Bacterial indicators of risk of diarrhoeal disease from drinking-water in the Philippines

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Inadequate measures of water quality have been used in many studies of the health effects associated with water supplies in developing countries. The present 1-year epidemiological–microbiological study evaluated four bacterial indicators of tropical drinking-water quality (faecal coliforms, Escherichia coli*, enterococci and faecal streptococci) and their relationship to the prevalence of diarrhoeal disease in a population of 690 under-2-year-olds in Cebu, Philippines.* E. coli *and enterococci were better predictors than faecal coliforms of the risk of waterborne diarrhoeal disease. Methods to enumerate* E. coli *and enterococci were less subject to interference from the thermotolerant, non-faecal organisms that are indigenous to tropical waters. Little difference was observed between the illness rates of children drinking good quality water (< 1* E. coli *per 100 ml) and those drinking moderately contaminated water (2–100* E. coli *per 100 ml). Children drinking water with > 1000* E. coli *per 100 ml had significantly higher rates of diarrhoeal disease than those drinking less contaminated water. This threshold effect suggests that in developing countries where the quality of drinking-water is good or moderate other transmission routes of diarrhoeal disease may be more important; however, grossly contaminated water is a major source of exposure to faecal contamination and diarrhoeal pathogens.*

Introduction

Seriously polluted water supplies are used by at least 1500 million people worldwide (*16*). Because of the magnitude of the health problems associated with water of inadequate quality and quantity, substantial efforts have focused on how to evaluate and maximize the health benefits derived from improved water supplies. In many developing countries the high incidence of waterborne disease and widespread use of untreated and often highly polluted water sources make the accurate assessment of faecal contamination of water particularly important.

The indicator bacteria commonly used to evaluate water quality in temperate climates may not be appropriate for the tropics, where water sources are typically warm (21–31 °C) and have high nutrient levels. These characteristics influence the survival and extra-enteral regrowth of indicator bacteria and encourage the growth of non-faecal, thermotolerant indigenous microorganisms. The latter can cause heavy background growth or give false positive reactions and thereby interfere with enumeration of the indicator bacteria.

Several investigators have reported difficulties in using total and faecal coliforms to measure the sanitary quality of tropical waters, including the ability of the bacteria to multiply, to survive for long periods, and to occur in high numbers in the absence of any identifiable source of faecal pollution (*21*).

Current WHO bacteriological guidelines (*38*) for drinking-water recommend zero faecal coliforms per 100 ml of water. However, some investigators hold that this is not feasible for untreated, unpiped water sources used in developing countries (*16, 39*). There is therefore a need for epidemiological–microbiological studies in tropical developing countries to define measures of water quality that accurately reflect the potential for disease transmission and to formulate realistic water guidelines applicable to the special problems and needs of such countries (*14, 21, 39*).

Previous epidemiological field studies that attempted to relate water supply and diarrhoeal disease have been reviewed by McJunkin (*28*), Blum & Feachem (*5*), and Esrey et al. (*13*), *inter alia*. In an analysis of data pooled from nine separate studies, Esrey et al. concluded that improvements in water quality alone caused a median reduction of 16% in diarrhoea morbidity (range, 0–90%) and

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that improvements in both water quality and availability resulted in a median reduction of 37% (range, 0–82%) (*13*). Among the common methodological problems observed by McJunkin (*28*) was the inadequate measurement of water quality. Most of 32 field studies of water supply and diarrhoeal disease he reviewed appeared not to have measured bacteriological water quality; instead, exposure to "good" or "poor" water quality was estimated by the type of water source or level of service. Only 10 of the studies reported the bacteriological quality of the water; and of these, seven measured total coliforms (*29–32, 34, 35, 37*), one measured faecal coliforms (*22*), and two measured faecal coliforms and faecal streptococci (*3, 15, 33*).

The present study used an epidemiological approach to evaluate four bacterial indicators of tropical drinking-water quality (faecal coliforms, *Escherichia coli*, enterococci, and faecal streptococci) by examining the relationship between the concentrations of these bacteria in drinking-water and the prevalence of diarrhoea in a population that consumed this water. Faecal coliforms and faecal streptococci were selected as standard indicator bacteria, while *E. coli* and enterococci were chosen because of their successful use in other studies, because of evidence that they are relatively faecal-specific, and because of new selective methods for their enumeration (*8, 39*).

Methods

General description of the study

A one-year study of the above-mentioned indicators of tropical drinking-water quality was carried out as part of two larger investigations on infant feeding practices and on the effect of water and sanitation on infant health in metropolitan Cebu, Philippines. Because the study shared the infrastructure and resources of these larger investigations, their design limited the type and frequency of the data collected to 2-monthly intervals.

One of the larger studies examined the effects of water quality and use, excreta disposal, wastewater disposal, and solid-waste disposal practices on diarrhoeal disease morbidity among 690 infants. These infants were selected from nearly 2800 aged 0–24 months who were being followed in a 3-year prospective study of the relationship between infant feeding practices and infant morbidity and mortality.[a] In developing countries, under-2-year-olds suffer the highest incidence of diarrhoeal disease (*36*), and are a sensitive population group for studying the effects of water quality on this disease.

Data were collected in a series of interviews of mothers or caregivers. A baseline questionnaire that was administered before the birth of the child was followed by an interview several days after the baby's birth and then by interviews every 2 months until the child was 2 years of age. All questionnaires were administered by trained field-workers and then edited.

