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Drivers of Water Quality Variability in Northern Coastal Ecuador

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Abstract

The microbiological safety of water is commonly measured using indicator organisms, but the spatiotemporal variability of these indicators can make interpretation of data difficult. Here we systematically explore variability in *E.coli* concentrations in surface source and household drinking water in a rural Ecuadorian village over one year. We observed more variability in water quality on an hourly basis (up to 2.4-log difference) than on a daily (2.2-log difference) or weekly basis (up to 1.8-log difference). *E.coli* counts were higher in the wet season than in the dry season for both source (0.42-log difference; $p < 0.0001$) and household (0.11-log difference; $p = 0.077$) samples. In the wet season, a one-cm increase in weekly rainfall was associated with a 3% decrease ($p = 0.006$) in *E.coli* counts in source samples and a 6% decrease ($p = 0.012$) in household samples. Each additional person in the river when source samples were collected was associated with a 4% increase ($p = 0.026$) in *E.coli* counts in the wet season. Factors affecting household water quality included rainfall, water source, and covering the container. The variability can be understood as a combination of environmental (e.g., seasonal and soil processes) and other drivers (e.g., human river use, water practices and sanitation), each working at different timescales.

Introduction

The microbiological safety of recreational and drinking waters is commonly measured using fecal indicator bacteria, with the ultimate goal of preventing the transmission of waterborne disease. However, concentrations of indicator organisms are spatiotemporally variable, and most sampling is too infrequent to transcend this granularity, making interpretation of data difficult (1). For example, in developing countries, large variations in concentrations of indicator organisms have been observed at different times of the year, especially for in-house drinking water storage containers (2). In the United States, indicator concentrations have been observed to be highly variable in near coastal waters on timescales ranging from minutes to decades (3). This variability presents challenges to the use of indicator organism concentrations to guide water quality interventions and regulations.

Seasonal changes are known to be a major source of variability in indicator data. Peaks of microbial contamination in waterways have been associated with rainfall in the nearshore marine environment (3), with rainfall and soil moisture content in subtropical rivers (4), with rainfall and suspended solids content in temperate streams (5), and with peak rainfall, peak

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streamflow events, and peak turbidity measurements in rivers in northern latitudes (6). Higher levels of fecal contamination of drinking water sources have been correlated with the rainy season or increased rainfall in Uganda (7), Malawi (8), and Gambia (9). In other locations, such as Nicaragua (10) and Sierra Leone (11), deteriorations in source water quality have been observed in the dry season, or in the transition from the dry season to the wet season (Sierra Leone (11), Nigeria (12)).

Seasonality is also known to be an important factor in determining the incidence of infectious diseases (13). Waterborne disease outbreaks have been associated with peak rainfall events in the United States (14), Canada (15), Guatemala (16), and with both high and low extremes of rainfall in Fiji (17). Increased waterborne disease has been associated with major floods in Bangladesh and elsewhere (18–20).

In addition to season, variability in water quality measurements has been noted on hourly, daily, weekly, and other timescales. For example, Boehm et al. (3) found that the concentration of fecal indicator bacteria in the nearshore marine environment in Southern California varied over time scales ranging from minutes to decades, determined by pulse contamination events, tidal processes, diurnal sun movements, lunar cycles, seasonal dynamics, El Niño dynamics, and changing human practices over time. Assessing microbial contamination at these different timescales helps distinguish true patterns from noise in water quality data. Yet this same type of study has not been undertaken in freshwater sources of drinking water, or in a developing country.

To interpret the data provided by indicator organisms it is important to understand and quantify sources of variability in their measurement in both space and time. Understanding this variability has implications for when and how often to collect water samples, for providing recommendations on whether water is safe to drink or bathe in, and for distinguishing true variability from uncertainty in measurement caused by limitations of testing technology. With a better grasp of the inherent variability in water quality samples one can ask more informed questions about how environmental drivers of water quality such as seasonal and soil processes interact with human practices such as water use, water storage, and community sanitation.

In this paper we systematically examine sources of variability in water quality measurements in source and household water samples at varying timescales over the course of one year in a rural Ecuadorian village, where high rates of diarrheal disease have been observed (21, 22). Specifically, we explore environmental drivers of seasonal, day-to-day, and hour-to-hour variability in water quality measurements of surface waters used as drinking water sources; we also examine seasonal and other drivers of variability of water quality in the home.

