H$_2$S as an Indicator of Water Supply Vulnerability and Health Risk in Low-Resource Settings: A Prospective Cohort Study

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Abstract. In this large-scale longitudinal study conducted in rural Southern India, we compared a presence/absence hydrogen sulfide (H$_2$S) test with quantitative assays for total coliforms and *Escherichia coli* as measures of water quality, health risk, and water supply vulnerability to microbial contamination. None of the three indicators showed a significant association with child diarrhea. The presence of H$_2$S in a water sample was associated with higher levels of total coliform species that may have included *E. coli* but that were not restricted to *E. coli*. In addition, we observed a strong relationship between the percent positive H$_2$S test results and total coliform levels among water source samples ($R^2 = 0.87$). The consistent relationships between H$_2$S and total coliform levels indicate that presence/absence of H$_2$S tests provide a cost-effective option for assessing both the vulnerability of water supplies to microbial contamination and the results of water quality management and risk mitigation efforts.

INTRODUCTION

Diarrheal diseases, including severe illnesses such as cholera and dysentery, are primarily caused by the ingestion of diarrhea-causing microbial pathogens, which include specific viruses, bacteria, and protozoa. Diarrheal pathogens are concentrated in the feces of infected individuals; as a result, their transmission is promoted by environmental fecal contamination. Approximately 88% of diarrhea cases worldwide are attributable to pathogen transmission by fecal contamination of water, food, or hands; these cases result in about 1.5 million deaths each year, mostly among children < 5 years of age.[1]

Water quality improvements can reduce diarrhea,[2–5] and monitoring microbial water quality is a primary activity of safe water management.[6] The diversity of diarrheal pathogens renders direct pathogen testing impractical, therefore water providers and public health surveillance agencies rely on water quality indicators to assess microbial drinking water quality: *process indicators* monitor the efficacy of water treatment and the integrity of distribution networks; *fecal indicators* directly identify the presence of fecal contamination.[7] Most national regulations, which are generally based on World Health Organization (WHO) guidelines, specify the broad group of coliform bacterial species (total coliforms) as process indicators; fecal indicators include thermotolerant coliforms, a subset of the total coliform group, and *Escherichia coli*, a subset of the thermotolerant coliforms.[8]

Disease outbreaks, however, have been associated with water that was free of fecal indicators,[9] and in tropical climates, fecal indicators may persist in the environment in the absence of recent fecal contamination.[10–12] These observations reflect the variable relationships observed between water quality indicators and actual fecal pathogens,[13,14] which may also contribute to the weak and inconsistent associations between water quality indicators and diarrheal disease.[2,13,15–19]

Most of the validated methods for quantifying total coliforms, thermotolerant coliforms, and *E. coli* in drinking water are costly and require both dedicated facilities and specialized training. To promote on-site microbial water testing in resource-poor settings, Manja and others[20] developed a simple presence/absence test for hydrogen sulfide (H$_2$S)-producing bacteria, based on the observation that coliform bacteria in drinking water were consistently associated with H$_2$S production. Improved versions of the presence/absence H$_2$S test have gained popularity as low-cost assays for fecal contamination. For example, the Indian government has supported the use of H$_2$S tests for community-level monitoring of common water supplies, accompanied by laboratory-based confirmation of positive results.[21]

A recent systematic review showed that the diagnostic accuracy of the H$_2$S test for the established fecal indicators, thermotolerant coliforms and *E. coli*, varied between studies and that the sources of this variation were not obvious.[22] Culture-based and molecular analysis of environmental water samples, however, have shown strong relationships between positive H$_2$S tests and fecal microorganisms, including coliform and non-coliform species.[23,24]

To directly evaluate the H$_2$S test as a drinking water management tool in low-resource settings, our study focused on two objectives: 1) to conduct a comparative analysis of drinking water quality indicators (H$_2$S, total coliforms, and *E. coli*); and 2) to estimate the associations between the water quality indicators and diarrhea among children < 5 years of age. We conducted this analysis as part of a 12-month longitudinal cohort study in rural Southern India, designed to measure the effect of a combined sanitation, water supply, and hygiene intervention program on child health.[25]

MATERIALS AND METHODS

Study setting. This study was conducted between January 2008 and April 2009 in 25 rural villages in the Tiruchirappalli district of Tamil Nadu, India. The study population mainly worked in agriculture (64% of households in the cohort). The climate is tropical and subject to heavy rainfall during seasonal monsoons (August–December). In all study villages, drinking water was provided by pumping groundwater into overhead storage tanks and then distributing it to public and

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private taps. Qualitative interviews suggested that chlorination of the drinking water was infrequent and, in most villages, probably limited to disease outbreaks. During the study, 93% of households relied on public or private taps for all of their daily water use (drinking, cooking, bathing, and washing).

Study design. Details of the study design and population have been reported.[25] In brief, the study included 12 villages that received a combined water supply, sanitation, and hygiene education intervention between 2003 and 2007, and 13 matched control villages that did not receive the intervention. We enrolled a random sample of up to 50 households with children < 60 months of age per village from a complete listing of all households in the villages in 2008 after the completion of the intervention. We visited households monthly over 12 months to collect water samples and caregiver-reported child health symptoms.

Water sample collection and testing. Beginning in the third month of the cohort study (Round 3), field staff collected water samples concurrently with diarrhea surveillance. The 25 study villages had between one and seven water sources, and all village sources were tested in survey Rounds 3–12. Field staff collected 125 mL of water from these sources according to local water retrieval practices.