The prevalence of diarrhoea morbidity in the study population was determined at the 2-monthly interviews, and rectal swabs were collected from all children who had diarrhoea at the time of the interview. Most of the cases of diarrhoea detected at home visits were relatively mild. All cases identified by the mother as diarrhoea were included, and no formal case definition was used. This approach has been used previously (*19, 36*), and is considered satisfactory. Of the 363 cases identified in the study, 82% involved the passage of three or more stools in 24 hours, which is similar to definitions of diarrhoea used in several other studies (*36*). The mean reported duration of the illness was about 4 days. The diarrhoea etiologies were determined and have been described elsewhere.[b]

Study site and population

Metropolitan Cebu has a population of slightly more than 1 million, and includes Cebu city, the second largest city in the Philippines. A total of 33 communities (*barangays*) were randomly selected to include a mixture of urban and rural, coastal and mountainous environments, and a considerable spread of social, economic, and health conditions.

Preliminary data indicate a birth rate of about 23 per 1000 and an infant mortality rate of about 54 per 1000 (Office of Population Studies, unpublished reports, 1987). Diarrhoea, pneumonia, and malnutrition are the leading causes of infant mortality, with diarrhoea being involved in approximately 17% of deaths during the first 2 years of life (Office of Population Studies, unpublished reports, 1987).

The 690 infants were chosen randomly from all live births that occurred from May 1983 to April 1984, in the 33 selected *barangays*, excluding multiple births. Their age distribution in January 1985 at the beginning of the study is shown in Table 1; 53% were male and 74% lived in urban *barangays*.

[a] Conducted jointly by the Carolina Population Center, University of North Carolina, the Office of Population Studies, University of San Carlos, and the Nutrition Center of the Philippines.

[b] **Moe, C.L.** *An evaluation of four bacterial indicators of risk of diarrheal disease from tropical drinking-waters in the Philippines.* Doctoral dissertation. The University of North Carolina at Chapel Hill, 1989.

Table 1: **Age distribution of study children, Cebu, Philippines, January 1985**

Age (months)	No. of children
9–10	204 (29.6)[a]
11–12	152 (22.0)
13–14	83 (12.0)
15–16	86 (12.5)
17–18	96 (13.9)
19–20	69 (10.0)
Total	690 (100.0)

[a] Figures in parentheses are percentages.

Water sampling programme

All water sources used by the study population were routinely monitored for bacteriological quality. The proportion of families using the various types of sources included in the study is summarized in Table 2. It was intended to sample different types of sources at a frequency based on the proportion of the study population using the source and the number of sources in each category. In practice, however, each of the boreholes, wells and springs was sampled approximately five times between January and December 1985. The city water supply was sampled approximately 138 times at various points in the distribution system.

Samples of water were collected in sterile 500-ml polypropylene bottles, employing methods that took into account those of a typical user of the source. For example, taps or spouts were not sterilized before sample collection; however, a sterile bucket was used to collect water samples from open wells with no pumps. All samples were transported to the laboratory in insulated containers that were chilled with frozen refrigerant packs, refrigerated overnight, and analysed the next morning. The water samples were examined for the four indicator bacteria using membrane filtration methods. For each indicator 1-ml, 10-ml, and 100-ml samples of water were passed through 47-mm diameter, 0.45 μm porosity cellulose filters.[c] Phosphate-buffered saline (pH 7.5) was used as a diluent for the filtration of the 1-ml samples and as a rinse.

Faecal coliforms were enumerated on mFC agar[d] and faecal streptococci on KF streptococcus agar (*1*).[c] During the last 6 months of the study not all water samples were analysed for faecal streptococci because of economic constraints and problems with heavy background growth and atypical colonies.

E. coli was enumerated on mTEC agar according to the method described by Dufour et al. (*12*), an approach that is particularly suitable for tropical waters since it singles out *E. coli* from the interfering background growth. However, the method assumes that the majority of thermo-tolerant, non-*E. coli* coliforms are urease-positive, and thus can be easily differentiated; this may not be true for tropical waters. The technique has, nevertheless, been used successfully by Carillo et al. in Puerto Rico (*9*).

Enterococci were enumerated on mE medium followed by testing for esculin hydrolysis on esculin–iron agar, as described by Levin et al. (*24*). This method has potential for the analysis of tropical waters because of its ability to differentiate enterococci (a more faecal-specific indicator group restricted to *Streptococcus faecalis* and *S. faecium*) from background growth.

Statistical analysis of the data

The statistical analysis examined whether there was a consistent and significant relationship between any of the indicator bacteria and the risk of diarrhoeal disease. Initially, estimates of the concentrations of these bacteria in each water sample were obtained from the number of colonies on the membrane filters. Their density per 100 ml was calculated from the filter colony counts of the three sample dilutions, as outlined below, assuming that the average density was independent of the volume of water filtered and that the number of colonies per plate followed a Poisson distribution (*20*).

- If the number of colonies on one filter was in the countable range (approximately 10–100 colonies for faecal coliforms, *E. coli*, and faecal streptococci; and 10–80 for enterococci), the density was estimated from the number of colonies on that filter.

Table 2: **Types of drinking-water sources used by the study population, Cebu, Philippines**

	No. of families	No. of sources
Springs	59 (8.6)[a]	34
Open dug wells	55 (8.0)	38
Wells with pumps	22 (3.2)	15
Boreholes	350 (50.7)	230
MCWD[b]	168 (24.3)	1
Other piped water[c]	36 (5.2)	1
Total	690 (100.0)	319

[a] Figures in parentheses are percentages.
[b] Municipal piped system; chlorinated groundwater.
[c] Untreated piped system in one rural neighbourhood.

[c] GN-6, Metricel, Gelman Sciences, Ann Arbor, MI, USA.

[d] Difco Laboratories, Detroit, MI, USA.

