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VARIABILITY OF MICROBIAL CONTAMINATION IN GROUNDWATER AND OPTIMAL SAMPLING FREQUENCY:

EVIDENCE FROM RURAL UGANDA

Valerie Bauza, Kelsey Reeves, Chloé Poulin, Ranjiv Khush, Rachel Peletz & Caroline Delaire

With funding from the Conrad N. Hilton Foundation (CNHF), The Aquaya Institute (Aquaya) supports government agencies in selected districts of Ghana and Uganda in their efforts to achieve 100% coverage of safe, sustainable, and equitable drinking water supplies.

SUMMARY OF KEY FINDINGS

Groundwater sources in rural Uganda, such as handpumps and springs, had high variability in microbial contamination, spanning multiple risk categories.

Variability was greater across longer time scales, like months or quarters, compared to shorter timescales such as days or weeks.

Rainfall, land cover, and temperature did not explain the observed variability in microbial contamination.

Sampling a groundwater source such as a handpump or spring only once every year (or every three to five years, as recommended by WHO) is unlikely to provide reliable information on microbial contamination levels. More frequent sampling (at least bimonthly) is necessary to obtain a representative estimate.

The optimal sampling frequency may depend on applications. Monthly sampling generally provides a representative range of contamination levels. Sampling every two months may be sufficient when less precise measurements of risk are acceptable. Sampling twice a month may be necessary to capture infrequent contamination events (occurring less than 25% of the time) in groundwater sources that are otherwise generally safe.

Although less frequent sampling may not provide representative information about an individual water source, aggregating measurements across a specific geographic area can provide useful information about risk levels in that area.

STUDY IMPLICATIONS FOR WATER QUALITY MONITORING OF COMMUNITY WATER POINTS

DO'S

- Continue to test drinking water sources for microbial contamination.
- Test water sources more frequently to capture representative contamination levels, if possible.
- Aggregate water quality information from many similar water points to understand district or regional levels of contamination.
- When reporting water quality results to community members, aggregate information from multiple sources in the vicinity, or from multiple tests over time. Aggregated results are likely more representative than single test results.

DON'TS

- Don't rely on a single water quality measurement to make decisions about a water source. Microbial contamination fluctuates and a single measurement may not be representative.
- Don't rank water quality across different waterpoints based on single measurements.
- Don't refrain from water treatment based on a water quality test result. A water quality test showing no or low microbial contamination does not justify lack of treatment. Water treatment is essential to protect consumers against occasionally high microbial levels and against contamination during transport and storage.

BACKGROUND

Drinking water contaminated with fecal contamination can result in diarrhea and other illnesses, particularly in children. *E. coli* is commonly measured as an indicator of fecal contamination. Higher levels of fecal contamination are associated with higher risk of illness, as reflected in the World Health Organization (WHO) risk levels in Table 1 [1].

Temporal variability of fecal contamination in drinking water sources is common. Source water quality often varies across seasons (e.g., wet season and dry season) and in response to specific events (e.g., rainfall) [2,3]. For groundwater, rainfall and seasonal variation may affect individual aquifers differently: some aquifers experience a dilution effect (i.e., decreased concentration of pollutants) and others experience a concentrating effect (i.e., increased concentration of pollutants) in response to increased rainfall and rise in groundwater levels [3].

Despite potential temporal variability, sampling of groundwater sources in Sub-Saharan Africa is often infrequent. For example, Multiple Indicator Cluster Surveys (MICS) provide one-off sampling of water sources [1] and the WHO Guidelines for Drinking Water Quality recommend sampling and testing for *E. coli* every 3-5 years for point sources (e.g., handpumps and springs) [4]. This may be insufficient to estimate the risk associated with drinking water from sources with high variability throughout the year. However, as increased sampling frequency requires increased cost and resources, it is important to understand the minimum

frequency of monitoring required for different applications. Additionally, most prior research focused on seasonal variability, and better understanding water quality variability over shorter time scales would be helpful to inform monitoring recommendations. Prior research provided recommendations related to sampling frequency for regulatory compliance of piped systems in sub-Saharan Africa [5]; our goal is to consider recommendations for non-piped systems based on different stakeholder needs.

