Section I. Abstract

**Title:** Impact of Chemical Water Quality on the Gut Microbiome of Infants in Piura, Peru

**Background:** A person can contract diarrhea by drinking unsafe water contaminated with disease-causing organisms, but how drinking chemically contaminated water impacts individual diarrheal risk and the gut microbiome has yet to be elucidated.

**Objective:** Geographically adjacent urban and rural households in Piura, Peru have mixed access to the municipal water distribution system due to infrastructure limitations of the city. This unique situation will enable us to determine the extent to which household drinking water quality influences the gut microbiome composition.

**Methods:** Household drinking water and infant stool samples will be collected from 100 households (25 from each sampling group). Household surveys will be used as a surveillance system for household diarrheal incidence. I will analyze water samples for the presence of various chemicals (pesticides, heavy metals and antibiotics). Stool samples will be assessed for their microbial community composition.

**Implications:** The results of this study will not only make a significant contribution to the scientific literature but will also help provide policy makers in Piura, Peru with the targeted information they need to demand further investments in the province’s water infrastructure.
Section 2. Detailed Proposal

A. Background

Often caused by organisms disseminated in unsafe drinking water (Kaper et al 2004), diarrhea is responsible for an estimated 1.4-1.9 million deaths worldwide (Lozano et al 2012, WHO 2013). Repeated diarrhea episodes in the first two years of life are associated with impairment of the gut (Guerrant et al 2013), and reductions in the diversity of the gut microbiome have been observed in children in regions with high diarrhea burden (Pop et al 2014, Lindsay et al 2015). Antibiotics, heavy metals and pesticides can reduce the microbial diversity of the gut microbiome (Thompson et al 2015, Dethlefsen et al 2008, Lu et al 2014) and increase the risk of diarrheal disease (Rahman et al 2011). This study seeks to examine the role of the chemical quality of drinking water in determining the gut microbiome of children, and the impact of this on their risk of developing diarrheal disease.

B. Project Objectives

In November and December of 2015, approximately 300 newborn infants in Piura, Peru were recruited and enrolled in a cohort study established by Dr. Robert Gilman at Johns Hopkins University. With a population of approximately 642,000 people living in both urban Piura and the outlying rural area, there are geographically adjacent communities in both the urban and rural areas that differ in their municipal water connection availability while maintaining similar socioeconomic status; those without a municipal water connection drink untreated water from heavily polluted canals. This situation that arose from infrastructure limitations uniquely lends itself to answer our overarching question concerning the effects of water quality on the gut microbiome,
“Does household drinking water quality influence gut microbiome composition in a cohort of infants in Piura, Peru?” Responses from the initial enrollment questionnaire will be utilized to ensure that a total of twenty-five households from each sampling site (rural or urban; connected or not connected to the municipal water supply) are selected for inclusion in the follow-up this summer of 2016. Follow-up will include additional household surveys on household diarrhea incidence, household drinking water collection, and infant stool collection.

This practicum opportunity will also provide the data necessary to answer my intended thesis question, “Does exposure to chemicals such as pesticides, heavy metals or antibiotics in drinking water impact the microbial community composition of the infant gut?” I am also interested in determining if the presence of these chemicals in the water affects enteropathogen prevalence in the water. This focus will complement that of another MPH student who I will alongside in Peru, Forest Altherr, who will focus on exposure to microbial contaminants in drinking water.

The results of the overall question and my own thesis question combined will make it possible to not only link overall water quality to gut microbial composition, but specifically chemical water quality. These findings have the potential to make a significant contribution to the scientific literature, as well as provide policy makers globally, and specifically in Piura, Peru, with the targeted information they need to demand further investments in the province’s water infrastructure.

C. Methods and Deliverables

Before departure for the field, my role for the project will involve the development of the water quality analysis protocols; this will include adjusting the chemical water
quality analysis protocols currently utilized in Dr. Barr’s laboratory and adapting the ultrafiltration protocol developed by Dr. Vince Hill at the Centers for Disease Control and Prevention for this developing country context. I will work alongside the other MPH student throughout this process; we will also work together to design a household survey intended to act as an informal surveillance system for diarrhea illness.