Field staff also collected household drinking water samples from a random, rotating sample during the study. Households within each village were randomly allocated into four groups: two of the groups were measured in survey Rounds 3 and 5, and the other two groups were measured in survey Rounds 4 and 6. Households in one of the four groups were tested in each survey round thereafter (Rounds 7–12). Household water samples were collected in 125 mL sterilized plastic bottles in a fashion that mimicked each household's water retrieval practices: by dipping a household cup into the vessel to transfer the water, by pouring water from the storage container into the sample container, or, if a household did not store drinking water, by retrieving water directly from the tap. Along with the water samples, field staff recorded basic characteristics of the water conditions at the time of collection (such as storage container type). The field team transported all the water samples to the laboratory in coolers containing ice. In the laboratory, samples were stored at 4°C before conducting assays for total coliforms, *E. coli* and H_2S. All assays were performed within 24 hr of sample collection. Autoclaved tap water samples were used as negative controls for each Round of water quality analysis.

Samples collected during survey Round 3 were diluted 1:10 before the analysis of total coliform and *E. coli* levels. In survey Rounds 4–12, the laboratory increased the dilution to 1:100 to optimize total coliform enumeration. After dilution, 100 mL of sample water was passed through a 0.45 μM membrane filter, which was then placed onto a rehydrated membrane nutrient pad impregnated with HiCrome Coliform medium with sodium lauryl sulfate (HiMedia MF026; HiMedia, Mumbai, India). The filters and nutrient pads were incubated at 37°C for 24 hr. HiCrome coliform medium contains two chromogenic substrates, Salmon-gal and X-glu. All coliform bacterial species produce the enzyme β-galactosidase, which cleaves Salmon-gal to generate red-purple colonies. *Escherichia coli* produce an additional enzyme, β-glucuronidase, which cleaves X-glu: the combination of Salmon-gal and X-glu cleavage by *E. coli* generates dark blue-green colonies. The number of red-purple colonies was counted and recorded as

total coliform bacteria. The number of blue-green colonies was counted and recorded as *E. coli*.

An undiluted 30 mL volume of each sample was also analyzed for H_2S producing bacteria, using the HiH2S test kit (HiMedia K020; HiMedia, Mumbai, India). Samples were left to incubate at room temperature for 24 hr and, if room temperature fell below 30°C, for an additional 12 hr. Samples were recorded as positive for H_2S, if they turned black.

Diarrhea measurement. The primary health outcome in this study was caregiver-reported diarrhea during the 7 days before the visit among children < 5 years of age at enrollment; we defined diarrhea as three or more loose or watery stools in 24 hr or a single stool with blood or mucus.[26,27] Field interviewers collected caregiver-reported symptoms during each monthly home visit with the aid of a health calendar.[28]

Statistical analysis. *Water quality descriptive analyses.* We summarized total coliform measurements using quantitative measures. The distribution of total coliform levels was right-skewed, therefore we imputed samples below the lower detection limit at 0.1 and conducted the analysis on the $\log_{10}$ scale. We calculated mean total coliform levels (on the $\log_{10}$ scale) and the proportion of positive H_2S samples by village source and household characteristics.

Comparison of H_2S to other indicators. Analyses that examined lower concentrations of *E. coli* (i.e., < 10 colony forming units (CFUs)/100 mL) were restricted to the 727 samples from Round 3 when the detection limit was 10 CFUs/100 mL. All other analyses included all samples from survey Rounds 3–12. We estimated the sensitivity and specificity of H_2S for total coliform and *E. coli* contamination based on the proportion of water samples that were positive for H_2S within pre-specified ranges of total coliform concentrations (< 100, 100–199, 200–499, 500–999, 1,000–4,999, 5,000–9,999, ≥ 10,000 CFUs/ 100 mL) and *E. coli* concentrations (< 10, 10–99, ≥ 100 CFUs/ 100 mL). We also calculated the positive predictive value (PPV) and negative predictive value (NPV) of H_2S for total coliform and *E. coli* contamination > 100 CFUs/100 mL.[29]

Association between water quality indicators and child diarrhea. We estimated the increase in diarrhea risk associated with a positive H_2S test result or with higher concentrations of total coliforms or *E. coli* from drinking water samples collected concurrently with disease surveillance. In our analysis, we only included diarrhea surveillance that was paired with a household water sample. Our parameter of interest for each comparison was the prevalence ratio (PR), comparing diarrhea prevalence among children at higher exposure levels of the water quality indicators with children in the baseline category. For H_2S, we compared diarrhea risk associated with a positive test versus a negative test; for total coliforms we compared diarrhea risk at concentrations > 10,000 CFUs/ 100 mL to concentrations below that value; for *E. coli* we compared diarrhea risk at concentrations of 101–1,000 and > 1,000 CFUs/100 mL to concentrations ≤ 100 CFUs/100 mL. For binary exposures (H_2S, total coliforms), we modeled the probability of diarrhea using a log-binomial model:

$$\log E[Y \mid X, \mathbf{W}] = \alpha + \beta_1 A + \gamma \mathbf{W}, \qquad (1)$$

where Y is a binary indicator of a child's diarrhea status, A is a categorical variable of the water quality indicator, and $\mathbf{W}$ is a vector of potentially confounding characteristics. We modeled the association similarly for *E. coli*, but included two categories

of exposure: $A_1 = I(E.\ coli$ 101–1,000 CFUs/100 mL) and $A_2 = I(E.\ coli > 1{,}000$ CFUs/100 mL).