Table 1. WHO risk levels for fecal contamination of drinking water.

E. coli/100 ml	Log *E. coli*/100 ml	WHO risk level
<1	<0	Low risk
1-10	0-1	Medium risk
11-100	1-2	High risk
>100	>2	Very high risk

RESEARCH OBJECTIVES

Specific objectives of this work included:

1. ***Characterizing the temporal variability of fecal contamination in shallow groundwater-supplied point sources using high frequency sampling in rural Uganda.***
2. ***Examining whether rainfall, land surface temperature, and land cover were directly linked to this temporal variability.***
3. ***Determining the minimum sampling frequency required to reliably characterize fecal contamination of groundwater point sources.***

METHODS

Data collection

We collected regular samples from five drinking water sources in Kabarole District of rural Uganda over four months and analyzed all samples for *E. coli*, an indicator of fecal contamination. We collected these samples four days a week (Monday through Thursday) for five weeks during the wet season (July to mid-August 2019) and nine weeks during the dry season (September to early November 2019). During this period, we collected 64-65 water samples from each water source, for a total of 324 samples. We purposively selected the five groundwater sources to capture diverse characteristics and hydrological parameters. The five sources included one well near a population center, one protected spring near a population center, one well near crop fields, one well near lakes, and one well near a hillslope (Table 2). None of the water sources provided any form of water treatment.

We followed standard water sampling, transport, and analysis procedures detailed in the MICS Manual for Water Quality Testing [6]. At each water point, we collected the sample after sterilizing the outlet and flushing the water for 30 seconds. We then stored water samples on ice and analyzed them within six hours of collection. In brief, we filtered 100 ml of source water or a dilution, placed filters on CompactDryTM media

plates (NISSUI Pharmaceutical Co. Ltd., Japan), incubated them, and counted *E. coli* colonies after 24 hours of incubation. We diluted samples when we expected high concentrations of contamination based on turbidity or the prior day's concentration results. We also processed daily blank and duplicate samples for quality control, with duplicate samples collected at the water source within minutes of each other. All blanks were free of contamination.

Table 2. Descriptions of sampled water sources

Photo	Description
	**HP-Fields:** Handpump, sourced by a shallow well, located near agriculture fields
	**HP-Slope:** Handpump, sourced by a shallow well, located near a hillslope
	**HP-Lake:** Handpump, sourced by a shallow well, located near a lake
	**SP-Pop**: Protected spring, located near a population center
	**HP-Pop:** Handpump, sourced by a shallow well, located near a population center

Data analysis

We transformed *E. coli* concentration results to a log-scale for analysis. For samples with no detectable *E. coli*, we assigned a value of half our limit of detection (i.e., 0.5 CFU/100 mL in the absence of dilution), which is a typical approach enabling non-detect datapoints to be used in analysis. We evaluated variability across timescales using the coefficient of variation (the ratio of the standard deviation to the mean). We examined bivariate correlations between *E. coli* concentrations and three parameters: rainfall (source: CHIRPS V.2, resolution: 6 x 6 km), temperature (source: CPC, resolution: 55 x 55 km), and vegetation index representing land cover (source: MOD13Q1.006, resolution: 0.25 x 0.25 km). We examined these parameters on the day of sampling as well as one, three, seven, and fourteen days preceding sampling events.

We subsampled *E. coli* concentration results from our full dataset to simulate different sampling plans, including fixed frequency plans (i.e., sampling at a fixed interval such as every week or month), random sampling plans (i.e., sampling at random times), and event-responsive sampling plans (i.e., sampling based on rain events). The sampling plans that we simulated are summarized in Table 3.

Table 3. Description of sampling plans simulated.

