PREVALENCE OF POTENTIALLY PATHOGENIC BACTERIA, PROTOZOANS, AND HELMINTHES IN DRINKING WATER FROM SOURCES LOCATED IN JACSONVILLE AND THROUGHOUT THE MATABONITE REGION OF CENTRAL HAITI

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Abstract

This report provides water quality data for water collected from eleven wells, two rivers, and one spring located in Jacsonville and throughout the Matabonite Region of Central Haiti. Water was tested for the presence of total and fecal coliforms, Escherichia coli, and Vibrio spp. using the membrane filtration technique and mFC, TEC, methylumbelliferyl-β-D-glucuronide (MUG), and thiosulfate-citrate-bile salts-sucrose (TCBS) agars. After incubation, bacterial colonies were counted and isolates chosen for tentative identification using the API 20E Identification System. Also, water and soil samples collected from two sites were microscopically examined for the presence of potentially pathogenic protozoans and helminthes.

The number of coliforms in water samples ranged from 3.0 to 3.1 x 10^5 CFU/100 ml. Bacterial isolates identified included Pseudomonas luteola, Enterobacter cloacae, and Aeromonas hydrophilia. E. coli was detected in seven of the fourteen water sources sampled suggesting that water from these sources had contact with contaminated waste. Giardia, Entamoeba, Endolimax, and Cryptosporidium trophozoites and cysts were found in eight of fourteen water sources and eggs of the helminth, Ascaris lumbricoides, were detected in one of the water and one of the soil samples. Based on drinking water guidelines set by the United States Environmental Protection Agency (USEPA) and the World Health Organization all water samples assessed during this study require treatment prior to consumption, particularly for populations most at risk for acquiring the associated diseases. Seven water sources met recreational standards issued by the USEPA and would be suitable without treatment for bathing purposes.

Included in this report are descriptions of accessible and cost effective means to treat water and suggestions for future water quality studies. With comprehensive water quality data, appropriate drinking water guidelines and recreational standards could be established for Jacsonville and the Matabonite Region to ensure that water supplies for these communities are safe.

1 Introduction

Access to clean and safe drinking water represents a primary public health concern for many countries including Haiti. In developing countries, diarrheal diseases are responsible for the majority of health risks associated with poor sanitation and consumption of poor-quality water. They are one of the leading causes of morbidity and mortality in the elderly, immune compromised, and children (Lui et al., 2012). Diarrheal diseases are caused by a variety of organisms, including viruses, bacteria, and parasites that contaminate potable water when water sources are exposed to the feces of infected animals and humans. Many waterborne pathogens are coliforms defined as facultative anaerobic, Gram negative, rod-shaped, non-endospore forming bacteria. Coliform bacteria are ubiquitous in nature and are found in a variety of environments including the intestinal tracts of warm-blooded animals. Coliform bacteria that inhabit the intestinal tract are more often referred to as fecal coliforms. Their presence in drinking water indicates that the water has been contaminated with feces and could harbor any number of pathogenic organisms.

An important fecal coliform is *Escherichia coli,* which is a member of the family *Enterobacteriacea,* and when ingested can cause a serious disease known as enterohemorrhagic diarrahea. For this reason, *E.coli* alone is often used as an indicator of water quality. Other clinically relevant bacterial pathogens and members of the *Enterobacteriacea* found in contaminated water include *Salmonella, Shigella,* and *Vibrio.* These bacteria are known to cause salmonellosis or typhoid fever, shigelliosis, and cholera respectively. While bacterial pathogens are the primary cause of waterborne diarrheal diseases in developing nations, another significant health threat and a primary cause of mortality of local populations are infections by parasites.

Parasites generally fall into one of two groups: protozoans and helminthes. Protozoans are a diverse group of unicellular eukaryotic organisms. In addition to being common inhabitants of animal and human intestinal tracts, they are often characterized by life cycle transmission stages (cysts and oocysts) that are highly resistant to external environments. Unlike bacterial infections, diarrheal infections caused by protozoans tend to be chronic (Okhuysen, 2001). Some examples of clinically relevant protozoa include *Giardia, Cryptosporidia,* and *Entamoeba.* Helminthes are parasitic worms comprised of trematodes (flukes), cestodes (tape worms), and nematodes (round worms). Like protozoans, they have complex life cycles that involve life stages (egg and larval stages) that can survive in water and soil for extended periods of time. Examples of helminthes include *Ascaris lumbricoides*, *Necator americanus*, and *Trichuris trichuria* (De Rochars et al., 2004). Individuals, particularly school aged children, experience malnutrition and impaired growth when infected with parasitic worms (Karabanow, 2013).