Experimental

Study Site

Water samples were collected in the village of Colon Eloy, a town of approximately 700 inhabitants living in 170 houses in the northwestern Ecuadorian province of Esmeraldas. Villagers use unimproved surface water from the Estero Maria, a small stream, as their primary source of drinking water. Our field staff carried out basic surveys about sanitation and water usage practices in the village. According to these surveys, 71% of people interviewed report using the stream as their primary water source, and more than half (54–69%, depending on the season) drink their water without treating it. Alternate sources of drinking water include harvesting of rainwater and use of private unprotected hand-dug wells (reported as the primary drinking water source by 10% and 13%, respectively, of

people surveyed). Some community members also import water from the larger Santiago River nearby during the dry season, when reduced flows are experienced in the Estero Maria. Inadequate sanitation infrastructure exists in this community and only 65% of houses have private latrines.

The difference in the mean temperature between the wet and dry seasons is minimal, with an average in the wet season of 27.2°C (range: 23.8–29.9 °C) and in the dry season of 26.6°C (range: 24.1–29.1 °C). Average daily precipitation, on the other hand, varies quite a bit between the wet (0.61 cm; range: 0–18 cm) and dry seasons (0.34 cm; range: 0–6 cm).

Water Sample Collection

Between January 2005 and March 2006, weekly samples were collected from five sites along the Estero Maria (shown in Figure 1), one just upstream of the village (Site 1), one upstream of the major population center (Site 2), two in the center of town (Sites 3 and 5), and one at the downstream end of the village (Site 6). One day per month, samples were taken three times on the same day at the three middle sites (Sites 2, 3, and 5). Weekly samples were also collected from seven households randomly selected using a block randomization scheme to ensure a distribution of houses throughout the village (Figure 1). To assess day-to-day variability, daily samples were collected for 11 consecutive days in the dry season from four stream sites at the same time each day at each particular site. Samples from Sites 1, 2, and 4 were collected between 9:00–12:00 and samples from Site 6 were collected between 14:00–16:00. To assess hour-to-hour variability, samples were collected every 90 minutes between 5:00 – 23:00 and every 180 minutes between 23:00–5:00 for four consecutive days, once in the wet season and once in the dry season, at Site 4. These different sampling schemes are summarized in Table 1.

Water Quality Testing

All samples were collected in Whirlpak bags (Nasco), immediately placed on ice, and processed within 24 hours. Petrifilm Coliform - *E.coli* count plates (3M) were used to detect and quantify *E.coli* colonies in the samples (23). A previous study comparing petrifilms to other established assays for *E.coli* found comparable results when 1-mL samples were consistently used (24). Petrifilms were inoculated with 1 mL of water and, because of logistical limitations of the study context, they were incubated at ambient temperatures (27°C ± 3°C) for 24 hours. If a sample was suspected of being particularly clean (rain water, treated drinking water), the test was carried out in triplicate and the results of the three tests were summed and divided by 3 mL; this occurred in 15% of the weekly samples and none of the daily or hour-to-hour samples. Non-detects were included in the analysis as one-half of the lower detection limit. The highest plate count recorded for the weekly samples over the course of the year (processed by one researcher) was 100 CFU/plate, and the highest value for the samples collected on a day-to-day and hour-to-hour basis (processed by a different researcher) was 450 CFU/plate. The plates that had too many colonies to count were therefore assigned a value of 100 CFU/plate for the weekly samples collected over the course of the year, and a value of 450 CFU/plate for the samples collected on a day-to-day and hour-to-hour basis. The number of samples above and below the detection limit for each of the sampling schemes is summarized in Table 1. In all analyses described herein that combines results from samples collected by the two different researchers an upper limit of 100 CFU/plate was assigned. The number of *E.coli* colonies/plate was multiplied by 100 to get a standardized total count per 100 mL. Possible results therefore ranged from 16.7 or 50 CFU/100 mL (halfway between zero and the lower detection limit of 33 or 100 CFU/100 mL) to 10,000 or 45,000 CFU/100 mL, depending on the analysis. These values were log₁₀-transformed for use in the analysis, because we were interested in order-of-magnitude scale variability in indicator counts.

Data Analysis

All analysis was carried out using STATA 9.0 (StataCorp LP, College Station, TX). Generalized estimating equations (GEE) were used to estimate the association between water quality (*E.coli* concentration (CFU/100 mL)) and various explanatory variables, controlling for correlation within sampling sites and within households, and using a log-linear (Poisson regression) model (25). Robust standard errors were specified to protect the inference against misspecification of this model. Explanatory variables were first modeled individually, and then combined into a multivariate model to produce adjusted coefficients.

For the source samples, environmental covariates included *weekly rainfall* (cm of rainfall summed over the calendar week), *river height* and *river clarity* (both measured weekly at Sampling Site 1), *pH*, *electroconductivity* (mS/cm), *water temperature* (°C), and *number of people in the river* at the time of sample collection. Water temperature, pH, and electroconductivity were measured at source waters at the time of collection using a handheld device (Hanna Instruments, Ann Arbor, MI). The number of people in the river was also noted. River level and water clarity were measured each week at the most upstream location in the village (Site 1). Distance from a fixed point above the river (a cement bridge) to the water level was measured to calculate river height, and a Secchi disk lowered into the water from the same point was used to determine water clarity. Values for precipitation were taken from a rain gauge (Onset Computer Corporation, Bourne, MA) in the town of Borbón, twelve kilometers away.