Sampling plan	Description of sampling plan	Number of unique combinations fitting the sampling plan*	Number of samples per combination
Fixed frequency sampling			
Weekly	Sampling weekly, on the same day of the week each time	4	16-17
Biweekly	Sampling every two weeks, on the same day of the week each time	8	8-9
Monthly	Sampling every month, on the same day and week of the month each time (e.g., 1st Tuesday of the month)	16	3-4
Bimonthly	Sampling every two months, on the same day and week of the month each time	27	2
Random sampling			
Twice (any 2x)	Sampling any two random days	2,080†	2
Three times (any 3x)	Sampling any three random days	43,680†	3
Rainfall-based sampling‡			
Twice (rain/dry)	Sampling one random day following§ a rain event and one random day following an absence of a rain event	231-312 depending on the source	2
Four times (2 rain/2 dry)	Sampling two random days following§ a rain event and two random days following an absence of a rain event	12,540-21,528 depending on the source	4

* For every sampling plan, we captured every possible combination of data points meeting sampling criteria.
† One water source (HP-Pop) had 2,016 combinations for random sampling any two times and 41,664 combinations for random sampling any three times due to only 64 samples being collected from it instead of 65 as collected from all other sources.
‡ We defined a rain event as two consecutive days of rainfall greater than 5 mm/day. We defined an absence of rain event as two consecutive days of rainfall smaller than 1 mm/day. Rainfall was different at every water source, resulting in a unique number of combinations at each water source.
§ "Following" means one or two days after an event.

FINDINGS

Variability of water sources

All groundwater sources had highly variable water quality, spanning at least two WHO risk levels (Figure 1). Two sources even spanned all four risk levels. In general, variability was larger over longer time frames (e.g., months) compared to shorter time frames (e.g., weeks; Figure 2). For example, for the HP-Fields water source shown in Figure 2, *E. coli* concentration (CFU/100 mL) ranged over 0.90 log on average within a week compared to 2.1 log within a month. The variability we observed over all time frames was also greater than variability between duplicate samples taken within minutes of each other. Across all water sources, the coefficient of variation, which measures dispersion of the data, increased from 9% for duplicates taken within minutes, to 72% for samples taken within the same week and 97% for samples taken within the same month. Higher percentages correspond to higher dispersion or variability of the data. This comparison suggests that the observed temporal variability over weeks and months was not intrinsic to the measurement method but rather reflected changes in groundwater contamination levels over time.

Bivariate analyses revealed no consistent association between *E. coli* concentrations and rainfall, temperature, or vegetation index (representing land cover). For example, higher precipitation correlated with higher *E. coli* concentration ($p<0.05$ in log linear regression) at two sources, with lower *E. coli* concentration at one source, and did not correlate at the remaining two sources. We obtained similarly diverse results for temperature and vegetation index.

Image 1. A community meeting to disseminate water quality results in Kabarole, Uganda.