Once in Peru, we will team up with two Peruvian students supported by the NIH-Fogarty GEOHealth Hub environmental health training program led by Drs. Kyle Steenland and Karen Levy at Emory. Together with these students, I will conduct household visits to carry out surveys and collect household drinking water samples and fecal samples from the children enrolled in the study. We will spend the first three weeks in Peru piloting these materials before finalizing them for use in the remainder of the summer.

During the first household visit we will collect the drinking water samples and administer a household survey; the second visit will occur three days later and will involve the collection of infant stool samples to allow us to establish a temporal relationship between water consumption and the gut microbiome. I will collect several 100mL samples to bring back to the lab for the chemical water quality analysis.

Data Collection

My unique contribution to this summer’s project involves the chemical processing of the water samples. The other MPH student will focus on the microbial analysis of the water samples. Together with the Peruvian students, we will carry out extractions from water and stool samples in Dr. Gilman’s lab in Lima, and ship the extracts to the U.S. to: Dr. Dana Barr and Dr. Barry Ryan’s lab at Emory; Dr. Baker at the University of Iowa, so
the samples may be analyzed for a suite of enteropathogens using the TaqMan (PCR) array card she adapted for environmental samples; and Dr. Konstantinidis at Georgia Tech for microbial community characterization using the V4 region of the 16S gene.

I will work this spring to finalize protocols, but briefly the procedures for the chemical water quality analysis will involve passing the 100mL water samples through solid phase extraction columns in order to pull out pesticides, heavy metals and antibiotics; these columns will then be shipped to the HERCULES lab at Emory for further analysis via GC-MS/MS (pesticides), ICP-MS (heavy metals) and LC-MS/MS (antibiotics). Prior to my arrival in Piura, Peru, I will be working in the HERCULES lab so I will become familiar with the protocols and adapt them to this study's use. In addition to the initial household survey, we will also carry out surveillance for diarrheal disease in the infants every 2-3 days for two weeks following water collection using the open source Android-based Open Data Kit (ODK) electronic data collection system.

A.B. PRISMA’s IRB has already approved the study protocols, excluding the water sampling collection; the protocol amendment including the collection of water samples will be resubmitted to A.B. PRISMA before the end of March, and to Emory’s IRB if necessary, during the same timeframe.

D. Learning Objectives and Career Goals Statement

Prior to enrolling at Rollins, I applied directly to the PhD program in Environmental Health at Emory with Dr. Karen Levy as my intended advisor. My research focus was and remains focused on waterborne disease and water quality, with a special interest in the impact of chemicals on waterborne pathogens. This summer’s practicum and thesis opportunity in Peru aligns perfectly with my research interests and will help me
develop the relevant chemical water quality analysis skills I have been working towards as part of my public health experience. It will also help to set me up to potentially continue into the PhD program at Emory.

Additionally, while I have significant laboratory experience that will be a strong asset to this study, I have not yet had the opportunity to work in the field or conduct research in a developing country. This summer's research project will provide ample opportunities to develop these skills in this unique setting while improving my Spanish skills.

### E. Timeline

<table>
<thead>
<tr>
<th>Method Development (Atlanta)</th>
<th>Pre-Departure</th>
<th>In-Country</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>April 13 to 17</td>
<td>May 18</td>
<td>June 19 to 21</td>
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<tr>
<td>Chemical Water Quality</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Ultrafiltration</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Household Survey</td>
<td>X</td>
<td>X</td>
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<table>
<thead>
<tr>
<th>Sample Collection (Piura, Peru)</th>
<th>Pre-Departure</th>
<th>In-Country</th>
<th>Post</th>
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<tbody>
<tr>
<td>Piloting of Survey materials</td>
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</tr>
<tr>
<td>Drinking Water Collection</td>
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<td>X</td>
<td></td>
</tr>
<tr>
<td>Stool Sample Collection</td>
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<tr>
<td>Survey Implementation</td>
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<tr>
<td>DNA extractions</td>
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<td>X</td>
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<td>SPE columns</td>
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<table>
<thead>
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<th>In-Country</th>
<th>Post</th>
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<td>X</td>
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</tr>
<tr>
<td>Water Quality Data</td>
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<td>X</td>
<td></td>
</tr>
<tr>
<td>Begin write-up (thesis)</td>
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### F. Budget