The association of weekly rainfall, river height and river clarity with water quality was tested with no lag and with a lag of one week in the univariate analysis, and the effect of rainfall with no lag was used in the final multivariate models. Because river level and river clarity lie in the causal pathway between rainfall and water quality, these variable were omitted from the multivariate analysis. The models were stratified by season to assess any interacting effects of season and rainfall. Based on rainfall patterns for the time period of collection, we considered July – December the dry season and January – June as the rainy season.

For the household samples, in addition to *weekly rainfall*, covariates included observed *container type* (small vs. large mouth), reported duration of *storage time* (hours), reported water *source* (rain, well, Estero Maria, or Santiago River), reported *treatment* (none, boiled, chlorinated, left to settle), and whether the container was *covered* at the time of collection. Container mouths were classified as <8 cm (plastic soda bottles and jerry cans) versus >8 cm (buckets, large water barrels, and cooking pots). Additionally, whether the stored water was used as *drinking water* in the home was tested univariately; because this variable is not a driver of water quality and because this question was only asked for half of the samples it was not included in the multivariate analysis. Information on household samples was either observed directly or reported by a key informant in the household. The Institutional Review Boards at U.C. Berkeley and the Universidad San Francisco de Quito approved protocols for interaction with human subjects.

Results

Geometric means for all samples, for source samples, and for household samples, stratified by season, are shown in Table 2. Further details for each sampling site and household are provided in Table S1 (Supporting Information). Water contamination was approximately 0.2 logs higher in the wet season than in the dry season ($p=0.0025$). A stronger seasonal difference was seen in source samples (0.42 log difference; $p<0.0001$) than in household samples (0.11 log difference; $p=0.077$). An overall trend of increasing contamination from Sampling Site 1 (upstream of the main human settlement) to Sampling Site 6 (downstream

end of the village) was also observed (nonparametric test for trend across ordered groups: $p < 0.0001$).

The variability at different timescales is shown in Figure 2. We observed seasonal differences, with overall lower counts in the dry season, but more variability occurred on an hour-to-hour basis than on a day-to-day or week-to-week basis (Figure 2a). The week-to-week sampling series ranged over a 1.5-log difference in the wet season and a 1.8-log difference in the dry season (from 164 to >10000 CFU/100 mL in the dry season (mean = 1,859; $\sigma = 2,792$) and from 308 to >10000 in the wet season (mean = 2,354; $\sigma = 1,744$)). In the day-to-day sampling series at four sites along the Estero Maria, ranges spanned a 2.2-log difference (from 17–2,800 CFU/100 mL (mean = 477; $\sigma = 502$)) (Figure 2b). Within any one given site, 1.0 to 2.2-log differences occurred from one day to the next. Counts of *E. coli* varied over the course of a single day even more than the day-to-day measurements taken at the same time each day. The hour-to-hour sampling series ranged over a 2.15-log difference in the wet season and a 2.35-log difference in the dry season. Mean counts for the hour-to-hour sample series were similar in both seasons, and ranged from 200 to $>45,000$ CFU/100 mL (mean = 5,383; $\sigma = 11,363$) in the dry season and from 300 to $>45,000$ CFU/100 mL (mean = 6,614; $\sigma = 11,401$) in the wet season (Figure 2c).

The stream overtopped its banks during the four-day sampling period in the wet season. During the flood, *E. coli* counts were high, despite its occurrence during nighttime hours, when lower counts are usually experienced (see Figure S1, Supporting Information).

The impact of various explanatory variables on water quality for source and household samples is shown in Table 3. A more complete table showing univariate analyses for all variables considered in the model selection process is shown in Table S2 (Supporting Information). As mentioned above, river level and river clarity are not included in the multivariate model because they are in the causal pathway between rainfall and water quality. The correspondence of these three variables can be seen in Figure S2 (Supporting Information). Controlling for the time of day when the sample was collected did not affect the outcome of the model, and was therefore not included.

When both seasons were combined, the adjusted coefficient of -0.03 for weekly rainfall suggests that a one-cm increase in rainfall was associated with a 3% decrease in *E. coli* counts (CFU/100mL) in source waters ($p = 0.006$). This effect was not seen in the dry season ($\beta = 0.01$, $p = 0.96$), but was evident in the wet season ($\beta = -0.03$, $p = 0.006$). Spikes of up to 25 cm in weekly rainfall occur during the wet season (Figure S2). Number of people in the river at the time of sample collection was associated with significantly elevated *E. coli* counts when both seasons were considered together ($\beta = 0.04$, $p = 0.004$), but again the stratified analysis revealed that this effect was only significant in the wet season ($\beta = 0.04$, $p = 0.026$), and not in the dry season ($\beta = 0.05$, $p = 0.315$). This suggests a 4% increase in *E. coli* counts in source waters for every additional person in the river.

