GRAHAM SUSTAINABILITY INSTITUTE
UNIVERSITY OF MICHIGAN

Gastric cancer and *Helicobacter pylori* infection in Lima, Peru: the role of water contamination

Final Project Report

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Background

Helicobacter pylori (HP) is a bacterium that, while asymptomatic in most people, is a Class 1 carcinogen because of its direct causal relationship to gastric cancer (Correa et al., 1990). After lung and liver cancer, gastric cancer is the most lethal cancer in the world, with an estimated 951,000 new diagnoses and 723,000 deaths from gastric cancer each year, mainly in Eastern Europe, Asia and Latin America. It has been suggested that 90% of gastric cancer cases are attributable to HP (Plummer, Franceschi, Vignat, Forman, & de Martel, 2014). In Peru, gastric cancer is the most common cancer and cause of cancer mortality men and women combined (Pilco, Payet, & Cáceres, 2006).

HP colonizes around 80% of people living in low socioeconomic areas of Latin America. By contrast, less than 20% of asymptomatic Caucasians carry *HP* in the US (Sari et al., 2008). HP infection is treatable with antibiotics, and is effective in approximately 80% of cases. However, treatment success varies according to the virulence and antibiotic resistance of the organism. Recurrence of HP infection after antibiotic therapy is more common in developing countries than in developed countries (13% vs. 2.67%, respectively), and is highly variable in Latin America [(Gisbert, 2005; Niv & Hazazi, 2008; Ramirez-Ramos et al., 1997). High rates of HP recurrence were reported from Peru: 73% at 8 months (Ramirez-Ramos et al., 1997) and 30% at 18 months (Soto et al., 2003)s; the latter reported after the 1991 cholera epidemic that led to additional chlorination of the drinking water. Reports from other Latin American countries reflect annual recurrence rates as high as 54% in Chile, 50% in Brazil and 37% in Mexico (Gisbert, 2005). Drinking water has long been suspected to be a source of infection in Lima: in 1991, 50% of 48 water samples from the municipal Atarjea (source of drinking water in Lima) tested positive for HP (Klein et al., 1991; Ramos & Sánchez, 2009). Similar observations have been made in Mexico, though at a lower rate of 10 to 20% (Mazari-Hiriart et al., 2005). The distribution of gastric carcinoma in Metropolitan Lima follows the rate of HP infection, being highest in lower socioeconomic areas such as Puente Piedra, Lince, Villa El Salvador, El Agustino, Breña, Rimac (21-25 cases per 100,000 people), and lowest in high socioeconomic areas such as San Isidro and Miraflores (9-13 cases per 100,000 people), where the source of drinking water is often derived from wells; see the attached map (Pilco et al., 2006).

Finally, multiple studies have shown strong epidemiological associations between HP infection and lack of access to improved drinking water and sanitation (Hulten et al., 1996; Klein et al., 1991; Rolle-Kampczyk et al., 2004). While no studies have provided direct evidence that waterborne transmission occurs in humans, multiple studies have detected and isolated HP in water with culture and non-culture techniques (Bahrami, Rahimi, & Ghasemian Safaei, 2013; Lu, Redlinger, Avitia, Galindo, & Goodman, 2002; Moreno & Ferrús, 2012a).

These previous observations led us to hypothesize that **surface and drinking water are major reservoirs for HP in the environment and one of the sources of HP infection.** Our project was initially supported by a grant from the Hope Foundation. In 2012, we were awarded a grant from Graham Sustainability Institute to examine links between contaminated water and HP infection in Lima, Peru. The objectives of this research were **to establish strong scientific evidence to support the direct linkage between drinking water contamination by HP and human gastric infection and to evaluate the efficacy of interventions focusing on clean and**

safe drinking water for combating HP infections in developing countries. These studies were conducted in parallel to those utilizing water samples from areas of higher socioeconomic strata who often do not share the same water source with poor areas in order to compare the differing water qualities and gain further knowledge about the health iniquities.