#### GFE Practicum Budget

<table>
<thead>
<tr>
<th>Item</th>
<th>Cost ($USD)</th>
<th>Units</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roundtrip Flight</td>
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<td>1</td>
<td>$1,000</td>
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<tr>
<td>Immunizations (tetanus, rabies, flu)</td>
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<td>1</td>
<td>$1,112</td>
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<tr>
<td>Antimalaria medications (for 2 weeks)</td>
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<td>1</td>
<td>$85</td>
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<tr>
<td>In-country travel (bus; per month)</td>
<td>$50</td>
<td>4</td>
<td>$200</td>
</tr>
<tr>
<td>Room and Board (per month)</td>
<td>$250</td>
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<td>$1,000</td>
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</table>

**Total Requested from GFE** $3,397

#### Additional Budget Items (from Personal Funds)

<table>
<thead>
<tr>
<th>Item</th>
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<tbody>
<tr>
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<td>15</td>
<td>$1,575.00</td>
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<tr>
<td>Entertainment (per month)</td>
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<td>$800</td>
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<td>Transportation (per month)</td>
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<td>Utilities (per month)</td>
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<tr>
<td>Spanish classes (3 weeks total)</td>
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<td>$200</td>
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**Expected Total Cost to Personal Funds** $2,975.00
Section 3. Letter of Invitation

Dear Committee Members,

I am pleased to welcome the Emory University students, Student A (A) and her associate, to conduct field research for our project based in Piura, Peru. These students will provide valuable data on how water quality influences the bacteria that reside in the gut of newborn children. We expect that the students will provide high quality water collection, sampling, and laboratory analysis. I will serve as the primary contact for these students throughout their field experience as the project’s supervisor for the Asociación Benéfica Prisma.

My writing of this letter shows my complete and thorough support to host these students at our field site in Piura between May and August of 2016. Student A (A) and Forest will reside at our student house and will have access to laboratory space to conduct their experiments. They will be responsible for the costs associated with room, board, and in-country transportation. Peruvian students from the GeoHealth Hub will provide additional support to aid Student A (A) and associate in their objectives to ensure the success of their study.

We look forward to welcoming the students from your university, as they will make significant contributions to our study.

Sincerely,

Lilia Cabrera Rojo
Project Supervisor
A.B. PRISMA
01 2920999
Section 4. Current curriculum vitae
Section 5. Appendices

Appendix A: First draft of Household Survey questions

House ID.__________________________  Interviewer__________________________
Date ____________________________  Household Informant__________________________
Community: 0 Rural 0 Urban  Water Connection: 0 Connected 0 None

---

Household Diarrhea Survey

HOUSEHOLD CHARACTERISTICS:

1. How long have you lived in this house?
   - ☐ >5 years
   - ☐ 2-5 years
   - ☐ 1-2 years
   - ☐ <1 year

2. Do you have a cell phone?
   - ☐ No
   - ☐ Yes, the number is: ____________________________

3. Do you have running water in your house?
   - ☐ No
   - ☐ Yes

4. Do you share your water access point with any neighbors?
   - ☐ No
   - ☐ Yes

5. How long have you had running water in your house?
   Date (month/year) ____________________________

6. In the last two months, how many times has the water service been cut? ________

---

HOUSEHOLD DRINKING WATER SOURCE AND STORAGE:

7. What is the principal source of water in your house?
   - ☐ River, irrigation ditch, ravine
   - ☐ Rainwater
   - ☐ Public water station
☐ Water truck  
☐ Neighbor’s water  
☐ Gift  
☐ Well (tubular) (protected)  
☐ Household connection (24 hour access)  
☐ Household connection (<24 hour access)  
☐ Connection outside of the house  
☐ Other

8. How many water access points are there in the house? ____________________

9. What family member is responsible for the collection of the household water?  
☐ Adult female  
☐ Adult male  
☐ Female under 18 years old  
☐ Male under 18 years old  
☐ Other  
☐ Don’t know/no response

10. How long does it take them to collect the household water and bring it back to the house?  
__________________ horas

11. Usually, what do you do to clean the water used for drinking or eating?  
☐ Boil  
☐ Add chlorine  
☐ Leave it/let it settle  
☐ Use water filter  
☐ Other  
☐ Don’t know

12. When do you treat the water?  
☐ Immediately before storage  
☐ Before storage and before drinking  
☐ Only before drinking  
☐ Once before storage  
☐ Don’t treat

WATER STORAGE:

13. How many water storage containers do you use to store water? ________________

14. Are these the same containers the water was collected in?  
☐ Yes  
☐ No  
☐ Not relevant
15. Did you wash the water storage container before filling it?
   □ No
   □ Yes, with water
   □ Yes, with soap
   □ Yes, with chlorine and soap
   □ Other (please specify)

16. How do you remove the water from the container?
   □ Pouring
   □ Spigot on bottom
   □ Drink directly from container
   □ Dip
   □ Hose
   □ Not relevant
   □ Other (please specify)

17. What has this water been used for since collection?
   □ Washing dishes
   □ Drinking
   □ Cooking
   □ Cleaning (hygiene)
   □ Animals (pets, farm animals)
   □ Washing clothing
   □ Nothing
   □ Other (please specify)

---

**HOUSEHOLD HYGIENE:**

18. Do you use treated or boiled water for food preparation?
   □ Never
   □ Rarely
   □ Sometimes
   □ Always

19. At what times do you wash your hands?
   □ Before preparing food
   □ Before feeding the baby
   □ After helping your child defecate
   □ After going to the bathroom
   □ Other

20. Do you use soap when washing your hands?
   □ No
   □ Yes
21. How do you deal with your excrement?
- Open defecation
- River
- Other “open”
- Storage pit
- Well
- Shared latrine
- Personal latrine
- Household drain
- Drain outside of the house

22. Do you have any animals at the house?
- No
- Yes. What kind(s)?

23. Are any of these animals allowed in the house?
- No
- Yes

24. In the last week, did any members of the household have diarrhea?
- No
- Yes. #__________ Ages ____________ Sex _______

25. Did you breastfeed your child?
- Yes
- No

26. When did you stop breastfeeding your child? Date (mth/year) ____________
- Still breastfeeding
- Not relevant

CHEMICAL EXPOSURES:

27. Do you wash your fruits and vegetables before eating them or using them for cooking?
- Never
28. Where do you typically buy the food for the household?
☐ Grocery store
☐ Soup kitchen
☐ Restaurant
☐ Street Market
☐ Other
☐ Unsure

29. How often do you eat rice in this house?
☐ 1-2 meals per week
☐ 3-4 meals per week
☐ 5-6 meals per week
☐ Every day
☐ Twice a day
☐ Never. I don’t eat rice

30. Has anyone in the household taken any antibiotics or other medications in the past three months?
☐ No
☐ Yes. The medications are: ______________________________

31. Is anyone in the household exposed to chemicals such as pesticides as part of their occupation?
☐ No
☐ Yes. If known, they are: ______________________________

32. Do you use any chemicals for any reason within the home?
☐ No
☐ Yes. They are: ______________________________

33. Do you feed any chemicals to the animals at the house?
☐ No
☐ Not relevant
☐ Yes. They are: ______________________________

Fed to the animals: ______________________________
Appendix B: First Draft of Observational Survey

House ID: ___________________________  Interviewer ___________________________
Date: ___________________________  Household Informant ___________________________
Community:  ☐ Rural  ☐ Urban  Water Connection:  ☐ Connected  ☐ None

---

**Household Water Quality Observations**

**WATER MEASUREMENTS:**

<table>
<thead>
<tr>
<th>Turbidity (NTU)</th>
<th>pH</th>
<th>Temperature (°C)</th>
</tr>
</thead>
</table>
| _______________ | _______________ | _______________

**General Observations of Water in Containers:**

- Visible contamination, cloudiness, discoloration, odor...

