The Water and Health in Limpopo Innovations Fellowship Program

Richard Guerrant, MD
Rebecca Dillingham, MD, MPH
Pascal Bessong, PhD
Liz Olmsted, Program Administrator

University of Virginia &
University of Venda

Framework Programs for Global Health Innovation Network Meeting
Fogarty International Center
NIH, Bethesda MD
June 26 – 27, 2014
Fellows-Trainees/Disciplines

- Year 1 (8/13/12 – 8/31/13)

- Year 2 (9/1/13 – 8/31/14)

Lufuno Mavhandu - Prog. Coordinator - UNIVEN  May-Jun 2014

Recruitment underway (deadline 30 Jun 2014) for

- Year 3 (9/1/14 – 8/31/15)
1. Teri Dankovich, PhD  
– Chemistry, McGill University (2012)  

Presentations:
Presented abstract and poster at ASTMH: “Inactivation of bacteria from contaminated rivers in Limpopo, S Africa by silver- or copper- coated nanoparticle paper filters.”

Publications:


Patents: A provisional filed on the copper nanoparticle paper in April 2014.

Other Activities:
Participated in competitive teaching training workshop during which she developed a module for training students in design thinking combining chemistry and engineering

Urls:  https://drinkablebook.crowdtilt.com/the-drinkable-book  
INACTIVATION OF BACTERIA FROM CONTAMINATED RIVERS IN LIMPOPO, SOUTH AFRICA BY SILVER- OR COPPER-NANOPARTICLE PAPER FILTERS

THERESA A. DANKOVICH, CORINNE CLINCH, HANNAH WEINRONK, REBECCA DILLINGHAM, AND JAMES A. SMITH
DEPARTMENT OF CIVIL AND ENVIRONMENTAL ENGINEERING, CENTER FOR GLOBAL HEALTH, UNIVERSITY OF VIRGINIA, CHARLOTTESVILLE, VA

Objectives
- To evaluate the performance of silver- and copper-nanoparticle paper filters in the field with water samples obtained from contaminated rivers in Limpopo, South Africa.

Performance Criteria:
- Bacteria inactivation
- Turbidity reduction
- Metal release from the papers

Novel Ag or Cu nanoparticle paper point-of-use water purifier

- The filters were tested in a lab-scale model with a capacity of 250 mL.
- The filters were tested at 100 mL of water per filter with a single type of bacteria (E. coli in the early growth phase).
- The bacteria were exposed to 0.1 mg/L of Ag or Cu nanoparticles for 5 minutes.
- The paper filters were able to kill bacteria, even after being rinsed.

Laboratory evaluation of antibacterial papers

- The paper filters were assessed for their ability to inactivate bacteria.
- The filters were tested against various bacteria, including Escherichia coli and Salmonella typhimurium.
- The results showed that the paper filters were effective in inactivating the bacteria.

River water samples from Limpopo, South Africa

- The samples were collected at different locations along the river.
- The samples were tested for bacteria and turbidity.

Field test results

- The field tests were conducted at various locations along the river.
- The bacteria inactivation rates were monitored at different times.
- The results showed that the paper filters were effective in inactivating bacteria in the field.

Turbidity Reduction

- Turbidity was reduced by 73% in the treated water.
- The results were consistent at all test sites.

Bacterial Inactivation Results:

- E. coli: 4 log reduction
- Salmonella typhimurium: 4 log reduction

Projected paper filter capacity

- The paper filters were projected to have a capacity of 250 mL.
- The filters were expected to be used for up to 100 people.

Ag release in effluent water

- The concentration of Ag released in the effluent was measured.
- The concentration was found to be below the limit.

Cu release in effluent water

- The concentration of Cu released in the effluent was measured.
- The concentration was found to be below the limit.

Conclusions

- The paper filters were effective in inactivating bacteria in the field.
- The filters were effective in reducing turbidity.
- The filters were cost-effective and easy to use.

Acknowledgements

- The authors acknowledge the support of the University of Virginia and the National Institutes of Health.
- The authors thank the local communities for their cooperation.

