



# Virus Concentrations in Non-Disinfected Groundwater Used for Drinking:

## Association with Community Rates of Acute Gastrointestinal Illness

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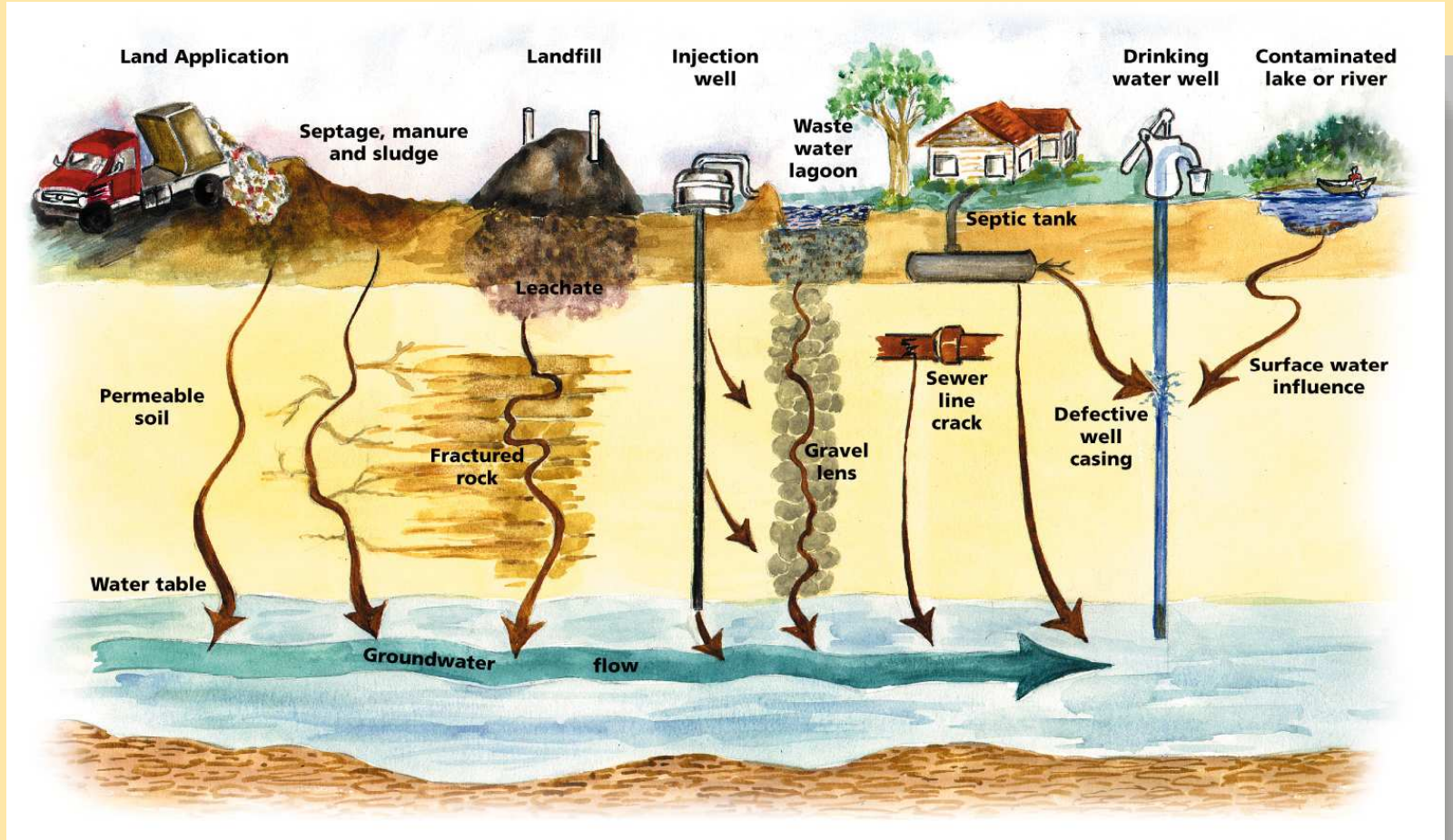


# Virus Occurrence in Groundwater in the USA

- Tested 448 municipal wells in 35 states, 31.5% positive for viruses (Abbaszadegan et al 1998)
- Tested 30 municipal wells in 17 states, 23% positive for enteroviruses (Lieberman et al 1999)
- Tested 29 wells and 72% were virus-positive (Fout et al 2003)
- Viruses in Wisconsin groundwater: Private domestic wells (8%), City of La Crosse wells (83%), and City of Madison wells (66%) were virus-positive (Borchardt et al 2003, 2004, 2007)
- Occurrence and Monitoring Document for the Final Ground Water Rule: at some point in time 27% of public water supply wells are virus-positive (EPA 2006)



# Virus Sources and Infiltration Routes into Groundwater





## Wisconsin WAHTER Study

# Health Risk or Non-Issue?

- So viruses are present in public water supply and domestic wells ...
- Does it matter?
- Is there any effect on public health?



