Viruses at the nexus of water and human health

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Global access to safe drinking water

- 748 million people lack access to improved drinking water
- 1.8 billion people use a drinking water source that is fecally contaminated
- 2.5 billion lack access to improved sanitation facilities

(World Health Organization and UNICEF, 2014)
Water-borne pathogens and their impact on health

- Pathogenic organisms from contaminated water is the largest global human health hazard
  - Responsible for 10% of global health burden

- Annually, 4 billion episodes of diarrhea & 2 million deaths from diarrhea (most of which are children under 5) (WHO, 2012)
The effects of unsafe water on health

- **Direct impact**
  - Sicknesses and death of children
  - Increased morbidities and mortalities in women, impoverished people, immunocompromised people (HIV/AIDS, malaria, pregnant women)

- **Indirect effects**
  - Repeated severe diarrhea results in malnourishment, stunting, decreased mental capacities in people
  - Sickness decreases productivity, increases social and economic burdens on families, communities and governments
Microbial pathogens in the water

- Helminth eggs
  - tapeworm
- Protozoa (oo)cysts
  - *Cryptosporidium parvum*
- Bacterial spores and vegetative bacteria
  - *Bacillus anthracis, E. coli*
- Viruses
  - Adenovirus, norovirus, rotavirus

Bondo, Kenya
Safe water and health

- Waterborne viruses are of particular concern
  - Resistance to disinfectants
  - Detection of infectious viruses is a challenge
    - Cell culture is necessary
    - No method currently exists to test for infectious viruses onsite
      - PCR
      - ELISA

Bondo, Kenya

Tigray, Ethiopia

Ntisaw, Cameroon
Adenovirus is re-emerging as a pathogen of concern

**EPA Contaminant Candidate List 3: Microbial Contaminants**

- Adenovirus
- Caliciviruses (*Norovirus*)
- Enterovirus (*Coxsackievirus*)
- *Mycobacterium avium*
- *Campylobacter jejuni*
- *Escherichia coli* (0157)
- *Helicobacter pylori*
- Hepatitis A virus
- *Legionella pneumophila*
- *Naegleria fowleri*
- *Salmonella enterica*
- *Shigella sonnei*

**Viruses:** Treatment technique for 99.99% removal/inactivation

- **Free Chlorine**
- **Monochloramine**
- **Low Pressure Ultraviolet Light**

**Water at pH 8, 14°C: Add 2 mg/L as Cl₂**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free Chlorine</td>
<td>6 seconds</td>
</tr>
<tr>
<td>Monochloramine</td>
<td>17 hours</td>
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(U.S. EPA, 2006)
Adenovirus disinfection

- Characterize virus inactivation for a range of disinfectants and water chemistries
- Elucidate the mechanism of adenovirus inactivation
Experimental Protocol

- Disinfection experiment (Free chlorine, pH 9.2, 14°C)
  - Plaque assay to determine inactivation
- Attachment: 4°C on A549 T-25s
- Wash off unattached viruses
- Incubate at 37°C to synchronize entry

Collect Sample (Cells+ virus) at 4, 12, 24, 36 hours post infection

Isolate DNA
Isolate RNA
DNase Digestion
RT Reaction (cDNA)
Run qPCR: E1A, Hexon, B-actin
Inactivation kinetics of adenovirus by free chlorine at pH 9.2, 14°C
Adenovirus disinfection
Free chlorine inactivation of adenovirus causes a delay in early mRNA production (E1A)

36 hours post infection

**E1A mRNA copies/0.5 ug total RNA**

- **Time post infection (hours):** 4, 12, 24, 36
- **N/N₀ =**
  - 1
  - 0.27
  - 0.084
  - 0.0078
  - 0.0064
  - 0.0036
  - 0.0018

**Log decrease**

- **CT (mgCl₂*min/L):** 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7

**N/N₀**

- **E1A/E1A₀**
Adenovirus disinfection
After free chlorine inactivation, adenovirus is unable to replicate its genome.
Adenovirus disinfection
Adenovirus is able to attach to host cells after free chlorine inactivation.
Conclusions and Future Work

- Life cycle inhibition
  - Integrin binding/attachment affinity?
  - Endosomal lysis?
  - Nuclear entry?
- Protein integrity
- Other disinfectants: UV
- Overall goal: Providing safe global water
  - Development of robust treatment technologies
How do we neutralize viruses in a meaningful/helpful way for society?

testing potential solutions in Uganda
collaborating with Oruchinga Refugee Camp and Uganda Rural Farm

capacity building with Makarere University

Professors

Graduate students

Undergraduates
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