Household and source water quality were loosely correlated over time (regression of weekly geometric means across sampling sites/households: $r^2 = 0.15$; $p = 0.006$) (Figure 3), although on average approximately 0.7 logs less contamination (dry season: 0.5 logs; wet season: 0.8 logs) was observed in the household samples than in the source samples (Table 2, Figure 3). In the households, rainfall was also associated with reduced *E. coli* counts ($\beta = -0.03$, $p = 0.049$). The significance and magnitude of this effect increased in the wet season ($\beta = -0.06$, $p = 0.012$) but was not observed in the dry season ($\beta = 0.11$, $p = 0.273$). This analysis suggests that a one-cm increase in rainfall was associated with a 3% decrease in *E. coli* counts in water stored in the household (6% decrease in the rainy season only).

Other factors affecting household water quality included source of water (rain and well water were protective in both the dry and wet season, whereas only rain water was protective in the wet season) and whether or not the container was kept covered (which predicted increased contamination in the dry season). A significantly higher percentage of households reported treating their water during the dry season (mean = 46%, range: 24–64%) than during the wet season (mean = 31%, range: 0–71%); (one-sided t-test: $p=0.0003$).

Discussion

Explaining Variability in Surface Source Water Quality

We observed large variations in concentrations of *E.coli* at different times of the day, week, and year in this rural Ecuadorian village. More variability was seen in water quality on an hour-to-hour basis (Figure 2c) than on a day-to-day (Figure 2b) or week-to-week (Figure 2a) basis. Despite this variability, seasonal, diurnal and spatial patterns can be discerned and explained by environmental (e.g., seasonal weather and soil processes) and other drivers (e.g., human river use, water practices), suggesting that weather variables can interact with human practices to determine water quality at the source and in households in this village.

Strong seasonal differences in microbial contamination were seen in source waters, with significantly higher average *E.coli* counts in the wet season than in the dry season (Table 2). Interestingly, despite higher contamination levels in the wet season, increased weekly rainfall was significantly associated with *reduced* contamination levels in the wet season (Table 3). This seemingly inconsistent result can be understood via a consideration of microbial processes related to soil moisture conditions, as well as an examination of the effects of rainfall at different timescales. We explore three mechanisms for how weather affects variability in microbial contamination of source waters—the runoff effect, the dilution effect, and the concentration effect—and the timescales over which they act.

The first way in which weather and season might affect *E.coli* counts in source waters is through what we call the “runoff effect.” This effect is characterized by increased microbial contamination associated with wet conditions as caused by direct flushing of fecal material from the surrounding environment into the stream following heavy rainfall, and/or mobilization of bacteria resident in soil. These phenomena can act at seasonal and short-term (hourly) timescales. *E.coli* counts in source water samples were on average 0.4 logs higher in the wet season than in the dry season. We posit that the higher overall contamination levels observed in the rainy season can be explained by transport of *E.coli* via shallow subsurface flows and scouring of riverbank soils due to variable river flows in the wet season that may release bacteria that are associated with sediments or that have survived or grown in the soil.

Several authors have found that *E.coli* and other indicators of fecal contamination can survive and regrow in tropical (4, 26–28) and even temperate soils, (29) and the ability of *E.coli* to multiply in the soil is dependent on soil moisture content (4, 27). While this microbial contamination may not all be human-sourced, poor sanitation in the village suggests the potential for at least some of it to originate from human fecal material, and more work on source tracking is necessary to establish the human health risk.

We also observed some evidence of the runoff effect on the shortest timescale measured (hours), with the immediate impact of a heavy pulse of rainfall. During the four-day intensive (hour-to-hour) sampling, increased levels of microbial contamination were observed at the onset of the flood event, during the hours of the day that normally experience lower contamination. This spike in contamination was followed by a decrease

before the flood had subsided, suggesting that existing contamination might have been flushed out of the system in the short term (Figure 2c).

The effects of flushing by rainfall is also seen in the results of the GEE analysis. This comprises the second effect we believe to be working in this system, which we term the “dilution effect,” whereby higher overall water flows dilute the concentration of *E.coli* in the stream. This effect occurs at an intermediate timescale, on the order of days or weeks. Despite the overall higher contamination levels in the rainy season, we observed a significant inverse relationship between rainfall and *E.coli* counts in source water samples. For every cm of weekly rainfall in the wet season, a 3% decrease in *E.coli* counts in source waters was observed. Thus, while soil moisture content coupled with subsurface transport may lead to overall higher levels of *E.coli* in the stream, rainfall pulses still have the effect of diluting the concentrations of these microorganisms.

