### The Incidence of Water-borne Pathogens in Rural Coastal Ecuador Erin E. Crain & Catherine L. Woodward

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#### Introduction

Water quality is a leading global environmental issue that has important human health implications. About 2.2 million people die every year due to dehydration caused by diarrheal illness, the majority of which are children (WHO "Water Sanitation Health," 2014). Major transmission pathways of water-related pathogens include consumption of contaminated water, household transmission, and community-level transmission due to inadequate sanitation (Eisenberg, Scott & Porco, 2007; Bhavnani, Goldstick, Cevallos, Trueba, and Eisenberg, 2014). Additionally, seasonal changes can affect infection rates by some contaminated water-related pathogens. For example, Domínguez-Bello et al. (2002) in Venezuela found *Helicobacter pylori* bacteria, a cause of gastric ulcers, to be much more common during the wet season than in dry months. Likewise, *Giardia lamblia* was found to be much more common during the wet season than the dry season in Ethiopia (Ayalew et al., 2008).

In the Ecuadorian coastal villages of Tabuga, Camarones, Tasaste, and Don Juan (Jama County, Manabí province), people rely on rivers for bathing, washing, and drinking, but also irrigation and watering livestock. This leads to the transmission of various diarrheal and gastro-intestinal diseases, such as cholera, giardia, and amoebic dysentery (WHO "Diarrhoeal disease," 2013). The rivers flow from forested coastal hills through a landscape dominated by pasture and agricultural land, becoming contaminated by manure and agricultural pollutants along the way. High levels of fecal coliform bacteria, notably *Escherichia coli* (*E. coli*), have been found at many sampling sites and indicate a public health risk (Levy, Nelson, Hubbard, & Eisenberg, 2009).

The first goal of this project was to strengthen local capacity for water quality monitoring. The second objective was to gather information on preventable water-borne diseases from healthcare providers in the regional hospital in Jama and to assess their incidence in order to effectively educate local communities on water-related health risks and their prevention. My community partner is the Ceiba Foundation for Tropical Conservation, a U.S. non-profit organization based in Ecuador that has been working in Jama County since 2000. My mentor, Dr. Catherine Woodward, is the current president and co-founder of the Ceiba Foundation. Ceiba focuses on the conservation and rehabilitation of tropical forests and involves local people and U.S. students and volunteers in habitat protection, environmental education, and research. In 2004, Ceiba established the Lalo Loor reserve near Tabuga on Ecuador's Pacific coast to protect highly threatened tropical dry forest habitat. Deforestation has reduced forest cover in this area by 98% and caused reductions in stream flow and water quality (Dodson & Gentry, 1998; C. Woodward, personal communication, January-June, 2014). In 2011, Ceiba initiated a citizenscience water monitoring project in Jama County (based on UW-Extension and the Wisconsin DNR's Water Action Volunteers) aimed at collecting data and educating local communities about the links between land use, water quality, and human health. The long-term intended impact of this project was to maintain and improve the ongoing water quality monitoring, which will benefit the local communities through continued education in monitoring water quality so they will have the power to improve water quality and to detect and prevent health risks.

#### Methods

Jama County is located on the Pacific Coast in the Manabí province of Ecuador. Western Ecuador has a distinct dry season from about June to November and a wet season from about December through May. According to the 2010 Ecuador census, Jama County has a population

of 23,253 people (Resultados del Censo, 2010). The Hospital de Jama (Jama Hospital), located in the town of Jama, the county seat, was originally built to function as a hospital but is used solely as a clinic since it did not pass hospital certification. However, it is commonly referred to as the hospital. The next closest medical clinic is in the city of Pedernales, about 50 kilometers to the north. The Jama Hospital offers several services, including a laboratory for hematological, urological, coprological, serological, and bacteriological exams.

I visited the hospital twice prior to beginning data collection: first in May 2014 and again in early June 2014. During these visits, I spoke with a doctor at the hospital as well as the laboratory director to explain the purpose of my project, obtain permission, and review the amount, type and format of data available. The Jama Hospital does not have a computerized patient records system, so all records that are logged are documented on paper medical forms. However, much of the patient information was incomplete or illegible. For example, patient hometown was frequently listed as the location of the hospital or simply not recorded. Also, gender was not specifically recorded, and had to be deduced from first name, which was often illegible or unfamiliar. Records were presented to me as unorganized piles in a cardboard box. I tried to organize based on date and month, but records were available for only several days for many of months in 2013 and 2014.