- If more than one filter had counts in the countable range or if all three filters had counts <10, the density was estimated using a maximum likelihood function in which the total number of colonies on these filters was divided by the total volume of water filtered (*20*).
- If one or two filters had counts <10 and the other filter(s) had colonies too numerous to count, a maximum likelihood function was used to estimate the number of colonies on the latter filter(s) (*20*).
- If for all three dilutions the colonies were too numerous to count, the concentration was assumed to be twice the maximum of the countable range of the highest dilution, i.e., 200 colonies per ml.

Each estimate was given a reliability rating (A–D) based on the following criteria: the relation of the colony counts to the countable range; the amount of background growth; the progression of increased counts at higher volumes; and the colony characteristics (typical or atypical). The following proportions of the approximately 2000 water samples in the study had high reliability ratings: *E. coli* (85%), enterococci (79%), faecal coliform (77%), and faecal streptococci (60%). Low ratings were assigned to estimates calculated from less than 10 colonies, filters with heavy background growth, or filters with atypical colonies.

If a water source was sampled more than once in a 2-month period, the estimate with the best reliability rating was taken. Also, if in this period several measurements of a water source had the same reliability rating, the highest estimate was used, since it reflected the worst case potential exposure for the population using that source. Although this may bias the data towards higher estimates of pollution, such extremes are more likely to cause disease than minor day-to-day variations in water quality. In the USA there is evidence that unexpectedly heavy contamination of water supplies is responsible for the occurrence of many outbreaks of waterborne diseases (*11*).

The data obtained in the study were analysed as a series of six cross-sectional studies, rather than as a follow-up study. Each cross-sectional analysis included information for all the study households that were interviewed and whose water source was sampled during a 2-month period. This analysis assumes that the occurrence of diarrhoea in each 2-month period is an independent event. However, each study child was followed for more than one 2-month period (depending on how often his or her water supply was sampled), and his or her risk of diarrhoea may have been influenced by diarrhoea morbidity in the previous 2-month period.

This approach also assumes that the level of water pollution reflected by the concentration of indicator bacteria at the time of sampling is representative of the level that the study child was exposed to during a given 2-month period. For sources with highly variable water quality, this assumption has limited validity and may have misclassified the exposure of some study children. However, because this misclassification is likely to be independent of disease status it tends to bias the results conservatively by underestimating the effect of water quality on diarrhoea morbidity (*23*).

In the analysis of the disease–exposure relationship, the outcome (diarrhoeal disease at the time of the longitudinal interview) was considered to be a dichotomous variable (presence or absence of disease) for each study child at each 2-monthly interview. The exposure of interest was the concentration of indicator bacteria in the drinking-water sources used by the study families. This exposure was considered as follows:

— as a continuous variable ranging from <1 per 100 ml to >20 000 per 100 ml;
— as a categorical variable with initially five categories (<1, 2–10, 11–100, 101–1000, and >1000 per 100 ml); and
— as a categorical variable with two categories (<1000 and >1000 per 100 ml based on the results of the preliminary stratified analysis).

When exposure was considered as a continuous variable, estimates of the indicator bacteria level per 100 ml were $\log_{10}$-transformed to reduce skewness.

Consideration of the risk factors for diarrhoea is complicated by its multiple transmission routes and by underlying determinants that affect behaviour and health. In our analysis the effects of age, gender, urban or rural environment, and season on diarrhoeal disease were examined. Any potential confounding by the type of water source was analysed by stratifying for this. These results have been described elsewhere.[e]

Results

Water quality

Heavy background growth on membrane filters was a frequent problem in the analysis of faecal coliform and faecal streptococci that influenced the reliability of the data. The greater proportion of reliable estimates of *E. coli* and enterococci concentrations reflects fewer problems with background growth and atypical colonies. The mTEC and mE methods for

[e] See footnote *b*, p. 306.

E. coli and enterococci, respectively, were advantageous because they allowed a clearer separation of the target organism. Random indole tests for presumptive *E. coli* on the mTEC plates were usually positive, indicating that the method was reasonably specific.

The temperature of 37 water samples measured in the field ranged from 24 °C to 30 °C (mean, about 27 °C).

Table 3 shows the distribution of the densities of indicator bacteria by type of water source for those samples that were used in the disease–exposure analysis, i.e., one sample per water source per 2-month period. All the indicators exhibited the same general distribution pattern in terms of the overall quality of different types of water sources. For example, more samples (33–40%) of springwater were in the category 101–1000 indicators per 100 ml than in any other density category for all four indicator bacteria.

Boreholes, which ranged in depth from about 12 m to 37 m, were generally better protected sources and usually provided water of relatively good quality. More borehole samples (38–77%) had <1 indicator organism per 100 ml for all four indicators. A greater proportion of borehole samples had higher densities of enterococci and faecal streptococci than of faecal coliforms and *E. coli*; this may reflect the longer survivability in water of enterococci and some species of faecal streptococci (*27*).

Compared with open dug wells, dug wells with pumps had a greater proportion of samples in the two lowest indicator density categories (<1 and 2–10 indicators per 100 ml). At least 65% of water samples from open dug wells had faecal coliform, enterococci, and faecal streptococci densities of >100 indicators per 100 ml. *E. coli* densities were somewhat lower.

The high contamination level of the majority of water samples from wells is not surprising in view of the geology of the study area, the construction of the wells, and local sanitation practices. Most of the wells were relatively shallow (<5 m deep) and either unprotected or only poorly protected. Parts of Cebu island consist of porous marine limestone, which results in rapid groundwater flow rates. The siting of pit latrines and communal bathing and laundry activities near water supplies creates conditions with a high potential for groundwater contamination.

