
  - *esp* gene is cited in the literature as being a good marker for Enterococci from a *human* source (MST application)



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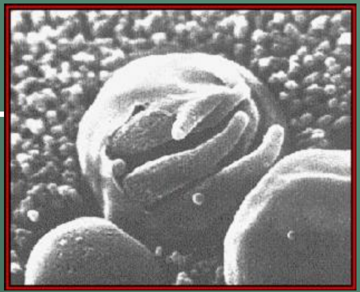
Giardia and Cryptosporidium

- Analysis done by PA-DEP Bureau of Labs
- Protozoan parasites
- USEPA Method 1623
- Giardia
  - Causes Giardiasis, (diarrheal disease)
  - Exist as cysts in the environment (moderately chlorine resistant)
  - 6-19 microns
- Cryptosporidium
  - Causes Cryptosporidiosis (diarrheal disease)
  - Exist as oocysts in the environment (chlorine resistant)
  - 3-5 microns



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Pathogen and MST Approach

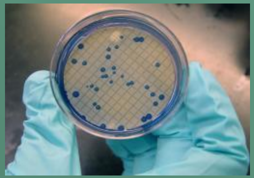
- Use of fecal indicator bacteria cultures as platform
  - Growth/viability of the target organisms
    - Original Indicator Sample
  - Target pathogens and source tracking markers within those cultures
    - Enrichment PCR
    - Improved detection limits over direct PCR
      - Most direct PCR assays reported in the literature have high detection limits (e.g., 20 target CFU/ml) due to matrix interference and small volumes
  - Relevance to water quality criteria/standards
    - Pathogen occurrence can be related to concentrations of fecal indicator bacteria

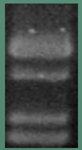
Pathogen and MST Gene Targets

Organism	Gene Name	Gene Product	Gene Function
E. coli	*eaeA*	Intimin protein	Allows *E. coli* to tightly bind intestine
E. coli	*stx1*	Shiga Toxin 1	Causes less severe disease in humans (more common in ruminants than human)
E. coli	*stx2*	Shiga Toxin 2	Causes severe disease in humans (HC, HUS)
E. coli	*E. coli 16s*	*E. coli* ribosome	Universal marker of all *E. coli,* control
E. coli	*rfbO157*	O157 surface protein	Marker of *E. coli* O157 (hamburger outbreak *E. coli*)
E. coli	LTIIa	Heat Labile Toxin Subunit IIa	Bovine Sources, gastroenteritis in humans
E. coli	STII	Heat Stabile Toxin Subunit II	Swine Sources, swine and human disease
Enterococci	esp	Enterococcus surface protein	Human Sources, associated with human disease (skin and bladder infections)

