Preliminary Report: Bacteriological Testing of 2 wells in Bonaire

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Introduction

Contamination of ground water by bacteria is a significant public health concern. The current practice of dumping untreated sewage in unlined ditches at LVV in Bonaire is of particular interest. In addition to the ditches, in many households sewage is put directly into the earth in cesspits that contaminate groundwater. Open sewage pits will contaminate ground water and surface water through runoff and percolation events (Campagnolo et al. 2002; Krapac et al. 2002). In Bonaire, there is little surface water to contaminate in terms of fresh water, but the nearshore marine waters of the Bonaire National Marine Park are at risk of contamination by way of groundwater seepage. The central area of the island where the ditches are located is characterized by shallow soils that overlay either limestone (fossil coral reefs) or the volcanic rock of the Washikemba formation. Although percolation rates will differ depending on the underlying rock, groundwater can move to the sea very quickly on small islands through cracks and fissures in otherwise impervious rock. Subsurface particles, including bacteria, move with flowing groundwater. So in addition to human health concerns from the contamination of wells used for livestock and other uses, there are implications for any area where contaminated groundwater is entering the marine environment.

Enterococci are found in high numbers in the feces of humans and other warm-blooded animals. The presence of this type of bacteria in the water is an indication of fecal contamination and the possible presence of enteric pathogens (US EPA 2003). The density of enterococci in water is positively correlated to swimming-associated gastrointestinal disease (Cabelli 1979). As the number of enterococci in water increases, the number of cases of acute gastrointestinal illness increases. Although some types of bacteria do not live long when exposed to seawater, enterococci bacteria are very adaptable to marine waters and are monitored by public health officials in many countries to ensure the safety of recreational users of the marine and freshwater environment (Brudnick et al. 1996; Cabrelli 1982).

Methods

The Enterolert™ system (IDEXX Laboratories, Westbrook, USA) is accepted by the United States Environmental Protection Agency as a quantitative test for determining the number of colony-forming enterococci bacteria in a 100 ml sample of marine or freshwater. A ditch at LVV, a pond by Well # 1 and Well #1 was sampled on 14 November 2009 (Figure 1). Well #1 is located 1.4 km from LVV to the southeast. Well #1 was sampled again on 15 November 2009 to confirm the positive results. Well #2 is located 3.7 km from LVV, also to the southeast, and was sampled on 15 November 2009. A 100 ml sample of well water was collected in lab-certified, sterile plastic sample bottles at the LVV ditch, the pond by Well #1, and Well #1. Three samples were taken the following day at Well #1 and 3 samples were taken at another well further away from LVV, Well #2. Taps at the wells were turned on and samples were taken after a minimum of 2 minutes of water flow from the pipe. Samples were placed in a small ice box to keep them cool but were not in direct contact with the ice. Tests were run within 2 hours of collecting samples.

Figure 1. Google earth map of the central region of Bonaire indicating the well sites that were sampled on 14 and 15 November 2009.

The procedure for detecting enterococci in well water samples is as follows: one package of powdered Enterolert reagent was added to the 100 ml sterile, non-fluorescing sample bottle containing the well water sample. The powder was dissolved by shaking the bottle and the sample-reagent combination was then poured into a Quanti-Tray, a sterile plastic disposable panel containing 48 large and 48 small wells. The tray was then mechanically sealed with a machine that seals and distributes the sample into the cells. The trays are placed into an incubator at 41 degrees C for 24 h. Test results were read under a black light in a box in the lab. Each well was inspected for the presence of fluorescence, which indicated the presence of enterococci. Non-fluorescing cells were considered a negative result. Most probable numbers (MPN) were determined based on the number of positive large and small cells using the table provided by the manufacturer. In this way the number of colony forming units of enterococci was determined per 100 ml of sample water.

Results

LVV

The water sample from the ditch at LVV was positive for enterococci bacteria. The most probable number was 123.6 colony forming units (cfu)/100 ml.

Pond by Well #1

The water sample from the pond adjacent to Well #1 was positive for enterococci bacteria. The most probable number was 31.8 cfu/100 ml.