Currently Haiti does not have established guidelines for safe drinking water standards. In the United States, to protect the public from exposure to contaminated water, a set of standards known as the National Primary Drinking Water Regulations (NPDWR) have been set by the United States Environmental Protection Agency (USEPA). The maximum contaminant level goal (MCLG), the level of a contaminant in drinking water below which there is no known or expected risk to health, is set to zero for biological contaminants (the protozoans *Cryoptosporidium* and *Giardia*, enteric viruses, heterotrophic bacteria, total coliforms including *Legionella*, and fecal coliforms*)* in surface and groundwater drinking sources. The maximum contaminant level (MCL), the highest level of a contaminant that is allowed in drinking water is set as close to the MCGL's as possible depending on the best available technology (for example, disinfection or filtration) for treating water. For protozoan pathogens the permissible MCL remains at zero. For water sources that are sampled fewer than 40 times per month, no more than 5% of the samples should test positive for total coliforms. If water samples test positive for total coliforms they are required to be re-tested for fecal coliforms or for *E. coli*. If samples contain fecal bacteria or *E. coli*, water is considered to have violated the MCL criteria and is not recommended for consumption. Guidelines for the verification of microbial quality of drinking water used by the World Health Organization (WHO) are similar to those published by the EPA: *E.coli* or thermotolerant coliform bacteria must not be detectable in any 100 ml water sample. These guidelines were generated in conjunction with a metric known as disability-adjusted life-years (DALYs), a time-based measurement that takes into account the burden of disease across diseases, risk factors, and region (WHO, 2006).

Water samples from 11 wells, 2 rivers, and a spring located throughout the Matabonite region of Haiti were examined for the presence of total and fecal coliforms, *E. coli*, and *Vibrio* spp. in July of 2013. In addition, water samples from all sites and two surface soil samples from two locations were microscopically inspected for the presence of potentially pathogenic protozoa and helminthes. This report summarizes the results of the sampling event and provides a snap shot of the water quality at the time of sampling.

2 Materials and Methods

2.1 Sampling Sites and Water Collection

Fourteen water samples were collected throughout the Matabonite Region of Central Haiti and assessed for the presence of waterborne pathogens. Approximately 500 ml of water was taken from community wells located near Jacsonville Mission (Back Well and Front Well), Jacsonville Regional School, the town of Pignon (Pignon #2 and Pignon #3), Boekuero, Bouqueronne, Matabonite (Matabonite #1 and Matabonite #2), Terre Blanche, and Savanette. Samples were also collected from Bouyara and Guape Rivers and Tetechange Spring. Samples were brought back to Jacsonville Mission and processed as described below.

2.2 Enumeration of Enteric Bacteria

Figure 1. Filtering apparatus for the membrane filtration technique.

The membrane filter technique (see Fig. 1) was used to determine the number of colony forming units per 100 ml (#CFU/100ml) of total and fecal coliforms in water. Undiluted water samples (100 ml) or serially diluted (10^{-2} - 10^{-4}) water samples (100 ml) were filtered through a 0.45 μm pore-size membrane filter. The filter was aseptically placed onto mFC agar for the detection of total and fecal coliforms, 4-methylumbelliferyl-β-D-glucuronide (MUG) agar for the detection of *Escherichia coli*, TEC for the isolation and detection of thermotolerant *Escherichia coli*, and thiosulfate-citrate-bile salts-sucrose (TCBS) media for the isolation of *Vibrio* spp. Plates were incubated in the dark for 18-24 h at ambient temperatures (approximately 37 ℃). After incubation, resulting colonies were counted and the number of colony forming units (CFU) per 100 ml water determined (see Fig. 2 below).
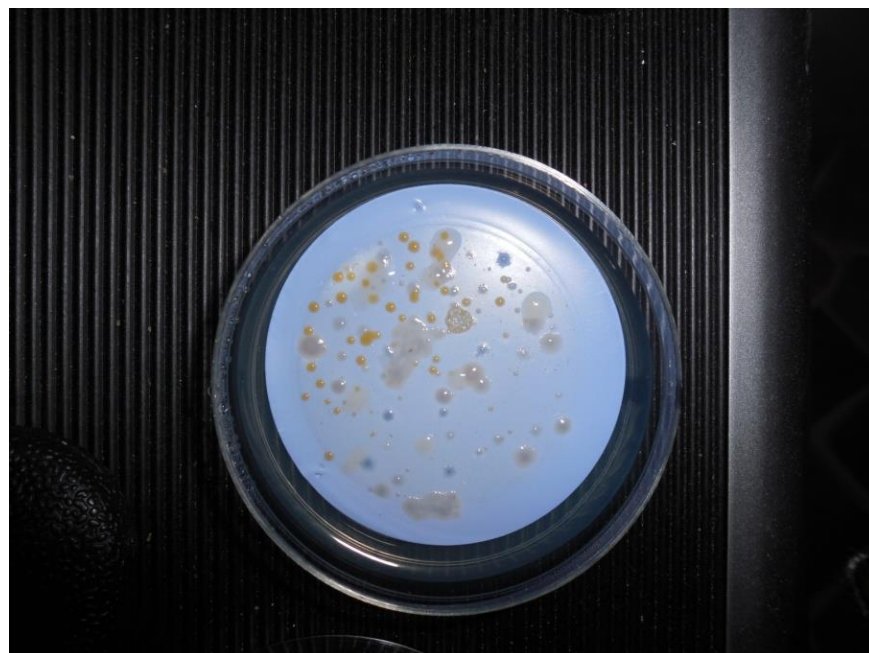
Figure 2. Colony forming units (CFU) resulting after filtering 100 ml water and placing membrane filter on mFC media.