## Study Objective

Determine the association between virus concentrations in non-disinfected tap water and community rates of acute gastrointestinal illness



# Wisconsin Study Communities



**Populations: 1,200 – 8,300**

**Number Wells: 2 – 5**

**Pumpage: 0.13 – 2.1 MGD**

**Hydrogeology: sand, sandstone, limestone**

**No surface water influence**

**No disinfection**



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# Epidemiological Study Design

- Acute gastrointestinal illness (AGI) surveillance for four 12 week periods, spring and autumn 2006 and 2007
- Participants submitted an illness symptom checklist every week
- AGI defined as  $\geq$  three episodes loose watery stools OR  $\geq$  one episode vomiting in 24 hour period
- Person-time estimated from nights slept away from home, self-reported on symptom checklist
- Outcome measure: Number AGI episodes/person-year for each community and surveillance period





# Participating Households' Characteristics

Characteristic	Number	%
<b>Household size (no. of persons)</b>		
2	17	(3)
3	159	(26)
4	246	(40)
5	136	(22)
≥6	63	(10)
<b>Residence type</b>		
Single family home	572	(92)
Apartment or condo	43	(7)
Other	6	(1)
<b>Faucet or plumbing filtering device</b>		
Yes	73	(12)
No	547	(88)
Don't know	1	(<1)
<b>Primary drinking water source</b>		
Municipal	1546	(93)
Bottled water	58	(3)
Other	1	(<1)
Missing	54	(3)

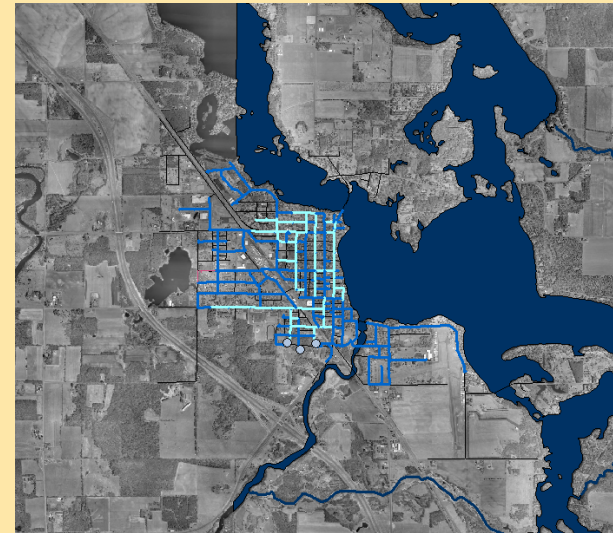
- **Beginning enrollment: 621 households**
- **Ending enrollment: 440 households**
- **Beginning enrollment: 1,079 children, 580 adults**
- **Ending enrollment: 765 children, 413 adults**





# Tap Water Sampling Plan

Monthly samples from household taps, 8 households per community



Viruses captured by glass wool filtration

Liters sampled

Mean = 860

Range: 76 – 2,067

N = 1,204





## Virus Laboratory Analyses

- Water samples analyzed for six virus groups: enteroviruses, adenoviruses, GI and GII noroviruses, hepatitis A virus, and rotavirus
- Viruses quantified by qRT-PCR and qPCR using LightCycler 480 system and TaqMan probes
- Extensive QA/QC procedures and PCR inhibition quantified and corrected for every sample
- Adenovirus and enterovirus infectivity evaluated by cell culture
- Adenovirus and enterovirus serotype determined by sequencing