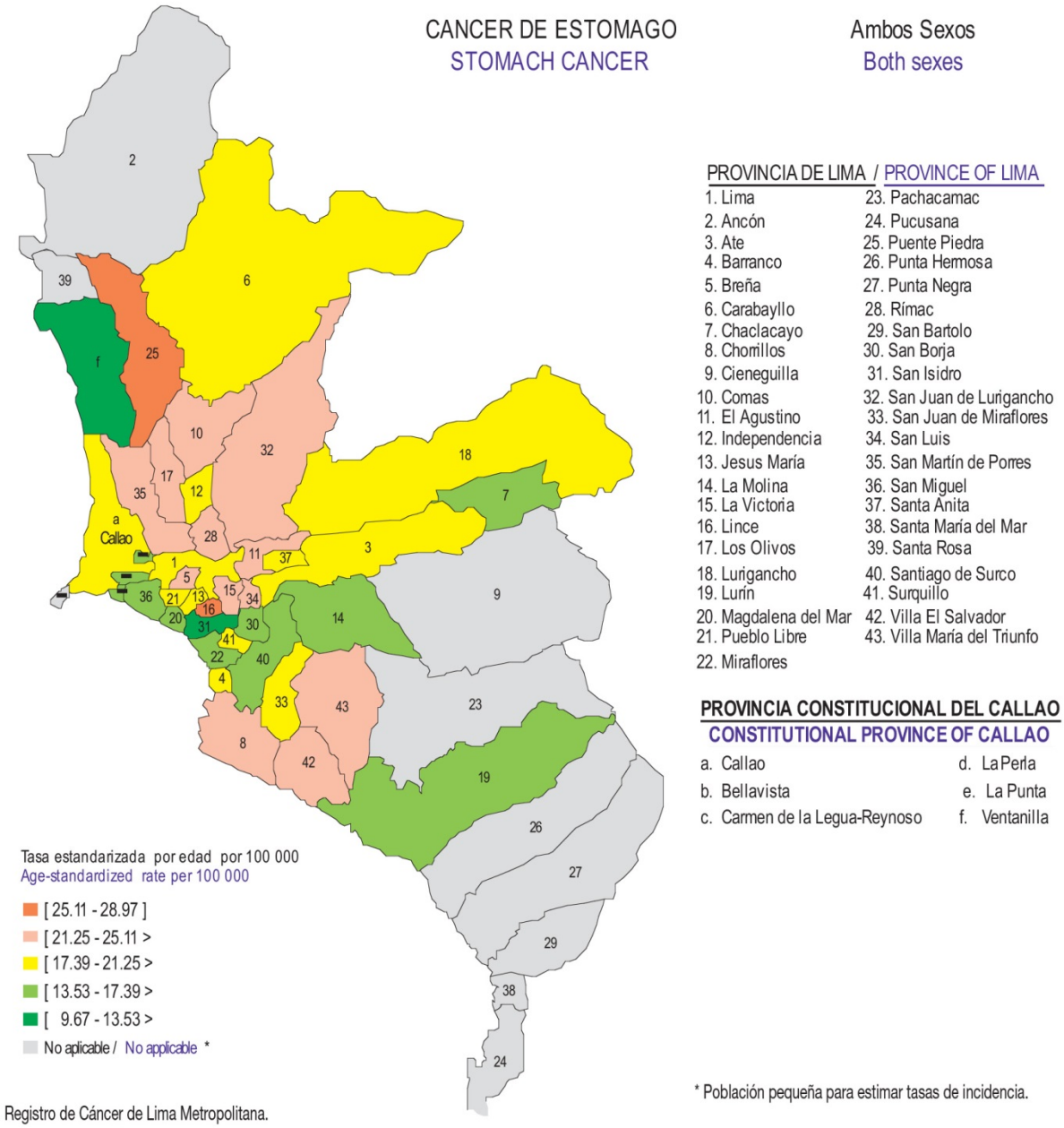

Figure 1. Gastric cancer incidence per district in Lima, Peru. From Pilco et al, 2006.