The purpose of data collection was to assess the incidence of and identify the most common water-borne disease pathogens present in the human population of Jama County. I proposed multiple research questions for data collection and analysis: "what are the three most common pathogens in both fecal and blood exams?," "how common is co-infection and which pathogens are more common with co-infection?," and "what ages were the patients with positive fecal and blood exam results for water-related pathogens?." I spent 15 days during June 2014 collecting

data at the Jama Hospital. I recorded the date of treatment, age, gender, community, county and province for every patient, and I did not record any personally identifiable information for any of the patients. When a fecal exam had been performed, I recorded presence/absence of bacteria, protozoans, cysts, and worms and other parasites. I also noted the results of blood tests for *Hepatitis A* virus, *Dengue* Virus, and *Helicobacter pylori* when these were performed. I recorded data from patient visits over three days in September 2013, 21 days in October 2013 and 21 days in November 2013 (dry season) as well as 7 days in March 2014 and 17 days in April 2014 (wet season). I recorded data from these days in particular since they were the only dates in the 2013 dry season and the 2014 wet season with available records.

#### Data analysis

The goal of this project is to use data analysis to plan a constructive way to communicate disease threats and possible preventive measures to the local communities. In order to answer the previously posed questions, I determined the frequency of pathogen occurrence without co-infection, the frequency of co-infection by more than one pathogenic micro-organism, and the frequency of pathogen occurrence alone or with co-infection in relation to the total number of patients, the number of patients with a fecal exam, and the number of patients with a positive fecal exam for the wet season, dry season and total (wet and dry). Co-infection is infection of an individual by more than one pathogenic micro-organism at one time. I then arranged the data by age and calculated the frequency of visits for 11 age groups for all patients, the number of patients with a positive fecal exam for pathogen presence, and the percent of total number of patients with a positive fecal exam for wet and dry seasons combined.

#### Health Education

Before leaving Ecuador at the end of June 2014, I created and gave a PowerPoint presentation in Spanish to students in Jama County to teach them about water-related disease prevention and the dangers of contaminated water. I presented twice at the Tabuga high school and three times at the Jama high school. Several students from the University of Wisconsin – Madison in the Ceiba Tropical Conservation Spring semester program will present at additional high schools and community centers in Jama County in April and May of 2015.

Finally, I returned to Madison, Wisconsin and worked through Fall 2014 with my mentor to conduct literature and internet research on the most common pathogens found in the Jama Hospital patient sample and create an educational campaign to teach community members about the causes and prevention of major water-borne diseases. The Ceiba Foundation initiated a project several years ago for a group of women in the Camarones community to create and sell biodegradable soap as a source of income and as a way to replace harmful detergents that are released into the environment by community members through bathing and washing clothes and dishes. These soap bars will be disseminated at educational presentations given by several students from the University of Wisconsin – Madison and the Ceiba Tropical Conservation Spring semester program throughout community centers, medical clinics and schools in Jama County during April and May of 2015

#### **Results**

Of the total 2,808 patients whose records I viewed, 492 (17.5%) had a fecal examination and 224 (45.5%) of those fecal examinations had a positive result for infection by a water-related pathogen or parasite. Assuming those who did not have a fecal exam did not have an intestinal pathogen, this translates to an estimated 7.98% of Jama Hospital visitors suffering from a water-borne parasite or pathogen (Table 1). Most of the patients whose records contributed to the data

were from Cantón Jama, while several were from Cantón Pedernales, Rocafuerte, Atahualpa, or Chone, all in the Manabí province of Ecuador.