Springs tended to be less contaminated than open dug wells. For all four indicator bacteria, more than 50% of water samples from springs had >100 indicators per 100 ml. Most springs were unprotected from surface run-off.

The small, untreated, piped system that served one rural *barangay* did not provide high quality water. All samples had at least 11–100 indicators per 100 ml and several had >1000 indicators per 100 ml.

Also, the municipal piped system (MCWD) did not deliver water of consistently high quality. Table 4 shows the distribution of all samples taken from this system, according to the density of indicator bacteria. Frequent fluctuations in pressure and leaky pipes resulted in contamination in areas where water pipes lay in open gutters. The majority of samples had <1 indicator per 100 ml for faecal coliforms, *E. coli*, and enterococci; however, depending on the microorganism, 4–13% of the samples had >100 indicators per 100 ml. The MCWD system was sampled in several city *barangays* in each 2-month period. Families who reported using the system, either through a standpipe or a direct connection in their home, were assumed to have been exposed to the indicator density measured in MCWD samples from their *barangay*.

Table 3: **Percentage of water samples in the indicator density categories 0–4, according to water source type**

	Faecal coliforms[a]						*Escherichia coli*[a]						Enterococci						Faecal streptococci					
Type of water source	*n*[b]	0	1	2	3	4	*n*[b]	0	1	2	3	4	*n*[b]	0	1	2	3	4	*n*[b]	0	1	2	3	4
Springs	123	12	9	25	33	21	123	13	14	21	33	20	123	5	7	30	40	18	75	19	8	23	35	16
Open dug wells	131	2	7	26	44	21	129	3	14	40	30	13	127	0	6	25	43	26	75	16	8	11	40	25
Wells with pumps	52	14	17	25	31	14	52	19	21	21	31	8	49	8	16	37	22	16	31	32	3	36	13	16
Boreholes	751	74	11	5	4	6	748	77	11	5	3	4	738	65	20	8	4	3	485	38	32	18	7	5
Other piped water	5	0	0	20	20	60	5	0	0	40	20	40	5	0	0	20	40	40	2	0	0	50	0	50

[a] 0 = ≤ one indicator per 100 ml; 1 = 2–10 indicators per 100 ml; 2 = 11–100 indicators per 100 ml; 3 = 101–1000 indicators per 100 ml; 4 = > 1000 indicators per 100 ml.
[b] *n* = number of water samples

Table 4: **Distribution of water samples from the municipal piped system (MCWD), by indicator density**

Indicator	No. of samples	% of samples with indicator density per 100 ml of:				
		< 1	2–10	11–100	101–1000	> 1000
Faecal coliforms	138	73	12	3	8	5
Escherichia coli	130	80	8	4	6	2
Enterococci	138	69	20	7	4	1
Faecal streptococci	74	42	30	24	1	3

Correlations between bacterial indicators

The correlation coefficients between the $\log_{10}$-concentrations of the bacterial indicators in all the water supplies studied are shown in Table 5 for each 2-month period and for all time periods combined. All four indicators correlated reasonably well with each other. Not surprisingly, the correlation was strongest between faecal coliforms and *E. coli* (a subgroup of faecal coliforms) ($r = 0.85$). Enterococci (a subgroup of faecal streptococci) were also well correlated with faecal streptococci ($r = 0.72$). The correlations between all the indicator bacteria decreased in May and June, but this did not coincide with any substantial change in rainfall or temperature.

Prevalence of diarrhoea

No significant seasonal differences in the prevalence of diarrhoea were observed ($P = 0.124$) (Table 6). Age-specific seasonal analysis also showed no significant differences. This may reflect the lack of extreme seasonal differences in rainfall in Cebu or suggests that the dominant transmission routes of diarrhoeal disease in this study were not very sensitive to the effects of rainfall.

An analysis of the prevalence of diarrhoea by age among 10–12-month-olds, 13–18-month-olds, and 19–24-month-olds revealed no significant differences. However, children aged about 14 months or younger had slightly higher diarrhoea rates than those aged 19 months or older. Higher rates of diarrhoea may have been associated with weaning, but we did not investigate this. There were no significant differences in the prevalence of diarrhoea between males and females.

Disease–exposure relationship

Table 7 shows the distribution of diarrhoea cases over five exposure categories of the density of the indicator bacteria. The lowest exposure category (<1 indicator per 100 ml water) meets WHO guidelines for faecal coliforms in drinking-water. Because no significant seasonal differences in illness rates were observed, Table 7 shows combined data for diarrhoea cases and children at risk for all six study periods.

The pattern of disease rates for the first four exposure levels was similar for all four indicators but somewhat greater disease rates were associated with the highest category (>1000 indicators per 100 ml).

Table 5: **Correlations between $\log_{10}$-indicator densities per 100 ml, by 2-month interval, and for all study intervals combined**

	Correlation coefficient (r) for the period:						
	Jan.–Feb.	Mar.–Apr.	May–June	July–Aug.	Sept.–Oct.	Nov.–Dec.	Combined intervals
Faecal coliforms versus:							
Escherichia coli	0.89	0.85	0.76	0.92	0.81	0.92	0.85
Enterococci	0.81	0.74	0.44	0.84	0.64	0.70	0.68
Faecal streptococci	0.48	0.70	0.50	0.65	0.59	—[a]	0.58
E. coli versus:							
Enterococci	0.77	0.80	0.48	0.85	0.75	0.76	0.74
Faecal streptococci	0.46	0.74	0.54	0.71	0.62	—[a]	0.63
Enterococci versus:							
Faecal streptococci	0.58	0.82	0.60	0.85	0.72	—[a]	0.72

[a] No faecal streptococci analyses were performed in Nov.–Dec.