2.3 *Isolation of Pure Cultures*

A loopful of bacteria from a colony was transferred to a new agar plate and a pure culture isolated using the streak plate technique. This procedure was repeated for 2 or more colonies per sample site (with the exception of the Mission Back and Mission School sites) per medium. Once pure cultures were obtained, isolates were Gram stained and their oxidase reaction determined in preparation for further identification using the API 20E Identification System (bioMerieux). Oxidase reaction was determined by adding a drop of tetramethyl-p-phenylenediamine reagent (oxidase reagent) onto pure cultures of bacterial growth. The development of a purple to black color indicated that the organism was oxidase positive.

2.4 *Identification of Coliform Bacteria: API 20E System*

The API 20E Identification System was used to tentatively determine the genus and species of the 38 enteric bacteria isolated from selective media. An incubation tray was prepared for test strips by distributing approximately 5 mL of filter-sterilized water into the incubation tray. API 20E test strips were removed from their packaging and placed in the incubation tray. A bacterial suspension was prepared by transferring a loopful of culture to a tube of sterile 0.85% saline solution. The resulting suspension was added to the test strip wells (see Fig. 3) by filling the tube of each test strip as follows: underlined tubes (ADH, LDC, ODC, H2S, and URE) were slightly under-filled to allow addition of oil, bracketed tubes (CIT, VP, and GEL) and their cupules were filled completely, and all other tubes (but not their cupules) were filled completely. Sterile mineral oil was added to the surface of the under-filled, underlined tubes to create an anaerobic environment. The tray was incubated at approximately 37 °C for 18-24 h. After incubation, the following reagents were added to the indicated compartments: 10% ferric

chloride to TDA, Kovac's reagent to IND, Barritt's reagent A and B to VP, nitrate reagents A and B and zinc to nitrate reduction compartments. Readings were made immediately after addition of reagents for TDA and IND, 10 min after addition of reagents for VP, and 5 minutes after addition of reagents for nitrate reduction. After recording results (positive and negative reactions) (see Fig. 4) and totaling the necessary numbers, the resulting seven-digit code was used to determine the likely organism using the Analytical Profile Index. Confirmation of the presence of Escherichia coli in water samples (Pignon #3, and Savanette) were made after culturing on MUG media and exposure to UV light (see Fig. 5).

Figure 3. Preparation of API 20E microbial identification strips.

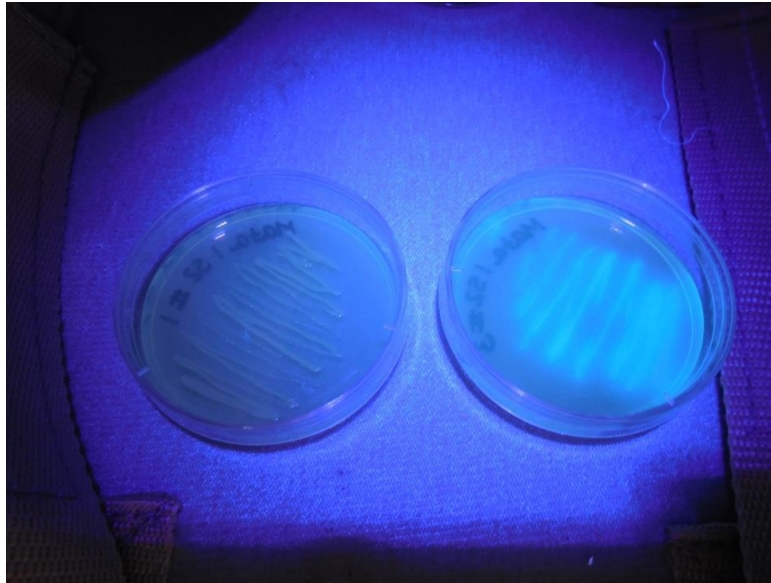**Figure 4.** Scoring inoculated API 20E microbial identification strips after 18-24 h incubation.