Wisconsin WAHTER Study

# Virus Types, Frequencies, and Concentrations in Tap Water

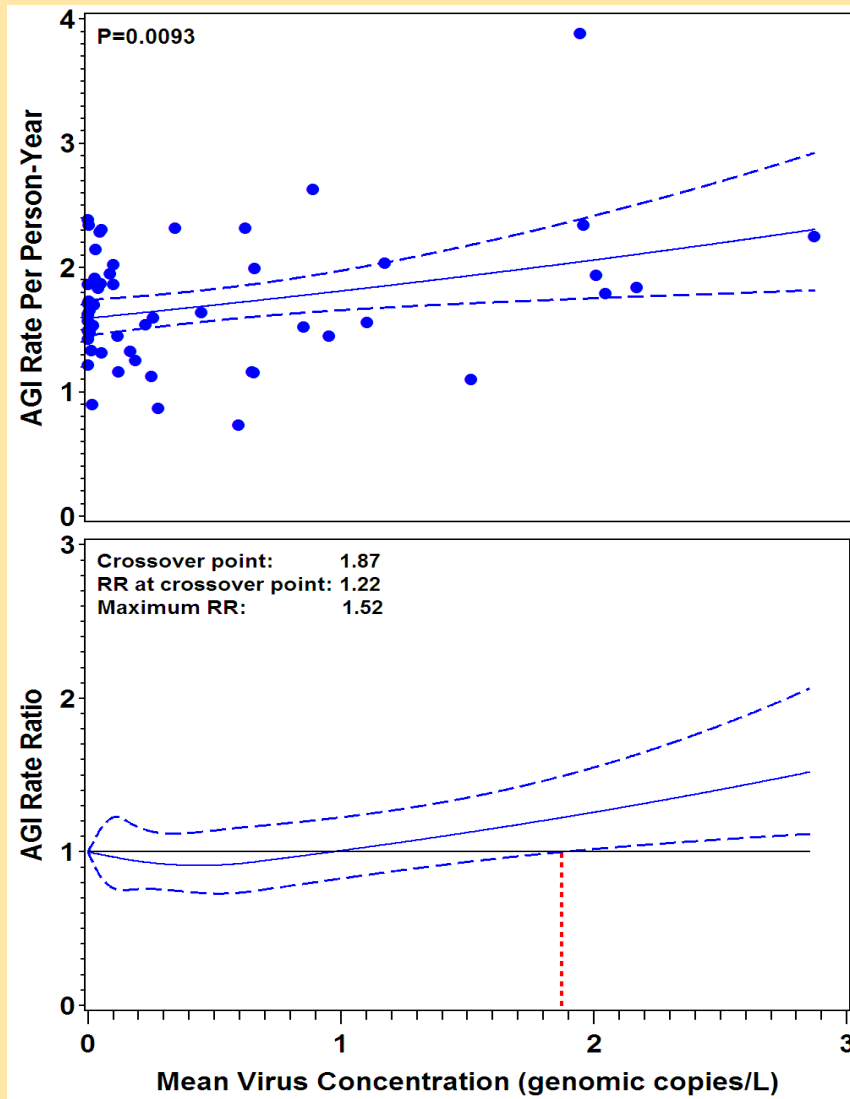
Virus Type	Number qPCR Positive Samples (%)	Virus Concentration Genomic Copies/L		
		Mean	Median	Maximum
Adenovirus	157 (13)	0.07	0	9.5
Enterovirus	109 (9)	0.8	0	851.1
GI Norovirus	51 (4)	0.60	0	115.7
GII Norovirus	0 (0)	0	0	0
Hepatitis A Virus	10 (1)	0.006	0	4.1
Rotavirus	1 (0.1)	$2 \times 10^{-5}$	0	0.03
All Viruses	287 (24)	1.5	0	853.6