$$\log E[Y \mid X, \mathbf{W}] = \alpha + \beta_1 A_1 + \beta_1 A_2 + \gamma\mathbf{W}, \qquad (2)$$

The exponentiated coefficient estimates for the exposure variables in the models (A) estimate the PR.[30] In adjusted models, $\mathbf{W}$ included fixed effects for survey round and village and potentially confounding child (age, age squared, sex, currently breastfeeding) and household characteristics: water source, confirmed baseline boiling, water storage container type, latrine ownership, dedicated location to wash hands with water and soap, housing characteristics (soil floor, thatch roof), durable goods ownership (television, motorcycle, mobile phone), socioeconomic characteristics (has a bank account, participation in a Self Help Group, household head works in agriculture, Scheduled Caste membership, education level of the child's primary caregiver). All analyses (descriptive, PPV/NPV, and regression models) estimated standard errors and 95% confidence intervals (CIs) using the robust sandwich estimator, clustered at the village level.[31]

As a robustness check, we repeated the analyses that estimated the association between water quality indicator levels and child health using two caregiver-reported respiratory symptoms (constant cough, congestion/coryza) that were unlikely to be caused by contaminated water but could be biased by similar confounding or measurement error as diarrhea and could, thus, serve as negative control outcomes.[32]

Human subjects research protection. All data collection followed protocols approved by the institutional review boards at the University of California, Berkeley, CA and Sri Ramachandra Medical College and Research Institute, Chennai, Tamil Nadu, India. All study participants provided informed consent.

RESULTS

Study population and sample collection. We enrolled 900 households and 1,284 children < 5 years of age. Of these, 1,220 (95%) children completed the 12 months of follow-up, and the study included 14,259 child-weeks of observation. Table 1 summarizes child and household characteristics in the study population. All households had access to an improved water source as defined by the WHO/United Nations Children's Fund (UNICEF) Joint Monitoring Program, with 93% of households obtaining water from public or private taps fed by ground water-supplied overhead tanks.[33]

Field staff collected and tested 695 village source water samples and 3,026 household drinking water samples during the study. Household water quality samples were collected concurrently with 4,166 child-weeks (H$_2$S) and 4,152 child-weeks (total coliform and *E. coli*) of diarrhea surveillance. Of the 900 households in the study, field staff collected water samples three ($N = 422$) or four ($N = 435$) times from 95% of households during the follow-up period.

Water quality descriptive analyses. On average, source water quality was best when obtained directly from hand pumps (mean Log$_{10}$ total coliforms 2.579, SE 0.099; percent positive H$_2$S 36.3%, SE 4.8%) and poorest in the smaller, public mini-tanks (mean Log$_{10}$ total coliforms 3.286, SE 0.071; percent positive H$_2$S 75.4%, SE 3.8%) (Table 2). When we aggregated water quality data by village, we observed a

TABLE 1
Child and household characteristics in the 25 study villages

	Mean	(SD)
Child characteristics ($N = 1{,}284*$)		
Age at enrollment, mo	28.5	(18.3)
Female, %	49.8	
Currently breastfeeding, %	32.1	
Diarrhea, last 7 days, %	1.8	
Fever, last 7 days, %	11.8	
Cough, last 7 days, %	8.1	
Congestion, last 7 days, %	31.6	
Household characteristics ($N = 900$)		
Mother's age, y	26.8	(5.0)
Caregiver's education, %		
None	14.6	
Primary	40.3	
Secondary	30.8	
Post-secondary	14.1	
Missing	0.2	
Primary water source, %		
Public tap	63.8	
Private tap	28.8	
Public or private well	7.4	
Boil drinking water, %	16.8	
Own private toilet, %	41.6	
Hand washing location, water and soap present, %	31.2	
Human/animal feces observed in living area, %	26.8	
Work in agriculture, %	63.7	
Scheduled caste, %	13.0	
Women members in Self-Help Group, %	34.4	
House has soil floor, %	31.4	
House has thatch roof, %	24.4	
Total persons in house	4.8	(1.3)
Total rooms in house	2.7	(1.6)
House has electricity, %	90.0	
Own their house, %	92.9	
Have bank account, %	21.7	
Own radio, %	55.9	
Own television, %	65.3	
Own mobile phone, %	32.7	
Own motorcycle/scooter, %	25.3	
Own bicycle, %	76.3	
Own mosquito net, %	13.3	

*$N = 14{,}259$ child-weeks of observation for symptom prevalence estimates.

consistent deterioration in microbial water quality between village source and household drinking water samples, and, although we observed heterogeneity in village source water quality, household water quality was uniformly poor across villages based on both total coliform and H$_2$S indicators (Figure 1). Self-reported boiling was infrequent in this study population: 9.6% of samples (291 of 3,026) were reported to be boiled (Table 2). Boiled water was of higher quality based on total coliform and H$_2$S indicators, but contamination levels in reportedly boiled samples remained high (mean log$_{10}$ total coliforms 3.162, SE 0.060; percent positive H$_2$S 66.7%, SE 3.6%) (Table 2).

