CHOOSING APPROPRIATE INDICATORS OF WATER QUALITY FOR PUBLIC HEALTH RESEARCH IN THE TROPICS

A Proposal to the Center for Occupational and Environmental Health
Student Project Award Program

Submitted By:

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ABSTRACT

Despite the dedication of a decade (1981-1990) by the United Nations General Assembly to water and sanitation issues, waterborne illness is still the third leading cause of mortality and morbidity in the developing world (WHO 2002). Yet waterborne illness is largely preventable, especially with a more sophisticated understanding of how it is spread.

Elucidating transmission patterns of waterborne pathogens requires an understanding of environmental contamination. Measuring pathogens directly in the environment, however, is inefficient and costly. Surrogate organisms have therefore been used to indicate the presence of fecal contamination. There exists extensive literature on identifying appropriate indicator organisms for temperate regions, but little is known about which water quality indicators are most useful in the tropical zones. Because of these climate differences, the tests most commonly used in industrialized countries may not accurately predict fecal contamination in tropical developing areas.

In response, we propose to carry out a concurrent study in the tropical regions of Ecuador and Haiti, the study regions where we will be carrying out our dissertation research. Our research during the summer field season will allow us to assess which indicator (or panel of indicators) is most suitable for our own future research in these regions on assessment of health risk factors in the environment (Karen) and effective and appropriate water treatment strategies (Sarah). What makes an appropriate indicator of water quality differs in our respective research areas. Working together will help broaden the applicability of our results, to elucidate which indicators are most effective for assessing both efficiency of disinfection and public health risk. The comparative assessment of our two sites will also allow us to contribute to the literature addressing appropriate water quality indicators for the tropics as a whole.

STATEMENT OF SIGNIFICANCE

The World Health Organization estimates that diarrhea causes 4% of all deaths and 5% of health loss to disability worldwide (WHO 2003). Most of the burden falls on children, who account for 90% of the deaths and suffer most from the aggravated malnutrition, stunting, and cognitive problems associated with waterborne illness. According to the Pan-American Health Organization, acute diarrheal disease accounts for 9% of annual registered deaths in Ecuador (PAHO 2002a) and 15% of registered deaths of children under five in Haiti (PAHO 2002b). Much of this disease is transmitted through contaminated water.

In order to combat the excessive morbidity and mortality toll inflicted by these waterborne and water-related infections, researchers need effective tools to detect the presence of pathogens and fecal contamination in waterways. Our study will help elucidate which water quality tests are most biologically, technically, and economically appropriate for use in tropical regions of the world.

This research project is a necessary first step in planning our respective dissertation and research projects. Karen, whose background is in ecology and epidemiology, is studying environmental determinants of disease in northern coastal Ecuador. Sarah, a mechanical engineer now studying environmental engineering, is studying low-tech water treatment devices in Haiti. Both of us will need to use microbiological water quality assays in our future research, and we also believe that an assessment of different water quality indicators in the tropics will make a significant contribution to the literature.

RELEVANCE TO OCCUPATIONAL OR ENVIRONMENTAL HEALTH AND SAFETY

Waterborne disease is a clear example of a factor in the environment that affects human health. This study will allow us to more effectively study the pathways by which pathogens are transmitted in waterways in order to determine the most effective measures to control their spread.

Background

Hundreds of different microorganisms have been shown to be involved in waterborne disease, making it impractical to look for every specific potential pathogen in water samples (Toranzos et al. 2002). Water quality indicators provide proxy assays to test for the presence of microorganisms or compounds that are typically found in the gastrointestinal tract of humans and other mammals. Several different microbiological indicators of water quality exist and are in common use throughout the world. However, most of these techniques were developed in temperate climates, and many of the traditional organisms that practitioners have used to assess fecal contamination of waterways (total coliforms, fecal coliforms, *E.coli*) have been found to survive in the environment (soils, streams, plant systems) in tropical areas (e.g., Hazen and Toranzos 1990, Rivera et al. 1988, Solo-Gabriele et al. 2000), bringing into question their utility as indicators of fecal contamination. Thus, there is a need to test the utility of various alternative indicators in a tropical setting.

We describe several such indicators in Appendix A, and the advantages and disadvantages of each. In addition to which indicator organisms to choose, there are also a variety of methods to test for these organisms.