Table 6: **Prevalence of diarrhoea among the study children by 2-month periods, Cebu island, Philippines**

	Jan.– Feb.	Mar.– Apri.	May– June	July– Aug.	Sept.– Oct.	Nov.– Dec.
No. of cases of diarrhoea	40	62	65	49	44	17
No. of children interviewed	555	646	651	577	480	324
% prevalence	7.2	9.6	10.0	8.5	9.2	5.2

These results suggest there was an exposure threshold effect on disease rates for all four indicators.

To further analysis this effect, we defined two exposure categories: low (≤1000 indicators per 100 ml); and high (>1000 indicators per 100 ml). Even though no significant time effect was observed, the Mantel–Haenszel χ^2 test for association was repeated, treating time as a stratification factor. This analysis tests for overall association and gives a corresponding overall P-value and estimate of overall effect (risk ratio). Table 8 shows the prevalence of diarrhoea associated with low and high exposure for all four indicators during the entire study period, and summarizes the results of these analyses.

All four indicators showed a positive relationship between exposure and disease. For *E. coli* and enterococci the overall P-values when time was controlled (*E. coli*, $P = 0.02$; enterococci, $P = 0.03$) were significant at the 0.05 level. For faecal streptococci the overall P-value (0.08) was not significant at the 0.05 level, and the 95% confidence interval (CI) of the risk ratio (0.95–2.28) includes unity. Faecal coliforms had the smallest overall risk ratio and were not significant predictors of diarrhoeal disease at the 0.05 level.

When the density of *E. coli* was taken as a predictor, the relative risk of diarrhoeal illness for a child in the high exposure group was 1.52, i.e., 52% greater than that for a child in the low exposure group. The 95% CI of the risk ratio (1.06–2.18) does not include unity. For enterococci exposure levels, children in the high group had a relative risk of 1.56 for diarrhoeal illness (95% CI: 1.04–2.33), i.e., 56% greater than that of children in the low category.

The results of the Breslow–Day test for interaction (*23*), i.e., the homogeneity of risk ratios over time, showed that for all four indicators the relationship between exposure and disease was relatively consistent over all six periods.

Stratified analysis of the relationship between water quality and risk of "highly credible diarrhoea", i.e., those cases from which a diarrhoea pathogen was isolated from the rectal swab (34% of all diarrhoea cases) indicated that faecal coliforms and faecal streptococci were again not significant predictors of risk of diarrhoeal disease (Table 8). *E. coli* were marginally significant when the data were combined over time ($P = 0.06$), but not when time was included as a stratification variable ($P = 0.12$). Enterococci were highly significant predictors of illness both in the combined analysis ($P = 0.005$) and when time was controlled ($P = 0.01$). For enterococci, the overall risk ratio was 2.13, i.e., children in the high exposure group had a 113% greater risk of diarrhoeal disease than those in the low exposure group.

Logistic regression analysis, as described by Kleinbaum et al. (*23*), was used to examine the disease–exposure relationship after controlling for the effects of age, gender, and urban/rural environment. Although age and gender were not statistically significant confounders, controlling for them increased the precision of the overall estimate of effect—the risk odds ratio. The urban/rural environment was controlled because of evidence of mild confounding.

Two logistic models were examined for each indicator microorganism. In the first model, the logarithm of the odds of having diarrhoea was

Table 7: **Stratified analysis of disease–exposure relationship for five exposure levels of the indicator bacteria for all time periods combined**

Indicator density per 100 ml	No. of diarrhoea cases/ population at risk
Faecal coliforms:	
< 1	106/1139 (9.3)[a]
2–10	16/204 (7.8)
11–100	16/196 (8.2)
101–1000	26/279 (9.3)
> 1000	36/296 (12.2)
Escherichia coli:	
< 1	104/1125 (9.2)
2–10	13/196 (6.6)
11–100	20/235 (8.5)
101–1000	25/248 (10.1)
> 1000	33/220 (15.0)
Enterococci:	
< 1	86/1001 (8.6)
2–10	37/339 (10.9)
11–100	26/281 (9.3)
101–1000	26/297 (8.8)
> 1000	25/175 (14.3)
Faecal streptococci:	
< 1	32/373 (8.6)
2–10	41/407 (10.1)
11–100	27/285 (9.5)
101–1000	13/141 (9.2)
> 1000	22/149 (14.8)

[a] Figures in parentheses are percentages.

Table 8: **Results of a stratified analysis of the disease–exposure and highly credible disease-exposure relationship using two exposure levels**

	Disease exposure				Highly credible disease exposure			
	Faecal coliforms	*Escherichia coli*	Enterococci	Faecal streptococci	Faecal coliforms	*Escherichia coli*	Enterococci	Faecal streptococci
Low exposure[a]								
No. of diarrhoea cases	164	162	175	113	63	64	64	44
Population at risk	1818	1804	1918	1206	1717	1706	1807	1137
Diarrhoea prevalence (%)	9.0	9.0	9.1	9.4	3.7	3.8	3.5	3.9
High exposure[b]								
No. of diarrhoea cases	36	33	25	22	14	13	13	8
Population at risk	296	220	175	149	274	200	163	135
Diarrhoea prevalence (%)	12.2	15.0	14.3	14.8	5.1	6.5	8.0	5.9
No. of people[c]	2114	2024	2093	1355	1991	1906	1970	1272
P-value (Mantel–Haenszel χ^2 test; combined over time)	0.087	0.004	0.026	0.038 0.251	0.062	0.005	0.254	
Overall *P*-value (Cochran–Mantel–Haenszel χ^2 test; controlling for time)	0.285	0.023	0.032	0.084	0.448	0.117	0.01	0.354
Overall risk ratio[d]	1.22 (0.85–1.74)	1.52 (1.06–2.18)	1.56 (1.04–2.33)	1.47 (0.95–2.28)	1.26 (0.69–2.30)	1.61 (0.89–2.91)	2.13 (1.20–3.80)	1.44 (0.68–3.07)
P-value (Breslow–Day test)	0.441	0.667	0.436	0.885	0.685	0.891	0.265	0.750