Comparisons of H$_2$S to other microbial water quality indicators. Our comparisons of H$_2$S test results and total coliform contamination showed that H$_2$S was increasingly sensitive to total coliform levels that fell in the intermediate to high range in this setting: 90% (328 of 363) of village source samples and 96% (2,148 of 2,232) of household drinking water samples that contained total coliform CFUs $\geq 1{,}000$/100 mL were also H$_2$S positive (Figure 2). For total coliform concentrations between 200 and 4,999 CFUs/100 mL, however, H$_2$S was consistently more sensitive to total coliform contamination

TABLE 2

Summary of total coliform and H$_2$S indicators for village source and household water samples measured over a 12-month period in rural Tamil Nadu, India

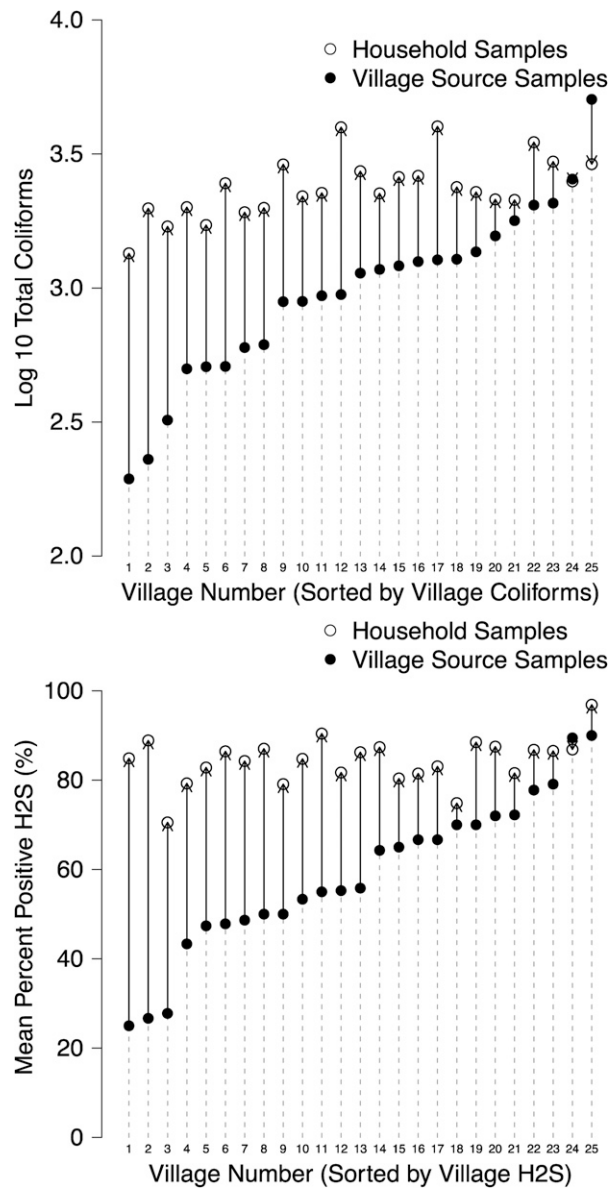| Water sample Characteristics	N	Log$_{10}$ total coliforms		H$_2$S proportion positive	
		Mean	SE	Mean	SE
Village source samples					
Overall	695	2.974	0.062	0.586	0.034
Village water source					
Hand pump (tube well)	201	2.579	0.099	0.363	0.048
Large overhead tank	304	3.059	0.070	0.641	0.042
Public mini-tank	122	3.286	0.071	0.754	0.038
Other	68	3.194	0.126	0.691	0.058
Household samples					
Overall	3,026	3.386	0.023	0.843	0.010
Household water source					
Private tap	875	3.381	0.026	0.826	0.013
Public tap	1,920	3.385	0.029	0.848	0.013
Private well	78	3.487	0.088	0.962	0.015
Public well	129	3.396	0.079	0.860	0.032
Other	24	3.286	0.128	0.542	0.090
Storage time, hours					
0–1	71	3.326	0.087	0.803	0.042
2–3	106	3.296	0.077	0.821	0.036
4–5	949	3.478	0.033	0.863	0.009
6–7	1,254	3.391	0.031	0.829	0.018
8–9	499	3.262	0.052	0.856	0.024
≥ 10	139	3.277	0.083	0.849	0.039
Unknown	8	3.039	0.160	0.375	0.124
Storage container type					
Directly from tap	11	2.920	0.286	0.455	0.136
Narrow mouth	488	3.303	0.036	0.814	0.019
Wide mouth	2,527	3.404	0.025	0.850	0.010
Self-reported boiling					
Not boiled	2,735	3.410	0.025	0.862	0.011
Boiled	291	3.162	0.069	0.667	0.036

FIGURE 1. Village-level mean water quality indicators in village source and household samples. Total coliforms (top panel) and H$_2$S (bottom panel). Arrows between points indicate the direction of change between household sample means and village sample means. N = 3,026 household samples; N = 695 village source samples.

in household samples than in village source samples (Figure 2). H$_2$S also showed specificity for total coliforms, with the proportion of positive H$_2$S tests dropping to 10% in samples containing < 100 CFUs/100 mL total coliforms (Figure 2). Village-level aggregations of water quality data showed a strong relationship between the percentage of positive H$_2$S tests and mean total coliform levels for village source samples in the concentrations observed (R^2 = 0.87); household samples did not vary greatly in their average levels of contamination (Figure 3).

In contrast to total coliforms, H$_2$S was sensitive to low levels of *E. coli* contamination: 96.4% (95% CI 89.9, 99.3) of household water samples with 10–99 *E. coli* CFUs/100 mL and 100% (95% CI 91.4, 100.0) of household water samples with ≥ 100 *E. coli* CFUs/100 mL tested positive for H$_2$S (Table 3). The H$_2$S test, however, was not specific for *E. coli* contamination: 53.3% (95% CI 48.7, 57.9) of household water samples containing < 10 CFUs/100 mL were also positive for H$_2$S (Table 3).