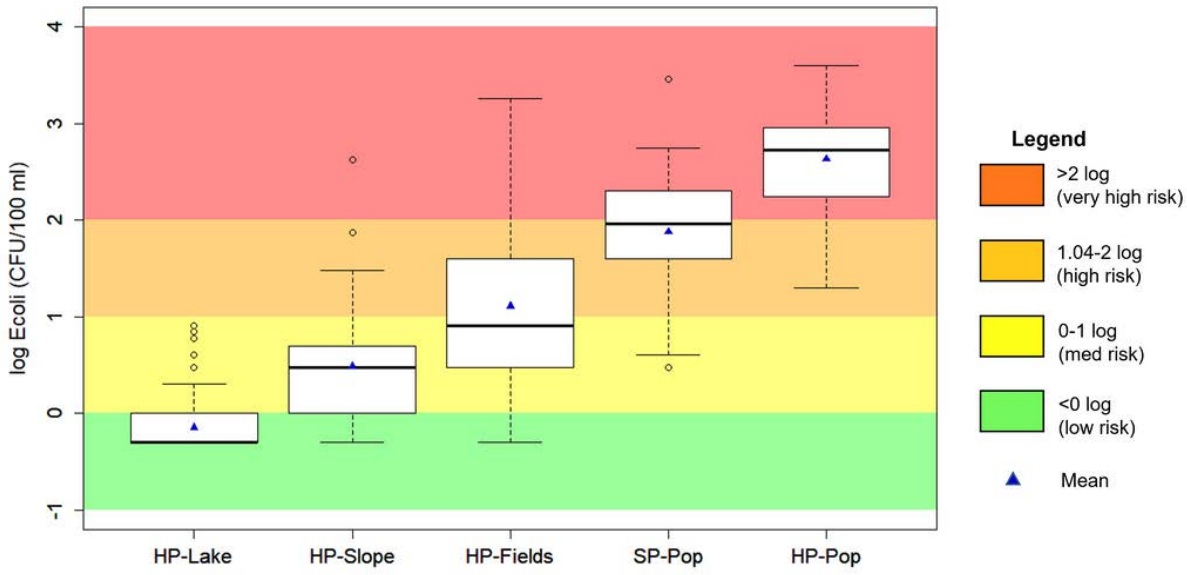

Figure 1: Boxplots of *E. coli* concentration data collected per source. 65 data points were collected for all sources except HP-Pop, which had 64 data points. The black lines within each box represent the median, the whiskers on the plots extend to 1.5 times (150%) the interquartile range, and the open circles represent outliers.

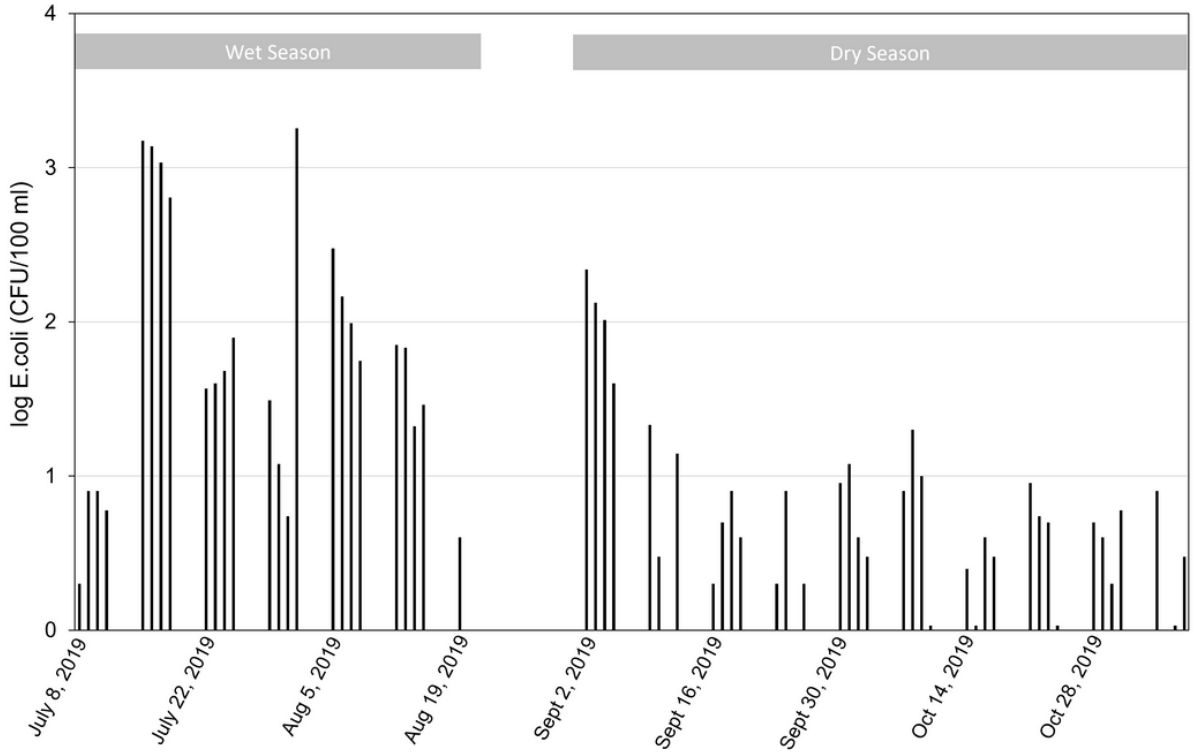

Figure 2: *E. coli* concentration data over time for one example water point (HP-Fields). Each bar represents the *E. coli* concentration measured on one day, with samples collected four days per week (Monday-Thursday) except for the second half of August when only one sample was collected over a two-week period. Dates are shown for every other Monday. Variability was higher over larger time frames such as months.