[a] ≤ 1000 indicators per 100 ml.
[b] > 1000 indicators per 100 ml.
[c] No. of people who were interviewed and had their water source sampled during the study period.
[d] Figures in parentheses are 95% confidence intervals.

regressed against a continuous exposure variable ($\log_{10}$ of the indicator density per 100 ml). In the second model, a dichotomous exposure variable (1 for >1000 indicators per 100 ml; 0 for <1000 indicators per 100 ml) was used. Both models used data combined from all six periods.

The estimated model parameters for all four indicator bacteria are shown in Table 9. A better fit to the data was obtained with the categorical model since it reflects the threshold effect of exposure on the risk of diarrhoeal disease. For this model the odds ratios for being exposed to >1000 indicators per 100 ml were 1.49, 1.92, 1.94, and 1.81, respectively, for faecal coliforms, *E. coli*, enterococci, and faecal streptococci. *E. coli* and enterococci were strongly significant predictors ($P < 0.01$) and faecal streptococci were also significant predictors ($P < 0.05$) of disease risk. Faecal coliforms were marginally significant ($P = 0.053$). However, the 95% confidence intervals of the odds ratios for all four indicators overlap.

The results of the logistic regression analysis support the conclusions drawn from the stratified analysis (Table 8). The odds ratios from the logistic regression analysis are similar to the risk ratios calculated in the stratified analysis and the 95% CI of these estimates overlap.

The odds ratios were used to predict the probability of a typical member of the study population (an 18-month-old female living in an urban *barangay*) having diarrhoea (Table 9). The results indicate that drinking-water with >1000 *E. coli* per 100 ml produced a 15% chance of having diarrhoea on any given day, compared with a 9% chance for drinking less polluted water. Consumption of the more contaminated water was associated with a 1.9 times greater probability of having diarrhoeal disease than that of drinking-water with <1000 *E. coli* per 100 ml.

Discussion

Relationship between water quality and diarrhoeal disease

Our results have important implications for assessing and attempting to control waterborne diarrhoeal illness in tropical developing countries.

The prevalence of diarrhoea among children who drank good water (<1 *E. coli* per 100 ml) was about the same as that among children exposed to moderately contaminated water (2–100 *E. coli* per 100 ml). When water quality is good or moderate,

Table 9: **Estimated parameters for logistic regression models using continuous or categorical exposure variables**[a]

	Faecal coliforms ($n = 2114$)		*Escherichia coli* ($n = 2024$)		Enterococci ($n = 2093$)		Faecal streptococci ($n = 1355$)	
	Continuous	Categorical	Continuous	Categorical	Continuous	Categorical	Continuous	Categorical
Intercept	−2.18	−2.12	−2.29	−2.15	−2.13	−2.09	−2.45	−2.32
Slope	0.10	0.40	0.16	0.65	0.13	0.66	0.17	0.60
Standard error of slope	0.05	0.20	0.06	0.21	0.07	0.25	0.07	0.26
P-value	0.060	0.053	0.004	0.002	0.063	0.007	0.021	0.020
Odds ratio	1.10	1.49	1.18	1.92	1.14	1.94	1.19	1.81
	(1.00–1.23)[b]	(1.00–2.22)	(1.05–1.31)	(1.27–2.91)	(0.99–1.31)	(1.20–3.16)	(1.03–1.37)	(1.10–3.00)
Probability of diarrhoea in 24 hours[c]								
Low exposure[d]	0.08	0.08	0.08	0.09	0.08	0.09	0.08	0.09
High exposure[e]	0.12	0.12	0.15	0.15	0.13	0.16	0.14	0.15

[a] Data were combined over time, controlling for age, gender, and urban/rural environment.
[b] Figures in parentheses are 95% confidence intervals.
[c] Calculated for an 18-month-old female in an urban *barangay*.
[d] ≤ 1000 indicators per 100 ml.
[e] > 1000 indicators per 100 ml.

these findings suggest that other transmission routes of diarrhoeal disease are more important.

This threshold effect of indicator density on risk of diarrhoeal illness tends to support Feachem's proposal for more flexible water quality guidelines for developing countries (*14*). Use of moderately contaminated water containing up to 1000 *E. coli* per 100 ml did not appear to cause an increased risk of diarrhoeal disease, and may be acceptable in areas where water treatment is not feasible and better quality sources are not accessible.

Probably several transmission routes for diarrhoeal disease were important in this study environment. The households of the study population were typical of those in many developing countries in that few had running water and that water usually was stored in large containers. Excreta disposal facilities (generally pit latrines, water-sealed toilets, and flush toilets) were usually shared by large numbers of people. Also, many households did not have access to any such facilities and used open fields, riverbanks, or seashores.

Relatively poor personal hygiene and food preparation and storage practices were not uncommon in the study population, and may have contributed to disease transmission. Very few households had refrigerators, and food was often cooked once a day and then eaten at various times thereafter with little or no reheating. Analysis of a common infant weaning food (rice pudding) left at room temperature revealed that it contained very high levels of bacteria by aerobic plate count.