No universal indicator exists to assess the presence of all of the different possible pathogen organisms in water. Viral, bacterial, parasitic protozoan and helminth pathogens are unlikely to all behave in the same way at the same points in time (Ashcroft et al. 2001). It is therefore important to understand the characteristics of each of the different tests available before employing them in research.

Also, some tests are technically or economically unfeasible for fieldwork at this point. Water testing laboratories are scarce and poorly equipped in much of the developing world. Tests requiring electricity or refrigeration are difficult to conduct in rural areas, which lack public utilities. Unfortunately, many of the simplest tests are cost prohibitive.

Specific Objectives

We propose a concurrent, collaborative research project in Ecuador and Haiti to assess the utility of various water quality indicators in these two different tropical regions.

Methodology

We will test several different types of water samples with a panel of indicators of water quality to see which indicators most effectively capture fecal contamination.

In Ecuador Karen will choose at random four different households within a single village. She will sample the following four different types of water associated with that household on two different occasions over the course of the summer.

(1) Surface water running off from the house, or water from the nearest drainage ditch;
(2) River water for household consumption (in the bucket in which it is collected);
(3) Rain water for household consumption (in the bucket in which it is collected); and
(4) Drinking water stored within the household.

In Haiti, Sarah will choose at random four community water sources for each of the following four types of water. She will sample each source on two different occasions during the summer.

(1) Well water from community wells;
(2) Uncapped spring water in the nearby hills used by community members;
(3) Public spigots; and
(4) Drinking water stored within a household.

The sampling plan is summarized in Table 1. When appropriate, water sources will be mixed before samples are collected. Each water sample will consist of three pooled sub-samples collected at the same time. All samples will be compared to a negative control (autoclaved distilled water) and a positive control (fecally contaminated water). This plan allows us to capture both spatial and temporal variability in water quality within the village. Including positive and negative controls, we will have a total of 96 tests (48 each in Haiti and Ecuador).

Table 1: Water sampling Locations

	Ecuador—4 Households	Haiti—4 Community Sources	
1.	Surface water around house	Well water	
2.	River water	Uncapped spring	
3.	Rain water	Public Spigot	
4.	Stored drinking water	Stored drinking water	
5.	Autoclaved distilled water	Autoclaved distilled water	[Negative Control]
6.	Fecally contaminated water	Fecally contaminated water	[Positive Control]

Each of the water samples will be tested using a panel of indicators. More than one testing method exists for some indicators. We have attempted to include a representation of the available methods, though some methods have been precluded from our proposed fieldwork because of extensive expense or equipment requirements. The indicators (*in italics*) and tests we will use include:

1. *E. coli and total coliforms*
   a. Membrane Filtration
   b. 3M Petrifilms
   c. Readycult Defined Substrate
2. *Fecal Streptococci and Enterococci*
   a. Membrane filtration
3. *Somatic Coliphage*
   a. Somatic phage test
4. *H_2S Producing Bacteria*
   a. H_2S presence-absence test
5. *Clostridium perfingens*
   a. Direct plating method

We will also filter 10 liter of water in the field and treat and freeze the filters for future work in Berkeley to isolate potential pathogens via the polymerase chain reaction technique.

We will follow the standard methods for each of the tests listed above (Clesceri et al. 1998). In cases where a standard method has not been specified we will follow procedures identified in the literature (e.g., Nair et al. 2001, Vail et al. 2003, Fujioka and Shizumara 1985) or manufacturer recommendations. We will practice and work out the details of each test in Kara Nelson's environmental engineering lab this spring before commencing fieldwork.

In our analysis, we will test the sensitivity and specificity of each of the different tests individually, and different combinations of groups of tests, using the positive control (fecally contaminated water) as the gold standard. Results will be presented with ROC curves to compare each indicator, or panel of indicators.

TIMELINE

All field research and water quality testing will be carried out during the summer field season, June – August 2004. Data analysis will take place upon our return to Berkeley, and should be complete by December 2004.

RESEARCH SITES

Ecuador

In Ecuador, Karen will carry out her research in the Santiago-Capayas-Onzole river system in the Ecuadorian province of Esmeraldas, an area that contributes to the high prevalence of diarrhea in the country. A new highway recently constructed along the coast has begun to connect previously remote villages in this area to the outside world, causing ensuing changes in resource extraction, land use, and human migration patterns. Construction of the road has led to intensified logging and subsequent deforestation, as well as increased migration into and out of the region.