Minimum required monitoring frequency

For every combination of samples fitting a given sampling plan, we calculated the median *E. coli* concentration, which is what practitioners would use as an estimate of typical contamination. Figure 3 represents the distribution of these contamination estimates across sampling plan simulations and allows comparing with the true median contamination level. As expected, more frequent sampling resulted in higher precision: contamination estimates were more closely grouped around the true median. Fixed frequency sampling performed better than both random sampling and rainfall-based sampling plans across all water sources. This is shown for the water source with highest variability in Figure 3A, though trends were similar across all water sources.

We evaluated the suitability of different sampling plans at estimating typical contamination by considering three performance criteria:

- *IQR:* The full distribution of contamination estimates should fall within the interquartile range (IQR; i.e., middle 50%) of the water source. This criterion is more conservative (i.e., stricter) for water sources that have low variability or variability skewed in one direction (e.g., HP-Lake).
- *Bias:* The full distribution of contamination estimates should be within +/- 0.5 log of the water source's true median. This criterion is more conservative (i.e., stricter) for water sources with high variability (e.g., HP-Fields),
- *Risk level:* The distribution of contamination estimates should not extend below the true median's WHO risk level category.

For us to recommend a sampling plan, it had to meet at least one of the above criteria, with plans that met two or three of the criteria offering greater precision in estimating typical contamination. Outliers (i.e., values outside 1.5 times the IQR) can be excluded from the above evaluations.

Across all water sources, monthly sampling performed well for estimating the median level of contamination (Figure 3B). Specifically, monthly sampling met the risk level criteria for all water sources and generally met or almost met the bias criteria (HP-Lake, SP-Pop, HP-Slope). It met the IQR criteria at only one site (HP-Lake). However, biweekly or bimonthly sampling may be more suitable for stakeholders that need either more or less precise contamination estimates for certain water sources. Biweekly sampling met all three criteria at all water sources whereas bimonthly sampling met at least one criteria at three out of five water sources.

