A Follow-up Study of Gastro-Intestinal Diseases Related to Bacteriologically Substandard Drinking Water

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Abstract: In a prospective follow-up study conducted in 52 French alpine villages, one weekly water sample was taken in each village provided with untreated ground water and analyzed as to the presence of four indicator bacteria: total plate count, total coliforms, thermotolerant (fecal) coliforms, and fecal streptococci. Cases of acute gastro-intestinal disease (AGID) occurring among 29,272 inhabitants were reported through physicians, pharmacists, and primary school teachers. A loglinear model identified fecal streptococcus (FS) as the best predictor; the presence of fecal coliforms enhanced the effect of FS. The total bacteria count and the total coliforms had no independent contributions. A threshold analysis suggested that any level of indicator bacteria above zero was associated with an excess of AGID. (*Am J Public Health* 1987; 77:582–584.)

Introduction

Microbiology standards for drinking water are periodically evaluated.[1] The indicator bacteria used in the European community to assess whether a 100 ml water sample is potable are fecal coliforms (FC) and fecal streptococci (FS).[2] Total coliforms are also used as indicator bacteria in many countries, such as the United States.[3] The dramatic decline of "classical" waterborne diseases in developed countries has lessened the concern of local authorities with these issues. The frequency of substandard water samples is relatively common in the alpine regions, but water-related disease outbreaks are rather rare.[4] Alpine health authorities are highly concerned, however, about the increasing costs and constraints of the water policies.

This study had the following objectives: to assess the risks related to the consumption of drinking water that does not meet current bacteriology standards; to identify the indicator bacteria that best predict this risk; to determine whether there is an indicator bacteria threshold other than zero above which water should be declared "non-potable".

Methods

The study design is presented in detail elsewhere.[5] Two parallel surveys were conducted: 1) a weekly water sample was taken in each of 52 villages and analyzed the same day in a single central laboratory; and 2) a day-to-day count of the acute gastro-intestinal diseases occurring among inhabitants of the study villages and identified through 119 physicians, 52 pharmacists, and 118 primary school teachers (for children aged 7 to 10 years). Physicians and pharmacists were located in the villages or within a 10 km surrounding area. None of these sources was informed of the findings of the weekly water samples.

Villages were chosen to meet several criteria: population between 100 and 3,500; non-tourist areas (to prevent wide variations in village population across seasons); public water systems with untreated ground water; sufficiently far from large cities so that the cases of AGID seeking help could be assumed to be completely enumerated by all the participating professionals.

The limits of the public water system in each village were known to the survey coordinators. Any case reported for an individual not using the municipal water system was excluded from the analysis. The village population was restricted accordingly to users of the public water system.

Although 64 weekly samples were available for most villages, because of technical problems, only 58 samples could be analyzed for some villages. Of the 52 villages available for study, two were excluded prior to examination of the data because of especially large numbers (>14) of missing observations, and two villages were excluded because the ground water they provided was subjected to treatment half-way through the survey, leaving 48 villages in the study group.

The bacteriologic assays conducted to ascertain the presence of indicator bacteria were as follows: 1) "standard plate count" (aerobic bacteria): 1 ml water cultivated on standard medium (PCA Institut Pasteur Production), with a 24-hours incubation at 37° ± 1°C; 2) "total coliforms": 100 ml filtered on cellulose membrane and cultivated on Tergitol TTC during 24 hours at 37° ± 1°C; 3) "fecal coliforms" (thermotolerant coliforms): same technique as for total coliforms but incubated at 42° ± 1°C for 24 hours; 4) "fecal streptococci: 100 ml filtered through cellulose membrane and cultivated 48 hours at 37° ± 1°C on Slanetz and Bartley medium (Institut Pasteur Production).