Figure 5. Identification of *Escherichia coli* (on right) after growth on MUG media and exposure to UV light. Isolate on the left is a non *E.coli* coliform.

2.5 *Identification of Protozoa and Parasites in Water*

Direct microscopic examination of water was used to determine the presence of trophozoites, cysts, and egg stages of parasites in water samples. Two additional water samples (100 ml – 200 ml) from sample sites were filtered as previously described. One filter was stained for 1 min with Lugol's Iodine Stain, the other filtered water sample was stained with 5% Geimsa Stain. Filters were examined immediately or permanent slides were made using Permount Mounting Media (Fisher Scientific) and viewed after returning to Rivier University.

2.6 *Extraction of Parasites from Soil*

In addition to assessing water, two soil samples collected from the Pignon #1 and #2 and two soil samples from Metabonite #1 and #2 sites were examined for the presence of parasitic eggs and cysts. The soil samples from each site were combined (approximately 50 g) and soaked in 750 mL of distilled water. After 24 h, the samples were stirred to re-suspend particulates and poured through two layers of muslin gauze stretched across a 1 L beaker. The contents in the beaker were left to settle for 3 h. The supernatant was drawn off and the sediment re-suspended in 500 mL of sterile distilled water. This step was repeated three times. The resulting sediment was mixed with saturated NaCl solution in a large 20 mL translucent centrifuge tube. The remaining sediment was allowed to settle to the bottom of tube while watching for a dark ring of material to appear on the surface of the solution. Small aliquots (50 μl) from the dark ring of material were placed on a microscope slide, mixed with Iodine Stain (10 μl), and examined under the microscope for the presence of parasitic eggs and cysts.

3 Results

3.1 *Enumeration and Tentative Identification of Enteric Bacteria*

Two well water samples (Mission Back and Matabonite #2) produced numbers below 10 CFU/100ml on all media used in this study (see Table 1). The remaining well-water and spring samples were found to produce numbers above 10 colonies for one or more media used (see Table 1) indicating that water from these wells has been impacted by human or animal waste. Of the 9 wells the highest number of coliforms were reported for water samples taken from the Mission School (mFC-890, TEC-1000, MUG-300, and TCBS-30 CFU/100 ml), Bouqueronne (mFC-360, TEC-900, MUG-48, and TCBS-30 CFU/100 ml), and Terre Blanche (mFC-340, TEC-160, MUG-18 CFU/100 ml) wells (see Table 1). The highest coliform counts were reported for water samples taken from Bouyara and Guape Rivers regardless of the type of media plated. Even though two water samples produced less than 10 colonies per media, based on the drinking water standards set by USEPA, none of the water samples would be deemed safe for human consumption.

Table 1: Number of colony forming units per 100 ml coliforms detected in water samples.

Sampling Site	Selective Growth Media			
	mFC	TEC	MUG	TCBS
Mission (Back)	2	3	1	0
Mission (Front)	24	26	3	47
Mission School	890	1,000	300	>30*
Pignon #2	34	8	3	0
Pignon #3	96	61	44	0
Boekuero	42	65	470	>30*
Bouqueronne	360	900	48	>30*
Matabonite #1	>200*	5	11	2
Matabonite #2	5	7	1	1
Terre Blanche	340	160	18	0
Savanette	130	12	38	4
Bouyara River	310,000	300,000	130,000	30
Guape River	>30,000*	>20,000*	>20,000*	>200*
Tetechange Spring	80	52	12	20

*plates were crowded or too numerous to count accurately.

Despite some water samples producing fewer than 10 colonies, between 1 to 3 colonies from all plates were re-streaked for isolation (with the exception of Mission Back and School wells) and prepared for tentative identification (see Table 2). All subcultured isolates were Gram negative. Seven of 12 isolates cultured from Mission Front, Pignon #2, and Pignon #3 wells were identified as *Pseudomonas luteola* comprising the majority of bacteria isolated from these wells. Another frequently identified enteric was *Enterobacter cloacae* isolated from Matabonite #1 (1 isolate), Matabonite #2 (2 isolates), and Pignon #3 (1 isolate). *Citrobacter, Klebsiella,* and *Aeromonas* spp. were isolated and identified in Bouyara and Guape River and Tetechange Spring samples, but not cultured from well water samples. The indicator organism, *Escherichia coli*, was identified by the API 20E or after exposure to UV light after plating on MUG. *E. coli* was isolated from Boekuero well water and found in water samples from Pignon #3, Matabonite #1, and Savanette wells, and Guape River and Tetechange Spring (see Table 2). While greater than 10 CFU/100 ml were reported for TCBS media (Mission Front, Mission School, Boekuero, Bouqueronne wells, Bouyara and Guape Rivers, and Tetechange Springs), none of the bacteria cultured from this media were identified as *Vibrio cholera*.