Flushing is a common phenomenon in hydrology, describing a situation where solutes or other substances undergo a period of accumulation, followed by a period of flushing by a snowmelt or rainfall event. What distinguishes the processes in this system is the constant source of fecal material available in the environment due to poor sanitation. As opposed to a “first flush” effect, the runoff and dilution effects in this village are not limited to the first heavy rains of the year. The collective flushing effects observed here are thus more transport-than supply-limited (30).

Lastly, another competing, but not mutually exclusive, phenomenon is the “accruing-input effect” (a.k.a., the “concentration effect”), whereby dry conditions cause an increase in the density of microorganisms due to lack of dilution by higher flows during storm events (11). This effect occurs at a seasonal timescale. These bacteria may originate from poor sanitation, from survival and/or growth in riverbank soils, or from people bathing and washing in the stream. The concentration effect has been used to explain cases of dry season epidemics of diarrheal disease (31). We observe some evidence of this effect in the dry season, since microbial contamination is not associated with rainfall (Table 3). During the dry season (during which it still rains periodically), peaks in contamination are therefore independent of rainfall pulses, and likely driven by local contamination events that do not get flushed out of the river, as they would during higher wet rains (on a shorter timescale). *E.coli* does not regrow in the soil or get transported through subsurface flows to the extent that it occurs during the wet season. Further research quantifying the dynamics of *E.coli* populations in the soil and subsurface flows during wet and dry seasons is needed to test these hypotheses.

Interestingly, we found a significant positive association between the number of people in the river at the time of sample collection and *E.coli* counts in the wet season only. People in the river may contribute localized pulses of fecal contamination through bathing and washing in the stream, and if the accruing-input (concentration) effect were at play in this system we would expect to observe these impacts in the dry season. However, more people use the river for bathing and washing in the wet season than in the dry season, and during this particular period of study, a community-enforced ban on bathing and washing in the river was put into place in the sampling months when the river was extremely low (mid-July through mid-November). Mean number of people in the river at the time of weekly sample collection in the dry season was 0.79 ($\sigma=1.95$) versus 2.15 in the wet season ($\sigma=3.62$) (χ^2 : $p = 0.02$), which may explain why we did not observe a significant effect of people in the river during the dry season. Still, the significant relationship between people in the river and contamination levels in the wet season suggest that bathing and washing clothes in the river are likely primary sources of fecal contamination in these surface waters. This effect occurs on a short (hourly) timescale, and is supported by our observation that peak *E.coli* counts

occurred during daytime hours (Figure S1), despite the fact that solar radiation is known to reduce the concentration of fecal indicator bacteria (3).

The interaction of environmental drivers and water quality of source samples can therefore be understood as a complex interaction of various effects under different conditions at different timescales. The runoff, dilution, and concentration effects are all likely contributing to the patterns observed. It is important to note that both the runoff and accruing-input (concentration) effects are exacerbated in areas with poor sanitation, where a constant input of fecal material is available for environmental transport. Areas with poor sanitation often also have inadequate protection of drinking water sources such as surface water and shallow wells, which increases the impact of storm flows on drinking water quality.

Explaining Variability in Stored Household Water Quality

For household water samples, both seasonality (which represents temporal variability) and household-level factors (which represents a sort of spatial variability) appear to affect variability in water quality. Water source, container type (large or small-mouthed), and whether the container was kept covered were consistently significant factors affecting household water quality in both seasons, whereas rainfall was significant in the wet season, but not in the dry season.

Treatment of water by boiling, chlorination, or settling did not show a significantly protective effect against microbial contamination in the samples taken from the seven households surveyed (Table 3). In fact, chlorination was actually associated with an increase in *E.coli* concentrations. This inverse association may be due to misclassification due to respondent error (since treatment practices were recorded as reported by key informants in the household), poor post-treatment storage practices leading to recontamination of water after treatment (32), or, in the case of chlorine, incomplete disinfection due to rapid dissipation of the chlorine residual due to reactions with other compounds (e.g. organic matter) (33). In another study in the same village, the concentrations of indicator organisms in stored water of households chlorinating their drinking water were not significantly lower than in households that did not treat their water (unpublished). Boiling has also been shown to be an ineffective form of treatment, likely due to recontamination during water handling (34).

Household water samples were approximately 0.7 logs less contaminated than source water samples, both in the wet and dry seasons (Table 2), and we saw a loose correlation over time between household and source water quality (Figure 3). This correlation was likely weakened by the fact that drought conditions led some villagers to import their drinking water from the nearby Santiago River, a larger and faster flowing water source, during the dry season period of study. While the use of Santiago River water in the dry season may affect the comparison of source versus household water, the result seen here is also similar to what we have observed throughout this study region, where we have recorded significantly higher concentrations of indicator organisms in source waters than in at its point-of-use (35). This decrease may be due to settling or die-off of bacteria in the turbid source waters. This result contradicts other studies that have found water stored in the home to be significantly more contaminated than source water (32). However, most studies of contamination between source and point-of-use have been carried out in areas with improved water supplies. In locations with highly contaminated source waters, this result is less apparent (36–38).