Our project aimed to help better understand the relationships between water, HP, and human health, asking the following questions:

1) How much HP are people in Lima exposed to via drinking water?

2) Can HP be found in biofilms in drinking water pipes in Lima? (Note: Biofilms are groups of microbes, including bacteria, which aggregate together to stick to a surface. They typically exist anywhere where there is moisture.)
3) Is HP in drinking water infectious?
4) Should we be concerned about recurrence of HP infection and antibiotic resistance among HP strains in Lima? (Note: Strain refers to a sub-type or specific genetic variation of a bacterium.)
5) Are current water treatment options suitable to eradicate/remove HP in water, and what are the most cost effective options for use in Lima?

Since then, we have worked on this project in Lima, Peru under the paradigm of an integrated assessment (IA). IAs are interdisciplinary projects that seek to bridge the gap between research and policy (Lund, Dinse, Callewaert, & Scavia, 2011). We followed the IA process provided by the Graham Institute (Fig 2.), collaborating with clinicians, researchers, and public health officials from the University of Michigan, the Peruvian Department of Environmental Health, and the Universidad Peruana Cayetano Heredia, to address waterborne HP in Lima through research and outreach to policy makers.

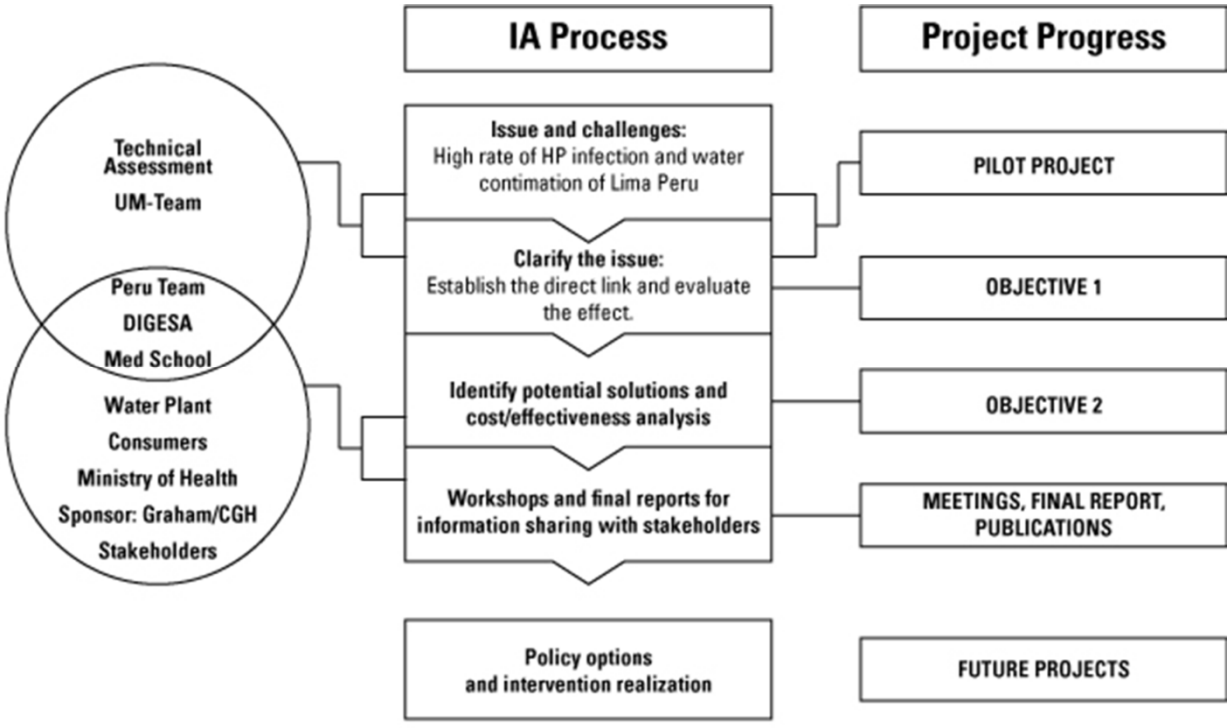

Figure 2. Paradigm for Integrated Assessment study in Lima, Peru.

