

Sources of Fecal Contamination in Groundwater in Rural Northeastern Wisconsin

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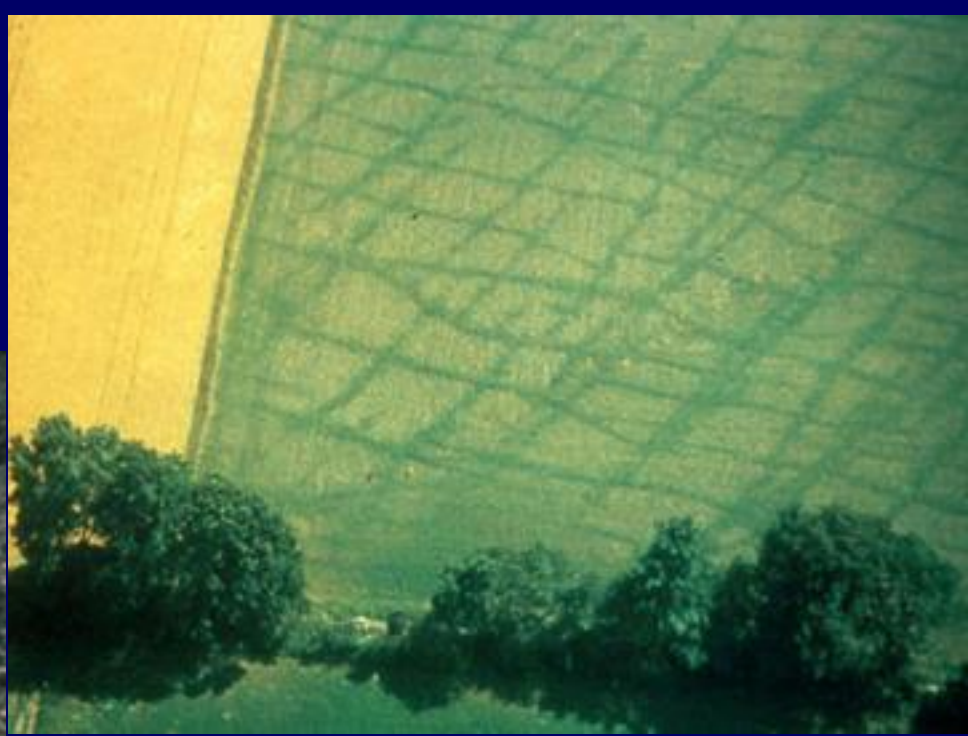
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Silurian Dolomite Aquifer



Bedding-plane Fractures



Kewaunee CAFOs

- In WI, Concentrated Animal Feeding Operations (CAFOs) are defined as those farms having > 1000 animal units
- Kewaunee County has 15 CAFOs (14 dairy and one beef operation)
- Town of Lincoln
 - Three CAFOs
 - $\sim 13,500$ cattle
 - 334 households



Brown Water

- Recharge, especially spring snow melt, can generate “brown water” events
- These events create public health risks
 - at least 2 documented cases of near-fatal hemorrhagic *e. coli* illness in infants
- This particular well:
- code compliant well (123 ft deep, cased to 63ft)
- Persistent bacteria problems
- Nitrate level has fluctuated from high teens to 39 ppm.
- State regulators often state that they cannot determine a specific source for these brown water events

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Microbial Source Tracking – The Principle

- The friendly microbes present in feces are specific to the animal source, (e.g., an *E. coli* from humans is different from an *E. coli* from cattle)
- These same microbes can be measured in water that has fecal pollution and the animal source identified.
- The Bacteroides group is the most common bacterial target for MST

Microbial Source Tracking – Current Limitations

- Gut microbes are not 100% specific to animal type
- Assay specificity is evaluated by comparing fecal specimens from ~ 40 different animals, but there are 1000s of bacteria taxa with similar genetic sequences that could cross-react with the assay
- Microbes from different animals die-off in the environment at different rates
- Microbe abundance and types in the host animal can change with time, geographic location, and diet so the signal in the environment changes
- Current best assay for human bacteroides is HF183

Using Viruses for MST – The Principle

- Enteric pathogenic viruses are more host-specific than commensal bacteria
- Virus gene targets are not present in indigenous microbes in the environment, increasing assay specificity
- Viruses are especially useful markers for fecal contamination in groundwater because they are highly mobile in the subsurface
- Primary limitation is pathogenic viruses are not always present in the host population and therefore can be absent in fecal material