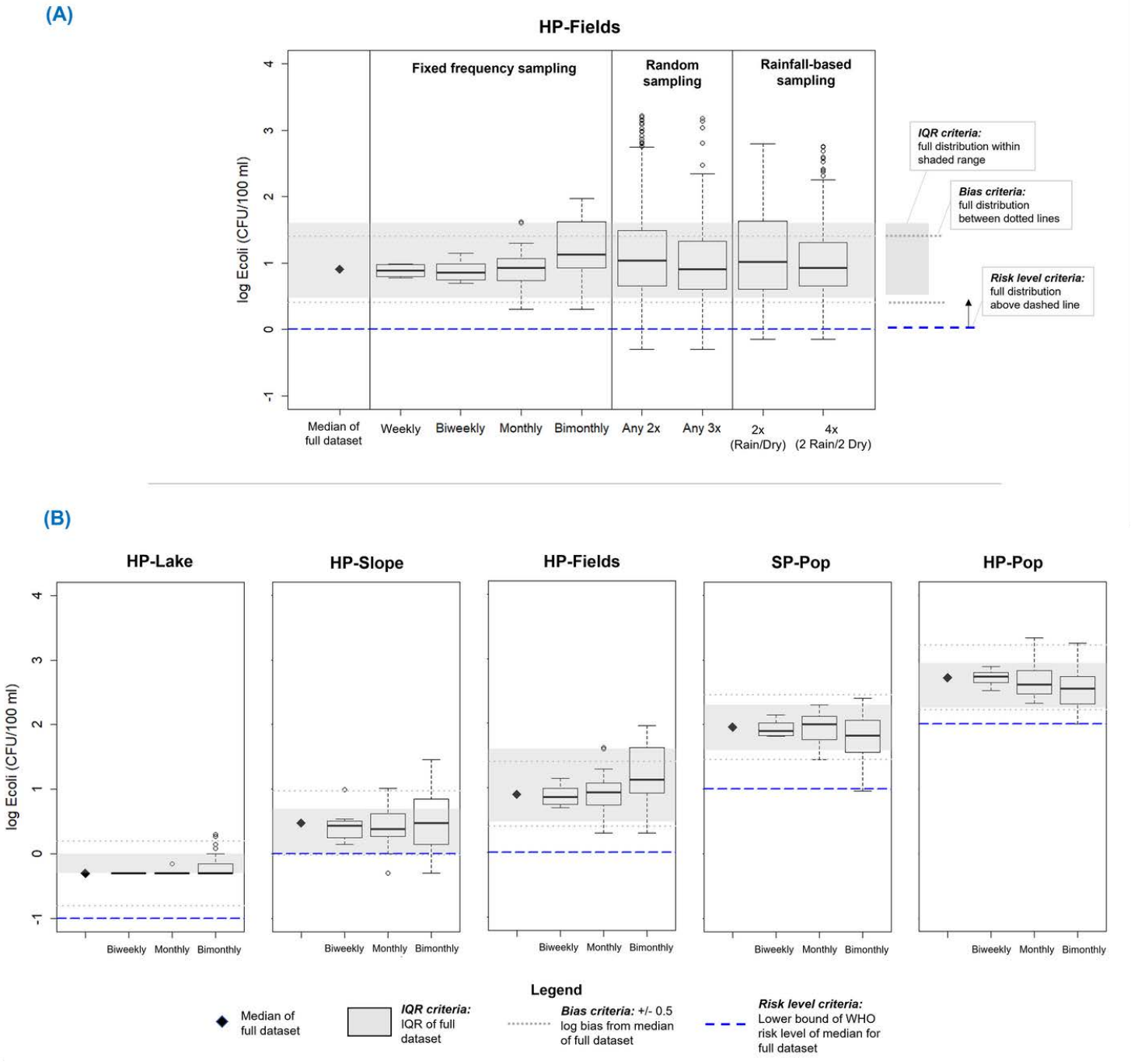

Figure 3. Distribution of estimated contamination levels for each simulated sampling plan and comparison with true median contamination. Shown for (A) all simulated sampling plans for one water source (HP-Fields), and (B) the three best sampling plans across all water sources. Optimal sampling plans have the full distribution of contamination estimates within the shaded interquartile range (IQR) (IQR criteria), within the +/- 0.5 log bias range (bias criteria), and/or above the blue dashed line representing the lower bound of the true median's WHO risk level (risk level criteria).

For water sources with infrequent contamination events, capturing the top quartile (25%) of *E. coli* measurements may be more important for decision making than capturing the median. For example, HP-Lake had detectable contamination only 28% of the time. For this source, monthly sampling would accurately estimate median contamination (Figure 3B), but it would miss the top quartile of contamination levels half of the time (Figure 4). Bimonthly sampling would almost always miss it. In contrast, weekly and biweekly sampling would reliably capture the top quartile of contamination levels (Figure 4). Therefore, while we generally recommend monthly sampling for groundwater sources in rural Uganda based on our analysis, specific water source characteristics should be considered; in some cases bimonthly or biweekly may be preferred depending on stakeholder needs (Table 4).

