Sources of Fecal Contamination in the Dolomite Aquifer in Northeastern Wisconsin

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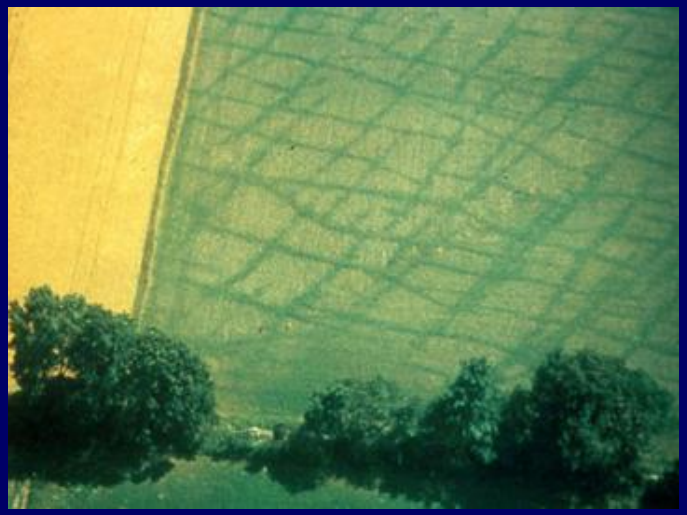
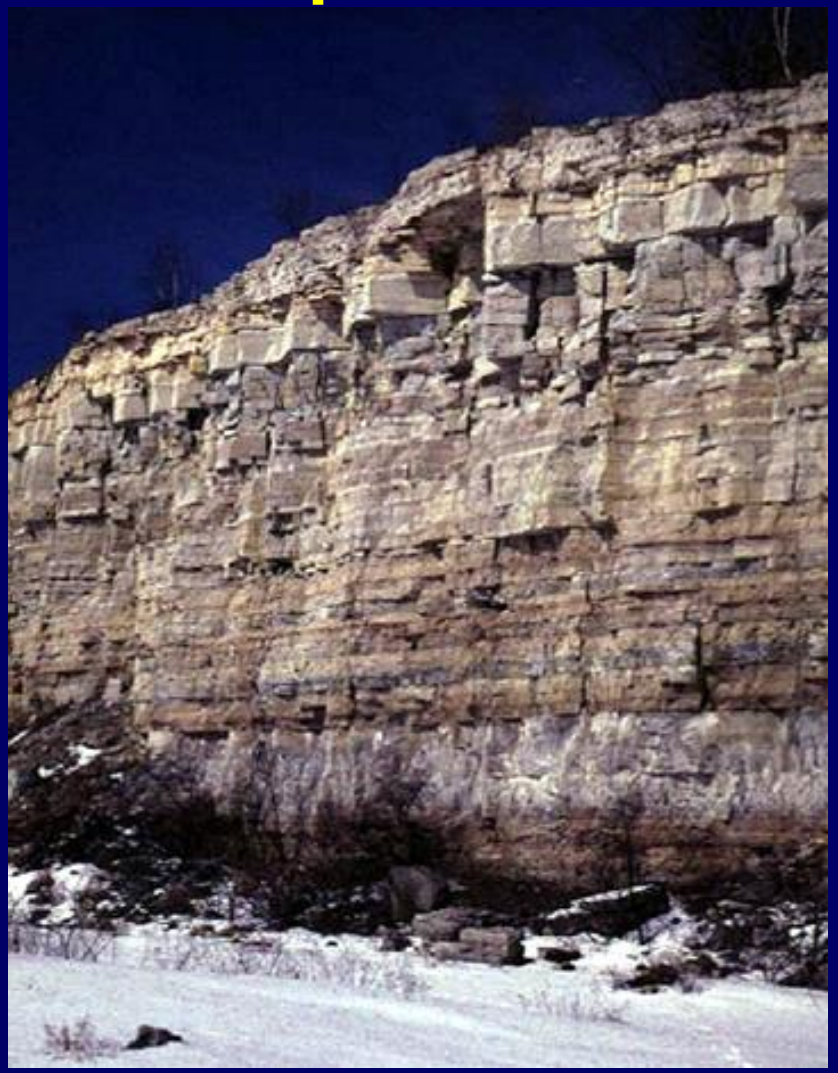
Silurian Dolomite Aquifer