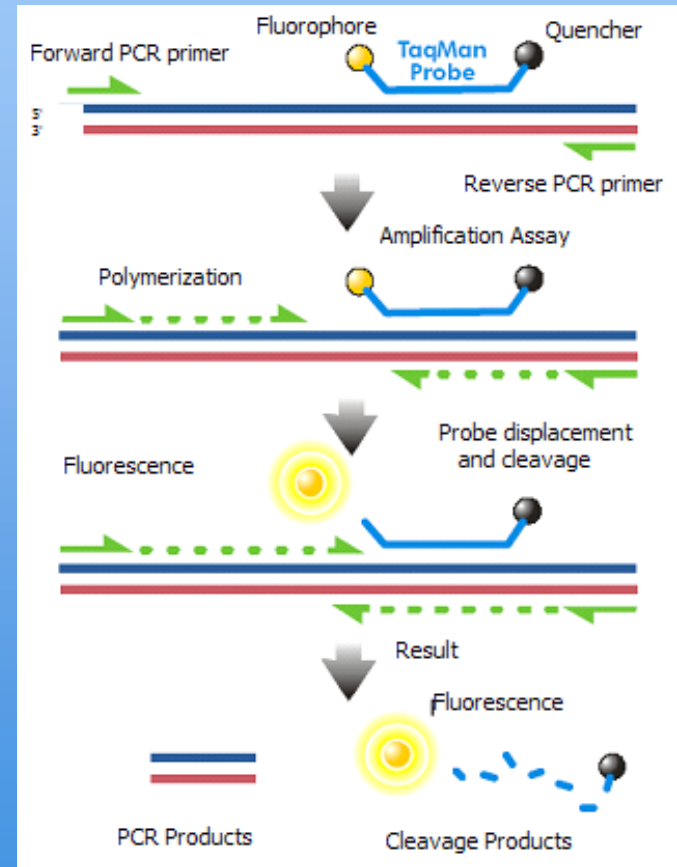
Measuring Pathogens in the Environment - Quantitative PCR

Strengths

- High specificity
- Absolute quantification by standard curves
- Low limit of detection
- Amenable to high-throughput

Limitations

- PCR inhibition
- No “live” “dead” distinction
- QA/QC procedures are crucial



Virus Sampling (May 2014)

- Sampled 10 household wells in Kewaunee County
 - 6 bovine-specific viruses plus bovine bacteroides
 - 7 human-specific viruses plus human bacteroides
 - pepper mild mottled virus
 - zoonotic pathogens
 - enterohemorrhagic *E. coli*
 - *Salmonella* species
 - *Campylobacter jejuni*
 - *Cryptosporidium* species
 - *Giardia lamblia*



- pump ~800 L through hemodialysis filters
- qPCR methods except protozoa by IFA
- Coliforms by Quanti-Tray

Kewaunee County Results

Host	Microbe	No. Wells Detected	Concentration Range (genomic copies/L)
Human-specific	Adenovirus C, D F	1	26
	Adenovirus A	1	3
	Enterovirus	2	21 and 21
	All	3	
Bovine-specific	Polyomavirus	2	16 - 18
	Bacteroides	1	6
	All	3	
Non-defined	Pepper mottle virus	2	22 and 22
	Salmonella spp.	4	2 - 24
	Campylobacter jejuni	1	11
	All	5	
Indicators	Total coliforms	1	3 MPN
	<i>E. coli</i>	1	1 MPN