Most households in both urban and rural *barangays* had domestic animals (dogs, cats, poultry, and pigs) living in close proximity in the yard or under the house. Direct or indirect contact with animal faecal material may be common and could result in transmission of diarrhoeal diseases with animal reservoirs, e.g., salmonella and campylobacter.

The results of the larger studies that included the present study confirm the importance of several transmission routes of diarrhoeal pathogens in the first year of life (*10*). The methodology used in the present study permits only the investigation of a unidirectional relationship, i.e., it assumes that water quality can affect health but not vice versa. In the larger studies the possibility that the health status of a child is not only determined by water quality but that water use choices may be affected by the health status of the child was also investigated. The results indicate that among the urban population poor water quality, poor excreta disposal practices, poor food hygiene, and high community density were all significantly associated with an increased likelihood of diarrhoea in the first year of life. Breast-feeding appeared to exert a strong protective effect against diarrhoea, perhaps by decreasing exposure to foodborne pathogens and by providing immunological benefits.

In our study, the type of water source used by each study family was examined to check possible confounding by socioeconomic status or geographical location. The type of water source had no significant effect on the risk of diarrhoeal disease after controlling for the indicator density.[f] However, the density of *E. coli* was still a significant

[f] See footnote *b*, p. 306.

predictor of diarrhoeal disease after controlling for the type of water source. Type of water source may not be a good surrogate for socioeconomic status in this study population, since the municipal water supply was widely used regardless of socioeconomic status. Children in rural areas were more likely to be exposed to contaminated water, but those in the urban environment were generally at greater risk of developing diarrhoea than rural children for all indicator exposure categories.[f]

Our results show that when the water supply was grossly contaminated (>1000 *E. coli* per 100 ml), the rate of diarrhoeal disease was significantly higher, and it appears that in this situation water becomes a major source of exposure to faecal contamination and diarrhoea pathogens. The use of such highly contaminated water to prepare food may also increase foodborne transmission of diarrhoeal illness. In the Gambia, microbiological examination of weaning foods in various stages of preparation has confirmed that highly polluted water (1700–11 000 *E. coli* per 100 ml) was a major source of faecal contamination, which was not completely eradicated by cooking (*4*).

Evidence for a threshold effect of water quality on diarrhoeal disease may explain some of the ambiguous results of previous studies of water quality and diarrhoeal disease in developing countries. It is generally assumed that provision of a safe water supply results in a corresponding reduction in waterborne disease. However, in some developing countries the installation of improved water supplies in villages did not produce a consequent measurable decrease in diarrhoeal disease morbidity (*35*).

Briscoe has proposed that the removal of a waterborne transmission route might not result in a substantial reduction in the rate of diarrhoeal disease because of the effects of other routes, and that improvements in water quality without concurrent improvements in sanitation and excreta disposal practices are not sufficient to significantly reduce the incidence of diarrhoea (*6*). This is supported by the findings of one study in the Philippines on the health impact of an improved water supply (piped water system) in two cities. Improved water quality had a positive impact on health, as indicated by the incidence of diarrhoea and the nutritional status of children aged 0–4 years in one city, but only among upper-income households, which already had adequate sanitary facilities and sound hygiene practices. Installation of an improved water source alone was therefore not sufficient to produce improved health (*25*). Also, two recent case–control studies of water supply, sanitation, and diarrhoea in the Philippines (*2*) and in Malawi[g] suggest that improved water supply in itself is not enough to reduce the incidence of diarrhoeal disease in children under 5 years of age; however, the combined effect of improved water and sanitation was associated with a reduction in diarrhoea morbidity.

Our results suggest that if a population is consuming moderately contaminated water (perhaps up to 100 *E. coli* per 100 ml), the introduction of an improved water supply that meets WHO guidelines (0 faecal coliforms per 100 ml) may not result in an appreciable reduction in diarrhoeal illness rates. However, if a population is exposed to a grossly contaminated water supply (>1000 *E. coli* per 100 ml), modest improvements in water quality to reduce *E. coli* levels by tenfold or more may significantly reduce diarrhoeal disease rates.

In developing countries it may therefore not be greatly beneficial to health or cost-effective to use limited financial resources to provide high quality water supplies in the absence of other improvements in hygiene and sanitation. Instead, low-cost improvements to existing water supplies should be made to eliminate grossly contaminated water sources, and consideration should be given to improving sanitation and excreta disposal facilities to help eliminate other environmental transmission routes of diarrhoeal diseases.

Comparison of the indicator bacteria

Although the densities of all four bacterial indicators showed a positive relationship with the prevalence of diarrhoea, because of laboratory methodology some were more useful than others. In accord with the findings of Cabelli et al. on recreational waters (*8*), we found that *E. coli* and enterococci appear to be better predictors than faecal coliforms of the risk of waterborne gastrointestinal illness. These results further support the contention that *E. coli* and enterococci are more faecal-specific indicators than faecal coliforms and possibly faecal streptococci (*8, 24*).

E. coli and enterococci may correlate significantly with the rates of diarrhoeal disease because the methods used to enumerate them are more specific and less subject to interference from background growth. Although faecal streptococci were significant predictors of disease in some of the analyses, substantial problems with background growth and atypical colonies detract from their value as indicator bacteria for tropical waters.

Our results support the use of *E. coli* and enterococci as indicators of the quality of tropical

[g] **Young, B. & Briscoe, J.** *Water and health in rural Malawi: aspects of the performance, utilization and health impact of the Malawi self-help rural water supply project.* USAID report, 1986.

freshwaters. The observed ability of *E. coli* to predict the risk of waterborne illness contrasts somewhat with the observations of Hazen that *E. coli* may be indigenous to tropical aquatic environments (*21*). However, if low levels of *E. coli* are indigenous to tropical waters, this may explain why only high densities of *E. coli*, where perhaps the majority are from faecal contamination, caused an increased risk of diarrhoea.