The American Society of Tropical Medicine and Hygiene National Meeting, November 13-17, 2013, Washington, D.C.
2. Nicola Bulled, PhD
– Anthropology, University of Connecticut (2012)
– Appointed at UVA - June 1, 2013 – August 31, 2014

Presentations:
• Presented abstract and poster at ASTMH, “Syndemics of Childhood Diarrhea”
• Oral presentation at SAA, “Citizenship and water insecurity: Understanding the Impact of Water Insecurity on Biomedical Citizenship in the Vhembe District of South Africa”
• Oral presentation at AAA, “Prescribing Risk Reduction: Implications of biocommunicability of HIV in southern Africa”

Publications:
• Dr. Bulled’s book on Global Health Education, “Prescribing HIV Prevention” is set to be released in October, 2014.

Other Activities
• Developed and taught a summer graduate course, Global Health Policy and Practice, enhancing her curriculum development skills relevant to planned career in academic global health.
The tendency in existing research is to look for and seek to resolve individual causes of diarrheal disease. Causal interaction is an understudied issue.

"the concentration and deleterious interaction of two or more diseases or other health conditions in a population, especially as a consequence of social inequality and the unjust exercise of power"  
SYNDEMIC, MERRILL SINGER, 2000

SYNDERMICS of CHILDHOOD DIARRHEA

What is the problem?

- Diarrhea 2nd leading cause of death in children under 5
- Malnutrition and early mortality rates are much higher within low-income countries
- Diarrheal diseases and stunting are major causes of illness and mortality
- Early childhood development and learning outcomes are negatively affected
- Children who suffer from multiple episodes of diarrheas experience a delay in cognitive development
- Children who suffer from multiple episodes of diarrheas are more likely to have a lower educational achievement

Imagine a solution

- greater involvement of diverse stakeholders
- strategies with equal or greater investments on improving social conditions

Brazil's Bolsa Familia Program (BFP), initiated in 2003, is the world's largest conditional cash transfer program for poverty reduction. Conditional cash transfers require that children are in school where they receive at least one meal per day. The program has been successful in reducing poverty, improving health outcomes, and increasing school enrollment. However, more research is needed to understand the long-term effects of the program.
3. Nicoline Fri Tanih, PhD
— Biochemistry & Microbiology, Univ. of Fore Hare, S.A.(2011)

Presentations:
• Abstract submission and poster presentation at ASTMH, “Genotypes of Enteropathogens in Rural South Africa: Impact on Child Growth”
• Poster presentation at ICID, “Prevalence of diarrheagenic Escherichia coli in young children from rural South Africa: The Mal-ED Cohort”

Activities:
• Learned to work with an animal model to evaluate relevant EAEC strains thanks to intensive laboratory mentoring.
• American Society of Microbiology National Conference
• Consortium of Universities for Global Health national conference
• International Society for Infectious Diseases national conference
Genotypes of Enteropathogens in Rural South Africa: Impact on Child Growth

Nicolene Tanih, Pascal Bessong, Amidou Samie, Theresa Dankovich, Nicola Bulled, Leah Barrett, Rebecca Dillingham, Richard Guerrant
University of Venda and Centre for Global Health, University of Virginia

Summary

Cryptosporidium spp., Giardia lamblia and pathogenic forms of E. coli are organisms recently associated with diarrheal outbreaks in several communities across the world. Their impact on child growth still remains poorly understood. A total of 2862 stool samples were collected monthly from 247 children recruited at birth and followed up for 12 months for anthropometric measurements and illness history. The stool samples were analyzed by standard microbiological methods and PCR for genetic diversity. Cryptosporidium was found in 70 (2.4%) of the samples. Giardia was observed in 77 (2.7%) of the stool samples. The pathogenic forms of E. coli found included EAEC (19.3%), EPEC (6.2%), STEC (1.3%) and ETEC (3.6%). Giardia was significantly associated with being underweight as well as low birth weight among the children. The importance of enteropathogens in child health cannot be over emphasized.

Background

Morbidity related to enteropathogens infections remains a significant health problem worldwide. Cryptosporidium spp, Giardia lamblia and pathogenic forms of E. coli are some of the pathogens most commonly associated with diarrheal infections.