Kewaunee Results Summary

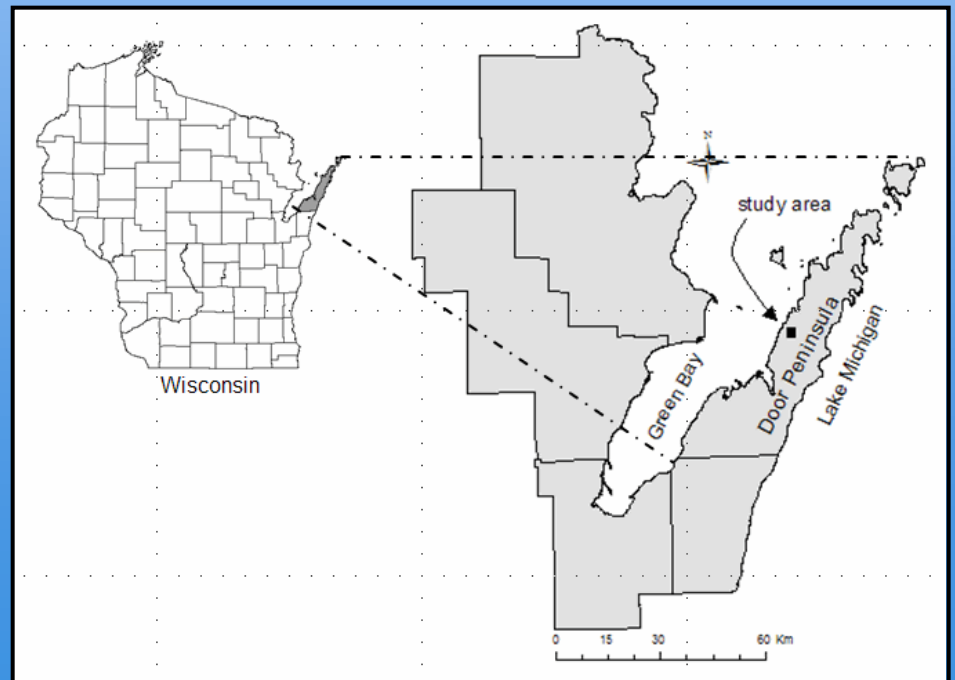
- 7 of 10 wells were positive for fecal contamination
- Human and bovine fecal sources contaminated 3 wells each; 1 well had both sources
- 6 wells were negative for total coliform or *E. coli* indicators even though pathogens or MST markers were present
- 4 wells had evidence of contamination of *Salmonella* bacteria and one well was positive for the bacterium *Campylobacter jejuni*

ground water

Case Study/

Norovirus Outbreak Caused by a New Septic System in a Dolomite Aquifer

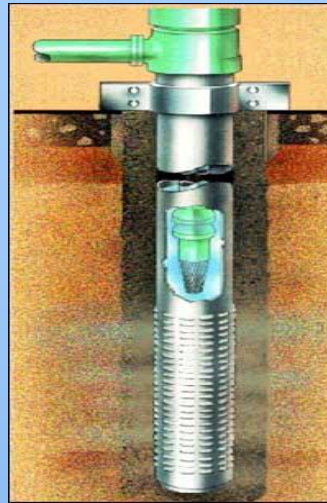
by Mark A. Borchardt¹, Kenneth R. Bradbury², E. Calvin Alexander Jr.³, Rhonda J. Kolberg⁴, Scott C. Alexander³, John R. Archer⁵, Laurel A. Braatz⁶, Brian M. Forest⁷, Jeffrey A. Green⁸, and Susan K. Spencer⁹



Outbreak Background

- In early June, 2007, 229 patrons and employees of a new restaurant in Door County were affected by severe acute gastrointestinal illness, 6 people hospitalized
- New well and conventional drain-field septic system, both conforming to State code
- Hydrogeologic setting: shallow soil over densely fractured dolomite
- Epidemiologic case-control analysis indicated the restaurant's well water was associated with illness