**N = 1,204 samples**



# Wisconsin WAHTER Study

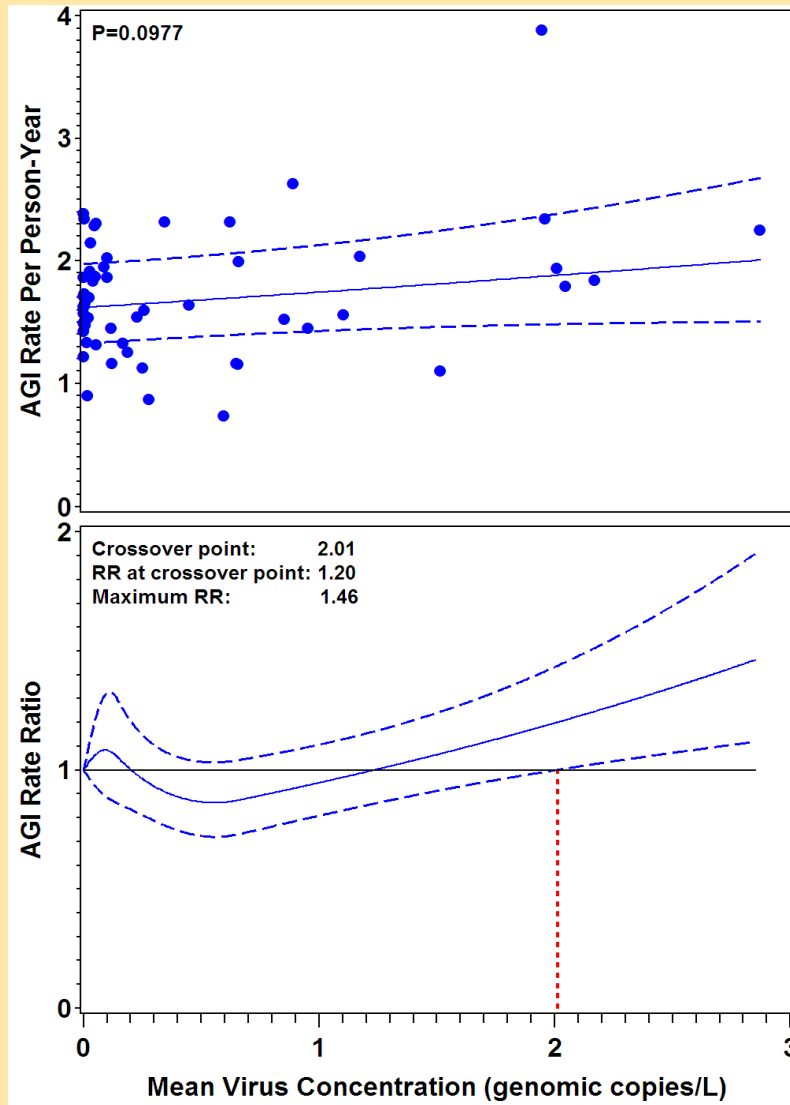
## Total Virus Concentration in Tap Water and AGI Incidence



