Salivary antibody responses as an indicator of waterborne infections: pilot community study before and after installation of UV treatment
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Study objectives

• Test and validate novel infection surveillance technique that uses salivary antibody as a biomarker of infection

• Apply this technique to assess health benefits of EPA water quality regulations in a selected community

• Identify sites for future studies utilizing this methodology
Study Design

• Study sites:
  – Lawrence, MA (population 70,000):
    • Water from a microbiologically-challenged river
    • In March 2007, the city replaced an old plant (built in 1938) with a new plant (ClO₂, UV) meeting LT2 requirements
  – Lowell - control community using the same river
• Participants: Local families with at least one 1 to 11 y.o. child
• Study cohorts: “before” (June 2006 – Jan. 2007) and “after” (planned in June 2008 – Jan. 2009)
  – Planned 100 families for a full year but the start was delayed. Recruited ~400 families to compensate for shorter follow-up
  – Monthly exposure and illness questionnaires
  – Monthly saliva samples
• Supplemental water monitoring project (Crypto, Giardia, viruses, aerobic endospores, other bacterial indicators)
Data analysis

• Immunoconversion (a steep increase in antibody response to a specific pathogen) as an indicator of infection

• Compare the incidence of immunoconversions before and after new water treatment
  – Compare temporal changes in Lawrence with temporal changes in Lowell
  – Asymptomatic vs. symptomatic infections (immunoconversion following diarrhea/vomiting)
  – Effect of non-boiled tap water consumption

• Compare the results of risk assessment with epidemiological results
“Before new treatment” cohort
Demographics of the study population in Lawrence

- 85% Hispanic (mainly Dominican Republic and Puerto Rico)
- Income
  - 79% had a household income below $25k
  - 94% had a household income below $50k
- Education
  - 36% of adults did not complete high school
  - 32% had only a GED or high school diploma
Summary of saliva sampling by month

<table>
<thead>
<tr>
<th>Month</th>
<th>Samples from Lawrence</th>
<th>Samples from Lowell</th>
<th>Total number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>June</td>
<td>8</td>
<td>36</td>
<td>44</td>
</tr>
<tr>
<td>July</td>
<td>39</td>
<td>33</td>
<td>72</td>
</tr>
<tr>
<td>August</td>
<td>206</td>
<td>47</td>
<td>253</td>
</tr>
<tr>
<td>September</td>
<td>698</td>
<td>80</td>
<td>778</td>
</tr>
<tr>
<td>October</td>
<td>1088</td>
<td>235</td>
<td>1323</td>
</tr>
<tr>
<td>November</td>
<td>948</td>
<td>204</td>
<td>1152</td>
</tr>
<tr>
<td>December</td>
<td>1282</td>
<td>256</td>
<td>1538</td>
</tr>
<tr>
<td>The entire “before” period</td>
<td>4269</td>
<td>891</td>
<td>5160</td>
</tr>
</tbody>
</table>

Total number of families: 391
Total number of individuals: 1398
24 hour liquid consumption
(average numbers of 8 oz glasses)

<table>
<thead>
<tr>
<th>City</th>
<th>Soda</th>
<th>Milk</th>
<th>Bottled water</th>
<th>Boiled water</th>
<th>Filtered non-boiled tap water</th>
<th>Non-filtered non-boiled tap water</th>
<th>All non-boiled tap water</th>
<th>All drinks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lawrence</td>
<td>2.3</td>
<td>1.9</td>
<td>1.7</td>
<td>1.0</td>
<td>1.1</td>
<td>0.7</td>
<td>1.8</td>
<td>8.8</td>
</tr>
<tr>
<td>Lowell</td>
<td>1.8</td>
<td>1.6</td>
<td>1.3</td>
<td>0.6</td>
<td>0.9</td>
<td>1.3</td>
<td>2.2</td>
<td>7.5</td>
</tr>
</tbody>
</table>

- Relatively low consumption of untreated tap water in Lawrence
- Extensive use of home water filters in Lawrence but not in Lowell:
  - Lawrence – 46 % of participants
  - Lowell – 22 % of participants
Objective 1

Test and validate novel infection surveillance technique that uses salivary antibody as a biomarker of infection
Salivary antibody – advantages and challenges

• Advantages:
  – Sampling well tolerated by children
  – Multiple samples are possible

• Challenges:
  – Substantial variability in antibody concentrations
  – Precipitation of antibody-protein complexes
  – Non-specific reactivity
  – Lower concentrations of antibodies than in serum
Saliva sampling