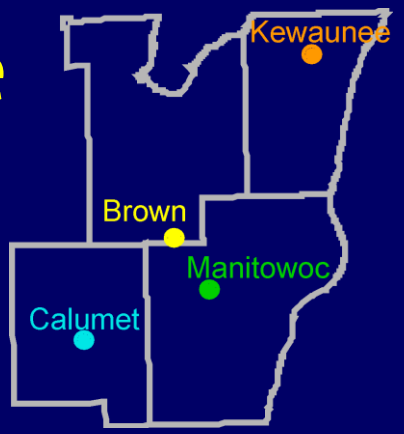


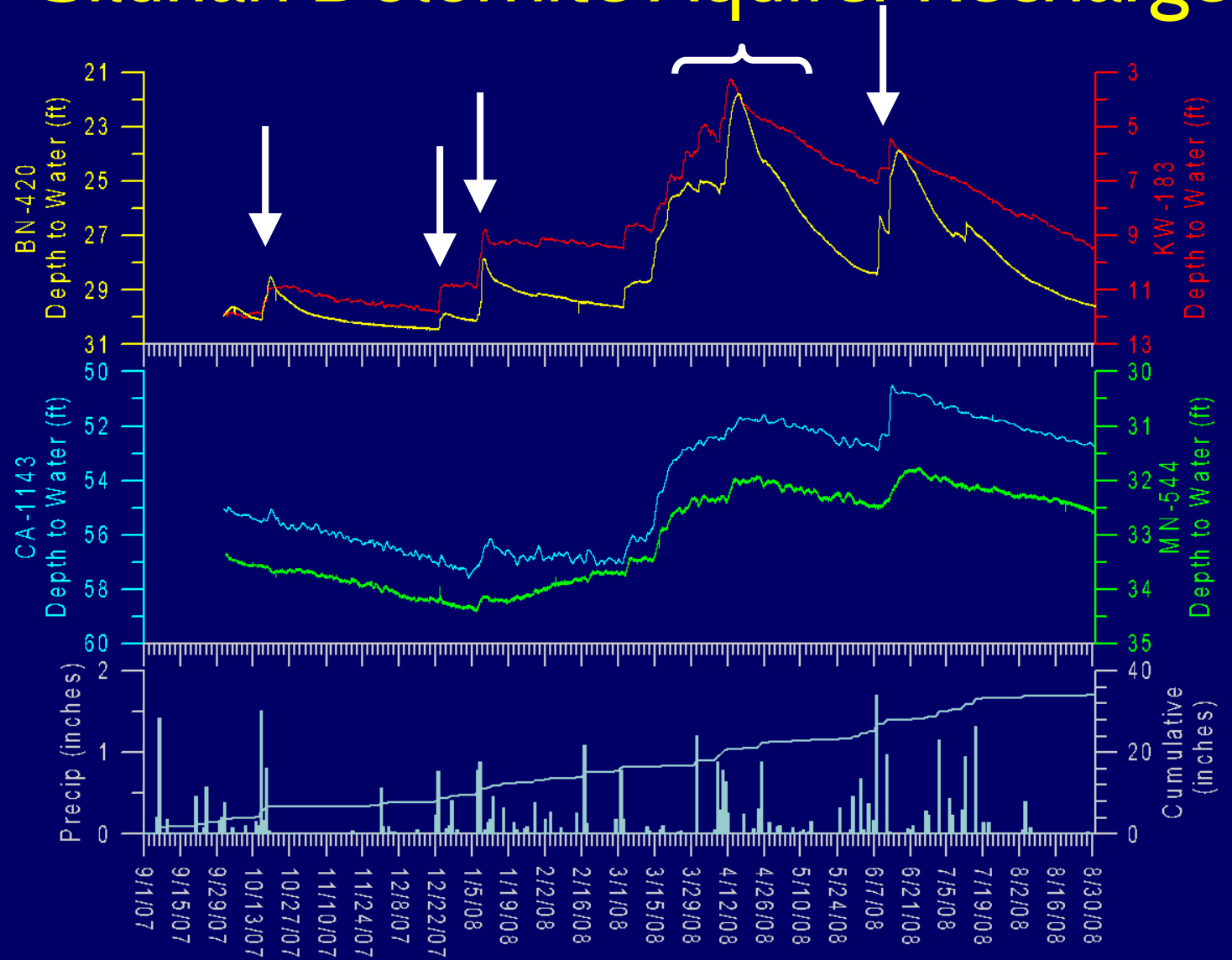
Photos courtesy of Ken Bradbury and Maureen Muldoon

Silurian Dolomite Aquifer Characteristics

- Dense and ubiquitous fracture network
  - little surface runoff
  - water easily infiltrates to subsurface
- Recharge
  - exceedingly rapid
  - carries surface contaminants to the water table
- Flow within the aquifer occurs primarily along bedding plane fractures
  - Little to no attenuation of contaminants within the aquifer
- Flow rates vary from 10's to 100's of ft/day

Silurian Dolomite Aquifer Recharge





Slide courtesy of Maureen Muldoon

Brown Water Events in Northeast Wisconsin

- Groundwater recharge, especially spring snow melt, can generate brown water events
- Several outbreaks associated with these events e.g., EHEC, *Campylobacter jejuni*
- This well is code compliant, 123 ft deep, cased to 63 ft