The ongoing ecological, social, and health changes in this region make it an ideal location for researching the connections between ecology and health. Her future dissertation research on environmental determinants of disease will inform the local situation, and also add to the growing body of literature that addresses the connections between environmental integrity and human health. It will also make a contribution to the public health literature on water, sanitation, and hygiene studies.

Her research will be carried out in conjunction with Principal Investigator Joe Eisenberg's larger U.C. Berkeley research project funded by the U.S. National Institutes of Health, in collaboration with Universidad San Francisco de Quito and Trinity College. This project is investigating the ways in which environmental and social change associated with road-building affect the distribution of waterborne human diseases in the region.

She will work with a team of researchers working on the larger umbrella project, and will share a field laboratory set-up with the Ecuadorian microbiologist who is carrying out bacteriological analysis of stool specimens in the field. She carried out preliminary research in the region during June-August, 2003, so is familiar with the study site and facilities available to her in the villages in which she will be working.

The testing of water quality samples proposed here will not involve human subjects, but she has submitted an application to the U.C. Berkeley Committee for the Protection of Human Subjects for her other research.

Haiti

Haiti is not only the poorest nation in the western hemisphere, but it also ranks last on the Water Quality Index according to a recent report that considered water supply, drinking water quality, water use and management, and the health of natural water bodies (CEH Wallingford, 2002). Only 45% of rural Haitians have access to WHO defined "improved" drinking water source and 16% have access to adequate sanitation facilities. The under-five mortality rate is 12% (UNICEF, 2002).

Since 1998, Sarah has been working in the rural town of Borgne, Haiti with Haiti Outreach: Pwoje Espwa (H.O.P.E.), a non-governmental organization that came to Borgne to help with development projects at the request of a former resident who had immigrated to the US. At a series of community meetings, the people of Borgne set their development priorities at health, education, and economic development. The first project was a health clinic.

Diagnostic data from the H.O.P.E. Health Clinic, which sees over 7,000 patients per year, indicate that at least a quarter of all patients come to the clinic suffering from preventable waterborne illnesses. In June 2003, Sarah helped establish the Sant Teknoloji: Bwase Lide (Idea Exchange Technology Center)—an attempt to attack waterborne illness at its source. The center's goals are to inspire community involvement and creativity in environmental problem solving by introducing people to technologies used in other parts of the world, inviting local people and international students to collaborate to invent and test new design ideas, providing a space for community discussion on how to implement technologies, and providing support for implementation and dissemination projects. The center houses educational materials and examples of simple, inexpensive technologies that can be used to break the waterborne pathogen cycle.

One of these simple technologies is the UV-Tube, an effective, simple, inexpensive, passive, fast, reliable method of water treatment that uses ultraviolet (UV) light to eliminate microorganisms and doesn't change the taste of the water. Sarah has been working at the Energy and Resources Group's Renewable and Appropriate Energy Lab at U.C. Berkeley to help develop the UV-Tube technology.

The Sant Teknoloji: Bwase Lide is very interested in testing community water sources for fecal contamination as part of their educational program to promote better hygiene, sanitation practices, and water treatment. They are currently using the H2S method for water testing, but are concerned about its accuracy. The UV-Tube project is also in need of testing techniques to determine if the UV-Tube technology is effectively inactivating microorganisms.

Sarah will be in Haiti this summer to conduct a design seminar for Sant Teknoloji staff and other interested parties to investigate and develop solutions to the local sanitation problems. The Sant Teknoloji is already undertaking water testing, so sample collectors could easily take the larger samples necessary for this comparison study if provided with appropriate containers. The H.O.P.E. clinic has offered the use of their laboratory to support more extensive water testing after clinic hours. The testing of water samples described here does not involve human subjects.

ROLE OF RESEARCH PARTNERS

Both research partners will carry out the same study concurrently at their respective field sites in Ecuador and Haiti, and they will collaboratively analyze the data and write up the results at Berkeley during Fall semester, 2004. There is a synergy in Karen's work on the assessment of risk factors for diarrheal disease through environmental sampling, and Sarah's work to test appropriate technology solutions for the treatment of contaminated drinking water. Both are extremely significant and necessary for the advancement of public health in tropical developing countries.

We believe that we have much to learn from one another through collaborating on this research. Not only will the results of a combined study help strengthen the robustness of each of our findings, but by working together we can gain insight into our own research plans. Karen has already been spending time this year learning laboratory techniques from Sarah and other members of Kara Nelson's lab group. Sarah will learn how to design proper epidemiological studies for her future intervention trial research with low-tech water treatment devices, and Karen will learn how to design research that will best inform engineers planning water treatment interventions.