Infections with these pathogens have been reported to be linked to malnutrition. However, their impact on child growth still remains poorly understood. Different genotypes of these organisms have been described.

This study aimed at determining the occurrence and genotypic distribution of these enteric pathogens among children one year old or less in a rural community in Northern South Africa and to determine their potential associations with child growth.

Materials and methods

Ethical clearance was obtained from relevant authorities and signed informed consent obtained from the mothers. A total of 247 children were enrolled for this study and 2862 stool samples were collected monthly from them at birth and followed up for 12 month for health measurements.

Giardia, Cryptosporidium and pathogenic forms of E. coli were detected from these stools samples. For Giardia lamblia and Cryptosporidium spp., samples were tested microscopically followed by enzyme linked immunosorbent assay (ELISA).

Genomic DNA was purified from samples positive either for microscopy or ELISA (for identification of Giardia (40) and Cryptosporidium) using the QiAamp DNA Stool Mini Kit from Qiagen following the recommendations from the manufacturer while DNA extracted by boiling of the cultured cells for identification of E. coli. The presence of these organism and their potential associations with child growth was determined

A PCR protocol targeting the conserved region of the small-subunit rRNA gene of Cryptosporidium. Cryptosporidium DNA in the samples was tested using first set of primers, SSU-1 (5'-GAT TAA GCC ATG CAT GTC TAA G-3') and SSU-2 (5'-TTG CAT GCT CCA GTA TAC AAC-3'), followed by

Table 2. Occurrence of pathogenic forms of E. coli based on genotypes and association with gender.

<table>
<thead>
<tr>
<th>E. coli pathotypes</th>
<th>Genes</th>
<th>Total</th>
<th>Stats</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETEC</td>
<td>LT</td>
<td>49</td>
<td>χ²=0.038; p=0.846</td>
</tr>
<tr>
<td></td>
<td>ST</td>
<td>54</td>
<td>p=0.824</td>
</tr>
<tr>
<td>ETECany12</td>
<td></td>
<td>103</td>
<td>χ²=0.942</td>
</tr>
<tr>
<td>EPEC</td>
<td>bfpA</td>
<td>166</td>
<td>P=0.462</td>
</tr>
<tr>
<td></td>
<td>Eae</td>
<td>141</td>
<td>P=0.862</td>
</tr>
<tr>
<td>EPEC any12</td>
<td></td>
<td>177</td>
<td>(6.2%)</td>
</tr>
<tr>
<td>STEC</td>
<td>STX1</td>
<td>18</td>
<td>χ²=2.101; p=0.147</td>
</tr>
<tr>
<td></td>
<td>STX2</td>
<td>21</td>
<td>χ²=0.476; p=0.490</td>
</tr>
<tr>
<td></td>
<td>STEC any 12</td>
<td>38</td>
<td>χ²=1.826; p=0.177</td>
</tr>
<tr>
<td></td>
<td>EIEC</td>
<td>IPAH</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>EAEC</td>
<td>aatA</td>
<td>245</td>
</tr>
<tr>
<td></td>
<td></td>
<td>aaiC</td>
<td>535</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Any of the 2 genes</td>
<td>552 (19.3%)</td>
</tr>
</tbody>
</table>

Figure 1. Distribution of Cryptosporidium species in the study population.

Figure 1. Distribution of Cryptosporidium species in the study population.
Prevalence of diarrheagenic *Escherichia coli* in young children from rural South Africa: The Mal-ED Cohort

N. F. Tanih\(^1\), A. Samie\(^1\), E. Nyathi\(^2\), L. Barrett\(^3\), R. Guerrant\(^3\), P. Bessong\(^1\)

\(^1\)University of Venda, Department of Microbiology, Thohoyandou/ZA, \(^2\)University of Venda, Animal Sciences, Thohoyandou/ZA, \(^3\)University of Virginia, Center of Global Health, Charlottesville/US.