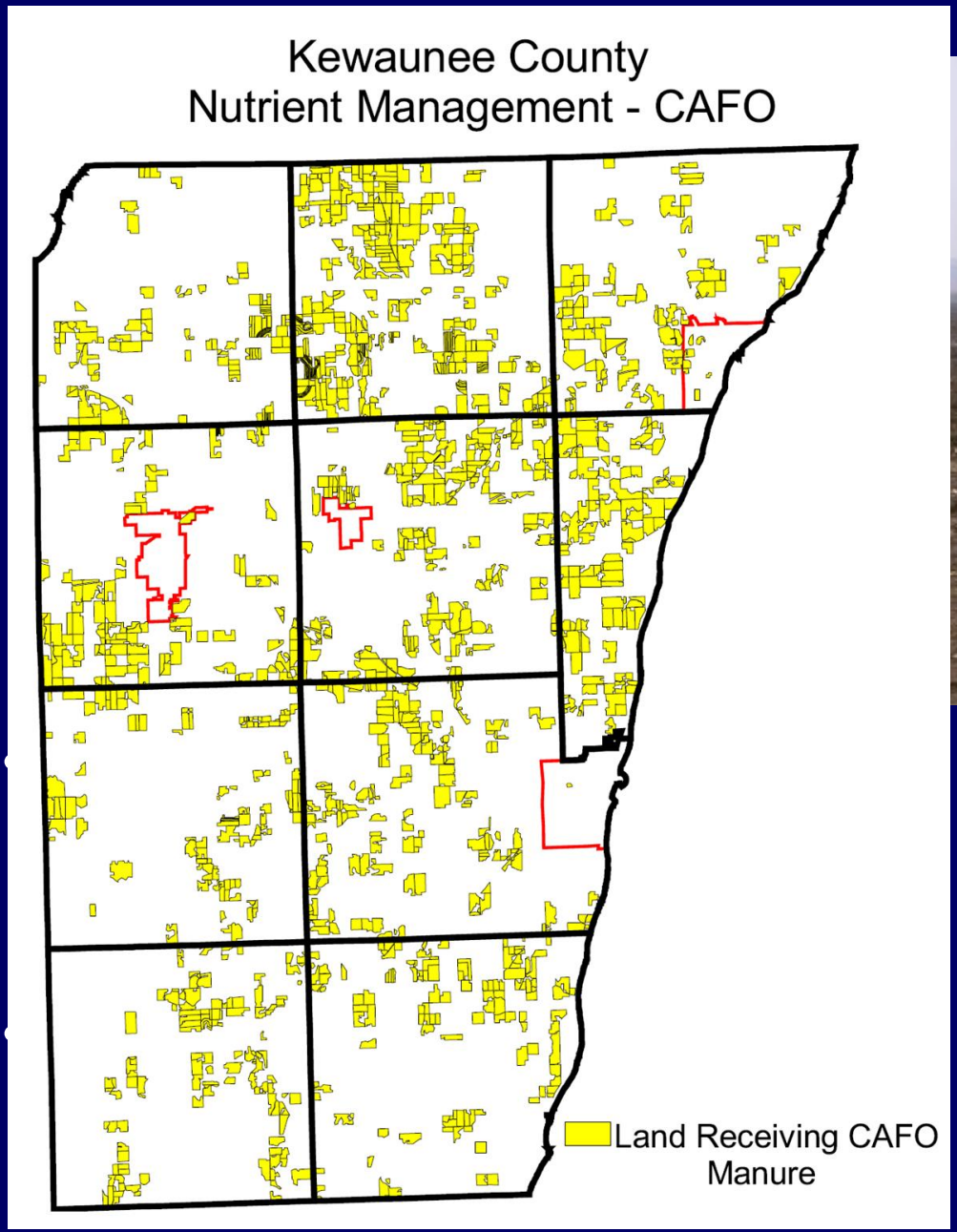
Photos courtesy of Chuck Wagner

Kewaunee County CAFOs

- In WI, Concentrated Animal Feeding Operations (CAFOs) are defined as those farms having > 1000 animal units
- Kewaunee County has 16 CAFOs (15 dairy and one beef operation)
- 98,000 cows, heifers, and calves in the county

Manure Application

- Timing
  - in Fall (after harvest) and Spring (prior to planting)
- Application rates
  - determined by Nutrient Management Plans (NMPs) that are designed to maximize crop yields while minimizing surface runoff
  - Protective rate of application in fractured rock areas is 25 tons/acre (or ~9000 gallons)



Political/Social Context

- Citizen activists hired attorneys to challenge a WPDES permit for a CAFO expansion
- Have petitioned U.S. EPA to intervene under emergency powers of SDWA

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Groups ask EPA to investigate pollution causes by cattle farms

By Lee Bergquist

Oct. 22, 2014

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Six environmental groups on Wednesday asked the U.S. Environmental Protection Agency to exercise emergency powers under the Safe Drinking Water Act to investigate groundwater contamination in cattle-intensive Kewaunee County in northeastern Wisconsin.

In a petition to the federal agency, the groups say the state Department of Natural Resources has failed to protect drinking water for county residents on two fronts: through its powers to regulate groundwater; and the agency's oversight of large-scale cattle operations.

The environmental groups estimate that the tens of thousands of cattle in the county produce the manure equivalent of more than 900,000 humans — or more than the city of Milwaukee.

Last year, 149 wells, or nearly 31% of the 483 wells tested in the

Current Research Objectives

- Design a county-wide randomized sampling plan, stratified by depth-to-bedrock, for nitrate and indicator bacteria
- Sample once per season a subset of wells for viruses and fecal markers capable of distinguishing septic versus bovine sources of contamination
- Install automated sampling systems on one or two wells to determine the timing of peak transport for viruses and indicator bacteria
- Identify spatial and temporal patterns of contamination

Objective 1

- County-wide randomized sampling of private wells stratified by depth-to-bedrock: <5 ft, 5-20 ft, > 20 ft
- Participation rate ~ 50%
- Several day "Synoptic" sampling
- Recharge
  - November 2015
  - 317 wells in analysis
- No recharge
  - July 2016
  - 400 wells in analysis

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One-third of wells in Kewaunee County unsafe for drinking water

Submitted photo

Deer Run Dairy LLC near the southern end of Sleepy Hollow Road in Kewaunee County is among many large dairy farms in the county, where a recent study found one-third of the wells exceeded safety standards for drinking water.

By Lee Bergquist of the Journal Sentinel

Dec. 21, 2015

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Nearly 100,000 cattle in county

Kewaunee County's cattle population is sharply higher than its resident population. Meanwhile, cattle numbers have soared since 1983.

Total cattle in Kewaunee County

100

More than one-third of wells in dairy farm-intensive Kewaunee County were found to be unsafe because they failed to meet health standards for drinking water, according to a new study.