Positive and negative predictive values of H$_2$S for total coliform and *E. coli* contamination. We analyzed the H$_2$S test's ability to predict total coliform and *E. coli* contami-

nation by comparing the levels of these indicator species between H$_2$S positive and H$_2$S negative water samples (Table 4). Almost all H$_2$S positive samples contained ≥ 100 total coliform CFUs/100 mL: the PPV was 99% for both village source and household samples (Table 4). Only about 50% of H$_2$S negative samples, however, contained < 100 total coliform CFUs/100 mL: the NPV for total coliforms < 100 CFUs/100 mL was 46% for village source samples and 51% for household samples (Table 4). In contrast, a positive H$_2$S result was not highly predictive of *E. coli* contamination: the PPV of the H$_2$S test for *E. coli* concentrations ≥ 100 CFUs/100 mL was 17% for village samples and 23% for household samples (Table 4). The NPV of the H$_2$S test for < 100 CFUs/100 mL *E. coli* was 97% for both village source and household water samples (Table 4).
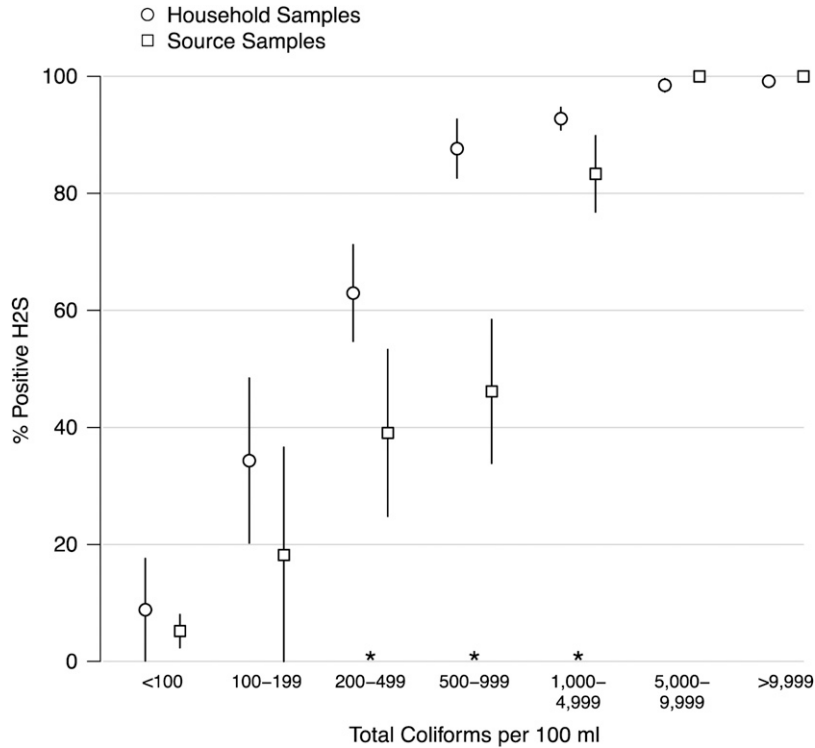
FIGURE 2.   Percent positive H_2S tests by total coliform concentration for household and village source samples. Vertical lines are robust 95% confidence intervals. Asterisks (*) mark statistically different means between household and village source samples at the 95% confidence level. $N = 3,026$ household samples; $N = 695$ village source samples.

Microbial water quality indicator associations with child diarrhea. Diarrhea prevalence among children < 5 years of age was 1.8% over the entire study period, 2.4% in the 4,166 random subsample of visits that included water testing, and 1.4% in household visits from the same time points but without water sample collection (2.4% versus 1.4%, $P < 0.0001$; seasonally adjusted difference = 2.3% versus 1.8%, $P = 0.06$).

We observed slightly higher diarrhea prevalence at times with a positive H_2S result compared with a negative H_2S result (2.5% versus 2.1%), but found no evidence for an association between a positive H_2S test result and child diarrhea (adjusted PR = 1.13; 95% CI 0.53, 2.40) (Table 5). In addition, we did not identify associations between high levels of total coliforms (> 10,000 CFUs/100 mL) and child diarrhea
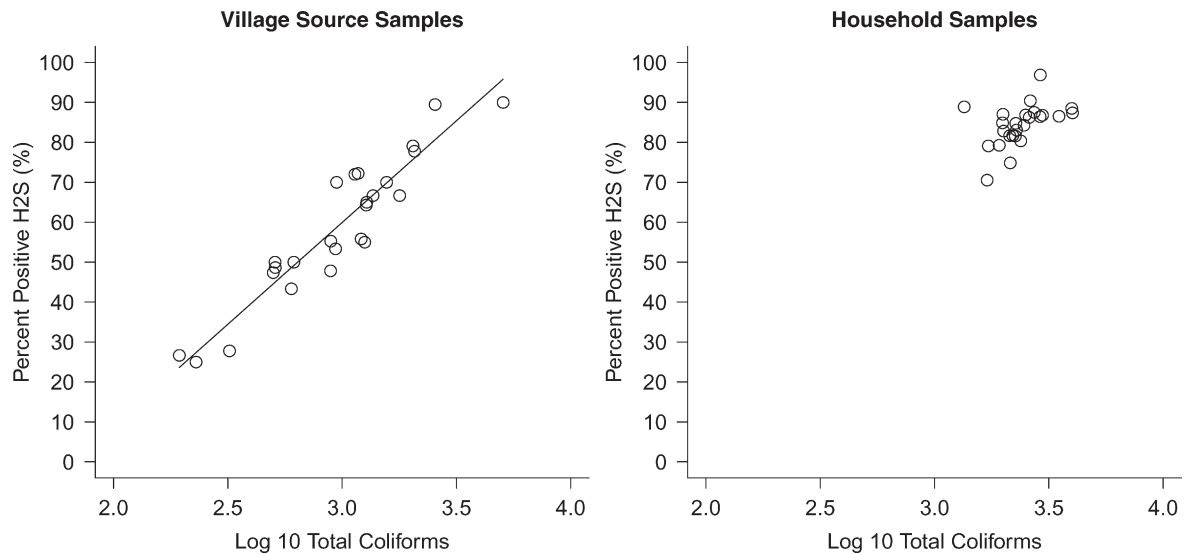