Studies for each objective performed to carry out IA objectives

Under the IA paradigm, we collaborated with our colleagues in Lima, Peru and the University of Michigan on multiple studies, checking in throughout the process to communicate results, come up with future directions, and plan ways to best disseminate our findings. This process was an

iterative, dynamic process, which was necessary due to the shifting political landscape in Peru (which could sometimes prevent us from achieving our objectives). However, this process also allowed for new collaborations and fruitful idea exchange, giving us new paths to pursue that made our results more translational. Throughout the course of this project, we performed the following studies:

Study 1: *Cohort of patients in Lima, Peru*
a) We completed a clinical study in Lima, Peru in which we recruited patients who were positive for HP gastric infection and provided them with treatment for HP infection. We collected stomach biopsy samples from these patients, and monitored them 6-8 weeks and 1 year after treatment to gauge the effectiveness of the antibiotic therapy. *Key Collaborators: Italo Novoa, Claudia Meza, Alejandro Bussalleu*

b) In the same cohort of patients from study 1, we collected bulk water samples and biofilm samples from their households to see if their water or faucets were contaminated with HP. We tested for HP contamination using molecular biology based methods that tested for the presence of HP DNA, as well as a culture based method (Degnan, Sonzogni, & Standridge, 2003; Nayak & Rose, 2007). We then examined whether there were any links between water/biofilm contamination and likelihood of antibiotic treatment failure/recurrence of HP infection in the cohort from study 1. *Key Collaborators: Claudia Meza, Soledad Osorio, DIGESA staff*

c) We collected different strains of HP from the stomach biopsy samples from patients in study 1, and examined whether any of these strains were resistant to antibiotics that are typically used to treat HP infection. *Key Collaborators: Kathryn Thompson*

Study 2: *Waterborne exposure in mice*
One gap in knowledge that was identified by both our colleagues in Lima and the literature was the question of whether HP in water was actually infectious. Since it is challenging and unethical to conduct dosing experiments with HP in humans, we decided to use mice as an animal model for our study. Mice have been commonly used for experiments involving HP, and are known to be susceptible to HP infection. For four weeks, mice drank water contaminated with known quantities of HP, with separate groups of mice given different amounts of HP and a control group, which was given uncontaminated water. After four weeks of exposure, we checked the mice for infection. We recently published the results of this experiment in *Helicobacter* (Boehnke, Eaton, Valdivieso, Baker, & Xi, 2015), and shared our results with our collaborators in Lima. *Key Collaborators: Kathryn Eaton*

Study 3: *Testing water treatment systems*
While many microbes in water are known to be killed or inactivated by conventional water treatment systems, little is known about how these water treatment systems are at handling HP. Further, many of the more advanced water treatment technologies would not be suitable for use in Lima. This is partially because these systems are meant to be used solely on water without much microbial contamination, but also

because of the expense of many of these systems, unfortunately rendering them an unusable treatment option. Thus, we examined whether two inexpensive and effective water treatment technologies (bleach exposure and boiling water) were suitable for killing HP in water. *Key Collaborators: Ariel Saulles*