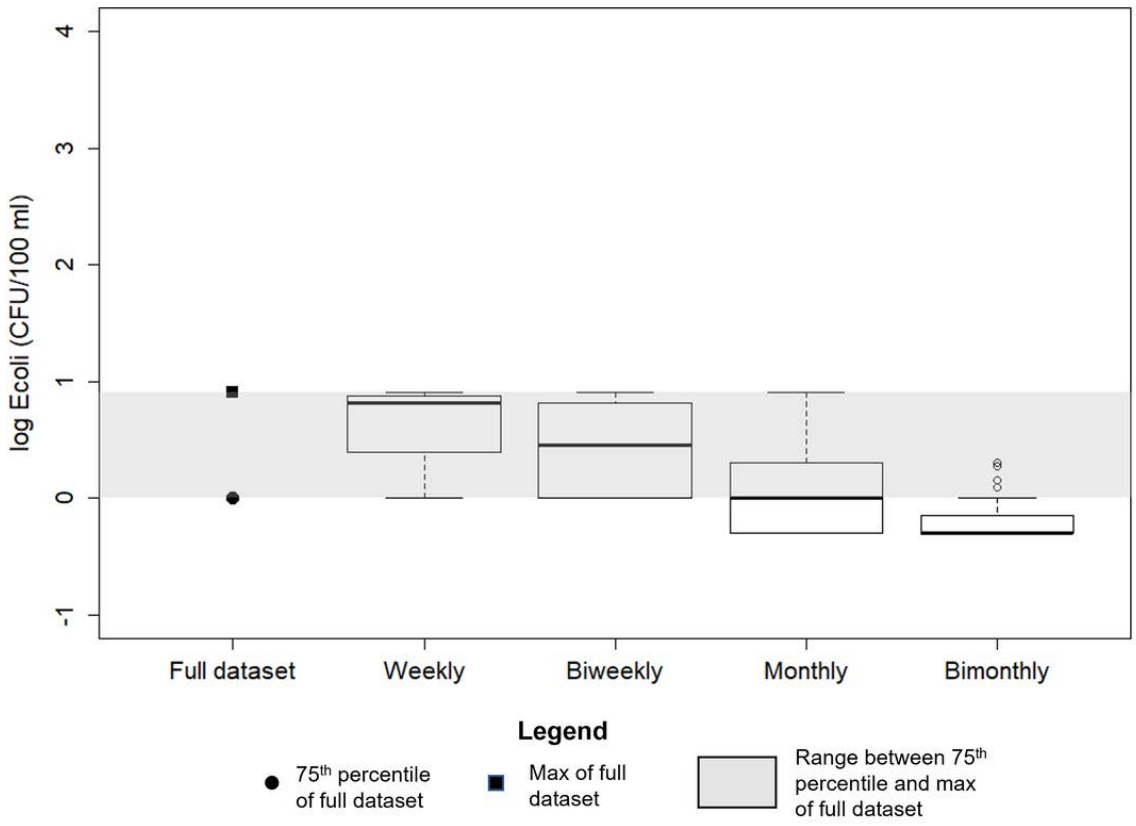

Figure 4. Distribution of estimated maximum contamination levels in four simulated sampling plans for HP-Lake, which had infrequent contamination events. Optimal sampling plans have the full distribution of estimated maximum contamination within the shaded region (which represents the top quartile of true contamination levels).

CONCLUSIONS AND RECOMMENDATIONS

Our analysis illustrates that collecting a single sample once a year or less frequently (as recommended in WHO Guidelines for Drinking Water Quality for point sources) is unlikely to provide reliable information on a groundwater source's typical risk level. More frequent sampling (at least bimonthly) is necessary to obtain a representative estimate (Table 4). One-off sampling of multiple water sources across a geographic area, such as in MICS surveys, does provide a representative estimate of contamination levels across the area. But one-off sampling only provides limited information on individual water source quality.

Table 4. Sampling frequency recommendations summary.

Sampling Plan	Summary of performance	Recommendations
Biweekly	Biweekly sampling provided a representative estimate of the median and range of *E. coli* contamination as well as the median WHO risk level category for all studied water sources.	• Recommended when it is important to capture infrequent contamination events for a water source that is usually free of contamination. • Can also capture the typical level of contamination with greater precision than less frequent sampling plans.
Monthly	Monthly sampling provided a representative estimate of the median *E. coli* contamination and WHO risk level category for all studied water sources, but not the range of *E. coli* contamination.	• Recommended when stakeholders desire to estimate median levels of contamination. • Not recommended for capturing infrequent contamination events.
Bimonthly or less	Bimonthly sampling provided a representative estimate of the median WHO risk level category for some (3/5) studied water sources. It provided less precise measurements of median *E. coli* contamination and did not capture contamination events occurring 25% of the time or less.	• Recommended when budgets do not allow more frequent sampling and/or when precise estimates of a water source's median or maximum risk level are not needed. • Less frequent sampling may still be useful to provide estimates of contamination in an area by aggregating data from multiple sources.

Limitations of this analysis

Several factors can drive the variability of *E. coli* contamination in groundwater sources. Although this analysis included water sources with diverse characteristics, it is only based on five water sources in one geographic area, which limits the generalizability of the results. Therefore, while these recommendations may apply to similar water sources in this context, they may not be representative of variability and minimum sampling frequencies required for water sources in other contexts. Additionally, as we only collected samples for four months out of the year, we may not have captured the entire annual variability in water quality.

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Image 2. Research Assistant Joan Mukugiza testing water for *E. Coli* using Membrane Filtration with Compact Dry Plates.

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