REFERENCES CITED

Clesceri, L.S., A.E. Greenberg, and A.D. Eaton, eds. 1998. Standard Methods for the Examination of Water and Wastewater, 20th Edition. Washington, D.C.: American Public Health Association.

Ashbolt, N.J., W.O.K. Grabow and M. Snozzi. 2001. Indicators of microbial water quality. Chapter 13 in Fewtrell, L. and J. Bartram, eds. Water Quality: Guidelines, Standards and Health. London, UK: IWA Publishing (for the World Health Organization).

CEH Wallingford (for the UK Department for International Development.) (2002) Using the Water Poverty Index to Monitor Progress in the Water Sector. Contract Number IUDDC24.

Fujioka, R.S. and L.K. Shizumara. 1985. *Clostridium perfringens*, a reliable indicator of stream water quality. J. Water Pollut. Control. Fed. 57: 986-992.

Hazen, T.C., and G.A. Toranzos. 1990. Tropical source water. Pages 32-54 in G.A. McFeters, ed. Drinking Water Microbiology. New York: Springer-Verlag.

Nair, J., R. Gibbs, K. Mathew, and G.E. Ho. 2001. Suitability of the H_2S method for testing untreated and chlorinated water supplies. Water Science and Technology 44(6): 119-126.

Pan-American Health Organization. 2002a. Regional Core Health Data System—Country Health Profile 2002: Ecuador. http://www.paho.org/English/DD/AIS/cp_218.htm. Accessed 9/19/2003.

Pan-American Health Organization. 2002b. Regional Core Health Data System—Country Health Profile 2002: Haiti. http://www.paho.org/English/DD/AIS/cp_332.htm. Accessed 3/25/2003.

Rivera, S.C., T.C. Hazen, and G.A. Toranzos. 1988. Isolation of fecal coliforms from pristine sites in a tropical rain forest. Applied Environmental Microbiology 54: 513-517.

Solo-Gabriele, H.M., M.A. Wolfert, T.R. Desmarais, and C.J. Palmer. 2000. Sources of *Escherichia coli* in a Coastal Subtropical Environment. Applied and Environmental Microbiology 66(1): 230-237.

Toranzos, G.A., G.A. McFeters, and J.J. Borrego. 2002. Detection of Microorganisms in Environmental Freshwaters and Drinking Waters. Chapter 18 in Hurst, C.J., R.L., Crawford, G.R. Knudsen, M. J. McInerney, and L. D. Stetzenbach, eds. Manual of Environmental Microbiology, 2nd edition. Washington, D.C.: American Society for Microbiology Press.

UNICEF. (2002, Accessed 03/11/04) Statistics At a Glance: Haiti. [on-line] http://www.unicef.org/infobycountry/haiti_statistics.html.

Vail, J.H., R. Morgan, C.R. Merino, F. Gonzales, R. Miller, and J.L. Ram. 2003. Enumeration of Waterborne *Escherichia coli* with Petrifilm Plates: Comparison to Standard Methods. Journal of Environmental Quality 32: 368-373.

World Health Organization (WHO). (2002). "The World Health Report 2002." [on-line] www.who.int/whr/2002/en. Accessed 2/25/04.

World Health Organization (WHO). 2003. Water-related diseases. http://www.who.int/water_sanitation_health/diseases/diarrhoea/en/. Accessed 9/19/2003.

BUDGET

Equipment	Cost Each	Number	Total
Incubator (Amy Smith)	$ 200.00	2	$ 400.00
Igloo Cooler with ice pack	$ 36.00	2	$ 72.00
Hand vacuum pump	$ 75.20	2	$ 150.40
Membrane Filtration Unit	$ 400.00	2	$ 800.00
Portable UV-A light	$ 30.00	2	$ 60.00
Thermometer	$ 14.30	2	$ 28.60
Bunson burners (pack of 6)	$ 129.93	1	$ 129.93
		Total Equipment=>	**$ 1,640.93**

Lab Supplies		Total
Pipettes (variety)		$ 389.90
Sampling and testing bottles		$ 302.27
Other labwear		$ 40.34
	Total Lab Supplies=>	**$ 732.51**