In both the dry and rainy seasons, *Entamoeba histolytica, Entamoeba coli*, and *Giardia lamblia* were the most frequently occurring pathogens occurring both alone and in co-infections with another organism. Overall infection rates out of the 2808 total patient records surveyed during the combined wet and dry seasons were 6.3% with *E. histolytica* (with 2.8% coinfected), 3.7% with *Entamoeba coli* (with 2.4% coinfected), and 0.8% with *G. lamblia* (with 0.4% coinfected) (Table 1). Infection with *Entamoeba coli* alone was more common during the wet than the dry season ( $X^2_{Calc}=4.57$ , df=1, p < 0.05).

Co-Infection with more than one pathogenic micro-organism was fairly common. The most frequently occurring co-infections were *E. histolytica* with *Entamoeba coli* (2.2% of the 2,808 records surveyed), *E. histolytica* and *G. lamblia* (0.4%), and finally *E. histolytica*, *Entamoeba coli*, and *G. lamblia* (0.1%) (Table 1). Other co-infections included various combinations of *E. histolytica*, *Entamoeba coli*, *G. lamblia*, *Hymenolepis nana* (tapeworm), *Trichuris trichiura* (roundworm), and *Ascaris lumbricoides* (roundworm) in both seasons. One patient during the dry season had a co-infection with four species: *H. nana*, *T. trichiura*, *E. histolytica*, and *G. lamblia*. *H. nana* was found in two patients during the rainy season. *Trichomona intestinalis*, a protozoan, was found in two patients and *A. lumbricoides* was found in one patient during the dry season.

Although the total number of patients having blood examinations was not recorded during data collection, incidence of water-related blood pathogens *Hepatitis A Virus* (causes Hepatitis A), *Dengue Virus* (causes Dengue Fever), and *Helicobacter pylori* bacterial infections were recorded. *H. pylori* was most common (2.6% of all 2,808 patients), and *Hepatitis A Virus* 

and *Dengue Virus* were each present in 0.3% of all patients. *H. pylori* was more frequent during the dry season than the wet season ( $X^2_{Calc}$ =6.72, df=1, p < 0.01), which is opposite of the results presented by Domínguez-Bello et al. (2002) in Venezuela, where *H. pylori* was more common in the wet season.

Recorded ages of all patients visiting the Jama Hospital during the wet and dry seasons ranged from one month to 94 years. 48.8% of all patients visiting the Jama Hospital were between the ages of 6 and 30 (Figure 1A). Patients under 10 years of age (n = 295) and between the ages of 80 and 94 (n = 44) had the highest proportions of incidence of diagnosed water-related pathogens or parasites (14.9% and 11.36%, respectively) (Figures 1B and C).

#### **Discussion**

The most common pathogenic organisms found in this study and their modes of transmission suggest that people are coming into contact with contaminated water in these communities. *Entamoeba histolytica*, the most frequently occurring pathogen in this study, is a parasitic protozoan transmitted by cysts transferred from feces to mouth via unwashed food and hands (Bray and Harris, 1977). Previous work in The Gambia, West Africa found higher *E. histolytica* prevalence at the beginning of the wet season than at other times of the year (Bray and Harris, 1977). Additionally, Amin (2002) found that *E. histolytica* prevalence is highest during the wetter summer months of July through October in 48 states and the District of Columbia in the United States. However we detected no such pattern; infection rates with *E. histolytica* were near 6% in both seasons (Appendix I). *Entamoeba coli*, the second most common pathogenic organism found in this study is also transmitted via cysts carried from feces to mouth (Hashmey, Genta & Clinton White, 1996). *Giardia lamblia* was the third most common pathogen and is a protozoan that has a high resistance to chemicals used in water

treatment (WHO "Water Quality," 2001). Consumption of water contaminated by feces is the main means of transmission of giardia cysts (Adam, 2001). The cyst transforms into the trophozoite form when exposed to the acidic environment of the small intestine, where it causes symptoms such as diarrhea (Adam, 2001).

Those patients who tended to have at least one pathogen were commonly infected with one or more additional pathogens, which is referred to as a co-infection. Several risk factors for diarrheal diseases include household demographic factors, socioeconomic factors, water, sanitation, and hygiene (Bhavnani et al., 2014). People in higher-risk environments are more likely to be exposed to pathogenic organisms, and those exposed to contaminated water more frequently likely are exposed to more than one pathogenic organism, resulting in infection by multiple pathogens at the same time. We did not collect personally identifiable information from patient records in this study, precluding further analysis of risk factors in infected and co-infected people.