**Summary**

Diarrheagenic *Escherichia coli* strains are major pathogens associated with diarrhea. Currently, five pathotypes of diarrheagenic *E. coli* have been unequivocally associated with diarrheal illnesses. A total of 2663 stool samples obtained from 274 children from birth to 12 months of age from the Dzimwati community in South Africa and were studied for five *Escherichia coli* pathotypes in a multiplex polymerase chain reaction. At least one *E. coli* pathotype was detected in 1249 samples (46.9%). Diarrhea was not common in our study population and most of the pathotypes were obtained from non diarrheal samples. EAEC 780/1249 (62.4%) was the most detected pathotype with 40/740 (5.4%) from diarrheal stools and 740/780 (94.8%) from non-diarrheal stools (P > 0.05) while EIEC 14/1249 (1.1%) were the least detected. However, the implications of the EAEC isolates observed in the diarrheal and non-diarrheal stools are yet to be determine in a mouse model as well as their implication in growth.

**Background**

Over 1.5 million deaths occur each year in children under the age of five in developing countries due to diarrhea diseases. A broad range of recognized micro-organisms such as viruses, parasites and bacteria are associated with diarrhea. Among the bacteria, diarrhoeagenic *Escherichia coli* (DEC) are some of the most frequently detected pathogens worldwide. There are five pathotypes of DEC: enterotoxigenic *E. coli* (ETEC) enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), enteroheamorrhagic *E. coli* (EHEC) or verocytotoxin-producing (STEC) *E. coli* and enteroinvasive *E. coli* (EIEC).

Differences have been noted to exist in the prevalence of diarrheagenic *E. coli* around the world. This study aimed at determining the relative prevalence of five *E. coli* pathotypes in children one year old or less with and without diarrhea in a rural community in Northern South Africa and to determine their potential associations with child growth.

**Results**

Of the 2663 stool samples analyzed, 1249 were positive for *Escherichia coli*.

Gene amplification results revealed the presence of different *E. coli* pathotypes; 1.1% (14/1249) were positive for EIEC, 10.1% (126/1249) for ETEC, 22.1% (276/1249) for EPEC, 4.2% (53/1249) for STEC and 62.4% (780/1249) for EAEC. There was statistical significant difference between diarrhea and non diarrhea stools with the different pathotypes.

Of the one thousand two hundred and forty-nine strains, at least one diarrheagenic *E. coli* was found in 69.9% (874/1249) had at least one of the pathogens, 13.1% (163/1249) had two genes, 1.2% (15/1249) had three genes, 0.1% (1/1249) had four genes. (Standard error of mean amongst the five pathotypes mentioned above was 1.01 ± 0.01)

**Materials and methods**

A total of 2663 stool samples (diarrheal and non-diarrheal) were longitudinally obtained from 274 children from birth to 12 months of age from the Dzimwati community in South Africa. Demographic data, clinical and epidemiological factors were obtained through a questionnaire. The children were

**Fig 2: No. of *E. coli* pathotypes per season.**

EAEC which recorded the highest prevalence amongst the pathotypes also showed significant variation across the seasons with respect to occurrence P =0.016. However, the implications of the EAEC isolates observed in the diarrheal and non-diarrheal stools are yet to be determined in a mouse model as well as their implication in growth.
4. Erin Intrevado Root, M.Arch/MLA
– Architecture & Landscape Architecture, UVa (2011-3)
– Appointed at UVA - November 1, 2013 – October 31, 2014

**Presentations:**
- Association of Collegiate Schools of Architecture national conference in Miami
- “Cumulus: Design with the other 90%,” Innovation design conference, Johannesburg, S. Africa

**Publications:**
2 pending

**Activities:**
- Developed replicable program for community sourced water planning
- As also a CERAMICIST, she optimized the pottery factory for water filter production.
- Launched her design for a new factory in an adjacent province.
Disruptive water use patterns, Innovative adaptations, and Vision for the future

The people living within the rural communities in the Limpopo Region construct and manage (with their hands and human scale tools) much of their own infrastructure. This design proposal utilizes skills that the people of this region already have, enabling them to construct and manage their own water resources. Instead of relying on the municipal government to supply these rural towns with water, this infrastructure becomes the responsibility of the town. This not only removes water as a tool for political control, but also creates and even more symbiotic relationship between citizens and the ecological processes that surround them, therefore making citizens inherently aware of seasonal ecological processes.
Erin Root Research Statement:

In the interest of catalyzing a broader conversation around alternatives to large scale damming, I am reconsidering how water is collected and stored for human and agricultural use in villages in the arid region of Limpopo, South Africa. This design intends to re-scale water infrastructure from large centralized constructions to human scale networks, and to shift this infrastructure from fixed to a more flexible, adaptable system.