Testing Supplies for	Cost per Sample	Number of Samples	Total
E.coli and Total Coliform			
3M Petrifilm	$ 1.28	96	$ 122.88
Ready Cult Defined Substrate MPN	$ 4.75	96	$ 456.00
Membrane filtration	$ 7.37	96	$ 707.52
Fecal Streptococci			
Membrane filtration	$ 2.48	96	$ 238.08
H2S Producing Bacteria			
H2S Test P/A	$ 1.40	96	$ 134.40
Somatic phages			
Somatic phage test	$ 5.66	96	$ 543.36
Clostridium perfingens			
Direct Plating Method	$ 3.07	96	$ 294.72
Polymerase Chain Reaction			
Sample storage	$ 1.35	96	$ 129.60
		Total Testing Supplies=>	**$ 2,626.56**

TOTAL REQUESTED
=> $ 5,000.00

BUDGET JUSTIFICATION

Equipment includes one-time purchases required to test samples that we do not currently have available to us for use in the field. Lab supplies include sample bottles and consumable supplies (pipettes, gloves, markers, etc.) that are used for more than one of the tests and therefore are listed separately from costs per sample. Costs per sample include the consumable costs for each test (reagents, filters, Petri dishes, etc.). We can supply a spreadsheet detailing the costs per sample upon request.

Travel and living expenses will be covered by other funding sources.

Appendix A: Indicators of Microbial Water Quality Currently in Use (Source: Toranzos et al. 2002)

WQ Indicator	Description & Advantages	Disadvantages
Total coliforms	Used as indicators of possible fecal contamination or water pollution from sewage. Can originate from the intestinal tracts of homeothermic animals.	- Not specific to fecal pollution - Can originate from nonenteric environments (biofilms, algal-mat communities, wood industry wastes)
Fecal coliforms (a.k.a. thermotolerant coliforms)	Subset of total coliforms that grow and ferment lactose with the production of gas and acid at 44.5 ± 0.2°C. Excellent positive correlation with fecal contamination from warm-blooded animals.	- Have been isolated from environmental samples in the apparent absence of fecal pollution
Escherichia coli	Subset of fecal coliforms more specific for the presence of fecal contamination from warm-blooded animals. Easy to identify due to unique enzymatic reactions.	- May survive in the environment in the tropics
Fecal streptococci and enterococci	Gram positive bacteria that show a high and close relationship with GI health hazards. Accepted indicators of WQ because they are not as ubiquitous as coliforms, are always present in feces of warm-blooded animals, are unable to multiply in sewage-contaminated waters, die off less rapidly than coliforms in water, and show persistence patterns are similar to those of potential waterborne pathogenic bacteria.	- Taxonomy of the group is still being debated
Somatic coliphages	Specific viruses of *E.coli* that show direct correlations with the presence of enteric viruses. Recommended by Toranzos et al. 2002 for the tropics	- More suitable for assessing viral contamination that bacteriological contamination
FRNA phages	F (male)-specific RNA bacteriophages that are unable to replicate in the water ecosystem since pili are not synthesized below 30°C. These viruses are similar in size, shape, and genetic makeup to human enteric viruses, are more stable than human enteroviruses in environmental waters and more reistant to disinfection, and concentrations found in environmental waters correlate with sewage contamination.	- Better as an index of sewage pollution rather than fecal pollution - Not suitable for the tropics because waters may reach temperatures above 30°C.

Phage of strain HSP 40 of *Bacteroides fragilis*	*B. fragilis* is a strict anaerobe found in high concentrations in the human GI tract, and dies rapidly in environmental waters. The phage of strain HSP 40 is human-specific, consistently isolated from polluted but not unpolluted waters, shows levels related to pollution degree, always outnumber human enteric viruses, and no replication has been seen in (simulated) environmental conditions	- Low occurrence in waters with low and moderate levels of fecal pollution - Complex methodology for their recovery
Clostridium perfringens	One of a class of sulfite-reducing clostridia that reduce sulphite to H_2S.	- Ubiquitous in aquatic sediments - Long persistence in the environment compared to enteric pathogens
H_2S Producers	Extremely easy test to perform. Does not require incubators, sophisticated equipment, or trained personnel, therefore good alternative for rural, remote areas	- No corellation with coliforms
Fecal Sterols	Specific saturated sterols that can be used as a molecular signature of fecal contamination in water because of the relationship between its concentration and the degree of fecal pollution. Specific to the feces of higher animals, and biodegradable.	
Molecular methods	PCR techniques can be used to detect gene sequences of particular pathogens.	- No regard for bacterial viability, because get fragments