We observed rainfall to be associated with reduced contamination in the home in the wet season. This effect may be due to reduced microbial contamination in original source waters,

a higher turnover rate of water stored in the home in the wet season, which would limit the opportunity for recontamination, or a combination of both. Interestingly, covering the storage container was associated with increased levels of contamination, especially in the dry season. This might also be related to lower turnover rates in the dry season, increasing the probability of recontamination during storage. Unfortunately we did not collect data on the frequency with which households refill storage containers in the wet and dry seasons, so we cannot comment definitively. Another possibility is that households may rely more on rain- and well-water in the wet season, both of which were significantly protective against microbial contamination (Table 3) in the wet season. Data on water treatment trends across the village show that a higher percentage of households treat their water during the dry season than during the wet season. However, as mentioned above, treatment is not always sufficient, which might explain why treatment did not improve water quality in the home during either season. Additionally, the correlation between environmental factors and household water samples may have been biased by the importation of water from the larger Santiago River, as mentioned above.

Uncertainty in Measurement

While much of the variability in contamination levels in the home and at the source can be explained by temporal variability, environmental factors, and water storage and usage practices, a large amount of uncertainty in measurement limits the conclusions drawn by any study using indicator organisms.

Measurement error may be due to the methods used to culture *E.coli*. Each petrifilm plate tests a 1-mL water sample, which limits the precision of the analysis. This limitation was especially important in assessing the lower contamination levels in the household samples (Table 1). However, studies have shown that results with petrifilms were comparable to standard methods when 1 mL samples were consistently used (24). Additionally, we found a high degree of variability in results when we quantified *E.coli* concentrations in the hour-to-hour (up to a 1.24 log difference) and day-to-day (up to a 1.88 log difference) samples using 10 mL sample volumes and processed using membrane filtration with mI agar (BD Difco) (Figures S3 and S4; see Supporting Information). We did not use these techniques on the samples collected on a weekly basis.

In this study, weekly samples were not collected at a consistent time each day because at the time we designed the study we did not consider the potential impact of the time of day of sample collection on results. Our methods were also limited by the logistical constraints of our field study site, which constrained us to incubating *E.coli* at ambient temperatures.

Applications

The results reported here have several practical applications. First, in measuring water quality, our results suggest that an analysis of more than one grab sample is needed to provide an accurate assessment of water quality at any given time. In the time period of this study, we might have come to drastically different conclusions about the quality of source waters depending on the time, day, or season when sampling occurred. From an examination of Figure 2a, it appears that the day-to-day sampling series took place during a dip in the levels of contamination during the dry season, whereas the hour-to-hour sampling series in the dry season occurred during a point of higher overall contamination. This may also be due to the consistent hour of day at which the day-to-day sampling occurred (in the morning in all but one site).

As suggested by Jensen et al. (2), the relatively low sampling frequency of water quality in epidemiological studies may fail to provide accurate estimates of the varying levels of fecal

contamination that people are exposed to through drinking water. This factor may explain in part why indicators of water quality are rarely correlated with diarrheal disease, even when interventions to improve water quality are seen to be effective in preventing diarrheal disease (39). We agree with Boehm et al. (3), who suggest that the geometric mean standard, in which the geometric mean of multiple samples collected over a specified window of time, is preferable to single samples for evaluation of water quality.

When using indicator organisms to characterize fecal contamination, it is important to characterize the variability inherent in the system, in order to be able to sample at a frequency in both space and time that provides the appropriate resolution to detect true differences between samples. For example, we were able to detect differences between seasons using our weekly data collected over the course of a year, but these seasonal patterns were not apparent when just comparing our hour-to-hour data collected over the course of four days in two different seasons, because of the high degree of variability inherent to this system. Additionally, the seasonal, diurnal, and spatial patterns we observed suggest that some sources of variability can be reduced to focus on the variable(s) of interest (e.g., samples can be collected at the same time of day, at the same location, or within a single season).

Second, while the results of this study show that water can potentially be contaminated at any time of day, there were certain hours of the day and conditions under which water was more likely to be contaminated. In the absence of more comprehensive interventions involving in-home water treatment, safe storage devices, and sanitation improvements, community health workers could encourage people to collect their water in the morning, before the onset of extensive human activity in the river, and also encourage the use of rain water when available. However, in this and other villages with inadequate sanitation that depend on untreated surface water, more significant interventions are clearly needed to prevent consumption of microbially contaminated drinking water in the village.