FIGURE 3.   Relationship between the percentage of H_2S positive tests and $\log_{10}$ total coliform concentration for village source samples (left) and household samples (right) using mean concentrations at the village level. $N = 695$ village source samples; $N = 3,026$ household samples. The fitted line in the village source samples is a weighted least squares fit with parameters $H_2S = -92.9 + 51.0 \times \log_{10}TC$, $R^2 = 0.87$. The parameters fit including both village (left) and household samples (right) was similar: $H_2S = -90.2 + 51.3 \times \log_{10}TC$, $R^2 = 0.81$, and the fit was not greatly improved using a logistic instead of linear model.

TABLE 3

H$_2$S positive test results for different *Escherichia coli* concentrations in survey round 3*

Escherichia coli (CFUs/100 mL)	N	H$_2$S+	% Positive	95% CI
Village source samples				
< 10	126	3	2.4	(0.5, 6.8)
10–99	8	0	0.0	(0.0, 36.9)
≥ 100	3	1	33.3	(0.8, 90.6)
Household samples				
< 10	465	248	53.3	(48.7, 57.9)
10–99	84	81	96.4	(89.9, 99.3)
≥ 100	41	41	100.0	(91.4, 100.0)

*Results are stratified by village source and household source water samples.
CFU = colony forming units; CI = confidence interval.

(adjusted PR = 1.01, 95% CI 0.57, 1.80), and although we observed higher diarrhea prevalence at higher concentrations of *E. coli*, the PRs were not different from 1.0 at the 95% confidence level (Table 5). When we re-estimated the association between *E. coli* levels and negative control respiratory symptoms, we found a similar increase in risk associated with higher *E. coli* concentrations, which suggests that at least part of the association observed between *E. coli* and diarrhea could be the result of incomplete confounding control (Table 6).

DISCUSSION

Summary of main results. In this large-scale, longitudinal study of water quality indicators and child health in rural Southern India, we found that microbial contamination of village water sources was common and that contamination levels increased after collection by households (Figure 1). The H$_2$S test was sensitive to microbial water contamination measured by total coliforms at concentrations ≥ 1,000 CFUs/ 100 mL (Figure 2) and by *E. coli* at concentrations ≥ 10 CFUs/ 100 mL (Table 3). Aggregation of water quality measures at the village-level showed a strong relationship between the percentage of positive H$_2$S tests and total coliform concentrations observed (Figure 3). Diarrhea prevalence was relatively low in this population (1.8% over 12 months), and we found no consistent association between water quality indicators and diarrhea among children < 5 years of age (Table 5).

Interpretations. *Comparison of H$_2$S with total coliforms and* E. coli. Our analysis found that water samples with ≥ 1,000 CFUs/100 mL total coliforms and/or ≥ 10 CFUs/100 mL

TABLE 4

Positive predictive value (PPV) and negative predictive value (NPV) of the H$_2$S test for village and household water samples using different indicators and a cutoff point of 100 CFU/100 mL, measured over a 12-month period in rural Tamil Nadu, India*

Indicator cutoff, sample type	N H$_2$S+	PPV %	(95% CI)	N H$_2$S−	NPV %	(95% CI)
Total coliforms > 100 CFU/100 mL						
Village samples	407	99	(98, 100)	287	46	(36, 55)
Household samples	2,546	99	(98, 100)	469	51	(45, 56)
Escherichia coli > 100 CFU/100 mL						
Village samples	407	17	(10, 24)	287	97	(95, 99)
Household samples	2,546	23	(20, 25)	469	97	(96, 99)

*CI = confidence interval; CFU = colony forming units.

TABLE 5

Association between water quality indicator levels and diarrhea among children < 5 years of age measured over a 12-month period in rural Tamil Nadu, India*

Indicator level	N†	Diarrhea %	Unadjusted PR (95% CI)	Adjusted PR (95% CI)
H$_2$S				
Negative	662	2.1	Ref	Ref
Positive	3,504	2.5	1.17 (0.54, 2.56)	1.13 (0.53, 2.40)
Total coliforms				
≤ 10,000	3,150	2.6	Ref	Ref
> 10,000	1,002	1.9	0.73 (0.45, 1.17)	1.01 (0.57, 1.80)
Escherichia coli				
≤ 100	3,509	2.3	Ref	Ref
101–1,000	488	2.7	1.14 (0.58, 2.23)	1.23 (0.67, 2.28)
> 1,000	155	3.9	1.66 (0.65, 4.21)	2.16 (0.82, 5.71)

*PR = prevalence ratio; CI = confidence interval; Ref = reference group.
†N = child weeks of observation collected from 1,284 children.