---

**HOUSEHOLD STRUCTURE:**

1. How many family members are in the home? ______

2. How many of the newborn’s brothers and sisters live in the house? ______

3. Is there a refrigerator in the house?
   - ☐ No
   - ☐ Yes

4. How many rooms are in the house? ______

5. How many windows are in the house? ______

6. Is there a bathroom in the house?
   - ☐ No
   - ☐ Yes. How many? ______

6a. If yes, what are the conditions of the latrine? (Select all that apply)
- ☐ Pit toilet (No cover)
- ☐ Tapped into a water connection
- ☐ Other (Explain):
7. Does the house appear to have good ventilation?
   - No, not adequate
   - There are open air windows
   - There are windows covered by glass or wood

8. How many taps are in the household? _____

9. Are there flies in the kitchen?
   - Yes
   - No

10. Are leftover foods protected from flies?
    - Yes
    - No

11. What is the cleanliness of the cooking surfaces?

    |                |            |            |            |            |
    |----------------|------------|------------|------------|------------|
    | Very Dirty     | Moderately | Neutral    | Moderately | Very Clean |
    | Dirty          | Clean      |            | Clean      |            |

12. What are the conditions of the dishes?

    |                |            |            |
    |----------------|------------|------------|
    | All washed     | Mostly clean | Mostly dirty |
    |                |             | All dirty   |

13. What hand washing resources are present? (Select all that apply)
    - Dedicated area
    - Soap
    - Chemical disinfectants (Hand sanitizer)
    - Water
    - Other (Explain):

14. Are there animals present inside the house?
    - No
    - Yes

14. Are there animals around the house?
    - No
    - Yes
14a. What animals are present?

<table>
<thead>
<tr>
<th>Animals</th>
<th>Count</th>
<th>Corralled</th>
<th>Free Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chickens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dogs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monkeys</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turkeys</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ducks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pigeons</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Pigs</td>
<td></td>
<td></td>
<td></td>
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<td>Goats</td>
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<tr>
<td>Rabbits</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other:</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

15. Are there any chemicals present in or near the house?

Name: ____________________________________________

Product Number: __________________________________

Household use for chemical: _____________________________

Hazard level: ________________________________________

16. Ask to see the household rice. Where does the rice come from?

Region/Country: ___________________________________

Type of rice (brown vs. white): _______________________

---

WATER STORAGE:

17. Is there a water storage container in the house? How many?
   - Yes
   - No If yes, how many? ________________

For each water storage container answer the following:

What type of storage container is it:
   - Plastic jug
   - Bucket
   - Gallon jug
   - Tank
   - Drum
Student A (A)_EH-EPI_PERU

- Cylinder
- Pot
- Garbage can
- Water cooler
- Water bottle
- Other (please specify)

Is the water storage container covered?
- Yes
- No

Is the water storage container stored on the ground?
- Yes
- No

How well is the water covered?
- Well Sealed
- Poorly Sealed
- No Cover

Is the water in the storage container protected from flies?
- Yes
- No

How is water removed from the container?
- Pouring
- Spigot on bottom
- Drink directly from container
- Dip
- Hose
- Not relevant
- Other (please specify)

18. Please describe any other relevant findings or potential sources of contamination:
Appendix C: Map of Research Site in Peru

Appendix D: Works Cited


Pop et al. Diarrhea in young children from low-income countries leads to large-scale alterations in intestinal microbiota composition. *Genome Biol* 2014; **15**: R76.

