Virus Concentrations in Non-Disinfected Groundwater Used for Drinking:
Association with Community Rates of Acute Gastrointestinal Illness

Mark Borchardt, Susan Spencer, and Burney Kieke
Marshfield Clinic Research Foundation

Elisabetta Lambertini and Frank Loge
University of California - Davis
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
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?
Wisconsin WAHTER Study

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
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

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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Household size (no. of persons)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>(3)</td>
</tr>
<tr>
<td>3</td>
<td>159</td>
<td>(26)</td>
</tr>
<tr>
<td>4</td>
<td>246</td>
<td>(40)</td>
</tr>
<tr>
<td>5</td>
<td>136</td>
<td>(22)</td>
</tr>
<tr>
<td>≥6</td>
<td>63</td>
<td>(10)</td>
</tr>
<tr>
<td>Residence type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single family home</td>
<td>572</td>
<td>(92)</td>
</tr>
<tr>
<td>Apartment or condo</td>
<td>43</td>
<td>(7)</td>
</tr>
<tr>
<td>Other</td>
<td>6</td>
<td>(1)</td>
</tr>
<tr>
<td>Faucet or plumbing filtering device</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>73</td>
<td>(12)</td>
</tr>
<tr>
<td>No</td>
<td>547</td>
<td>(88)</td>
</tr>
<tr>
<td>Don’t know</td>
<td>1</td>
<td>(&lt;1)</td>
</tr>
<tr>
<td>Primary drinking water source</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Municipal</td>
<td>1546</td>
<td>(93)</td>
</tr>
<tr>
<td>Bottled water</td>
<td>58</td>
<td>(3)</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>(&lt;1)</td>
</tr>
<tr>
<td>Missing</td>
<td>54</td>
<td>(3)</td>
</tr>
</tbody>
</table>
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
## Virus Types, Frequencies, and Concentrations in Tap Water

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

*N = 1,204 samples*
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
Virus Types Detected in the Communities’ Tap Water

![Graph showing number of positive samples for different virus types across different study periods.]

- **Adenovirus**
- **Enterovirus**
- **GI Norovirus**
- **Hepatitis A**

Study Period:
- Spring '06
- Fall '06
- Spring '07
- Fall '07

Number of Positive Samples:
- Spring '06: Low
- Fall '06: Low
- Spring '07: Moderate
- Fall '07: High
Period 1 Only
GI Norovirus Concentration in Tap Water and AGI Incidence

Unadjusted Children < 5 years old
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

Marshfield Clinic Research Foundation
Enterovirus Concentration in Tap Water and AGI Incidence

Adults
Adjusted for community and period

Wisconsin WAHTER Study
Enterovirus Serotypes by Surveillance Period in Tap Water

- Echovirus 30
- Echovirus 18
- Echovirus 13
- Echovirus 11
- Echovirus 9
- Echovirus 6
- Echovirus 3
- Coxsackie B5
- Coxsackie B4
- Coxsackie B3
- Coxsackie B2
- Coxsackie A9
- Coxsackie A16
- Unable to sequence
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.