Table 2: Tentative identification of bacterial isolates.

Water Sample	Tentative Identification
Mission Front	*Pseudomonas luteola* (4 isolates) *Acinetobacter baumanni* *Escherichia coli*[1]
Pignon #2	*Pseudomonas luteola* Non-fermenter *possibly Brucella* sp. *Enterobacter cloacae*
Pignon #3	*Pseudomonas luteola* (2 isolates) *Pseudomonas aeruginosa* *Escherichia coli*[1]
Boekuero	*Leclericia adecarboxylata* *Escherichia coli* *Pasteurella pneumonotropica*
Bouqueronne	*Erwinia* sp. *Pantoea* spp. 3
Matabonite #1	*Providencia alcalifaciens* *Serratia fonticola* (2 isolates) *Enterobacter cloacae* *Escherichia coli*[1]
Matabonite #2	*Enterobacter cloacae* (2 isolates) *Serratia rubidaea*
Terre Blanche	*Pseudomonas aeruginosa* (2 isolates)
Savanette	*Pseudomonas aeruginosa* Non-fermenter possibly *Brucella* sp. *Shigella* sp. *Escherichia coli*[1]
Bouyara River	Non-fermenter possibly *Brucella* sp. (2 isolates) *Providencia rettgeri* *Klebsiella oxytoca* *Aeromonas hydrophilia* or *Vibrio fluvialis*
Guape River	*Klebsiella pneumonia* *Serratia liquefaciens* *Citrobacter freundii* *Escherichia coli*[1]
Tetechange Spring	*Aeromonas hydrophilia* or *Vibrio fluvialis* *Citrobacter freundii* *Escherichia coli*[1]

[1]*Escherichia coli* identification after UV exposure when growing on MUG.
( ) the number of times the isolate was identified

3.2 *Observation of Protozoa and Helminthes*

The protozoan parasites *Giardia*, *Cryptosporidium, Endolimax*, and *Entamoeba* were observed in water samples obtained from Pignon #2, Boekuero, Bouqueronne, Matabonite #1, Terre Blanche, and Savanette wells (see Table 3). Ascaris eggs were found in the Guape River and in the Pignon soil samples.

Table 3: Detection of parasites in water or soil samples.

Organism	Sample
Giardia	Pignon #2 Boekuero Terre Blanche
Cryptosporidium	Boekuero Bouqueronne
Endolimax	Boekuero Bouqueronne Terre Blanche Savanette
Entamoeba	Matabonite #1
Ascaris	Bouyara River Pignon #3[1]

[1]Found in soil sample.

4 Discussion

A large number of people can be adversely affected by ingesting contaminated water making waterborne transmission of pathogenic organisms a serious health concern. Some disease-causing organisms are indigenous to aquatic habitats, but many others are introduced to water when feces of infected animals or humans contact water supplies. Because many of the organisms that have been associated with waterborne diseases are not easily cultured the presence of specific organisms in potable water are used as indicators of water quality. Among the indicator organisms are total coliforms. The presence of coliforms in water indicates possible contamination with feces and historically was the only indicator used to assess water quality. Because coliform bacteria were once thought to represent only a small threat to human health, a subset of coliform bacteria, fecal coliforms, are now used as a better predictor of contamination (Avila et al., 1989). mFC media was used to cultivate and enumerate total and fecal coliform bacteria and TEC and MUG were used for the isolation of *E. coli* from water.

In this study, coliform bacteria were detected in the majority of well water, river, and spring samples during this sampling event. Based on EPA and WHO drinking water guidelines, this water would not be considered safe for human consumption. Coliform bacteria are ubiquitous and typically have not raised concerns, but more recently several coliform organisms have gained attention because they have been implicated in causing opportunistic infections. For example *Pseudomonas luteola*, present in Mission and Pignon samples, has been associated with septicemia, meningitis, and peritonitis in patients with underlying diseases (Chihab et al., 2004). It has also caused bacteremia (Arnold et al., 2005) and cutaneous abscesses (Patil et al., 2012) in immune competent individuals. The normal habitat of *P. luteola* is not yet known, but it is frequently isolated from water and soil (Chihab et al., 2004). Similarly, *Enterobacter cloacae* identified in Matabonite water samples is ubiquitous in terrestrial and aquatic environments and is a pathogen of insects and plants including sugarcane (Hoffman & Roggenkamp, 2003; Gan et al., 2012). This organism has been gaining more attention because of its increased importance as a nosocomial pathogen (Yang et al., 2012). Recent studies found that this bacterium has been responsible for hospital acquired septicemias, urinary tract infections, pneumonias, and postsurgical peritonitis (Hoffman & Roggenkamp, 2003). Susceptible individuals, the young, elderly, or immune compromised, may be putting their health at risk through direct contact or ingestion with water that contain these bacteria.