Researchers say it's too early to blame cattle as the source of pollution.

But the findings are significant because the northeastern Wisconsin county — where cows far outnumber people — has become the center of a growing controversy in Wisconsin over manure's role in polluting ground and surface water.

The results are from the first phase of research funded by the Department of Natural Resources to study pollution problems. In Kewaunee County, cattle numbers have grown sharply over two decades, and the amount of manure exceeds the waste generated by the human population of Milwaukee.

Animal waste isn't cleaned by sewage treatment plants, however. Manure is spread on farmland as fertilizer and has long been an accepted practice of replenishing the soil for growing crops.

But manure use in Wisconsin has grown increasingly controversial, especially in areas with large-scale farms and where soil and local geologic conditions make groundwater more vulnerable to pollution. Kewaunee County has 15 large-scale dairy farms, known as CAFOs, or concentrated animal feeding operations, that have 700 or more milking cows per farm.

The county ranks among the highest in the number of CAFOs in the state, according to the DNR.

It also lies in a region where soil depth varies and sometimes is only a few feet above fractured bedrock where bacteria from manure and

Kewaunee County Contamination Rate by Depth to Bedrock

Indicator	Depth to bedrock (ft)	Recharge - Nov 15		No recharge – July 16	
		Estimated contamination rate (%)	P-value chi square test	Estimated contamination rate (%)	P-value chi square test
Total coliform	<5	46	0.047	23	0.43
	5-20	28		29	
	>20	19		21	
E. coli	<5	4	0.49	7	0.46
	5-20	1		1	
	>20	0.3		1	
Nitrate-N > 10 ppm	<5	7	0.11	10	0.026
	5-20	20		19	
	>20	6		5	
TC or E. coli or high nitrate	<5	50	0.019	33	0.088
	5-20	42		40	
	>20	23		26	

County-wide contamination, weighted analysis: 26% (recharge) to 28% (non-recharge)

Objective 2

- Determine source of fecal contamination
- Randomized stratified sampling from 234 wells positive for TC, E. coli, or N-NO3 > 10 ppm
- Five sampling rounds, all completed:
    - April, August, November, 2016
    - January and March, 2017

Microbes: Identifying the Fecal Source

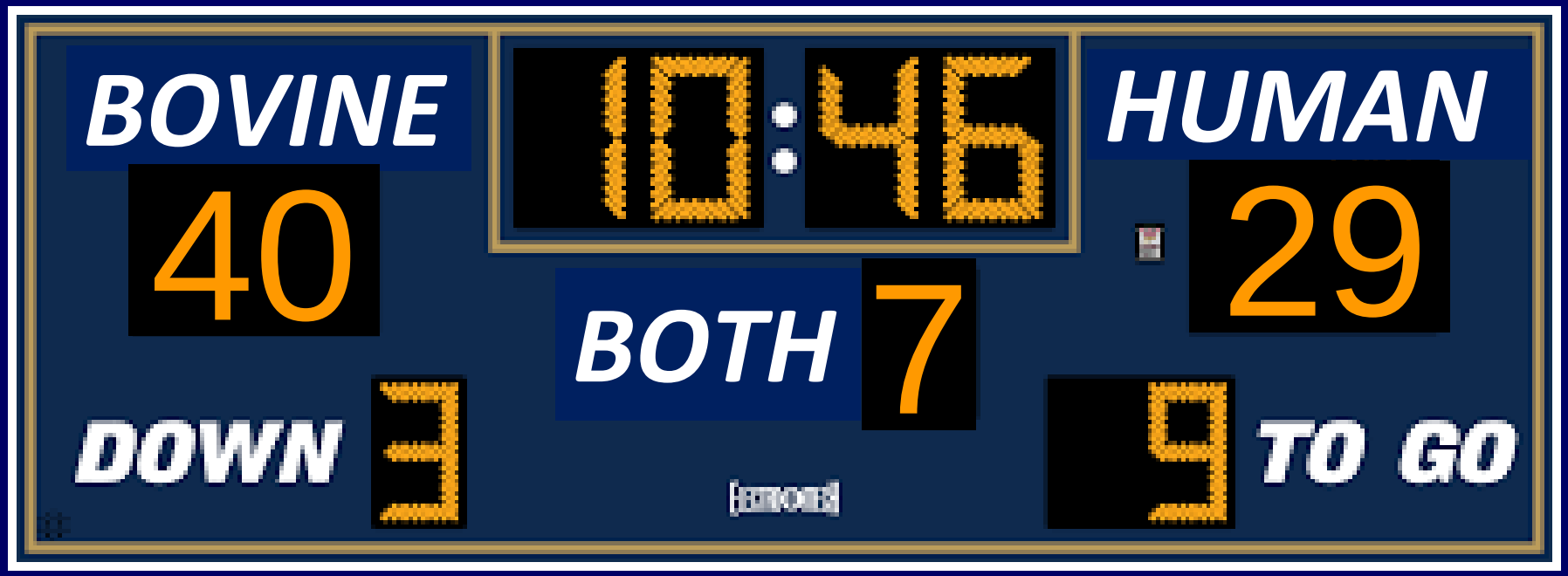
(n = 138 samples from 131 wells) (red font indicates pathogenic)