E. coli were likely to test positive for the presence of H$_2$S (Figure 2, Table 3). On the other hand, only samples with low total coliforms concentrations (< 100 CFUs/100 mL) were likely to be negative for H$_2$S (Figure 2): among household samples with low *E. coli* concentrations (< 10 CFUs/100 mL), the proportion of H$_2$S positive samples was 53% (Table 3). In addition, the PPV of the H$_2$S test was high for total coliforms and low for *E. coli* (Table 4). Together, these results suggest that the presence of H$_2$S in a water sample was associated with total coliform species (at levels ≥ 1,000 CFUs/100 mL) that may have included *E. coli* but that were not restricted to *E. coli*.

Our analysis of the NPV of the H$_2$S test, however, showed that a large fraction of H$_2$S negative water samples also contained total coliforms ≥ 100 CFUs/100 mL, although, they were unlikely to contain *E. coli* ≥ 100 CFUs/100 mL (Table 4). The low NPV of the H$_2$S test for total coliforms < 100 CFUs/ 100 mL is likely explained by the poor sensitivity of the H$_2$S test for total coliform levels < 1,000 CFUs/100 mL (Figure 2). The high NPV of H$_2$S for *E. coli* < 100 CFUs/100 mL may be caused by the relatively high percentage of samples with low levels of *E. coli* contamination (Table 3).

We also observed that under conditions where repeated H$_2$S presence/absence tests were recorded, the percentage

TABLE 6

The association between water quality indicators and negative control respiratory outcomes among children < 5 years of age measured over a 12-month period in rural Tamil Nadu, India*

Indicator level	N†	Cough %	Adjusted PR (95% CI)	Congestion/ coryza %	Adjusted PR (95% CI)
H$_2$S					
Negative	662	8.5	Ref	32.9	Ref
Positive	3,504	7.1	0.93 (0.70, 1.24)	31.2	0.97 (0.85, 1.10)
Total coliforms					
≤ 10,000	3,150	6.8	Ref	30.0	Ref
> 10,000	1,002	9.1	1.21 (0.94, 1.55)	36.2	1.09 (0.98, 1.21)
E. coli					
≤ 100	3,509	7.3	Ref	31.1	Ref
101–1,000	488	6.8	0.89 (0.60, 1.30)	32.6	1.01 (0.88, 1.16)
> 1,000	155	11.0	1.42 (0.80, 2.55)	38.7	1.15 (0.97, 1.38)

*PR = prevalence ratio; CI = confidence interval; Ref = reference group.
†N = child weeks of observation collected from 1,284 children.

that were positive for H_2S provided a quantitative measure of contamination that is strongly related to $\log_{10}$ total coliform concentrations (Figure 3). Our results suggest that in source monitoring applications, repeated presence/absence H_2S samples could be averaged to provide a quantitative measure of source water quality.

Differences between H₂S sensitivity for total coliforms in source versus household samples. The higher sensitivity of the H_2S test for total coliform contamination in household water samples versus village source samples suggests that the composition of total coliform species changed after water was collected and stored in households (Figure 2). This change in total coliform composition might be linked to the consistent increase in total coliform levels after collection (Figure 1), which likely included household-level fecal contamination resulting from poor hygiene and water handling practices. An increase in fecal contamination after collection was also indicated by the increase in the proportion of water samples with ≥ 10 *E. coli* CFUs/100 mL from 8.0% (11 of 137) in village source samples to 21% (125 of 590) in household samples (Table 3). Consequently, we hypothesize that the higher sensitivity of the H_2S test for total coliforms in household samples is caused by a greater proportion of fecal coliforms in these samples. Our hypothesis assumes that fecal coliforms are more likely to include H_2S producers than environmental coliforms, which is supported by a recent report of the consistent detection of multiple species of fecal microorganisms in H_2S positive environmental water samples.[24] A study that directly compares H_2S test results with fecal microorganism levels in village source and household water samples could test this hypothesis directly.

Poor microbial water quality was not associated with child diarrhea. In this study, we found no consistent association between water quality indicators and diarrhea among children < 5 years of age (Table 5). A lack of association between water quality indicators and child diarrhea is consistent with findings from studies in Bangladesh,[15] Pakistan,[17] and Ecuador[19] but stands in contrast to findings from studies in the Philippines[16] and Cambodia.[18] These variable results between study sites are not unexpected given regional differences in the non-fecal contributions to microbial indicator levels and in the relative importance of drinking water as a disease transmission pathway.[19] Our observation of low child diarrhea prevalence despite poor microbial water quality suggests that although the ground water sources in the study region are exposed to environmental microbial contamination, basic protection from fecal contamination may be sufficient to prevent the majority of water source-transmitted disease risk.[34] The consistent increase in household-level microbial contamination (Figure 1), however, indicates that water contamination during storage remains a potential transmission pathway for pathogens that could facilitate rapid disease spread during a diarrheal disease outbreak.