As expected the numbers of coliform bacteria detected in river samples were 1000-fold higher than the numbers detected in well water and spring samples (see Table 1). Surface waters are affected by storm events that cause direct run off from agricultural land and impervious surfaces providing conditions that expose surface waters to feces of infected animals and humans. The tentative identification of *Aeromonas* cultured from Bouyara and Gaupe Rivers and Tetechange Spring raises significant concerns for individuals who routinely use these water sources. *Aeromonas* is frequently isolated in fresh and salt-water environments and in sewage (Djuikom et al., 2008). It is currently recognized as a cause of several well-documented gastrointestinal illnesses (Janda & Abbott, 1998). Originally believed to cause disease only in immune compromised individuals, *A. hydrophila* is now known for aggressive infections of wounds in immune competent individuals (Grim et al., 2013). Wound infections caused by *A. hydrophila* are often indistinguishable from, and are as serious as, necrotizing-fasciitis caused by *Streptococcus* spp.

Ingredients in the media are specifically designed to select for fecal bacteria. Incubation at 44.5 ±0.2 ºC following inoculation enhances the growth of thermotolerant organisms like pathogenic *E. coli*. Because the 44.5 ºC incubation condition was likely not met, it is unclear how many thermotolerant bacteria of fecal origin are represented in these numbers, but the presence of *E. coli* provides evidence that several wells and river samples have been exposed to fecal matter. Compared to members of the genera *Klebsiella*, *Citrobacter*, and *Enterobacter, E. coli* is less likely to be found in the environment because it is a common inhabitant of the intestinal tracts of warm-blooded animals (Podschun & Ullmann, 1998). Many *E. coli* strains are commensals providing essential nutrients for their host, but some strains have acquired virulence factors that allow them to cause clinically significant disease. Combinations of virulence genes have led to the evolution of five distinct *E. coli* pathotypes: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli*, enterohemorrhagic *E. coli,* enteroinvasive *E. coli*, and enteroaggregative *E. coli* (EAEC) (Nataro & Kaper, 1998). These pathotypes cause diseases ranging from traveler's diarrhea to hemorrhagic colitis and hemolytic-uremic syndrome (Sidhu et al., 2013). Several studies have shown that these virulence genes are prevalent in *E. coli* isolates cultured from a variety of aquatic ecosystems and potable water sources and that treatment of these sources are strongly encouraged (Hamelin, et al., 2007; Ahmed et al., 2011; Sidhu et al., 2013).

In addition to isolating potentially pathogenic bacteria, parasitic protozoans (*Giardia*, *Cryptosporidium*, *Endolimax*, and *Entamoeba*) and eggs of the helminthe *Ascaris* were detected in several water and soil samples (Table 3). While the majority of acute diarrheal diseases are caused by bacteria, common chronic diarrheal infections are caused by protozoans. The protozoans identified in this study are among the most common protozoans that affect human health worldwide (Okhuysen, 2001). Protozoans are physiologically and morphologically diverse and therefore have differing effects on their human host. Ingestion of *Giardia* cysts (a flagellated protozoan) can result in varying infections that range from asymptomatic infections to chronic infections involving gastrointestinal complaints (abdominal cramping) with intermittent diarrhea (Okhuysen, 2001). *Cryptosporidia* are intracellular parasites belonging to the subclass Coccidiasina and are characterized by a complex life cycle that includes merogony, gametogony, and sporogony, the stage that takes place primarily in an environmentally resistant oocyst (Current & Garcia, 1991). Individuals become infected with *Cryptosporidium* when they ingest the thick-walled oocysts. Sporozoites excyst and invade the epithelium of the intestinal tract leading to profuse and watery diarrhea containing mucus and can be accompanied by abdominal pain, nausea, vomiting and a low grade fever. In most cases involving immune competent individuals, the infection is self-limiting, but the nutritional status of these individuals, particularly children, is a major factor that determines severity, length, and prognosis of this

disease (Current & Garcia, 1991). The pathogenesis of gastrointestinal *Cryptosporidiosis* in the heavily infected immune compromised (for example HIV-infected individuals) follows a pattern of increasing severity over time, including dehydration, malnutrition, and wasting that leads to death (Okhuysen, 2001). Finally, children infected with *Ascaris lumbricoides* have been linked to poor physical and cognitive development and morbidity (Chan, 1997). *Ascaris* is believed to infect more than a billion people world-wide and has been detected among Haitian school children (Fox et al., 2005). While *Ascaris* was the only parasitic helminthe detected during this sampling event, it raises concerns about the possible presence of other parasitic worms such as *Wuchereria bancrofti* and *Trichuri trichiura,* which are also debilitating diseases among young children (Fox et al., 2005).