Host	Microorganism	Wells	Concentration (gene copies/L)
Human-specific	Adenovirus A	1	1
	Bacteroidales-like Hum M2	7	< 1 – 1050
	Human *Bacteroides*	27	< 1 – 34
	Cryptosporidium hominis	1	qualitative
	All	**29**	
Bovine-specific	*Bacteroidales*-like Cow M2	2	29 - 915
	Bacteroidales-like Cow M3	4	3 – 49818
	Bovine *Bacteroides*	36	< 1 – 42398
	Bovine polyomavirus	8	< 1 – 451
	Bovine enterovirus	1	2
	All	**40**	

Not detected: [human-specific] adenovirus B & C, D, F, enterovirus, human polyomavirus, norovirus GI & GII
[bovine-specific] coronavirus, bovine diarrheal virus 1 & 2

Host	Microorganism	Wells	Concentration (gene copies/L)
Non-specific	*Campylobacter jejuni*	1	< 1
	Cryptosporidium parvum	8	qualitative
	Cryptosporidium spp.	16	< 1 – 3
	Giardia lamblia	2	< 1
	Pathogenic *E. coli* (*eae* gene)	1	4
	Pathogenic *E. coli* (*stx1* gene)	1	16
	Pathogenic *E. coli* (*stx2* gene)	1	1
	Pepper mild mottle virus	13	2 - 3811
	Rotavirus A (*NSP3* gene)	17	< 1 – 4481
	Rotavirus A (*VP7* gene)	7	< 1 – 732
	Rotavirus C	3	45 – 1301
	Salmonella (*invA* gene)	3	< 1 – 13
	Salmonella (*ttr* gene)	5	5 – 59
	All	**44**	
	Total positive wells	**79**	**< 1 - 49818**

Well Contamination Scoreboard



Not included: Rotavirus group A detections by qPCR are not human- or bovine-specific; subsequent typing will determine host specificity

Number of contaminated samples & wells

	Total	Negative	Positive	% Positive
Samples	138	56	82	59
Wells	131	52	79	60

Wells tested for microorganisms were selected from wells previously positive for total coliform or high nitrate (> 10 ppm N-NO_3^-)

Bacteroides Sequencing

Bacteroides dorei CL03T12C01, complete genome
Alignment statistics for match

#1 Score	Expect	Identities	Gaps	Strand
233 bits(126)	6e-58	126/126(100%)	0/126(0%)	Plus/Plus