Incidence of pathogens in blood exams also suggests that people are consuming contaminated water and practicing inadequate sanitation and hygiene. Hepatitis A, caused by an enterovirus, tends to occur in areas with low hygiene standards and is controlled in developed areas through sanitation, hygiene and appropriate water storage (WHO "Water Quality," 2001). Dengue fever, caused by DEN-1, DEN-2, DEN-3 and DEN-4 virus serotypes of the genus *Flavivirus*, is transmitted through a mosquito vector by the *Aedes aegypti* and *Aedes albopictus* (*Asian Tiger mosquito*) species (Gubler & Clark, 1995). Dengue fever is spread by *A. Albopictus* in Ecuador, and no literature was available supporting the spread of Dengue Fever by *A. aegypti* in Ecuador (Vasconcelos et al., 1999). Transmission of Dengue virus is facilitated by the

increase in population density of these species caused by inadequate water, sewer and waste management systems (Gubler & Clark, 1995).

Helicobacter pylori is a bacteria that causes gastrointestinal and duodenal ulcers. Infection has been shown to lead to an increased risk of gastric carcinoma (Klein & Graham, 1991). Although the method of transmission remains unknown, prevalence of *H. pylori* tends to correlate with unsanitary home water sources and is much higher in low-income than high-income families (Klein & Graham, 1991; US Dept. of Health, 2010).

Several difficulties regarding data collection arose during this study. Records were not computerized or organized chronologically, so I spent much of the first few days of data collection organizing piles of paper records and deciphering the handwritten information.

Furthermore, data was recorded inconsistently and there were temporal gaps in patient records, suggesting not all records were preserved. We cannot assume that data collected at the hospital, for example proportions of ages and genders, and of patients positive for blood and intestinal pathogens, are representative of the entire Jama population. Overall, the water-related pathogens pose a serious threat to human health in a substantial portion of the population, especially the young and the old, in Jama County and coastal Ecuador.

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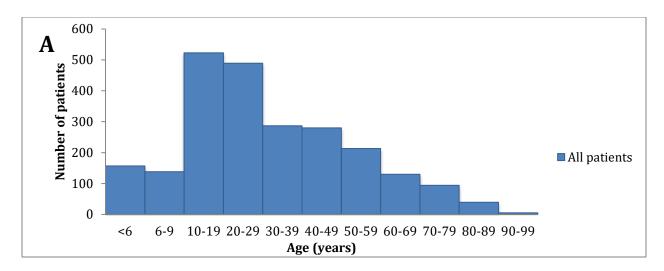
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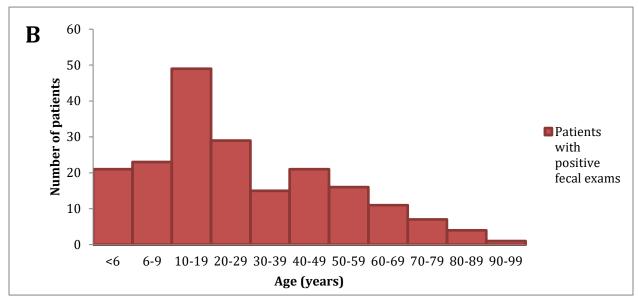
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**Tables and Figures** 





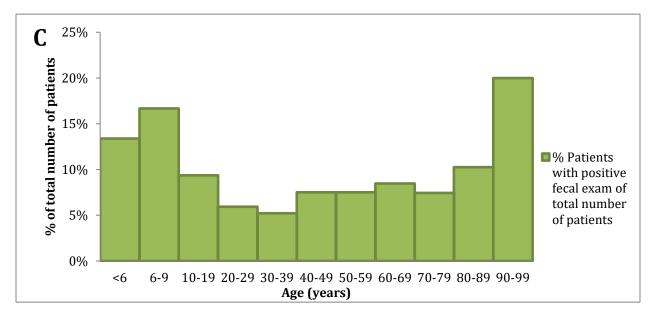


Figure 1. Histogram of the frequency of ages of all patients receiving medical attention as well as the frequency of ages of patients with diagnosed water-related pathogens or parasites at the Jama Hospital during the dry season months of September, October and November 2013 and the wet season months of March and April 2014 combined. Figure A shows the ages of all patients at the Jama Hospital, Figure B shows the ages of patients with positive fecal exams at the Jama Hospital, and Figure C shows the percent of patients with positive fecal exams at out of the total number of patients at the Jama Hospital in that age group.