Measured Parameters

- Presence/absence of Gene Markers (Pathogen & MST)
- Densities of *E. coli*, enterococci, *Cryptosporidium* oocysts, *Giardia* cysts
- Physical parameters
  - Discharge, total dissolved solids, total suspended solids, ANC
- Chemistry
  - Basic chemistry
  - Nutrients
  - Metals
  - Organics
    - Pharmaceuticals, antibiotics, hormones
- Site Characteristics
  - Land-use, proximal & catchment, basin slope, drainage area, % carbonate bedrock and glacial deposit at catchment level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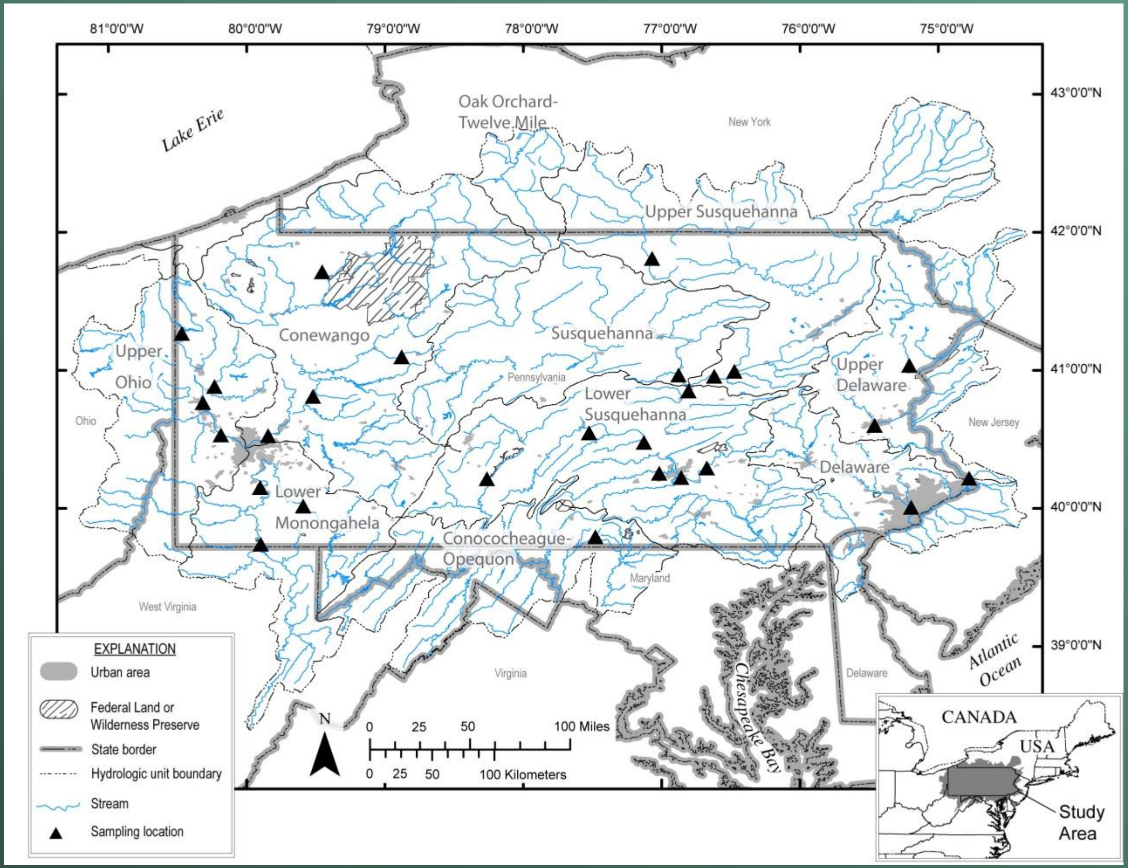
Study Area



- 27-stations
- Quarterly samples
- 2 years (2007-2009)
- Drainage areas from 5 km^2 to 49,000 km^2
- Within 5 miles of drinking water intake

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Study Goals

- Determine the frequency of occurrence of select bacterial and protozoan pathogens
- Determine relation of Indicator Bacteria to pathogens
- Determine factor(s) related to the occurrence of pathogens in Pennsylvania streams