All ages  
Unadjusted



# Total Virus Concentration in Tap Water and AGI Incidence

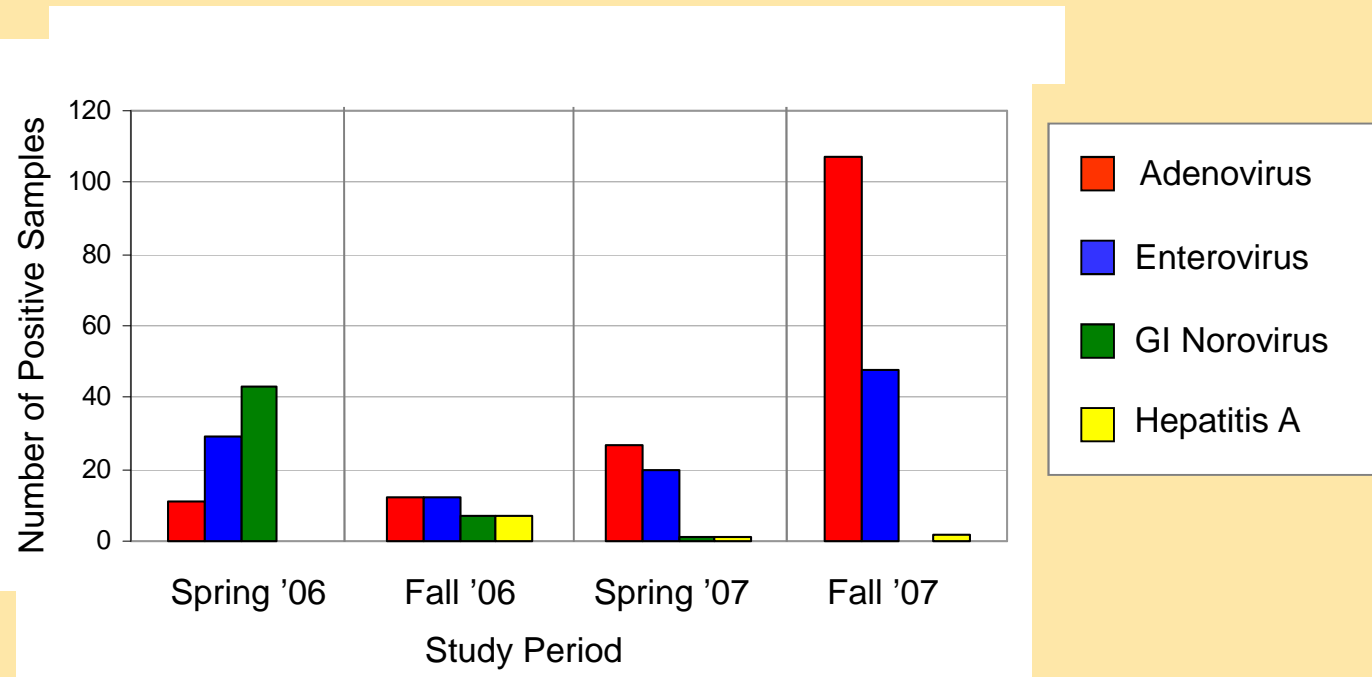


All ages  
Adjusted for  
community and  
period



# Wisconsin WAHTER Study

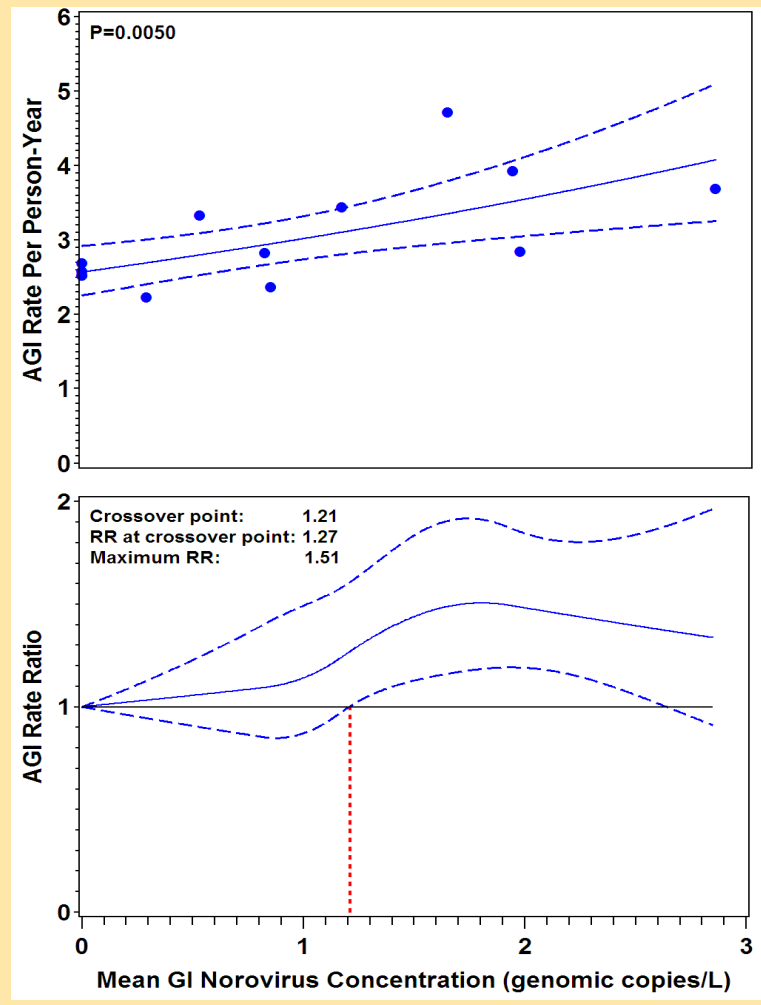
## Virus Types Detected in the Communities' Tap Water