Norovirus Transmission Cycle



Tap water from well:
50 genomic copies/L

Norovirus isolates from 3 sources
had identical
327 bp polymerase gene sequences

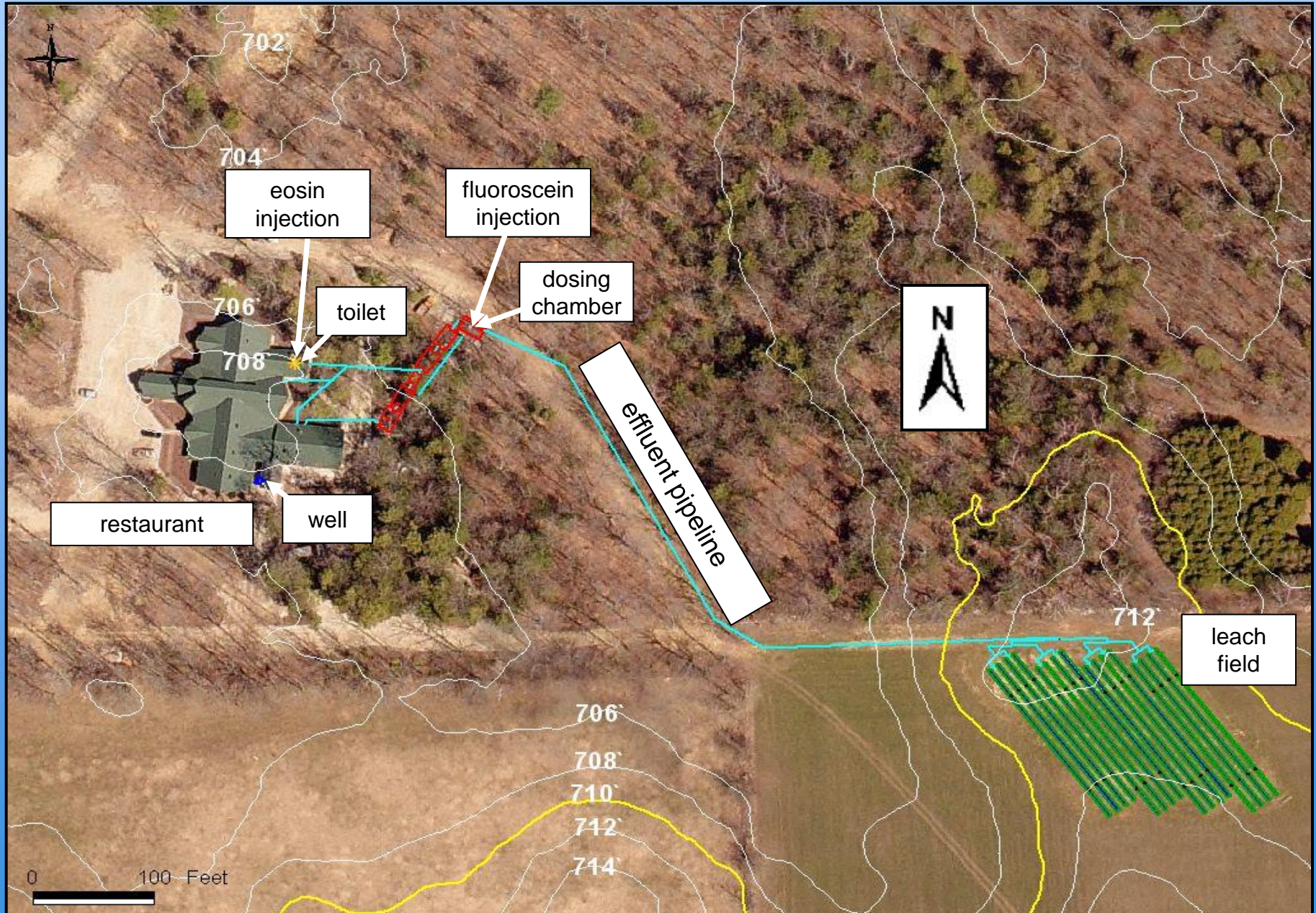