This study illustrates the importance of seasonal drivers on water quality at both the household and village scales. Heavy rainfall events, flooding, and increased temperature associated with global climate change are all expected to affect the incidence of waterborne diseases (40), and climate change is expected to increase the variability of rainfall. While we did not characterize the direct effects of sanitation on water quality, the main source of contamination in this village is likely feces, due to poor sanitation and use of the river for bathing and washing. Factors such as water supply and sanitation have the potential to modify the effect of seasonal weather differences on water quality in the home, and should therefore be considered when predicting the impact of climate change on diarrheal disease in areas with poor sanitation infrastructure. The effect of rainfall in other regions has been shown to vary depending on water source (10, 16). We believe that sanitation is likely interacting with rainfall in causing increased microbial contamination in unprotected wells and source waters, and should continue to be considered alongside other intervention strategies in efforts to reduce waterborne illness.

These results can also be considered in the larger context of anthropogenic changes in the landscape of this region. In 1996, a new highway was completed along the northern Ecuadorian coast to facilitate logging in the region, which is now considered one of the top 10 deforestation fronts in the world (41). Deforestation is known to affect local climatic and hydrological patterns (42, 43), and decreased vegetative cover may lead to flashier conditions, with higher incidence of both flooding and drought in the stream. In this region, higher rates of diarrheal disease, especially those with bacterial etiologies, have been observed in this region in villages closer to the new road (21), which correspond to the villages with the greatest loss of surrounding forests. The village of Colon Eloy, situated

along a secondary road that branches off of this main road, has suffered high amounts of forest clearing, and this loss of forest cover may indirectly affect water quality, by increasing variability of water flow patterns in the Estero Maria. In considering the interactions between seasonality, water quality, and human health, such upstream effects merit further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Map of Colon Eloy indicating Water Collection Sites. Source sampling sites are numbered and indicated in black boxes; Household sites are indicated with a large circle. Note that location of household sites is slightly offset in order to protect the identity of human subjects. Weekly samples were collected at Sites 1, 2, 3, 5, and 6. Daily samples were collected for 11 consecutive days at Sites 1, 2, 4, and 6. Hourly samples were collected for 4 consecutive days in the wet and dry seasons at Site 4. Arrows indicate direction of river flow.

Figure 2.

(a) Week-to-Week, (b) Day-to-Day, and (c) Hour-to-Hour Variability in Source Water Quality, as measured by *E. coli* Concentrations. (a) Geometric mean of weekly samples collected at five stream sampling sites, with results of daily and hourly sampling indicated. Note that the maximum value of log *E. coli* concentrations was capped at 4.0 for all sampling series. Geometric mean values for each season are shown with horizontal lines; (b) Daily samples collected during the dry season over the Course of 11 Days (8/12/05–8/22/05) for individual sites and overall geometric mean for all sites (shown with a thick solid line). Sites 1, 2, and 4 were sampled between 09:00–12:00 each day and Site 6 was sampled between 14:00–16:00; (c) Samples collected on an hour-to-hour basis over the course of four consecutive days, sampled at Sampling Site 4 in the dry season (Sept. 1–4, 2005) and in the wet season (Jan. 27–30, 2006). Note that in the wet season the stream flooded; the time for which it flowed at greater than bankful flow is indicated.

Figure 3. Variation in Geometric Mean Counts of *E.coli* in Water from Surface Source Waters and Household Containers over the course of the year. Geometric mean value for each type of sample is shown with a horizontal line. For log geometric mean counts of *E.coli*, upper limit of detection is 4.0 (10,000 CFU/100mL); lower limit of detection is 1.23 (17 CFU/100 mL).

Table 1

Summary of Datasets Used in the Analysis. Detection limit units are # *E.coli* Colony Forming Units per 100mL.

Dataset	Sampling Location(s)	Sampling Dates	Sampling Frequency	# Samples Analyzed	Lower Detection Limit	# Results Below Detection Limit	Upper Detection Limit	# Results Above Detection Limit
Hour-to-Hour: Dry Season	Site 4	9/1/05 – 9/4/05	Every 90 min *	82	50	0	45,000	6 (7.3%)
Hour-to-Hour: Wet Season	Site 4	1/27/06 – 1/30/06	Every 90 min *	76	50	0	45,000	5 (6.6%)
Day-to-Day	Sites 1, 2, 4, 6	8/12/05 – 8/22/05	Daily	51	50	1 (2.0%)	45,000	0
Week-to-Week: Source	Sites 1, 2, 3, 5, 6	1/28/05 – 3/2/06	Weekly	306	16.7	20 (6.5%)	10,000	50 (16.3%)
Week-to-Week: Household	7 Households	1/28/05 – 3/2/06	Weekly	980	16.7	349 (35.6%)	10,000	53 (5.4%)

* Between 23:00 - 5:00 samples were collected only every 180 minutes

Geometric Mean Values (*E.coli* CFU/100mL) by Season for weekly samples collected over 25 weeks in the dry season and 40 weeks in two wet seasons. Log₁₀ differences are calculated from the difference between the geometric mean values. Reported *p*-values are for the Mann-Whitney non-parametric test of equality of distribution between seasons.