# Wisconsin WAHTER Study

## Period 1 Only GI Norovirus Concentration in Tap Water and AGI Incidence



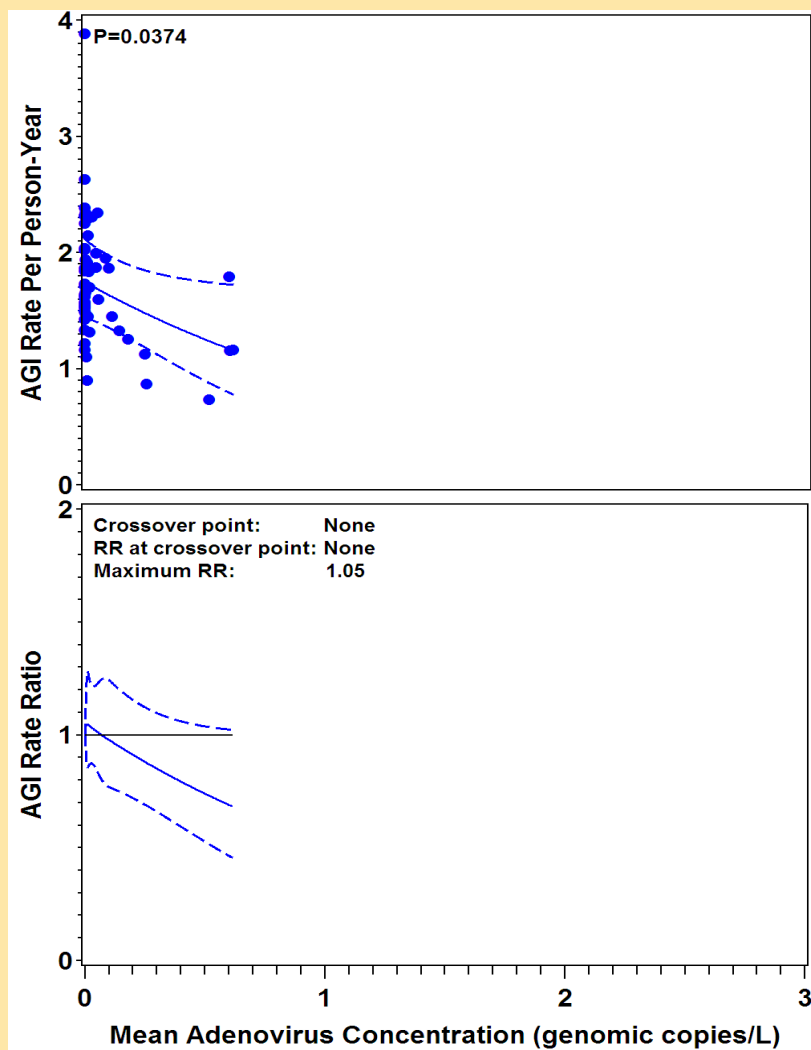
Unadjusted  
Children < 5 years  
old





# Wisconsin WAHTER Study

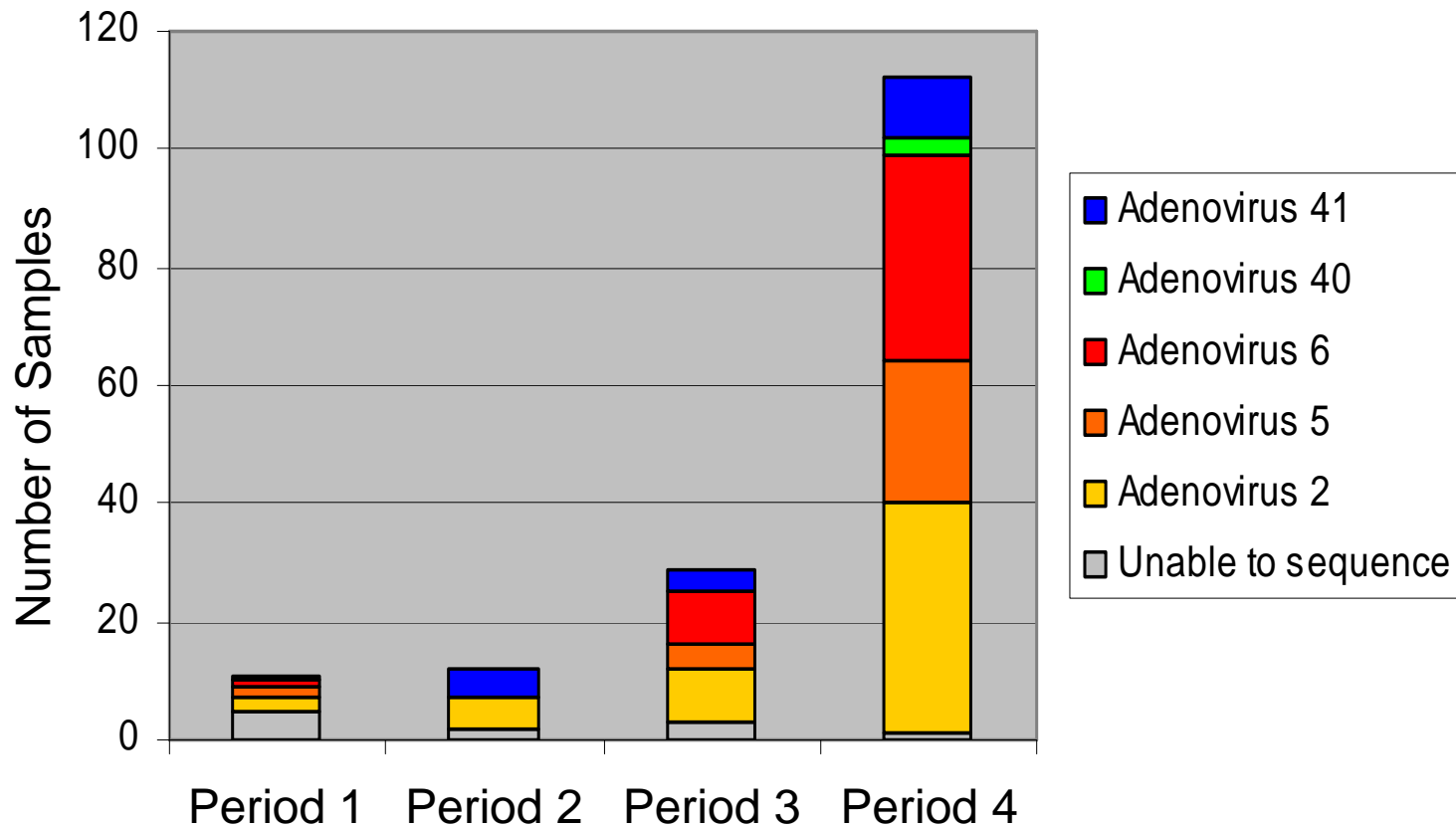
## Adenovirus Concentration in Tap Water and AGI Incidence