Restaurant patrons: $10^4 - 10^8$ gc /gm stool

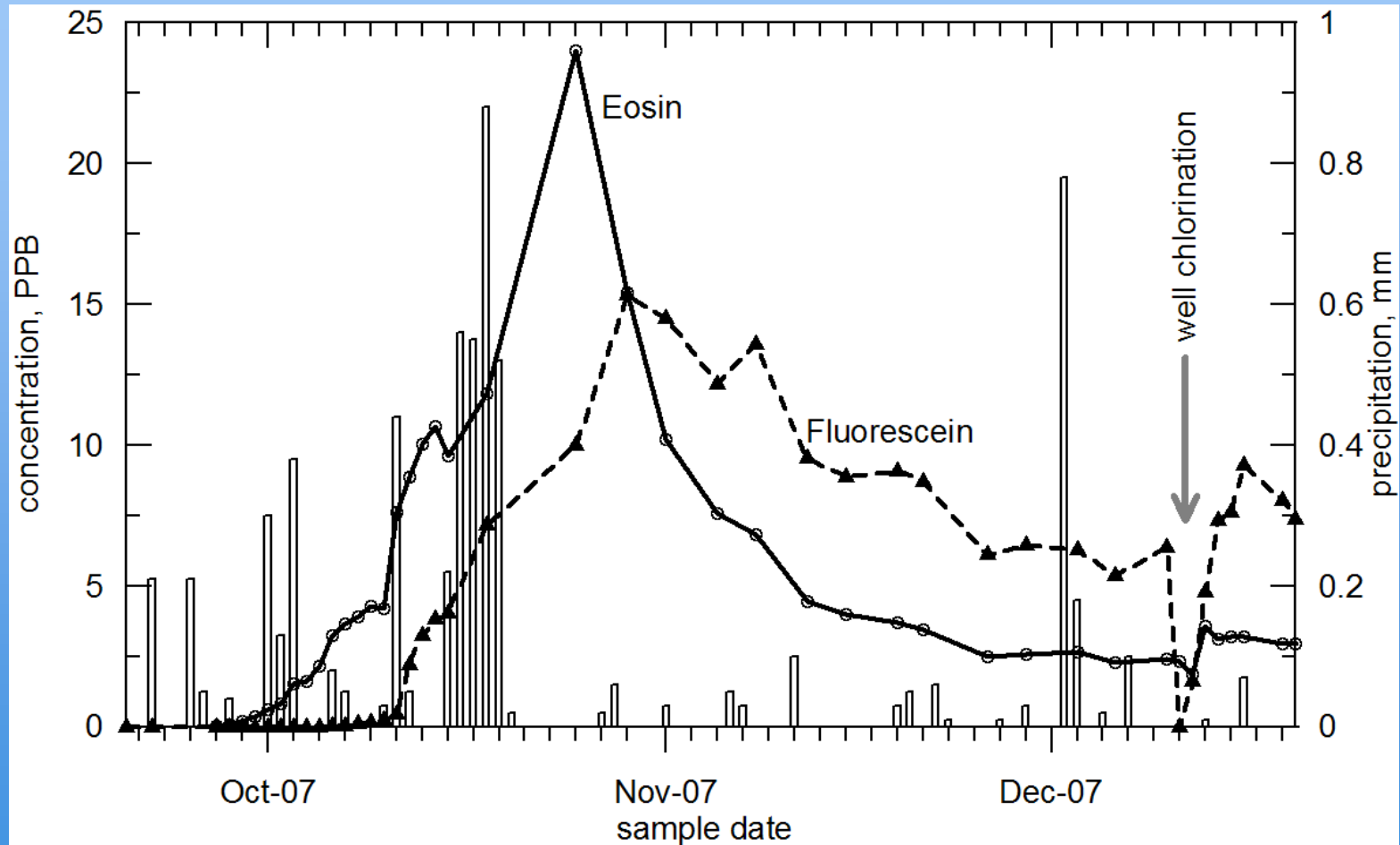


Septic tank: 79,600 genomic copies/L

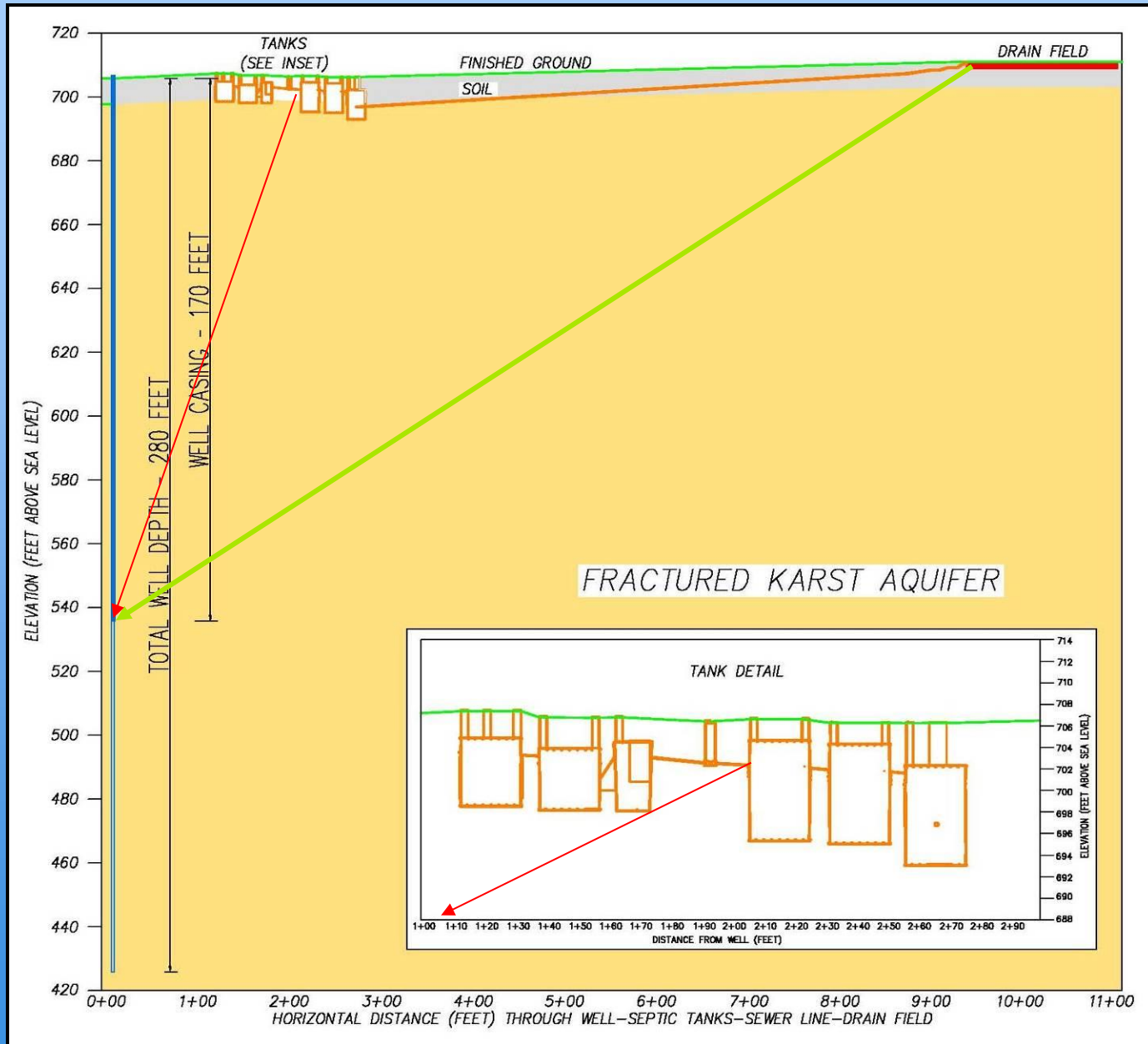
Restaurant - As Built Septic System and Well