Groundwater is less vulnerable to contamination by microbial pathogens than surface water and is often deemed safer for consumption, but there are several ways in which well water can be impacted by pathogens. Possible routes of microbial contamination of groundwater relevant to the Pignon Region include 1) poorly constructed wells that include faulty casings, inadequate covers, or lack of concrete pads such that contaminated surface water is introduced into the well, and 2) infiltration of contaminated recharge water (United States Environmental Protection Agency, 2002). Assuming proper well construction, a source of pathogens detected in well samples during this study may be the infiltration of contaminated recharge water to the shallow aquifer providing water to the wells. Well-water-borne disease outbreaks have been linked to extreme rainfall events particularly those preceded by rain deficits (Curriero et al., 2001). Pathogen contaminated water in saturated surface soils can readily mix with groundwater after substantial rain fall events particularly if the water table is shallow and approaching ground surface during the event. If extreme rainfall events are a contributing factor of well contamination, residents using these wells must be made aware that poor water quality may follow the event and treat the water accordingly before ingestion. Another source of contamination of well water is biofilms that develop over time in the distribution system of the well (pipes and pumps). Proper maintenance of the well's distribution system is required to remove biofilm and any potential pathogens that exist in these microbial communities.

Exposure to water-borne pathogens does not affect all individuals in the same manner. For individuals who have no underlying condition, are asymptomatic, or who are immune competent, repeated exposure to a pathogen can result in a less severe infection or in some cases acquired immunity. Unfortunately, these individuals can represent a secondary source of infection for at-risk individuals such as the malnourished, young children, the elderly, and the immune compromised populations (WHO, 2006). This study provides data for only a single sample during one sampling event for each source. It is strongly recommended that water should be routinely tested throughout the year in order to highlight trends in water quality that may be related to seasonal and climatic conditions. In the meantime, to avoid potential health affects related to the consumption of contaminated water, treatment of water before consumption is strongly encouraged.

The best way to treat microbially-contaminated water is the use of heat. The general recommendation when using heat for decontaminating water is a full boil for at least one minute (2 minutes for elevations above 2,000 ft). This treatment will kill viruses, bacteria and parasites (CDC, 2012). While this method will not deactivate bacterial endospores, most common pathogens are killed at boiling temperatures. Boiling provides an advantage over other heating methods because it does not require any other equipment (like a thermometer) to ensure that an appropriate temperature has been reached. Unfortunately, fuel for boiling water in Haiti is scarce.

The following currently are the most reliable, accessible, and cost-effective alternatives to boiling.

Solar Water Disinfection (SODIS): SODIS involves storing drinking water in transparent plastic bags or plastic or glass bottles in direct sunlight for time periods ranging from 8 h (during full sun conditions) to 2 days (during cloudy conditions) prior to consumption. SODIS has been deemed a simple and cost-effective means to render contaminated water safer for consumption by reducing the numbers of gastrointestinal disease-causing organisms. It is a combination of thermal heating, photo-oxidative destruction, and UV damage to DNA that is thought to bring about microbial killing (Heaselgrave and Kilvington, 2010). Boyle et al. (2008) were successful at reducing the number of *Campylobacter jejuni* in 20 min, enteropathogenic *E. coli* (EPEC) in 90 min, and *Yersinia enterocolitica* in 150 min to below the limit of detection (17 CFU/ml) under simulated strong natural sunlight conditions (approximately 1,050 $Wm^{-2} \pm 10\ Wm^{-2}$). Similarly, Heaselgrave and Kilvington (2010) found that the vegetative cells of *Escherichia* coli, *Fusarium solani*, *Candida albicans,* and *Acanthamoeba polyphaga* trophozoites were killed by SODIS after 2-6 h exposure to 150 Wm^{-2} irradiation, but cysts of *A. polyphaga* were not destroyed under these conditions. Only after treatment with 250 Wm^{-2} and with the addition of riboflavin (250 µM) were cysts deactivated after 2 h exposure. The mechanism by which protozoan cysts were deactivated in the presence of riboflavin is not clear, but it is believed that riboflavin induces the generation of oxygen radicals that attack the cyst wall (Heaselgrave and Kilvington, 2010).