All ages  
Adjusted for  
community and  
period



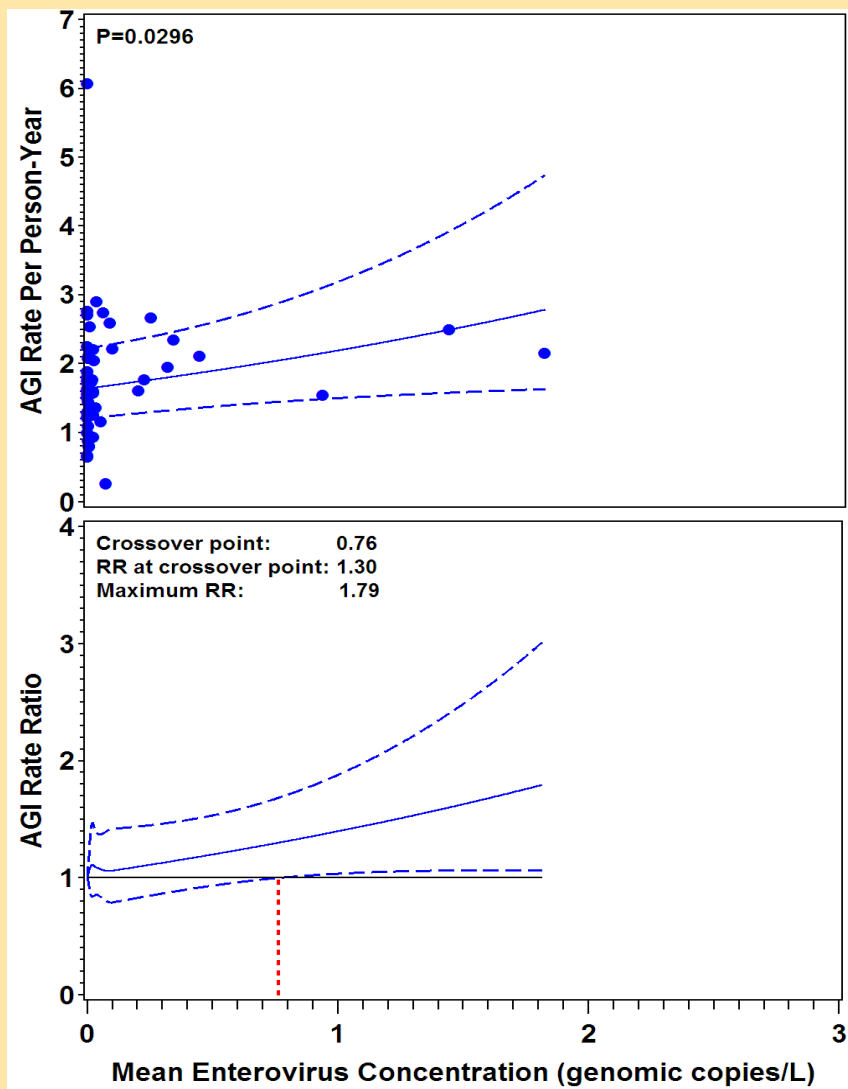
# Adenovirus Serotypes by Surveillance Period in Tap Water