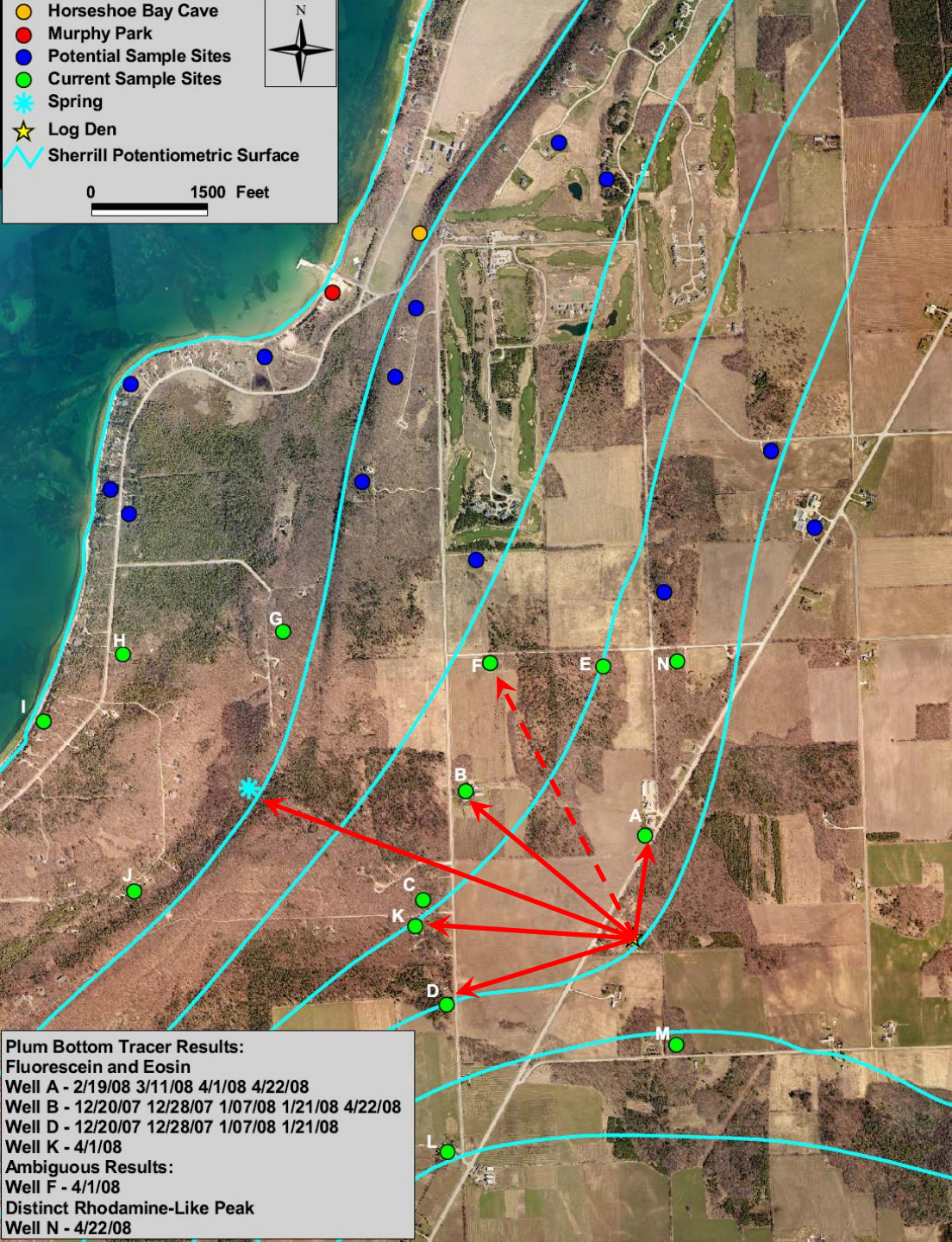
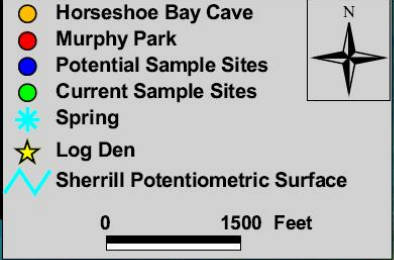


