Salivary antibody as a novel indicator of incident waterborne infections. Can it help to evaluate the beneficial health effect of improvements in water treatment?

Presenters: Al Egorov[1], GS Fout[2], TJ Wade[3]

Contributing Organizations: ORD: [1]NCEA, [2]NERL, [3]NHEERL; Region 1

LTG 4 Poster 03

Science Questions

- Is salivary antibody a feasible and cost-effective bioindicator of incident infections in longitudinal studies of waterborne infections?
- Can linkages be made between risk mitigation actions that bring drinking water treatment plants into compliance with drinking water regulations and reduction in the incidence of endemic waterborne infections? Specifically, is there a reduction in the incidence of selected common waterborne infections in a community following an LT2-driven improvement in treatment of water derived from a microbiologically contaminated river?

Research Goals

BACKGROUND

Waterborne infections can be caused by a variety of viral, bacterial and protozoan pathogens. The protozoan parasite *Cryptosporidium* is of major concern for water utilities which use unprotected surface water sources due to its extreme resistance to chlorination. Enteric viruses, such as noroviruses and rotaviruses, are also relatively resistant to disinfection. Recent regulatory actions, such as the Long Term 2 Enhanced Surface Water Treatment Rule (LT2), were developed to reduce the incidence of waterborne diseases. Assessing reductions in waterborne infections due to better water treatment remains a challenge for a number of reasons: 1) most cases of gastroenteritis are self-treated; 2) even when medical care is provided, fecal samples are rarely tested for viruses or *Cryptosporidium*; 3) these infections can be acquired from sources other than drinking water; 4) occurrence data on these pathogens in raw and treated water are limited; and 5) it can be difficult to demonstrate a statistical association (and even more difficult to show causality) between water quality and endemic infections in non-outbreak settings. Epidemiological studies that include collection and analysis of fecal samples, or testing of blood samples for antibodies to these pathogens have proven to be expensive and difficult to organize. Pathogen-specific antibodies in saliva can be used as a bioindicator of infection. Because collection of saliva is easy and painless, salivary immunoassays can be used in prospective studies involving children. A steep increase in specific salivary antibody between consecutive samples (immunoconversion) serves as a bioindicator of incident infections.

STUDY OBJECTIVES

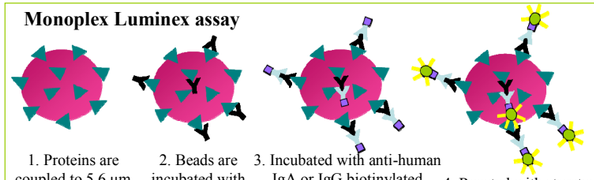
1) Develop a multiplex immunoassay for measurement of salivary antibody responses to selected waterborne pathogens

2) Apply this immunoassay to assess health benefits of EPA water quality regulations in a selected community

3) Identify future research projects utilizing this methodology

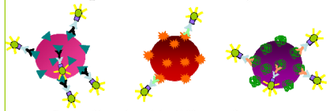
Methods/Approach

Monoplex Luminex assay



1. Proteins are coupled to 5.6 µm carboxylated beads
2. Beads are incubated with saliva samples
3. Incubated with anti-human IgA or IgG biotinylated detection antibody (Ab)
4. Reacted with streptavidin R-phycoerythrin (SAPE)

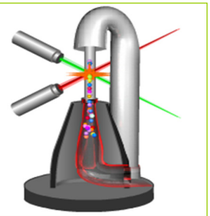
Multiplex Luminex assay



Sets of beads with different fluorescence properties are coupled to different antigens. Mixture containing 5000 beads of each type is incubated with saliva.

Luminex instrument

Samples are analyzed using Luminex dual-laser flow cytometer. Red laser is used for bead classification, green laser for assay quantification. Results are reported as Median Fluorescence Intensity (MFI) for each set of beads.