# Wisconsin WAHTER Study

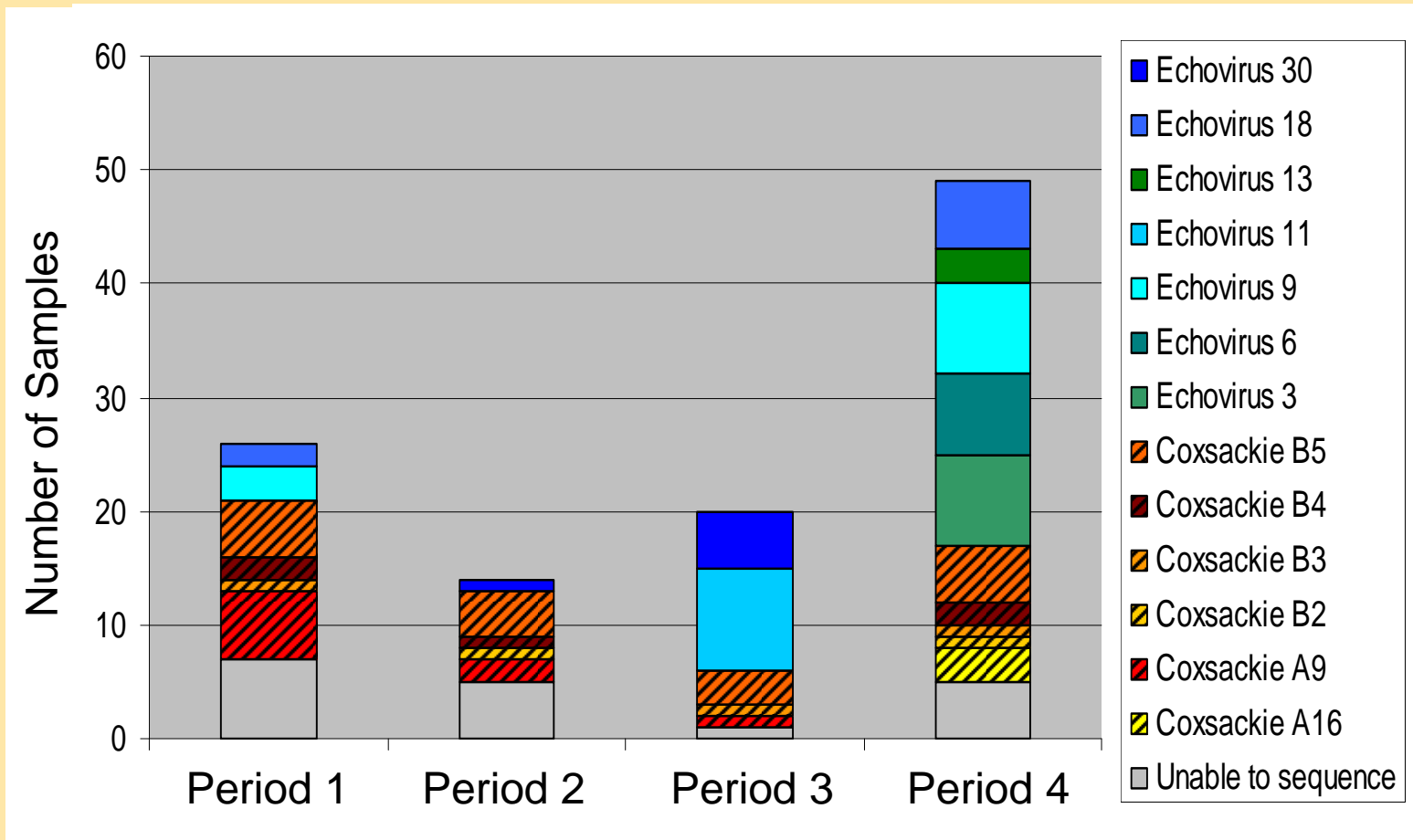
## Enterovirus Concentration in Tap Water and AGI Incidence



Adults  
Adjusted for  
community and  
period



# Enterovirus Serotypes by Surveillance Period in Tap Water



# Summary

- Virus levels in tap water were significantly associated with community rates of AGI; the higher the virus concentration, the more illnesses in the community
- GI Norovirus measures (mean, proportion positive, and maximum) were all strongly associated with AGI
- Adenovirus detects were common, but concentrations were low and unassociated or inversely associated with AGI
- Enteroviruses were associated with adult AGI in certain periods, when echoviruses were predominant
- The risk of AGI was significantly elevated when the 3-month average virus concentration was approximately 2 viruses/liter
- qPCR detection of viruses in non-chlorinated drinking water is meaningful and has value for understanding waterborne AGI risk