Features: rRNA-16S ribosomal RNA

```
Query  10      CTTCCTCTCAGAACCCCTATCCATCGTTGACTAGGTGGGCCGTTACCCCGCCTACTATCT  69
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  379235  CTTCCTCTCAGAACCCCTATCCATCGTTGACTAGGTGGGCCGTTACCCCGCCTACTATCT  379294

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Sbjct  379295  AATGGAACGCATCCCCATCGTCTACCGGAAAATACCTTTAATCATGCGGACATGTGAACT  379354

Query  130     CATGAT  135
               ||||||
Sbjct  379355  CATGAT  379360
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- Human-specific *Bacteroides:* 19 samples sequenced, all match human (9 samples still to be sequenced)
- Bovine-specific *Bacteroides:* 27 samples sequenced, all match bovine (3 samples still to be sequenced)
- No sequences matched qPCR positive controls (i.e., no lab contamination)

Rotavirus Group A Subtyping

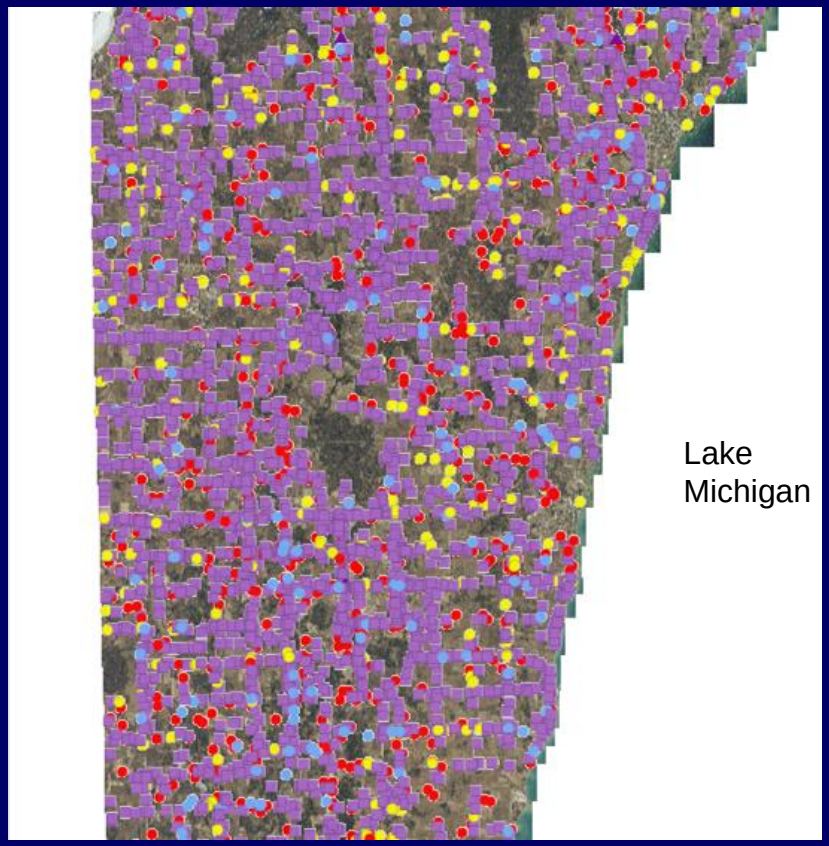
Source	G-Type	P-Type	Number of samples
Human	G1	P[8]	5
Bovine	G10	P[11]	6
Human and Bovine	G1/G10	P[8]/P[11]	2

Four rotavirus positive samples from the March sampling still need subtyping

Kewaunee County Septic Systems

- 4822 septic systems in the county
- 540 holding tanks, 155 abandoned

Personal comm. Lee Luft, Kewaunee County Supervisor, March 7, 2017



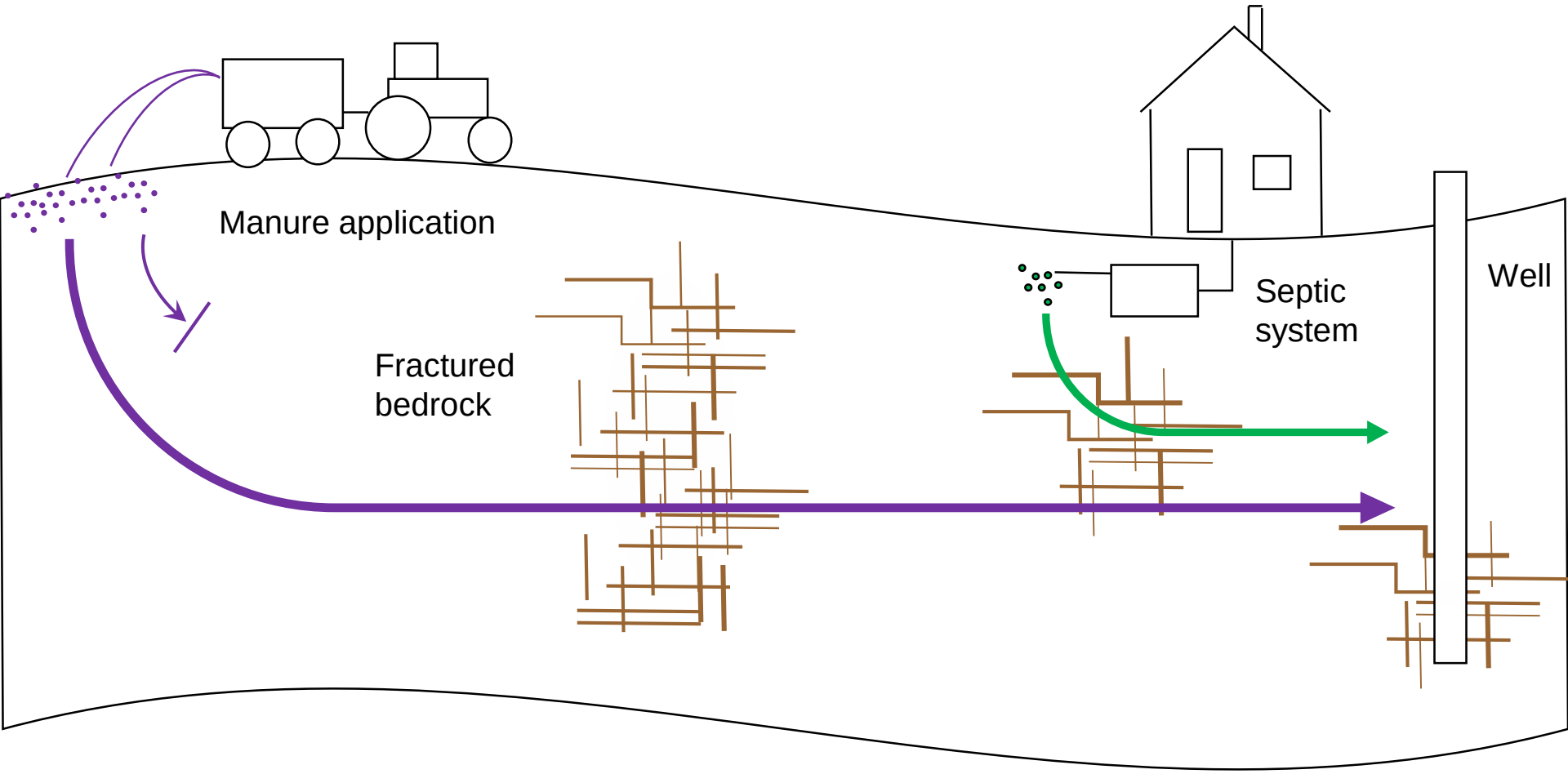
Legend
Purple = replaced or inspected