Tracer concentrations in the restaurant supply well



Restaurant Cross Section





Regional Scale Movement of Dye from the Restaurant

Tracer velocities to offsite wells B and D are in the range of 7 to 8 m/d.

Plum Bottom Tracer Results:
 Fluorescein and Eosin

Well A	2/19/08	3/11/08	4/1/08	4/22/08	
Well B	12/20/07	12/28/07	1/07/08	1/21/08	4/22/08
Well D	12/20/07	12/28/07	1/07/08	1/21/08	
Well K	4/1/08				

Ambiguous Results:
 Well F - 4/1/08

Distinct Rhodamine-Like Peak
 Well N - 4/22/08

Local Geology Is Illness Factor

'Summer Flu' Study Is Made By Scientists

Limestone Fissures Let
Wastes Get Into Wells.
Say the Researchers

Door county's geology is to blame for so-called "resort diarrhea" according to a U. S. Public Health Service team which thoroughly investigated the illness last summer. The study was made at the request of the Ephraim Men's club, which heard the preliminary report Thursday night. The report stated that purification of water through chlorination is the practical answer.

Door county is a sliver of top-soil covering the U.S. floor

Not Only Here

It was pointed out that the problem is not peculiar to Door county but occurs in many other places where a concentrated population uses well water.

Three officials were here to give the report, Don Mackel, bacteriologist, Norman Peterson, sanitary engineer, and Dr. Fred Payne. All work out of the USPHS main office at Atlanta, Ga.

More than 200 people cooperated in making the study this summer. There were 10 medical technologists and 200 college students who were working at the resorts.

Several factors were checked and it was found that diarrhea cases coincided with periods of

bad water. The degree of sickness depended on the degree of pollution and the amount of water drunk. The number of people in the area had a direct bearing on the amount of pollution. Another factor was rainfall. Rain dilutes the wastes, decreasing pollution.

Doesn't Make Bad Taste

Twenty-nine wells and 22 resorts were tested. Sixty-four per cent showed bad water at some time during the summer. Even the best constructed well can

1955!

Article in the
Door County Advocate

Policy "Lapse"

WI Septic System Code Comm 83 allows 24" minimum distance between drainfield and groundwater table or bedrock, regardless of bedrock type.

New Study: Assessing Groundwater Quality in Kewaunee County, Wisconsin

Investigators

Muldoon, Borchardt, Hunt, Bonness, Masarik, Stokdyk

Objectives

- Design a county-wide randomized sampling plan, stratified by depth-to-bedrock, for nitrate and indicator bacteria
- Sample bi-weekly (i.e., once every two weeks) a subset of wells from the sampling frame to assess seasonal variation in groundwater quality
- 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

Questions?
Comments?