Table 2

	OVERALL	DRY SEASON	WET SEASON	Log ₁₀ Difference	<i>p</i> -value
All Weekly Samples	375 (<i>n</i> =1,251)	293 (<i>n</i> =541)	451 (<i>n</i> =710)	0.19	0.0025
Weekly Samples: Sources Only	1,182 (<i>n</i> =238)	680 (<i>n</i> =106)	1,775 (<i>n</i> =132)	0.42	<0.0001
Weekly Samples: Households Only	260 (<i>n</i> =1,009)	224 (<i>n</i> =430)	291 (<i>n</i> =579)	0.11	0.0770

Table 3

Results of generalized estimating equation regressions testing the effect of various covariates on counts of E.coli/100 mL for a) source samples and b) household samples, controlling for correlation within sampling sites (a) and within households (b). Coefficients report results of multivariate analyses. Results of univariate analyses for the full set of variables tested can be found in Table S2 (Supporting Information). Results are shown for the entire year (Source samples: $N=291$; Household samples: $N=874$) and also stratified by season (Source samples: $N=119$ (dry season), $N=172$ (wet season); Household samples: $N=382$ (dry season), $N=492$ (wet season)).

a) SOURCE SAMPLES			BOTH SEASONS			DRY SEASON			WET SEASON		
Variable	Coeff.	p-value	95% C.I.	Coeff.	p-value	95% C.I.	Coeff.	p-value	95% C.I.	Coeff.	p-value
pH	0.04	0.770	-0.25 - 0.34	0.09	0.722	-0.41 - 0.60	-0.16	0.617	-0.78 - 0.46		
Electroconductivity (mS/cm)	-1.88	0.663	-10.33 - 6.57	0.36	0.962	-14.09 - 14.80	7.89	0.108	-1.73 - 17.50		
Water Temp (deg. C)	-0.04	0.301	-0.11 - 0.03	0.04	0.720	-0.18 - 0.26	-0.01	0.468	-0.05 - 0.02		
# People in River	0.04	0.004	0.01 - 0.06	0.05	0.315	-0.04 - 0.14	0.04	0.026	0.01 - 0.07		
Weekly Rainfall (cm)	-0.03	0.006	-0.05 - -0.01	0.01	0.961	-0.31 - 0.33	-0.03	0.006	-0.05 - -0.01		
b) HOUSEHOLD SAMPLES			BOTH SEASONS			DRY SEASON			WET SEASON		
Variable	Coeff.	p-value	95% C.I.	Coeff.	p-value	95% C.I.	Coeff.	p-value	95% C.I.	Coeff.	p-value
Container: Large-mouthed	-0.02	0.899	-0.38 - 0.33	0.35	0.300	-0.31 - 1.01	-0.32	0.094	-0.70 - 0.06		
Storage time (hours)	0.00	0.186	-0.01 - 0.00	0.00	0.897	-0.01 - 0.01	-0.01	0.111	-0.02 - 0.00		
Source: Estero Maria (baseline)											
Source: Rainwater	-0.62	<0.0001	-0.95 - -0.28	-1.10	<0.0001	-1.46 - -0.75	-0.80	<0.0001	-1.08 - -0.52		
Source: Well water	-0.93	0.001	-1.49 - -0.37	-2.49	<0.0001	-3.89 - -1.12	-0.01	0.981	-0.71 - 0.69		
Source: Santiago River	-0.27	0.390	-0.88 - 0.34	-0.13	0.772	-0.98 - 0.73					
Treatment: None (baseline)											
Treatment: Boiled	0.16	0.211	-0.09 - 0.41	0.11	0.652	-0.38 - 0.61	0.14	0.446	-0.22 - 0.49		
Treatment: Chlorinated	0.14	0.642	-0.46 - 0.74	0.43	0.102	-0.09 - 0.95	-0.19	0.723	-1.25 - 0.86		
Treatment: Left to Settle	-0.02	0.981	-1.32 - 1.29				-0.44	0.609	-2.13 - 1.25		
Uncovered (baseline) Covered	0.46	0.003	0.16 - 0.76	0.88	<0.0001	0.45 - 1.31	0.20	0.242	-0.14 - 0.54		
Weekly Rainfall (cm)	-0.03	0.049	-0.06 - 0.00	0.11	0.273	-0.09 - 0.32	-0.06	0.012	-0.10 - -0.01		