Results

Study 1: *Cohort of patients in Lima, Peru*
a) In our cohort study, antibiotic therapy was successful at rates similar to those found in the literature for other countries in Latin America. HP DNA was found via quantitative polymerase chain reaction (qPCR – a technique that amplifies and measures the quantity of DNA) in gastric biopsies of symptomatic patients who had negative gastric biopsies for HP

b) We were unable to culture HP from any of the water samples collected in Lima. Culturing HP from water is very challenging, and though it has been done, the methods are not standardized (Al-Sulami, Al-Edani, & Al-Abdula, 2012; Aziz, Khalifa, & Sharaf, 2013; Bahrami et al., 2013; Lu et al., 2002; Moreno & Ferrús, 2012b). We did, however, find that water in Lima is consistently contaminated with HP. 54% (97/180) of water samples and 38% (30/79) of biofilm samples were positive for HP DNA via qPCR. While we don't know whether this HP is viable, HP DNA rapidly degrades when it comes into contact with bleach (the disinfectant used in the Peruvian water treatment plant) (Sen, Acosta, & Lye, 2011), this is highly suggestive of contamination. Some of the quantities were also very high (similar to quantities that have been found to be infectious in humans), which was a cause of concern. Of 4 of samples of water from the main water treatment plant, all 4 samples were contaminated with HP.

c) From the gastric biopsies of study participants, we isolated 76 strains of HP. These isolates were tested for antibiotic resistance to antibiotics commonly used to treat HP infection. As the results are not yet published, a full summary cannot be included here. However, there was substantial resistance to the conventional antibiotics used to treat HP infection (clarithromycin and amoxicillin), lending support to the importance of primary prevention to maintain effectiveness of existing antibiotic therapies.

Study 2*: Waterborne exposure in mice*
Not only were some of the mice infected, but there was a clear relationship between the amount of HP in drinking water and the amounts of infection seen (Figure 3). This suggests that HP in water may be infectious in humans as

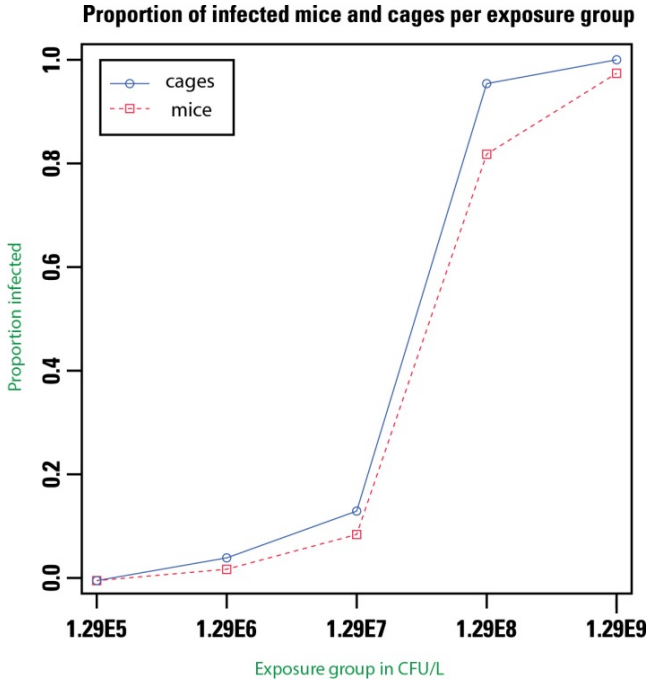

Figure 3. Percentage of infected mice and cages per exposure group. The infectious dose for the exposure paradigm appears to fall around 1.29E6 CFU/L in water. From Boehnke, KF et al, 2015.

well, and provides some additional evidence for the transmission of HP in water. The quantities ingested by mice are similar to those found to be infectious in human dosing trials (conducted for vaccine research) (Graham et al., 2004), suggesting that mice may be a suitable model for further examining infectivity of HP. Further experiments are now ongoing to better understand the infectivity of HP in water.

Study 3*: Testing water treatment systems*
As our results are not yet published, a full report cannot be provided here. However, both methods (boiling and bleach exposure) were relatively effective, causing membrane damage in 90-95% of *HP* in water.

Project successes and stakeholder engagement

Communication
We held annual workshops to disseminate our results to collaborators and stakeholders at University Peruana Cayetano Heredia, DIGESA (Peruvian Department of Environmental Quality and Health), and Peruvian Ministry of Health. At each meeting, we presented our results, got feedback, and made appropriate based on stakeholder input. These meetings provided the impetus for capacity building at DIGESA and the new monitoring program mentioned below under *Collaboration and capacity building*.