The design emphasizes the traditional role of water as a shaper of public space. Women in Limpopo spend one third of their lives collecting water. This time includes not only that spent at the community spigot and the rivers edge, but also the significant time it takes to walk to these sources. Both destination and path are public domains in constant flux. The destination and passage are to be considered in the redesign of this critical infrastructure. This strategy is a kit of parts that is adaptable to the landscape and the needs of the community. As opposed to the top-down construction of mega dams, this design will be implemented and maintained by the community, giving them independent and autonomous control of their water source.
5. Lindelani Mushaphi, PhD

— Nutrition, University of Free State (2012)
— Appointed at Univen January 1, 2014 – December 31, 2014

Planned Publications:
1. “Perceived Barriers to Brest-feeding in Rural Limpopo Province”
   Authors: Tjale C. Mahopo*, Noxolo C. Nesamvuni, Lindelani F. Mushaphi, Brenda Baloyi, Ellen Mashau, and Pascal O. Bessong, Department of Nutrition, Department of Microbiology, UNIVEN
2. “Feeding practices and growth status of infants aged 0 to 12 months in Dzimali village, South Africa: MAL-ED birth cohort study.”
   Authors: Mushaphi LF, Nesamvuni CN, Mahopo TC, Mashau E, Baloyi B and Bessong PO

Activities:
• “Fundamentals of Epidemiology”, a Public Health Sciences UVA Summer Course
• “Clinical Ethics”, a Public Health Sciences UVA Summer Course
• CDC Qualitative Methods Workshop, Atlanta, GA, August 2014
• Intensive one-on-one mentoring in SPSS
• Conducting Field work at UVa on breast-feeding promotion with the Thomas Jefferson Health Department
6. Angelina Maphula
PhD candidate, Psychology, University of Venda (2015)
– Appointed at Univen April 24, 2014 – March 31, 2015

Presentations:
MaLED and UVa meetings

Publications:
2 manuscripts in preparation.

Activities:
• Worked at UVa with Drs. Rebecca Scharf and Relana Pinkerton on SPSS and analyses of impacts of surprisingly common maternal depression on children’s pathogen burden, enteropathy, growth and development.
• Conducted field work at UVa on promotion of infant cognitive development by mothers in the homes.
Hypotheses being tested in MAL-ED

**Pathogen ingestion**

**Gut Function**
- L/M; A1AT
- MPO; LF
- Neopterin (“Microbial Enteropathy”)
- + Immunity

**Nutrition**
- Micronutrients;
- Breast feeding;
- Repair nutrients,
- Probiotics, etc.

**Impaired Growth**
- ΔHAZ; WAZ

**Impaired Cognition**

**Maternal Factors**
- Depression, cognition, caregiving,
  education, sociodemographic
Lufuno Mavhandu

PhD candidate, AIDS Research, University of Venda (2015)
  – Newly appointed Univen-based Program Coordinator

Background and Activities
  • Has worked closely with UVa faculty since 2008

  • Trained in HIV virology / AIDS research at UVA and UNIVEN

June 2014 - Trained in research administration
  with Liz Olmsted at UVA
  • Grant administration
  • Fellow mentorship
  • Federal policies and meeting the global standard
D43 Fogarty Fellows:
Angie Maphula
and Nicoline Fri Tanih
with Becca Dillingham,
Dick Guerrant and
Ruth Gaare-Bernheim

Lufuno Mavhandu and
Lindelani Mushaphi
with Liz Olmsted and
April Ballard