Red = not inspected
Yellow = holding tank
Blue = abandoned system

Roughly 200 million gallons septic effluent per year released to the subsurface

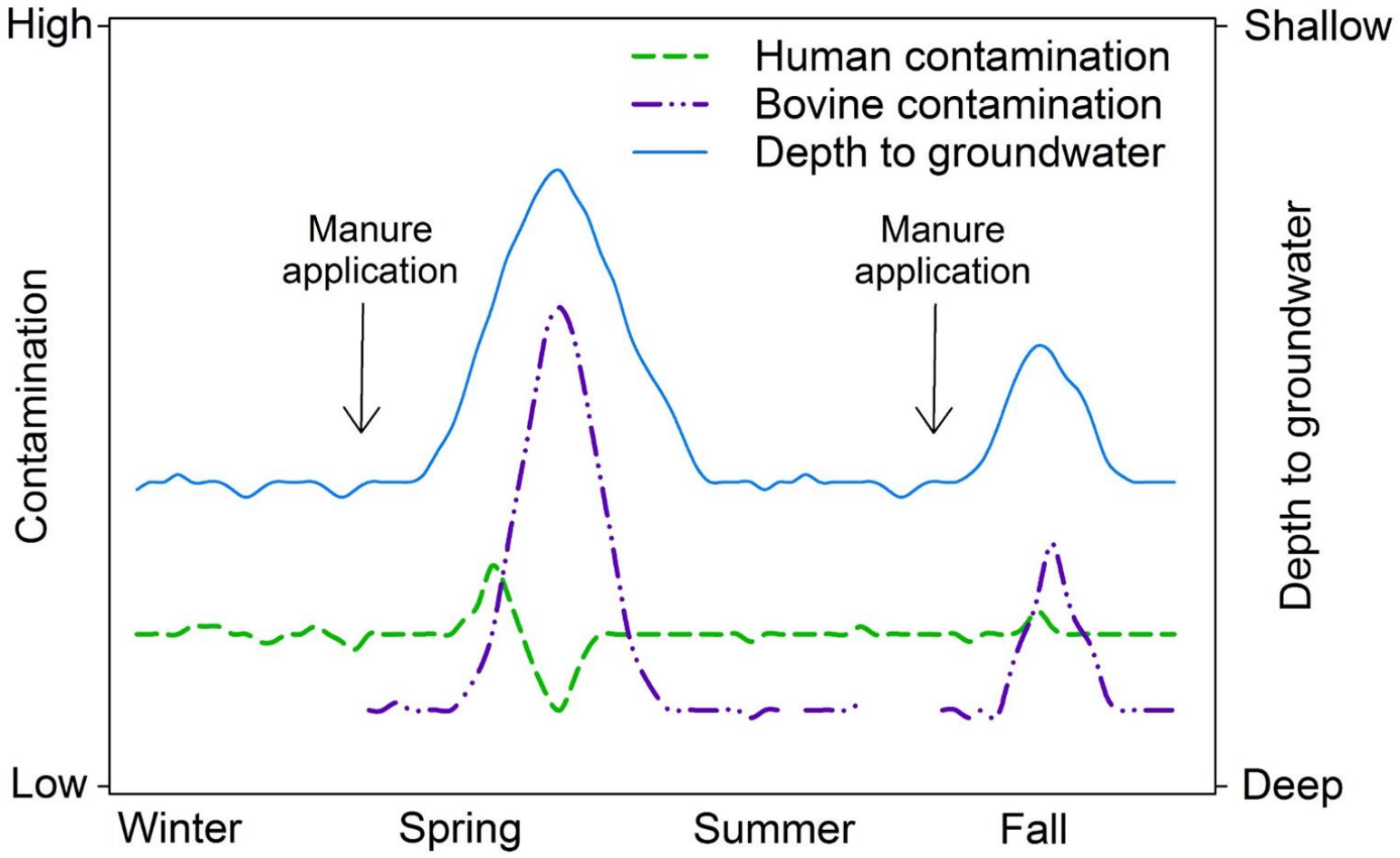
Conceptual Model of Fecal Contamination in Kewaunee County - 1

Bovine pathogen source
- Large fecal source
- Surface applied periodically
- Episodic infiltration

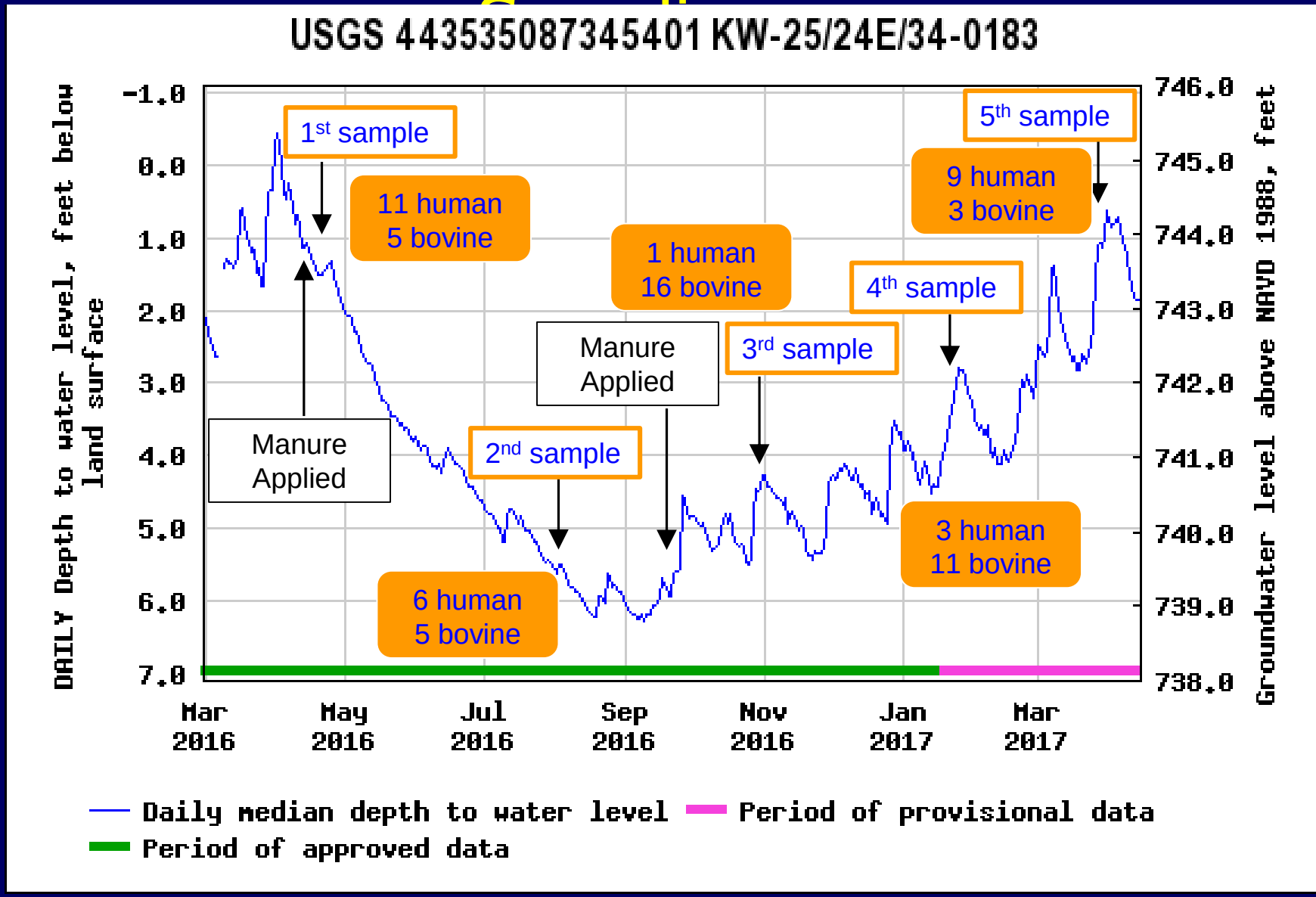
Human pathogen source
- Small fecal source
- Sub-surface release continuously
- Continuous infiltration



Conceptual Model of Fecal Contamination in Kewaunee County - 2



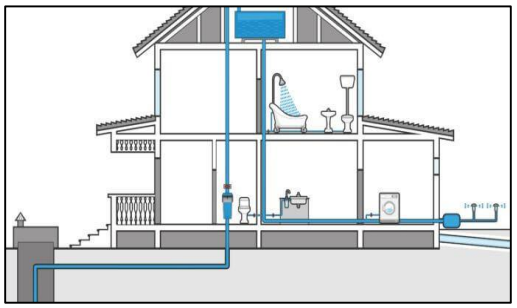
Groundwater Levels during MST



From Farm Field to Household Well

Manure applied Oct 25, 2016

> 1 inch rain Oct 26, 2016



House near field

Farm field sampled Oct 27, 2016

Bovine Bacteroides
Bovine enterovirus
Bovine polyomavirus
M2 Bacteroides-like
M3 Bacteroides-like

Rotavirus A NSP3
Rotavirus A VP7
Rotavirus C

Tap water Oct 27, 2016

Bovine Bacteroides
Bovine enterovirus
Bovine polyomavirus
M2 Bacteroides-like
M3 Bacteroides-like
Campylobacter jejuni
Cryptosporidium
Rotavirus A NSP3
Rotavirus A VP7
Rotavirus C

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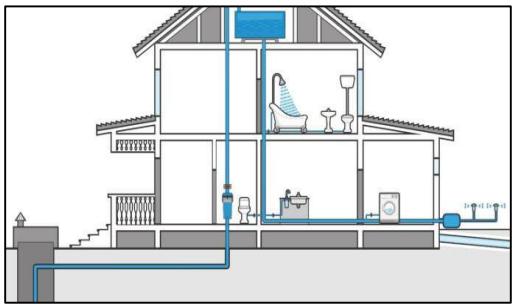
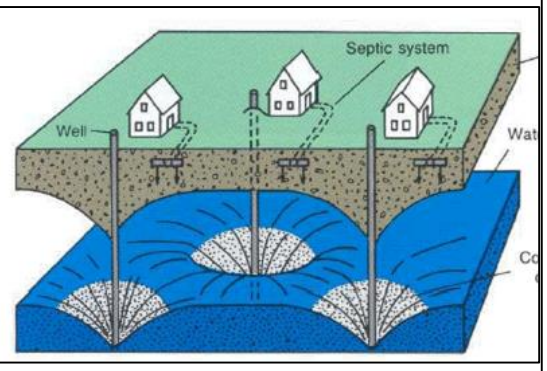


House near field

Neighbor's well sampled Oct 31, 2016



Bovine Bacteroides

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M2 Bacteroides-like
M3 Bacteroides-like

Rotavirus A NSP3
Rotavirus A VP7
Rotavirus C

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Bovine enterovirus
Bovine polyomavirus
M2 Bacteroides-like
M3 Bacteroides-like
Campylobacter jejuni
Cryptosporidium
Rotavirus A NSP3
Rotavirus A VP7
Rotavirus C

Objective 3

- Use automated samplers to analyze the time-series of fecal contamination to:
  - identify time periods/recharge conditions that lead to the highest pathogen loads
  - identify real-time measured parameters (e.g. Cl) predictive of pathogen contamination
  - relate pathogen loading to the manure runoff risk predictions for surface water

Summary

- By stratified random sampling it is estimated 26% to 28% of private wells in Kewaunee County are positive for TC, E. coli, or nitrate-N > 10 ppm
- Well contamination results from both human and bovine fecal sources
- Wells are contaminated with pathogens of significant concern: *Salmonella*, EHEC, *Cryptosporidium*, rotavirus
- Future work will determine how fecal source, pathogen types, and pathogen concentrations are associated with well construction, hydrogeological, and environmental variables

Questions?

Comments?