Figure 4. Presentation at the Peruvian College of Biologists.

These workshops also pushed us to examine laboratory models of infection, eventually leading to the mouse studies that have now been published. One of the main comments from stakeholders at our various meetings concerned viable but non culturable HP – the form in which it typically exists in the environment. HP is difficult to culture from water because it changes morphology and metabolic function when it enters water, likely as a survival mechanism. However, because we were unable to culture HP from water, we were unable to distinguish between bacteria that were viable but not-culturable and those that were dead (but whose DNA could still be detected). Because of the lack of knowledge about the infectiousness of this form of HP, stakeholders wanted us to perform more experiments to better characterize how well this form can colonize the stomach. Due to this, we are now testing the infectiousness of this form in mice.

Collaboration and capacity building
As a further result of this collaboration, DIGESA has now implemented a monitoring program for waterborne HP, collecting water samples 5 days/week to check for contamination. This

monitoring program will Mr. Boehnke has maintained contact with technicians at DIGESA, helping to provide technical advice about proper use of equipment, answering questions, and helping to provide reagents for laboratory studies.

Figure 6. Sampling in the Field. Left to right: Leopoldo Goetendio, Kathryn Thompson, Katharine MacDonald. Photo credit Samuel Shopinski

Continued funding for future research and collaboration

This work has also drawn interest from several other funding organizations, which will allow us to continue our work beyond the scope of this initial IA research. We have garnered funding from the Hope Foundation and SWOG (Southwest Oncology Group) – a leading cancer consortium in the USA. We have also obtained funding for several students to continue work on the project from various sources at the University of Michigan.

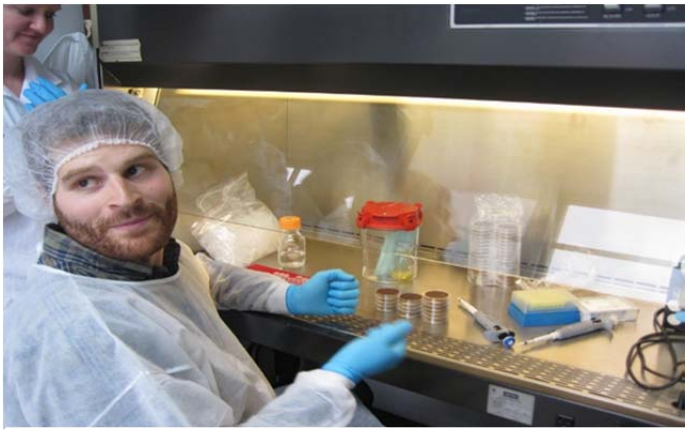

Figure 5. Teaching at DIGESA. Left to right: Katharine Thompson, Kevin Boehnke.

Barriers to full IA implementation

Our IA project has been faced several major challenges. First, there is not much available data in the literature that we could analyze for the purpose of this IA process. This meant that we needed to plan and carry out several field and laboratory studies to collect such data. Second, several changes in the top public health administration in Peru during the course of the study made it difficult to coordinate and perform certain studies. Of major significance was the unwillingness of authorities from the local water treatment plant to collaborate with us during this project.

Administration changes

While we initially began this project with the hope to provide data that could craft useful policy options in Lima, we quickly came to the realization that much needed to be done to have a chance to do so. Not only would relationships need to be built, but it would take time to establish trust between our institutions to allow for us to work together. Efforts to build collaborations with academics and the laboratory at DIGESA were quite successful: Drs. Valdivieso and Xi traveled to Lima multiple times to work with project stakeholders, and Drs. Bussalleu (Universidad Peruana Cayetano Heredia), Novoa (Jackson Memorial Hospital, Miami) and Osorio (DIGESA) effectively coordinated sampling and permitting through the proper channels. While the initial Minister of Health (Dr. Oscar Ugarte) was most supportive of the project, he

was changed with the arrival of a new government soon after. His successor (Dr. Alberto Tejada) was also supportive of the project. After one year in office, he was also replaced. The subsequent two Ministers of Health have not been supportive.