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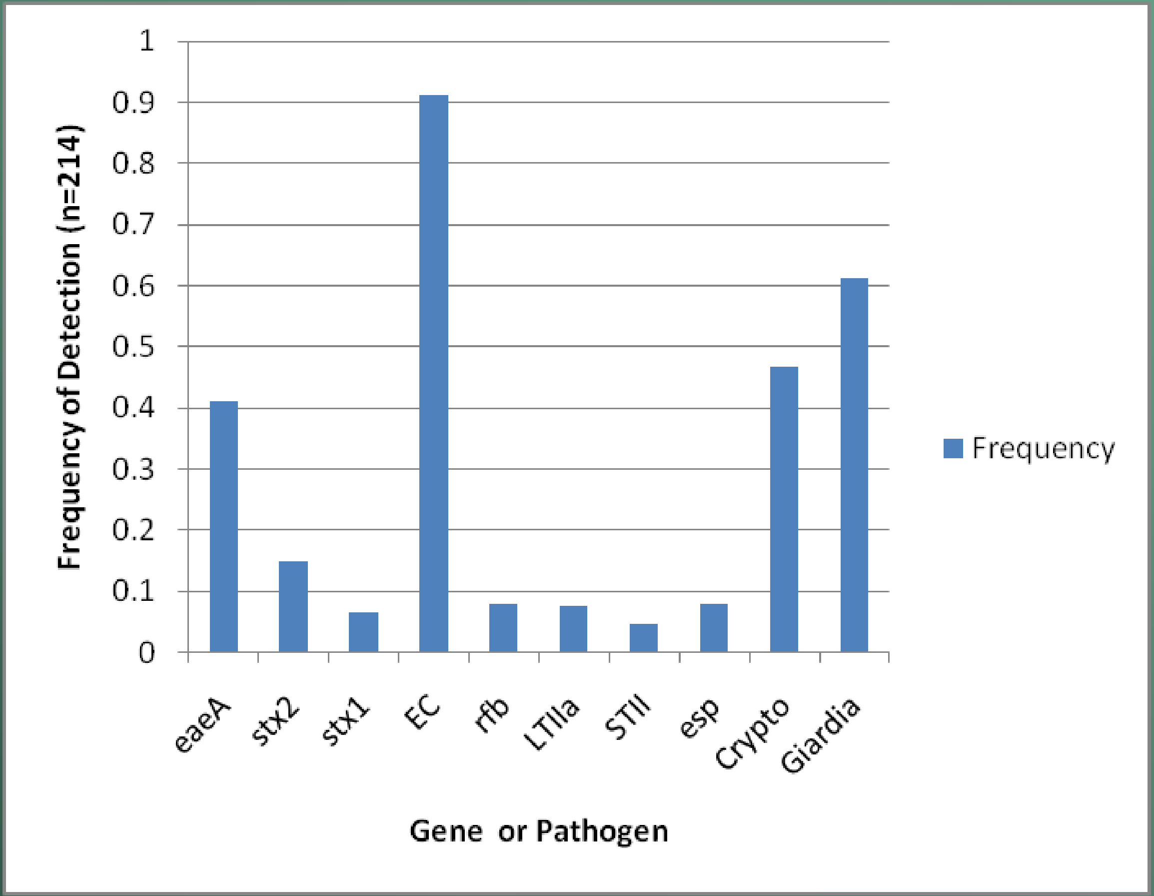
Frequency of Pathogen Detection



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Pathogen Relation to Indicator Criteria

	(n)	*eaeA* (%)	*stx2* (%)	*stx1* (%)	*rfb*O157 (%)	*LTIIa* (%)	*STII* (%)	*esp* (%)	*Crytposporidium* (%)	*Giardia* (%)
Meet *E. coli* criteria[a]	***186***	**32**	**7.6**	**3.2**	**3.8**	**3.8**	**1.6**	**2.2**	**44**	**58**
Exceed *E. coli* criteria	**31**	**97**	**53**	**26**	**32**	**32**	**23**	**42**	**61**	**77**
p-value[b]	**--**	**p<0.05**	**p<0.05**	**p<0.05**	**p<0.05**	**p<0.05**	**p<0.05**	**p<0.05**	**p=0.100**	**p=0.057**
Meet enterococci criteria[c]	**163**	**29**	**5.5**	**2.5**	**3.1**	**1.2**	**0.6**	**1.8**	**42**	**57**
Exceed enterococci criteria	**54**	**78**	**43**	**19**	**22**	**26**	**17**	**26**	**57**	**70**
p-value[b]		**p<0.05**	**p<0.05**	**p<0.05**	**p<0.05**	**p<0.05**	**p<0.05**	**p<0.05**	**p=0.076**	**p=0.115**

[a] Recreational Water Quality Moderate Full Body Contact Criteria for *E. coli*, 298 CFU 100 L^{-1}

[b] Pearson chi-square test with Yates' correction

[c] Recreational Water Quality Moderate Full Body Contact Criteria for enterococci, 78 CFU 100 L^{-1}

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Seasonal Differences in Indicator Bacteria and Protozoan Densities and Frequencies

		Concentration				Frequency (%)			
	(n)	*E. coli*[a]	*Enterococci*[a]	*Cryptosporidium*[b]	*Giardia*[b]	*E. coli*	*Enterococci*	*Cryptosporidium*	*Giardia*
Winter	**26**	**295**	**322**	**0.18**	**0.36**	**76.9**	**84.6**	**50**	**77**
Spring	**84**	**296***	**227***	**0.07**	**1.45***	**88.1**	**79.8**	**43**	**70**
Summer	**54**	**1186***	**1331***	**0.11**	**0.43**	**98.2**	**94.6**	**39**	**50**
Autumn	**50**	**435**	**749**	**0.19**	**0.63***	**96.1**	**82.4**	**57**	**47**
p-value[c]	--	p<0.05	p<0.05	P=0.270	p<0.05	<0.05	p=0.227	p=0.387	p<0.05