ASSAY DEVELOPMENT AND INITIAL VALIDATION

- More than 200 coupling experiments performed to select optimal conditions for coupling each protein to Luminex beads
- 200 saliva samples from 25 EPA volunteers in Cincinnati (7 person-years)
  - Selection of immunogenic proteins by comparing salivary antibody titers
  - Assay optimization (buffer, saliva dilution, detection Ab)
  - Effects of sampling and storage conditions
  - Temporal variability in salivary Ab responses and immunoconversions
- Validation using paired saliva and serum samples from ten EPA volunteers
  - Serological ELISA for *H. pylori, T. gondii*, *C. parvum* vs. Luminex saliva tests

EPIDEMIOLOGICAL STUDY

Study sites:

- Lawrence, MA - 70,000 residents, 85% of families with children are Hispanic
  - Water from the microbiologically contaminated Merrimack River
  - Court order to build a new plant due to turbidity violations at the old plant
  - New treatment plant using ClO_2 and UV completed in April 2007
- Lowell, MA - control community deriving water from the same river 15 miles upstream; data to characterize year-to-year variability in infections in the area

Study participants:

- Local families with children from 1 to 12 years-old
- Individuals who work or study in Lawrence or Lowell

"Before and after" panel design:

- Before new treatment (Phase 1): July 2006 – December 2006
  - 392 families, 1498 individuals, 5160 saliva samples
- After new treatment (Phase 2): July 2008 – December 2008
  - 392 new families, 1622 individuals, saliva collection ongoing in Nov. 2008

Data collection:

- Baseline demographic questionnaires
- Monthly data on illness symptoms, travel, other exposures; 24-hour liquid consumption data collected one per month
- Monthly saliva samples collected using Oracol™ samplers
- Samples are shipped overnight to EPA, processed and archived until analysis

Planned data analysis:

- Compare odds of specific infections before and after new treatment in Lawrence
- Stratify data by the level of non-boiled tap water consumption
- Use Lowell data to adjust for natural year-to-year variability in infections

WATER MONITORING

- Bi-weekly samples from Lawrence and Lowell
- *Cryptosporidium* and *Giardia* (EPA method 1623; 10-40 L raw water and 1,000-2,000 L finished water samples)
- Viruses (300 L raw water and 7,000 L finished water samples collected using NanoCeram® filters; samples archived for PCR and cell culture-PCR analyses)
- Aerobic endospores (heat treatment-culture method), *E. coli*, coliforms (Colilert), enterococci (Enterolert) in raw water, combined filter effluent and finished water

Assay Development Results

IMMUNOASSAY DEVELOPMENT STATUS

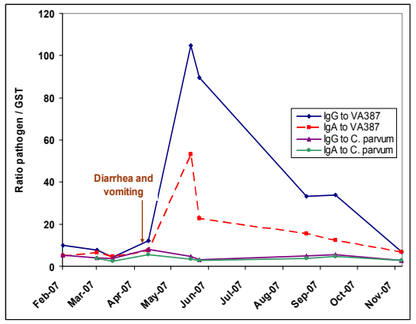
Completed:

- *Cryptosporidium:*
  - *C. parvum* recombinant 27 kDa protein
  - *C. hominis* recombinant gp15 protein
- Noroviruses:
  - Norwalk virus (GI) recombinant capsid protein
  - VA387 (GII) recombinant capsid protein
- Rotaviruses - recombinant VP6 protein
- *Helicobacter pylori* – cell sonicate and recombinant urease
- *Toxoplasma gondii* – soluble proteins and p30 protein
- Internal cross-reactivity controls:
  - Glutathione S-transferase for GST-tagged proteins
  - Bovine serum albumin for reactions with beads
- Duplex assay for total salivary IgA and IgG

Under development in Nov. 2009:

- Norovirus VA207 (GII) recombinant capsid protein
- *Giardia lamblia* recombinant α-1 and α-7.3 giardins