Well #1
The sample from 14 November 2009 indicated that enterococci bacteria were present at the well. The most probable number was 8 cfu/100 ml. The most probable numbers of the 3 samples taken on 15 November 2009 were 48.0 cfu/100 ml, 48.5cfu/100 ml and 75.4 cfu/100 ml.

Well #2
The samples from 15 November 2009 indicated that enterococci bacteria were present at Well #2. There were 571.7 cfu/100 ml, 755.6 cfu/100 ml and 791.5 cfu/100 ml. The counts were considerably higher than Well #1, even though the well is further from LVV indicating possibly that there are multiple sources of enterococci or that there is a crack of fissure in the rock that is allowing the movement of groundwater from LVV to the area of Well #2.

Discussion
Enterococci bacteria were found in all of the samples collected including the LVV ditches, the surface water (pond) and the water from the wells. A positive result for well water is reason for further examination of the bacterial levels and types of bacteria present in the surface water, ground water and marine waters of Bonaire. The 3 samples from Well #2 and one of the samples from Well #1 exceed the single sample standard of 61 cfu/100 ml for recreational waters (US EPA 2000), indicating that human contact with water from these well is not advisable. Kay et al. (1994) showed that the presence of fecal enterococci in water can be used to predict gastroenteritis among bathers in the United Kingdom. The positive relationship between enterococci and gastroenteritis has been confirmed by numerous other researchers (Wade et al. 2003, Prüss 1998). In addition, Fleischer et al. (1996) showed that exposure to fecal streptococci predicts acute febrile respiratory illnesses.

The results of this study are important to the health of the people of Bonaire. They indicate the presence of a harmful type of bacteria, occurring in very high numbers, in the surface water and groundwater. The groundwater is connected to the sea and movement of enterococci bacteria from land to sea is a significant concern as well because enterococci tolerate high salinity environments well.

References
Budnick, G.E., R.T. Howard and D.R. Mayo. 1996. Evaluation of enterolert for enumeration of enterococci in recreational waters. *Appl Environ Microbiol.* 62:3881-3884.

Cabelli, V. J., A. P. Dufour, M. A. Levin, L. J. McCabe, and P. W. Haberman, 1979. Relationship of Microbial Indicators to Health Effects at Marine Bathing Beaches. American Journal of Public Health. 69:690-696

Cabelli, V.J. 1983. Health effects criteria for marine recreational waters. EPA-600/1-80-031. US Environmental Protection Agency, Research Park Triangle, N.C.Prüss, A. 1998. Review of epidemiological studies on health effects from exposure to recreational water. International Journal of Epidemiology 27(1): 1-9.

Campagnolo ER, Johnson, K R, Karpati A, Rubin, C S, Kolpin, D W, Meyer, MT et al. 2002. Antimicrobial residues in animal waste and water resources proximal to large-scale swine and poultry feeding operations. Sci Total Environ 29 9:89-95.

Fleisher, J.M., Kay, D., Salmon, R.L., Jones, F., Wyer, M.D., Godfree, A.F. 1996. Marine waters contaminated with domestic sewage: nonenteric illnesses associated with bather exposure in the United Kingdom. American Journal of Public Health 86(9): 1228-1234.

Kay, D., Fleisher, J.M., Salmon, R.L., Jones, F., Wyer, M.D., Godfree, A.F., Zelenauch-Jacquotte, Z., Shore, R. 1994. Predicting likelihood of gastroenteritis from sea bathing: results from randomised exposure. Lancet 344(8927): 905-909.

U.S. Environmental Protection Agency. 2009. Review of published studies to characterize relative risks from different sources of fecal contamination in recreational water. Office of Water, U.S. Environmental Protection Agency, Washington, DC 20460. EPA 822-R-09-001

U.S. Environmental Protection Agency. 2003. Guidelines Establishing Test Procedures for the Analysis of Pollutants; Analytical Methods for Biological Pollutants in Ambient Water; Final Rule. Office of Water, U.S. Environmental Protection Agency, Washington, DC 20460. EPA-815-R-06

Wade, T.J., Pai, N., Eisenberg, J.N.S., Colford, J.M. 2003. Do U.S. Environmental Protection Agency water quality guidelines for recreational waters prevent gastrointestinal illness? A systematic review and meta-analysis. Environmental Health Perspectives 111(8): 1102-1109.