[a] *[E. coli]*,Geometric Mean CFU100 mL^{-1} ; *[Enterococci]* Mean CFU100 L^{-1};

[b] *[Cryptosporidium]* Mean oocysts, 1 L^{-1}; *[Giardia]* ,Mean cysts, 1 L^{-1}

[c]Pearson chi-square test with Yates' correction (frequency), or ANOVA on ranks of log transformed concentrations (concentrations)

Note: Dec-Feb, Winter; Mar-May, Spring; Jun-Aug, Summer; Sep-Nov, Autumn

Pathogen Relation to Season

Season	*(n)*	*eaeA* (%)	*stx2* (%)	*stx1* (%)	*rfb*O157 (%)	*lt*IIa (%)	*st*II (%)	*esp* (%)
Winter	26	38	15	3.8	3.8	7.7	7.7	15
Spring	84	24	9.5	4.8	7.1	6.0	2.4	7.1
Summer	54	59	23	12	11	11	7.1	8.9
Autumn	50	45	14	3.9	7.8	5.9	3.9	3.9
p-value[a]	--	$p<0.05$	$p=0.259$	$p=0.414$	$p=0.9127$	$p=0.874$	$p=0.815$	$p=0.625$

[a] Pearson chi-square test with Yates' correction

Note: Dec-Feb, Winter; Mar-May, Spring; Jun-Aug, Summer; Sep-Nov, Autumn

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Relation of Discharge to Indicator and Protozoan Densities

		Density			
Discharge Category[a]	*(n)*	*E. coli*[b]	*Enterococci*[b]	*Cryptosporidium*[b]	*Giardia*[b]
Low	35	49*	32*	0.08	0.41
Median	118	333	389	0.13	1.27*
High	61	1242*	1441*	0.15	0.37*
p-value[c]	--	$p<0.05$	$p<0.05$	$p=0.224$	$p<0.5$

[a] Low, discharge measured was less than 25th percentile of daily mean stream flow at sample location; Median, discharge measured was between 25th and 75th percentile of daily mean stream flow at sample location; High, Discharge measured was greater than 75th percentile of daily mean stream flow at sample location

[b] *[E. coli]*,Mean CFU100 L^{-1} ; *[Enterococci]* Mean CFU100 L^{-1}; *[Cryptosporidium]* Mean oocysts, 1 L^{-1}; *[Giardia]* ,Mean cysts, 1 L^{-1}

[c]Pearson chi-square test with Yates' correction (frequency), or ANOVA on ranks of log transformed concentrations (concentrations)

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Relation of Discharge to Pathogen Frequencies