Barriers to access

Unfortunately, efforts to collaborate with SEDAPAL (the main water utility in Lima) were unsuccessful. Despite many attempts to work with SEDAPAL – emailing questions and invitations to meet at annual workshops, setting up visits, reaching out through the Ministry of Health and DIGESA to try to work with them – they were not supportive of our work. Their stated reason was that we couldn't prove that HP was viable and infectious in water. This is true, but from our contacts we found other reasons as well, including a concern over public outcry if they weren't seen to be doing their job.

Future policy options

Due to the political challenges associated with this work, we are not yet at the point where we can discuss the feasibility of policy options. Any such suggestions would be speculation, based on our best judgment but not on the actual possibility of such options to be put into place. However, we will continue working with our partners on this research, and are planning to perform additional water treatment testing and cost-effectiveness analyses for each system (boiling water and bleach). We are also planning to perform a risk assessment with our existing data to better understand the annual risk of infection from drinking water in Peru. This approach will combine the quantitative data on water contamination, the effectiveness of water treatment options, and the data on the infectious doses from our mouse studies. The combination of these approaches will allow us to provide data on whether or not it is actually worthwhile to put policies into place to prevent HP exposure via water.

Summary

Helicobacter pylori (HP) is a stomach bacterium that is the primary cause of gastric cancer worldwide. Much evidence suggests that HP may be transmitted in water. Using the Integrated Assessment format, we carried out several studies to investigate water contamination with HP in Lima, Peru in collaboration with public health officials, clinicians, and basic scientists from Universidad Peruana Cayetano Heredia, DIGESA (Peruvian Department of Environmental Quality and Health), and the University of Michigan. With the input and help of all stakeholders, we found that municipal drinking water in Lima is often contaminated with HP (suggesting that drinking water may be a source of HP infection), that waterborne HP is infectious in mice, and that boiling water and bleach may be effective household interventions to prevent HP exposure via water. We also showed that HP is often resistant to the standard antibiotics used for

treatment, suggesting that further evaluation of alternative medications could be warranted. As a part of our work, we've raised awareness of this problem within DIGESA and in the Peruvian Ministry of Health, which has led to a new program in which drinking water is monitored weekly for HP in Lima. We have also helped build capacity within DIGESA – training them on how to use multiple new types of equipment, providing reagents for monitoring for HP, and being available for further queries from DIGESA staff. We are in the process of writing several papers for publication and (together with our partners) plan to use these data as leverage to help continue our conversations with policy makers in Lima to address this problem.

While we are not able to make any policy recommendations at this time we will continue our work with our collaborators in Lima to perform a cost-benefit analysis for different water treatment systems and to evaluate the costs of medical treatment vs. prevention of exposure.

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Appendix 1

Partners/client/research team

Our partners and research team included a broad range of actors. Drs. Chuanwu Xi and Manuel Valdivieso were the principal investigators on this project, coordinating funding, workshops, and research at the University of Michigan. From the Universidad Peruana Cayetano Heredia, Dr. Alejandro Bussalleu, Dr. Italo Novoa, and Claudia Meza RN coordinated the clinical aspects of the study – recruiting patients, testing for HP infection, collecting biopsies, providing treatment, and following up with patients over time. Drs. Soledad Osorio and Elena Gil (as well as the support staff at DIGESA) coordinated water and biofilm collection from patient homes in Lima, testing samples for HP status via the culture method, and were responsible for sending these samples to the University of Michigan for processing. Mr. Kevin Boehnke acted as the project manager at the University of Michigan, helping to train support stuff at DIGESA, processing samples and performing molecular biology tests at the University of Michigan. He also worked with Dr. Kathryn Eaton and Clinton Fontaine on the mouse studies. Multiple students aided with field collection and sample processing, including Kathryn Thompson, Ariana Wilkinson, Katharine McDonald, Samuel Shopinski, Michelle Long, Kaitlyn Leffert, Christine Greene, Rebecca Brewster, and Ariel Saulles.