Norovirus infection in an EPA volunteer



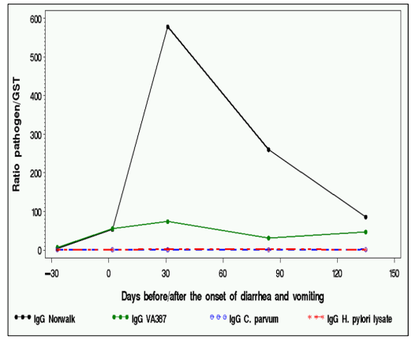
Preliminary Results of Phase 1 Epidemiological Study

- Incidence rate of self-reported acute gastroenteritis in Phase 1 cohort was 0.05 episodes per person-month
- Samples from most Phase 1 participants who reported episodes of gastroenteritis have been analyzed for IgA and IgG responses to noroviruses, *C. parvum, H. pylori*
- Analysis procedures were optimized:
  - Ratio of responses to microbial antigen and internal control is used in analysis of immunoconversions
  - Total antibody assay is used to detect samples with abnormally low or high concentrations of Abs
  - Samples from the same person analyzed on one plate
- 13 % of episodes of diarrhea and vomiting were followed by immunoconversion to VA387 or Norwalk viruses
- Incidence of immunoconversions to noroviruses in asymptomatic individuals was approximately 0.2/PY

Liquid Consumption in Phase 1 Cohort (8-oz glasses/day)

City	Bottled water	Boiled tap water	Non-boiled tap water	Soda	Milk	All drinks
Lawrence	1.7	1.0	1.8	2.3	1.9	8.8
Lowell	1.3	0.6	2.2	1.8	1.6	7.5

Norwalk virus infection in a study participant



Findings and Preliminary Conclusions

- Preliminary water monitoring data show a substantial improvement in removal of aerobic endospores and protozoa in Lawrence after the introduction of new treatment
- Demonstrated that salivary antibody can be used for detection of incident infections
- Preliminary data show that norovirus was the main cause of gastroenteritis; the total number of infections is projected to be sufficient for "before and after" comparison

Impact and Outcomes

- Development of a salivary antibody bioindicator that can be used for detection of incident waterborne infections
- Demonstration of the application of this non-invasive biomarker to link implementation of EPA's LT2 rule with a reduction in gastrointestinal infections

Plans and Future Directions

CURRENT STUDY

- Obtain series of control saliva samples from individual with and without infections (CDC study of norovirus infections in nursing homes, volunteer challenge studies)
- Determine sensitivity and specificity of immunoconversion tests
- Finish analysis of saliva samples (beginning of 2010)
- Finish data analysis and submit final manuscript (June 2010)

NEW STUDIES

- Use similar multiplex immunoassay to analyze a subset of serum samples from the NHANES study to identify sites of interest
- Conduct prospective salivary antibody studies in selected locations
- Expand this assay to include pathogens associated with recreational water
- Analyze saliva samples from beach-goers in Puerto Rico
- Expand this assay to include pathogens associated with distribution system biofilm
- Pilot distribution system study to assess spatial variability of exposures in Lawrence
- Epidemiological analysis of the effects of secondary contamination in the distribution system on specific infections in Lawrence

EPA Researchers Involved

NCEA: A.I. Egorov, S.M. Hunt; NHEERL: T.J. Wade; NERL: G.S. Fout, A. Dufour; Region 1: K. Reilly; OW: S. Shaw; NRMRL: L. Boczek.

Collaborations with Researchers outside EPA

- Jeffrey Priest (Division of Parasitic Diseases, CDC) provided *E. coli* cultures expressing recombinant 27 kDa *C. parvum* protein, α-1 and α-7.3 giardins of *Giardia lamblia*
- Honorine Ward (Tufts Medical Center, Boston, MA) provided purified recombinant gp15 protein of *C. hominis*
- Harry Kleanthous (Acambis) provided purified recombinant urease of *H. pylori*
- Richard Ward (Cincinnati Children's Hospital) provided recombinant VP6 rotavirus protein
- Jason Jiang (Cincinnati Children's Hospital) provided recombinant proteins of Norwalk virus, norovirus VA387 and VA206
- Mark Griffin (Lawrence Water Department) contributed to water monitoring

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