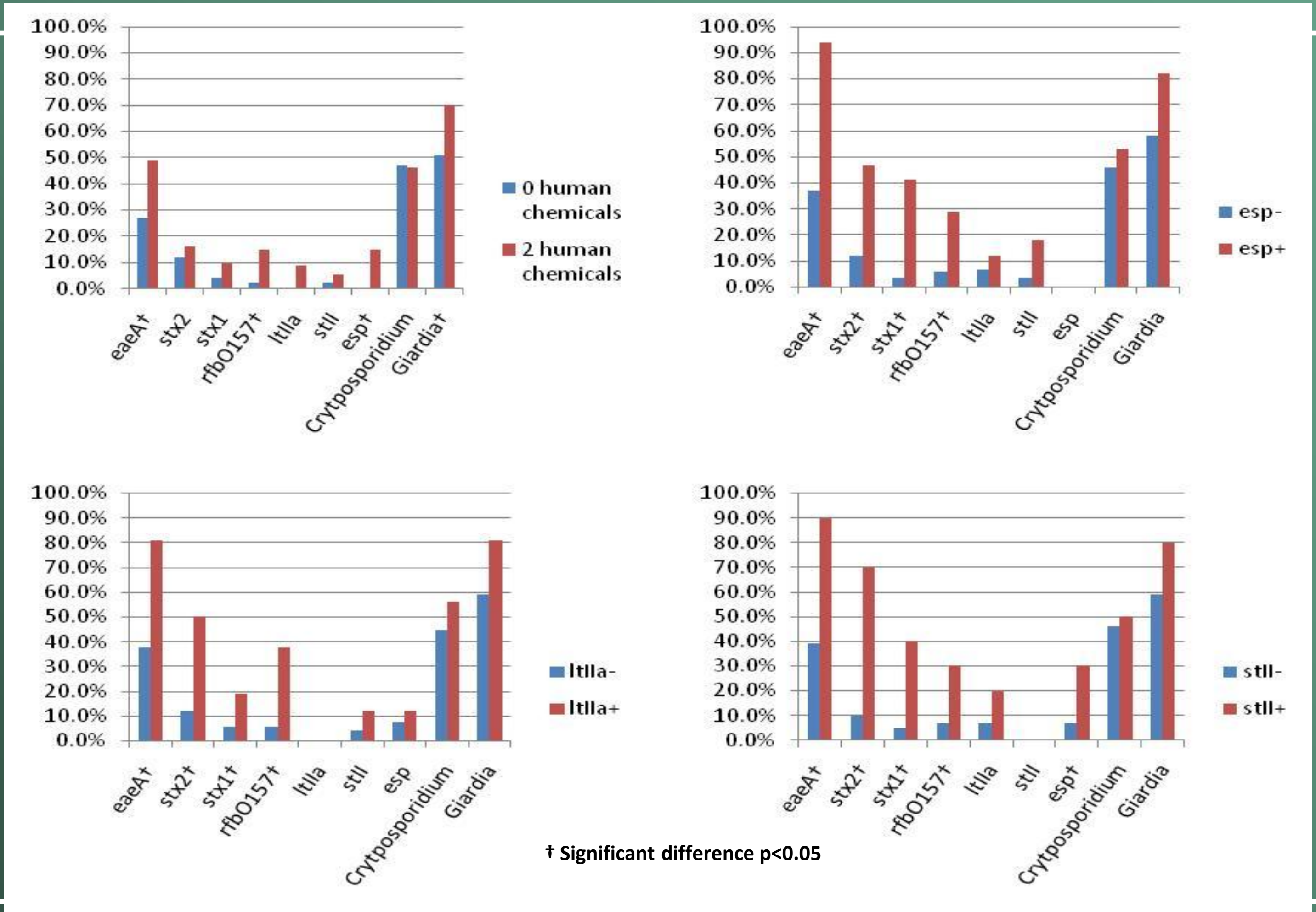
		Frequency (%)								
Discharge Category[a]	*(n)*	*eaeA*	*stx2*	*stx1*	*rfb*O157	*lt*IIa	*st*II	*esp*	*Crytposporidium*	*Giardia*
Low	35	31	5.7	2.9	2.9	5.7	0	0	34	60
Median	118	39	10	5.1	5.1	5.9	2.5	6.8	47	54
High	61	51	30	12	16	11	11	15	52	75
p-value[c]	--	p=0.139	p<0.05	p=0.164	p<0.05	p=0.580	p<0.05	p<0.05	p=0.222	p<0.05

[a] Low, discharge measured was less than 25th percentile of daily mean stream flow at sample location; Median, discharge measured was between 25th and 75th percentile of daily mean stream flow at sample location; High, Discharge measured was greater than 75th percentile of daily mean stream flow at sample location

[b] *[E. coli]*,Mean CFU100 L^{-1} ; *[Enterococci]* Mean CFU100 L^{-1}; *[Cryptosporidium]* Mean oocysts, 1 L^{-1}; *[Giardia]* ,Mean cysts, 1 L^{-1}

[c]Pearson chi-square test with Yates' correction (frequency), or ANOVA on ranks of log transformed concentrations (concentrations)

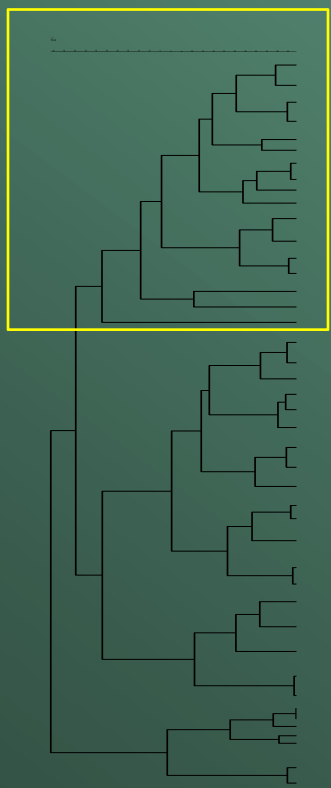
Pathogen Relation to Likely Source



“Data Exploration”