- Oracol™ oral fluid samplers
- Centrifugation to separate saliva from the sponge and debris from saliva
- Storage at -80° C until analysis
- Analysis at EPA using Luminex™ multiplex microbead immunoassay
Luminex xMAP microsphere suspension microplate immunoassay

1. Microscopic bead is coupled with one specific protein

2. Saliva is incubated with beads; salivary antibodies react with protein

3. Samples are incubated with biotinylated anti-human detection antibody

4. SAPE is added to wells to bind biotinylated detection antibodies

5. Microplates are analyzed using Luminex instrument
Multiplex assay

- Color-coded sets of beads coupled to different proteins
- Dual laser flow cytometer determines the type of bead and measures signal intensity
Selected potentially waterborne pathogens

- Cryptosporidium
- Noroviruses
- Rotaviruses
- *Helicobacter pylori*
- *Toxoplasma gondii*
Assay development

• Samples used:
  – Serum and saliva samples from EPA volunteers
  – Selected saliva samples from participants of main study
• Selection and acquisition of proteins
• Expression and purification of recombinant proteins
• Optimization of protein-bead coupling
  – Coupling confirmation tests using antigen-specific antibodies
• Selection of saliva dilution ratio and dilution buffer
• Internal controls (GST- and BSA-coupled beads)
• Total antibody and total protein concentrations
• Effects of sample volume, storage, freezing, etc.
• Validation of salivary tests for chronic infections
**Helicobacter pylori**

- Active chronic infection of the stomach
- Causes gastritis, ulcers, cancer
- >30% of US adults infected
- CCL2 pathogen
- Proteins:
  - Flagellin
  - VAC protein
  - CAG protein
  - Small subunit urease
  - Soluble antigen extract (strain)
Toxoplasma gondii

- Protozoan parasite of felines, forms environmental cysts
- Forms latent tissue cysts in muscles and brain of intermediate hosts including humans
- ~25% of US adults are infected
- Can infect human fetus and cause severe neurological damage
- Reported waterborne outbreaks

Proteins:
- Soluble proteins from tachyzoites
- P30 protein
  - Recombinant
  - Purified from HeLa human cells
  - Purified from mice
- GRA7 protein
- MIC3 protein

T. gondii cyst in brain tissue

http://www.dpd.cdc.gov
Cryptosporidium

- Oocysts resistant to chlorine
- Reported incidence 1 per 100,000 PY
- Major waterborne outbreaks
- LT2 is based on RA for endemic cryptosporidiosis
- Antigen extract from *C. parvum* oocysts
- Recombinant 27 kDa *C. parvum* protein
  - Transformed *E. coli* was provided by Jeffrey Priest (CDC)
  - Expressed and purified glutathione S-transferase (GST)-tagged protein
  - Use GST-coupled beads as internal control
Noroviruses

- The major cause of gastroenteritis in adults
- Severely underreported
- Highly infectious, resistant to chlorine
- Identified as cause of drinking water outbreaks
- Typical symptoms include vomiting and diarrhea

Proteins (provided by the Cincinnati Children’s Hospital):

- Genogroup II strain VA387 recombinant capsid protein
- Genogroup I Norwalk virus recombinant capsid protein
Rotaviruses

- Major cause of gastroenteritis in children
- Severely underreported
- Detected in surface and ground water
- Purified rotavirus particles procured from the Cincinnati Children’s Hospital:
  - DS1 strain:
    - Triple layered particles
    - Double-layered particles
  - WA strain:
    - Triple layered particles
    - Double-layered particles
Selected results of assay development and validation
H. pylori lysate: Serum ELISA vs. saliva Luminex, IgG

Saliva Luminex, 4x dilution (MFI)

Serum ELISA, 50x dilution (OD)

Sero-negative

Seropositive
Anti-Norovirus VA387 IgG in an EPA volunteer

Anti-Norovirus / anti-GST ratio at 4 x dilution

Diarrhea and vomiting

Study status and future directions

• Objective 1
  – Completed development of assays for all target pathogens
  – Developed multiple internal controls

• Objective 2 is underway
  – Detected immunoconversions to norovirus

• More antigens can be added to multiplex assay at relatively low cost
  – *Giardia lamblia* (acquiring recombinant giardins from CDC)
  – Recreational water pathogens
  – Biofilm-associated pathogens
Acknowledgments

• NCEA, NERL and OW TSC colleagues who donated their saliva and blood samples
• CDC for providing transformed *E. coli* culture expressing recombinant *C. parvum* protein
• Cincinnati Children’s Hospital for providing purified norovirus proteins
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