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.

<table>
<thead>
<tr>
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<th>Pre-Departure</th>
<th>In-Country</th>
<th>Post</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>April</td>
<td>May</td>
<td>June</td>
</tr>
<tr>
<td>Chemical Water Quality</td>
<td>X</td>
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</tr>
<tr>
<td>Ultrafiltration</td>
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<tr>
<td>Household Survey</td>
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<tr>
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<tr>
<td>Drinking Water Collection</td>
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<tr>
<td>Stool Sample Collection</td>
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<td>Survey Implementation</td>
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<tr>
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<tr>
<td>Water Quality Data</td>
<td></td>
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<tr>
<td>Begin write-up (thesis)</td>
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<tbody>
<tr>
<td>Item</td>
<td>Cost ($USD)</td>
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<td>Total</td>
</tr>
<tr>
<td>Food (per week)</td>
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<td>15</td>
<td>$1,575.00</td>
</tr>
<tr>
<td>Entertainment (per month)</td>
<td>$200</td>
<td>4</td>
<td>$800</td>
</tr>
<tr>
<td>Transportation (per month)</td>
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<th>Expected Total Cost to Personal Funds</th>
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Total Requested from GFE: $3,397

Additional Budget Items (from Personal Funds)

<table>
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<th>Item</th>
<th>Cost ($USD)</th>
<th>Units</th>
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<tr>
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<tr>
<td>Spanish classes (3 weeks total)</td>
<td>$200</td>
<td>1</td>
<td>$200</td>
</tr>
</tbody>
</table>

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 Benéficia 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

Cell: (302) 290-6560

EDUCATION:

MSPH in Environmental Health & Epidemiology, May 2017 – Emory University (Atlanta, GA)
Honors B.A. in Biological Sciences - University of Delaware (Newark DE) GPA: 3.71
Graduated May 2015. Studied abroad in Fall 2013 at the Danish Institute for Study Abroad.

WORK EXPERIENCE/INTERNSHIPS:

Center for Global Safe WASH - Graduate RA & Writer (Atlanta, GA) 9/15-present
Conducted literature review on water, sanitation and hygiene (WASH) in emergencies.
Helped design potential curricula on WASH in emergencies to supplement current Emory offerings

Centers for Disease Control and Prevention - ORISE Fellow (Atlanta, GA) 6/15-8/15
Worked in the smokeless team of the Tobacco and Volatiles Branch in the National Center for Environmental Health within the Division of Laboratory Sciences at the CDC
✓ Performed review of current literature regarding the monoamine oxidases isozymes and their known inhibitors using structural dimensions and other properties derived from chemical structures. Developed selection criteria and identified possible inhibitors within tobacco products for further testing
✓ Aided in microbial analysis of smokeless tobacco products for FDA funded project
✓ Developed a library of 30 bacterial families identified in smokeless tobacco products describing pertinent information such as gram-staining properties, nitrogen metabolism, and pathogenicity
✓ Performed library prep for next generation sequencing of DNA isolated from tobacco products

UD Food Science Department - Undergraduate Researcher (Newark, DE) 5/14-5/15
✓ Modeled point-source manure contamination of the water supplying a field of crops to determine the persistence of Escherichia coli populations on the leaves of lettuce and spinach plants
✓ Examined bacterial reduction ability of sand and ZVI filters on E. coli population levels in tomato plants
✓ Extracted DNA from and performed multiplex PCR analysis of all collected E. coli samples for their EHEC and ExPEC grouping, phylogenetic
✓ Maintained study field site by weeding, watering, planting and labeling plots

World Health Organization/Europe – Intern (Copenhagen, Denmark) 1/14-4/14
Main tasks involved the Central Asian and Eastern European Surveillance on Antimicrobial Resistance (CAESAR) Network
✓ Performed pre-assessment country profiles in preparation for country missions to Albania, Georgia, Moldova, Kazakhstan, Tajikistan, Turkmenistan and Ukraine
✓ Analyzed national AMR data submitted to CAESAR using SAS and Excel and wrote individual and comparative country feedback reports
✓ Wrote summary report on the 2013 External Quality Assessment for participating CAESAR countries
✓ Developed informational materials such as posters, brochures and postcards to promote the CAESAR network, as well as new content for the WHO/EURO AMR website
Reviewed literature on antibiotic policies, antibiotic stewardship, and alternatives to antibiotics

**Copenhagen University – Undergraduate Researcher (Copenhagen, Denmark)  9/13-12/13**
Analyzed data and published as first author a journal article in *Acta Radiologica* concerning breast cancer and mammographic density in women in Copenhagen's mammography screening program

**Summer Institute for Training in Biostatistics – Emory University (Atlanta, GA)  6/13 - 7/13**
Learned basics of SAS, R and JMP, along with statistical and epidemiological methods and techniques. Explored career and educational opportunities, faculty discussions, and shadowed public health professionals to formulate professional goals

**A.I. DuPont Children's Hospital – Clinical Research Assistant  Fall 2012**
Enlisted enrollment from ER families in research studies conducted at the hospital. Collected informed consent and other patient information and assisted in obtaining nasal swabs, nasal aspirate samples, and blood samples from patients

**University of Delaware – General Chemistry Workshop Leader  2012-2013**

**SKILLS AND TRAINING:**

**Computer/Technical Skills**
Proficient in Microsoft Word, PowerPoint, Excel, Adobe InDesign, Photoshop, SAS Statistical Software Program

**Laboratory Skills**
RNA and DNA extraction, autoclave operation, DNA quantification using Qubit and NanoDrop, pH meters, shotgun metagenomic library preparation for next generation sequencing on Illumina MiSeq, gel electrophoresis on Agilent 2100 Bioanalyzer, Polymerase Chain Reaction (PCR) and PCR clean up using AMPure, IDEXX, membrane filtration

**Languages -** French – Fluent; Danish – Conversational; Spanish – Beginner

**HONORS, AWARDS AND ACHIEVEMENTS:**
Emory University: RSPH Merit Scholar Recipient
University of Delaware: Dean's List; Cum Laude; Outstanding Academic Achievement Award in French

**ACTIVITIES:**

**Scholars in Action- Emory University  2015-present**
Helped facilitate a book discussion intended to connect scholars across disciplines with faculty and senior administrations

**ROTC – Air Force – University of Delaware  2011-2012**

**Haiti Family Initiative (Haiti) – Medical Clinic Volunteer  Summer 2012**
Aided in the diagnosis of patients, explained the prescribed medical treatments to patients and why they were necessary, and worked to teach patients the basics of sanitation and hygiene

**PUBLICATIONS:**

Section 5. Appendices

Appendix A: First draft of Household Survey questions

House ID_________________________________________ Interviewer______________________________
Date______________________________________________ Household Informant _______________________
Community: Rural Urban Water Connection: Connected 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
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:
Turbidity (NTU) ________________
pH ___________________________
Temperature (°C) ________________

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 Dirty  Neutral  Moderately Clean  Very 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?

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<thead>
<tr>
<th></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>
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<tr>
<td>Monkeys</td>
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<tr>
<td>Cats</td>
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<td></td>
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<tr>
<td>Other:</td>
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</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
○ 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?

<table>
<thead>
<tr>
<th>Well Sealed</th>
<th>Poorly Sealed</th>
<th>No Cover</th>
</tr>
</thead>
</table>

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.