- Original data set had 214 samples and over 220 measured parameters and sampling location observations
- Eliminated measurements that were mostly non-detects, or were incomplete across all sampling years
  - Some substitution of censored data according to established procedures.
- Cluster analysis and principle components analysis with remaining observations

Cluster Analysis



Low Pathogen cluster

- Less frequent pathogen gene detection
- Greater proximal forest, and less proximal urban land use
- Less carbonate bedrock area
- Fewer indicator bacteria
- Lower suspended solids
- Lower total nitrogen
- Lower caffeine concentrations

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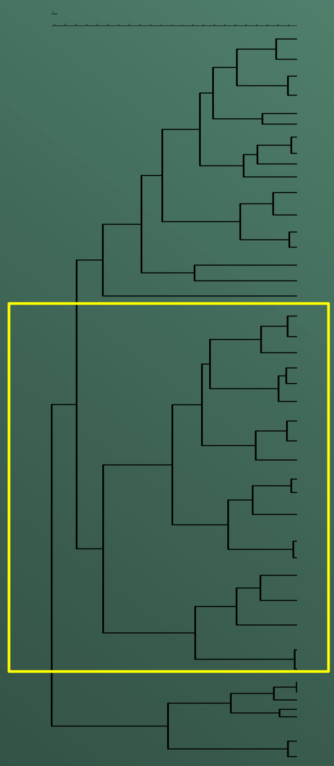
Cluster Analysis



Intermediate Pathogen cluster

- **Significantly greater upstream agricultural land use, and area of carbonate bedrock**
- **Significantly different (intermediate) indicator bacteria and suspended solids**
- **Significantly greater total nitrogen, carbamazipine, and sulfamethoxazole concentrations**

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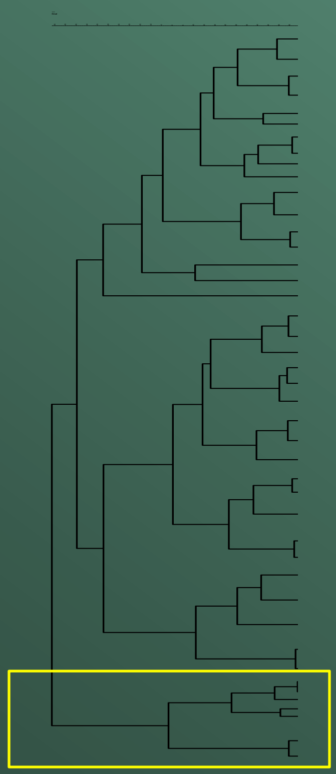
Cluster Analysis



High Pathogen Cluster

- Mixed land use
- Significantly different (intermediate) carbonate bedrock
- Significantly greater drainage area and unit discharge
- Significantly greater indicator bacteria and suspended solids
- Greater Fe, Mn, Al, and acetaminophen concentrations

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Conclusions

- **Bacterial and protozoan pathogens were frequently detected**
- **Samples exceeding standards are more likely to contain bacterial pathogens (not protozoa)**
- **Certain pathogenic populations of EC and ENT are affected by increased discharge while other populations of EC, most likely driven by source, are unaffected by increased discharge.**

Conclusions

- The presence of MST markers was a poor discriminator of EC pathogens
- *Giardia* frequency increased in winter and spring under median flow conditions.
  - *Giardia* occurrence is related more to non-point sources that are highly influential during seasonal overland transport resulting from snow melt and spring rain
- More frequent pathogen detection was associated with increased concentrations of acetaminophen, Al, Fe, and Mn
  - Suggests more frequent pathogen occurrence related to human waste and practices

Thank You.

- PA-DEP
- PA-DEP BOL
- USGS PA-WSC
- Co-authors:
  - Andrew G. Reif, USGS PA-WSC
  - Donna A. Krouse, PA-DEP BOL
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*Factors Related to the Occurrence and Distribution of Select Bacterial and Protozoan Pathogens in Pennsylvania Streams. In revision for submission to Water Research

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