## Appendix

									TOTA			
	DRY				WET				L			
	Patie nts	% of Patien ts	% of Fecal Exam	% of (+) Fecal Exam	Patien ts	% of Patien ts	% of Fecal Exam	% of (+) Fecal Exam	Patien ts	% of Patien ts	% of Fecal Exam	% of (+) Fecal Exam
Patients	1520				1288				2808			
	1320				1200				2000			
Fecal Exam	262	17.24 %			230	17.86 %			492	17.52 %		
Positive	202	70			250	70			7/2	70		
for pathoge			44.66				48.70				46.54	
n	117	7.70%	44.00 %		112	8.70%	48.70		229	8.16%	40.34 %	
			36.64	82.05			35.22	72.32			35.98	77.29
Eh total	96	6.32%	%	%	81	6.29%	%	%	177	6.30%	%	%
			19.08	42.74			23.04	47.32			20.93	44.98
Ec total	50	3.29%	%	%	53	4.11%	%	%	103	3.67%	%	%
Gl total	10	0.66%	3.82%	8.55%	11	0.85%	4.78%	9.82%	21	0.75%	4.27%	9.17%
Eh alone	53	3.49%	20.23	45.30 %	46	3.57%	20.00	41.07 %	99	3.53%	20.12	43.23
		0.700/	4.500/	10.26		1.040/	10.87	22.32		4.000/		16.16
Ec alone	12	0.79%	4.58%	%	25	1.94%	%	%	37	1.32%	7.52%	%
Clalene	_	0.220/	1 010/	4 270/	4	0.210/	1 740/	2 570/	9	0.220/	1 020/	2 020/
Gl alone	5	0.33%	1.91%	4.27%	4	0.31%	1.74%	3.57%	9	0.32%	1.83%	3.93%
Eh/Ec	36	2.37%	13.74	30.77	27	2.10%	11.74 %	24.11	63	2.24%	12.80	27.51
Eh/Gl	3	0.20%	1.15%	2.56%	7	0.54%	3.04%	6.25%	10	0.36%	2.03%	4.37%
Eh/Ec/Gl	2	0.13%	0.76%	1.71%	0	0.00%	0.00%	0.00%	2	0.07%	0.41%	0.87%
Hep A total	1	0.07%	0.38%	0.85%	8	0.62%	3.48%	7.14%	9	0.32%	1.83%	3.93%
Dengue total	3	0.20%	1.15%	2.56%	6	0.47%	2.61%	5.36%	9	0.32%	1.83%	3.93%
			17.94	40.17			10.87	22.32			14.63	31.44
Hp total	47	3.09%	%	%	25	1.94%	%	%	72	2.56%	%	%

**Table 1.** The most frequently occurring water-related pathogens and parasites during the dry season months of September, October and November 2013 and wet season months of March and April 2014. "Alone" = frequency of pathogen occurrence without co-infection by more than one pathogenic micro-organism. "Co-infection" = frequency of co-infection by more than one pathogenic micro-organism. "Total" = frequency of pathogen occurrence alone or with co-infection. "% of patients" = Percent of total number of patients (n of dry = 1520, n of wet = 1288, n of total = 2808). % of Fecal Exam = Percent of number of patients with a fecal exam (n of dry = 262, n of wet = 230, n of total = 492). "% of (+) Fecal Exam" = Percent of number of patients with a positive fecal exam (n of dry = 117, n of wet = 112, n of total = 229). "Eh" = Entamoeba histolytica, "Ec" = Entamoeba coli, "Gl" = Giardia lamblia, "Hep A" = Hepatitis A Virus, "Dengue" = Dengue virus, "Hp" = H. pylori bacteria