It should be noted that the threshold effect of indicator density on the prevalence of diarrhoea was observed for all four indicator bacteria. Low levels of any of these indicators might therefore reflect indigenous populations in the environment and not faecal contamination. Nonfaecal sources of faecal coliforms, some enterococci (*S. faecalis* var. *liquefaciens*), and faecal streptococci have been reported (*17, 18, 21*). The possibility that these indicators have nonfaecal sources should be further investigated in tropical regions where human and animal faecal contamination are absent.

In tropical countries the use of conventional indicators such as total or faecal coliforms is more likely to result in misclassification of some water sources as contaminated, in instances where high indicator levels may not reflect faecal contamination and a risk of waterborne illness. In such situations the effect of improved water quality on diarrhoeal diseases may be seriously underestimated. This may partly explain why several studies that relied on total or faecal coliforms to evaluate water quality found no differences in diarrhoeal disease rates between those exposed to "good" versus "poor" quality water (*7, 15, 25, 26, 29, 32, 35*). Our results demonstrate the importance of choosing an appropriate indicator of water quality in investigations of the effect of water quality on diarrhoea morbidity.

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Résumé

Indicateurs bactériens du risque de maladie diarrhéique due à l'eau de boisson aux Philippines

Dans de nombreux pays en développement, l'incidence élevée des maladies transmises par l'eau et l'utilisation très répandue de sources d'eau non traitées et souvent fortement polluées rendent l'évaluation précise de la contamination fécale de l'eau particulièrement importante. Il est nécessaire de disposer à la fois de mesures de la qualité de l'eau qui permettent de prédire le risque de transmission de maladies, et de directives réalistes concernant la qualité de l'eau qui soient applicables aux problèmes particuliers des pays en développement.

De nombreuses études ont examiné quelle était la relation entre l'approvisionnement en eau et les maladies diarrhéiques; cependant, la plupart de ces études ne mesuraient pas la qualité bactériologique de l'eau ou utilisaient des indicateurs bactériens qui ne constituaient peut-être pas une mesure appropriée du risque pour les eaux tropicales. Les sources d'eau dans les pays tropicaux sont caractérisées par des températures élevées et des taux élevés de nutriments, conditions qui influencent la survie et la multiplication extra-entérique des bactéries indicatrices et favorisent la croissance de bactéries thermotolérantes non fécales, qui peuvent modifier les résultats de l'analyse.

Nous avons utilisé une approche épidémiologique pour évaluer quatre indicateurs bactériens de la qualité d'eaux de boisson tropicales (coliformes fécaux, *Escherichia coli*, entérocoques et streptocoques fécaux) en examinant les relations entre leur concentration dans l'eau de boisson et la prévalence de la diarrhée dans la population consommant cette eau. Pendant une période d'un an, des échantillons ont été régulièrement recueillis dans 319 sources d'eau de boisson (sources, puits, forages et canalisations) utilisées par une population d'étude de 690 enfants (âgés de 6 à 24 mois) et analysés pour recherche des quatre indicateurs bactériens. La prévalence de la diarrhée a été mesurée tous les deux mois, et des écouvillonnages rectaux, pratiqués chez les cas de diarrhées et chez des enfants sains témoins, ont été analysés pour savoir quelles étaient les étiologies de la diarrhée et les taux d'infection asymptomatique. Une méthode avec analyse stratifiée et régression logistique a été employée pour examiner la relation entre la qualité de l'eau de boisson et la prévalence de la diarrhée.

Peu de différences ont été observées entre les taux de maladie chez les enfants qui avaient bu de l'eau de bonne qualité (définie suivant les normes de l'OMS) et ceux qui avaient bu de l'eau modérément contaminée. Cependant, les enfants exposés à de l'eau fortement contaminée (>1000 *E. coli* par 100 ml) avaient un risque de diarrhée supérieur de 52% à ce qu'il était avec de l'eau moins contaminée. Cet effet de seuil de la qualité de l'eau sur les maladies diarrhéiques laisse à penser que lorsque la

qualité de l'eau est bonne ou moyenne dans les pays en développement, d'autres voies de transmission des maladies diarrhéiques peuvent jouer un plus grand rôle. Cependant, un approvisionnement en eau fortement contaminée est dans tous les cas une source majeure d'exposition à la contamination fécale et aux agents pathogènes de la diarrhée.

Ces résultats confirment l'importance de choisir un indicateur approprié pour évaluer le risque de maladie diarrhéique associée à la consommation d'eau contaminée par des pathogènes fécaux. Bien que la concentration des quatre indicateurs bactériens ait fait preuve d'une relation positive avec la prévalence de la diarrhée, *E. coli* et les entérocoques étaient de meilleurs prédicteurs du risque de maladie transmise par l'eau. Les méthodes de numération de *E. coli* et des entérocoques donnaient de meilleurs résultats dans des conditions tropicales qu'avec les coliformes et les streptocoques fécaux.

Ces observations permettent de penser que dans les pays en développement, des améliorations même modestes apportées aux approvisionnements d'eau fortement contaminée peuvent sensiblement réduire la prévalence des maladies diarrhéiques dans la population. Quand les ressources économiques sont limitées, des améliorations peu coûteuses doivent être apportées aux approvisionnements d'eau pour éliminer les sources fortement contaminées, et il faut s'attacher à améliorer l'hygiène et l'évacuation des excréments pour parvenir à éliminer d'autres sources de transmission présentes dans l'environnement.

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