Is the H₂S test appropriate for monitoring microbial water quality in low resource settings? As shown in this and other studies, microbial water quality indicators are often poor surrogates for the actual health risks associated with drinking water.[2,14,19] Given the diversity of pathogens that cause diarrhea and the multiple exposure pathways to these pathogens (drinking water, food, hands, and other contaminated surfaces), the inconsistent associations between a limited set of microbial water quality indicators and disease risk is not sur-

prising. It is likely that accurate evaluations of the health risks associated with a drinking water supply will require direct assessments of pathogen levels. Methods for detecting and quantifying the full spectrum of waterborne pathogens, however, are difficult to implement in low-resource settings, and water quality management is increasingly focused on minimizing risk by reducing the vulnerability of drinking water supplies to contamination.[35,36]

Culture-based and molecular analysis of the bacteria present in positive H_2S tests indicate that H_2S production is correlated with species of fecal origin, however these species are not limited to the coliform group.[23,24,37] Nevertheless, many evaluations of the H_2S test as a microbial water quality indicator, including this one, have evaluated H_2S test results based on their correspondence with assays for total coliforms, fecal coliforms, or *E. coli*.[22,38] Interpreting the results of these evaluations is difficult because they do not consider the impact of non-coliform fecal species on H_2S test results. For example, positive H_2S test results in the absence of detectable coliform contamination are classified as "false positives," despite the possibility that non-coliform fecal bacteria may have contributed to H_2S production.[22,38–40] In addition, culture-based assays for coliform species, particularly methods developed for field use, do not always produce comparable results.[19] It is likely that differences in the microbial ecology of water supplies and in the performance of coliform assays have both contributed to the variable associations reported between H_2S and coliform test results.[22]

Despite the challenges of evaluating H_2S as a fecal indicator based solely on comparisons with coliform assays, our results show that in rural southern India, H_2S was associated with higher levels of total coliform contamination (Figure 2). In addition, the percentage of positive H_2S water samples from water sources showed a strong relationship with total coliform levels (Figure 3). Total coliforms are the accepted process indicators for water quality management: rather than providing a direct measure of health risks, they are used to determine the vulnerability of water supplies to possible pathogen contamination.[7] However, quantitative comparisons of coliform contamination between different water supplies or before and after supply improvements are often not feasible in low-resource settings because of the cost and complexity of approved diagnostic methods. Our results indicate that presence/absence H_2S tests provide a viable alternative for assessing both water supply vulnerabilities and improvement efforts.

Limitations. A limitation of our water collection approach was that field staff collected water samples during child disease surveillance visits. This design and analysis assumes that water quality conditions, as measured at the time illness is ascertained, reflect water quality conditions that led to the illness episode. This design limitation is shared by all other studies that have estimated the association between water quality indicators and child health to date.[16–19] The lack of temporal ordering between exposure and outcome could be subject to bias, if caregivers boil water or change their water management in response to the onset of illness.

In such a scenario, the association between water contamination and illness could be biased toward the null. In this study, 21.8% of drinking water samples were reportedly boiled when collected during visits when study children had diarrhea versus 9.7% of samples collected during visits

without diarrhea ($P < 0.0001$ for difference). Although our adjusted estimates of the PR conditioned on whether the water was reportedly boiled (in an attempt to block this potential pathway), regression adjustment is an imperfect solution to this problem and residual bias could exist. Indeed, water samples were also more likely to be reported boiled when children had cough (17.3% versus 9.4%, $P = 0.012$) and congestion/coryza (13.1% versus 8.5%, $P = 0.001$), yet we observed a positive association between household water quality and these negative control outcomes suggesting the potential for residual bias (Table 6).

Furthermore, diarrhea prevalence was significantly higher in the random subsample of household visits where water samples were collected, after adjusting for season (2.3% versus 1.8%), despite the water sample collection occurring at the end of the interview. This suggests that caregivers may have over reported diarrhea in visits with water sample collection, leading to possible misclassification bias in the outcome. A more rigorous design would collect water samples and then measure child illness in the subsequent 7–14 days to avoid a potential reversal of the cause and its effect and to reduce the chance for biased outcome measurements.

The low prevalence of child diarrhea in our study population limited the statistical power of this study to estimate associations between microbial water quality indicators and diarrhea. Although caregiver-reported illness could be subject to under-reporting,[27] we validated the community-based diarrhea surveillance with clinic records in the study villages.[25]

With respect to water quality analysis, we conducted a few initial side-by-side comparisons of our total coliform and *E. coli* diagnostics with Coliscan membrane filter medium (Micrology Laboratories CMFK2; Micrology Laboratories, Goshen, Indiana). However, we did not conduct a thorough comparison with approved methods (e.g., Standard Methods in the United States) for detecting total coliforms and *E. coli*. Similarly, we did not compare the performance of our selected H_2S assay with other H_2S tests. As a result, it is possible that poor performance of our reagents generated under- or over-estimates of the levels of each indicator. Nevertheless, we note that relative levels would have remained consistent through our study.

We also note that as a result of variability in water characteristics, sample handling, and analysis, assaying a single water sample will not always provide an accurate measurement of microbial water quality. We attempted to reduce the effects of this measurement variability by analyzing a large number of water samples over a 12-month study period. The consistent relationships that we observed between source and household water quality and between total coliform levels and H_2S presence suggest that measurement errors were limited. Nevertheless, it remains possible that inaccuracies in water quality results reduced our ability to detect associations between indicator levels and diarrhea, particularly since diarrhea prevalence was low.

Conclusions. Child diarrhea prevalence was surprisingly low in our study population (1.8%) despite poor microbial drinking water quality, as measured by H_2S presence and by total coliforms and *E. coli* indicator levels. These findings suggest that waterborne pathogens were not a principal cause of diarrhea during the study period. However, the poor microbial drinking water quality indicated that water supplies were vulnerable to fecal contamination and could facilitate patho-

gen transmission during disease outbreaks. The relationships between total coliform contamination and H_2S test results indicate that verified presence/absence H_2S tests provide simple and cost-effective alternatives to quantitative total coliform assays, both for assessing the vulnerability of water supplies to fecal contamination and for evaluating water quality management efforts. Because of the increasing emphasis on maintaining drinking water quality through risk mitigation, our results support the use of presence/absence H_2S tests for water supply management in low-resource settings.

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