Publications

1. Boehnke, K. F., Eaton, K. A., Valdivieso, M., Baker, L. H., & Xi, C. (2015). Animal Model Reveals Potential Waterborne Transmission of Helicobacter pylori Infection. *Helicobacter*, *20*(5), 326-333.
2. Manuel Valdivieso, Alejandro Bussalleu, Kevin Boehnke, Rachael Sexton, Kate Thomoson, Soledad Osorio, Italo Novoa, John Crowley, Gary Goodman, Laurence Baker, Chuanwu Xi. "*Clinical studies of Helicobacter pylori in Lima, Peru*". In preparation
3. Kevin Boehnke, Soledad Osorio, Manuel Valdivieso, Alejandro Bussalleu, Rachael Sexton, Italo Novoa, John Crowley, Gary Goodman, Laurence Baker, Chuanwu Xi. "*Survey of drinking and well water for Helicobacter plyori in Lima, Peru*". In preparation
4. Kevin Boehnke, Kathryn Eaton, Manuel Valdivieso, Chuanwu Xi. "*Reduced Infectivity of Viable but non-culturable Helicobacter pylori in mice.*" In preparation.

Posters

1. January 2016: Boehnke, K. F., Eaton, K. A., Fontaine, C., Valdivieso, M., Baker, L. H., & Xi, C. "Reduced infectivity of viable but non-culturable *Helicobacter pylori* in mice" (*poster*) at the American Society of Microbiologists general meeting in New Orleans
2. June 2015: Boehnke, K. F., Eaton, K. A., Valdivieso, M., Baker, L. H., & Xi, C. "Animal Model Reveals Waterborne Potential of Helicobacter pylori" (*poster*) at the American Society of Microbiology general meeting in New Orleans.

Presentations and workshops

Stakeholder workshops held:

1. October 9-11th, 2012
2. June 15th, 2013
3. June 9th, 2015

List of selected presentations:

1. June 2015: “Integrated Assessment of water contamination with *Helicobacter pylori*” at the *Helicobacter pylori* symposium in Lima, Peru.
2. June 2015: “Molecular studies of *Helicobacter pylori* in gastric mucosa, drinking water, and biofilms in Lima, Peru” at the *Helicobacter pylori* symposium in Lima, Peru.
3. June 2015: “Experimental transmission of *Helicobacter pylori* through drinking water” at the *Helicobacter pylori* symposium in Lima, Peru.
4. June 2015: “Clinical laboratory Correlations in the clinical genomic study of *Helicobacter pylori*: role of contaminated water” at the *Helicobacter pylori* symposium in Lima, Peru.
5. November 2014: “Climate change and an emerging waterborne pathogen: *Helicobacter pylori*” at the College of Peruvian Biologists in Lima, Peru.
6. November 2014: “Obtaining a Biology degree from the University of Michigan” at the College of Peruvian Biologists in Lima, Peru.
7. November 2013: “*Helicobacter pylori* and water contamination in Lima, Peru” for the Tinker Field Foundation at the International Institute, University of Michigan, Ann Arbor.
8. September 2013: “Summer 2013 *Helicobacter pylori* Integrated Assessment Research in Lima, Peru”, at School of Public Health, University of Michigan, Ann Arbor.
9. July 2013: “Detecting, quantifying, and culturing *Helicobacter pylori* from water samplesat the College of Peruvian Biologists in Lima, Peru.
10. July 2013: “Molecular Epidemiology and Genomic Evaluation of *Helicobacter pylori*: The role of water contamination at the Red Interamericana de Laboratorios de Análisis de Alimentos in Lima, Peru.