It is not clear if regular SODIS conditions would be sufficient to deactivate bacterial endospores or other protozoan cysts, but achieving and maintaining temperatures of 55 ℃ for a 7 h time period rendered *E.coli* (2.0 x 106 cfu/ml) contained in a 2L uncolored plastic bottle unviable (Joyce et al., 1996). Mendez-Hermida et al. (2005) found that exposing 10 ml distilled water containing 107 *Cryptosporidium* oocysts in polystyrene containers to 840 Wm^{-2} for 6 or 12 hours (water temperature reached and maintained was 40 ℃) was sufficient to reduce oocyst infectivity from 100% (controls) to 7.5% and 0.0%, respectively.

The efficacy of SODIS is significantly affected by turbidity, a measure of the relative clarity of a fluid, and represents a challenge for decontaminating water using SODIS. Turbidity is caused by suspended particulate matter. Particulate matter reduces the amount of light that can penetrate into the water negatively affecting the extent of heating and therefore decontamination (Joyce et al., 1996; Caslake et al., 2004). Turbid waters can be decontaminated if temperatures greater than 55 ℃ can be attained and maintained for longer periods of time (Joyce et al., 1996) or pretreatment by filtration and/or flocculation (process of precipitating particulates out of solution into small clumps) (CDC, 2012).

Chemical Disinfection: The most common chemical for treating water is chlorine. A 1.5% v/v (final solution) of sodium hypochlorite (household bleach) in clear water is the treatment method for developing countries promoted by the CDC and WHO. This is approximately equivalent to adding 10 drops of a 5.25-8.25% (v/v) solution of house hold bleach to 1 liter of clear water. Like SODIS, successful decontamination of water using hypochlorite is dependent on the turbidity of the water. Under highly turbid conditions, additional chlorine and contact time is required to successfully decontaminate water. While bacterial pathogens are easily killed by chlorination, some viruses and protozoan cysts, specifically *Cryptosporidium parvum* oocysts, are resistant to routine chlorination regardless of the degree of turbidity (Powers et al., 1994, Kahler et al., 2010). If water is contaminated with protozoans, water must be filtered prior to chemical treatment and ingestion.

Iodine is another commonly used chemical for disinfecting water. Its mode of action is similar to that of chlorine, but it is less effective under turbid water conditions (Powers et al., 1994). In combination with flocculation and filtration, both chemical treatments represent reliable, cost effective, and easily implemented methods for decontaminating water. Both chemicals are available in easy to use

tablet forms which may include a flocculant, but there are potential health risks associated with both treatments. Long-term exposure effects of chlorine and chlorine by-products are thought to pose a serious risk to human health (Nieuwenhuijsen et al., 2000) causing bladder and rectal cancer (McGeehin et al., 1993; Hildesheim et al., 1998). Iodine is a biologically active chemical and can cause thyroid issues. Extended use of iodine as a disinfectant for water is not recommended and pregnant women are advised to not to drink iodine-treated water (CDC, 2012). It should also be noted that if biofilms are the source of contamination, chemical treatment may have little effect on the viability of organisms associated with the biofilm community because the biofilm matrix creates an environment that protects the microbial inhabitants (De Beer et al., 1994: Chambless et al., 2006).

Conclusions

Based on the data collected during this study, none of the water sources sampled meet the EPA and WHO Drinking Water Standards. Several of the sources (Mission Front, Mission Back, Pignon #2, Matabonite #1, Matabonite #2, Tereblanche, and Tetechange Spring) do meet the USEPA guidelines for recreational use, suggesting that the water is suitable for bathing. Organisms isolated and tentatively identified in this study represent potential pathogens and individuals most at risk for acquiring diseases associated with these organisms should consider treating water before ingestion, and in some cases before bathing. It must be emphasized that this study provides data for only one sampling event. To better assess the quality of water throughout the Matabonite Region and in Jacsonville, identify trends in water quality as result of seasonal and climatic events, and provide specific details on how to best treat the water, the following actions are suggested:

1. Acquire a comprehensive dataset that details water quality over time by routinely sampling and enumerating fecal coliforms, *Enterococci* and/or *E. coli*, from water sources in the region at least quarterly and after significant rainfall events:
2. Collect turbidity data during water sampling; and
3. Analyze water samples for the presence of other contaminants (for example, nitrate, phosphate, metals, and halogens).

The regular sampling and assessment of water quality could be performed by members of the community with minimal training and equipment. Data collected after routine sampling could be used to determine if water treatment is necessary, and if warranted, what pretreatments (filtration and/or flocculation) are needed. It would also be advisable to bring in well drilling expertise to inspect the drinking water wells and make repairs or disinfect the wells, as necessary. This would allow for an assessment of whether the wells could be rehabilitated to provide better quality drinking water. With a comprehensive water quality program that integrates data related to disease outbreaks and incorporates health statistics of individuals (particularly with the identification of at-risk members of the community), appropriate drinking water and recreational standards could be developed and implemented by the members of the community of Jacsonville and the Matabonite Region.

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