The 48 villages were followed for 64 weeks and contributed 3,072 "village-weeks". There were 1,807 cases of gastro-intestinal disease reported during the study period. This relatively small number of cases precluded analyzing each village separately across weeks. We assumed that each village-week constituted an independent observation. To avoid being misled by errors in this assumption (i.e., in a given village the water quality was liable to be correlated across weeks), we used a variable that indicated whether cases had occurred the previous week in the same village. This variable was included in the multivariate model to adjust for the experience of the previous week. The unit of observation was the village-week: the cases occurring during a given week in a given village were related to the population of the village (person-week) exposed to the water quality indexed by the weekly sample.

Because of the sparseness of AGID and of the large denominator (each week, 29,272 person-weeks were accrued in the whole set of villages), the events were modeled using a Poisson distribution.

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TABLE 1—Risk of Acute Gastro-intestinal Diseases According to the Fecal Coliforms Exposure, in Absence of Fecal Streptococci, by Village Size

				Relative Risk*	
Village Population	Fecal Coliforms	Cases	Person-weeks	Point Estimate	95% Confidence Interval
<400	Present	3	20 594	.27	[.14; .51]
	Absent	127	237 188		
≥400	Present	17	52 438	1.14	[.67; 1.92]
	Absent	221	777 252		

*RR = rate-ratio

TABLE 2—Relative Risk (and 95% CI) due to Increasing Concentration of Fecal Streptococci, by Level of Fecal Coliforms and Village Size

		Fecal Streptococci Count/100 ml			
Village Population	Fecal Coliforms	0*	1–5	6–10	11+
<400	Present	1	4.25 (2.1–8.6)	4.74 (1.6–9.6)	5.05 (2.8–9.0)
	Absent	1	1.40 (1.0–2.0)	2.46 (.8–7.7)	2.27 (.8–6.7)
≥400	Present	1	2.72 (1.6–4.1)	1.83 (.9–3.8)	3.21 (1.8–5.6)
	Absent	1	.89 (.7–1.2)	.71 (.3–1.6)	1.44 (.6–3.2)

*Level 0 of FS is the referent category; Relative Risk = 1

Log-linear analysis can be used with Poisson models and accommodate categorical covariates[6–8] yielding estimates of relative risk. Further details on methods and data analysis are discussed elsewhere.* The following covariates were included as potential predictors or confounders of the AGID incidence rate: FS count had four levels (0, 1–5, 6–10, 11 and over, per 100 ml); FC was a binary variable (absent, present); so were total coliforms, total plate count, and village size (under or over 400 inhabitants); and indicator of at least one case the previous week.

The data were restricted to the cases occurring within a three-day period centered on the water sampling day. This restriction, which removed 60 per cent of the total cases from the analysis was implemented to assess and control for a possible misclassification of exposure. Cases far from the sampling day, say the day before the next week or just after the previous week, were liable to result from water pollution as indexed by the water sample of the next week (or the previous week).

The confidence intervals of the relative risks should be computed using the covariances between various pairs of variables. Unfortunately, the Loglin package* used on a Vax 11/780 computer does not generate covariance matrices. Therefore, the confidence intervals of the relative risks presented below were approximated using the appropriate crude (i.e., unadjusted) data tables and the usual formulas for the variance of rate ratios.[9]

Results

The total plate count and total coliforms had no independent predictive value. When the water met the standards (no FC nor FS), the incidence rate was 3.44 per 10,000 person-weeks when at least one germ was observed on the total plate count; it was 3.43 per 10,000 person-weeks when no germ was present. The corresponding incidence rates in the presence of at least one coliform was 3.74 per 10,000 person-weeks, and 3.32 per 10,000 person-week when no coliform was observed.

On the other hand, when both FS and FC were absent, 354 cases occurred, out of a total of 698 (51 per cent). Out of these, 198 cases (53 per cent) occurred when the total plate count was positive, the other half of the cases occurring while no germ was found.

Table 1 presents the raw data for the effect of FC, in absence of FS. This effect is somewhat unclear, and depends on the village size category: in large villages, FC are not related to AGID risk, whereas they seem protective in small villages.

The incidence rates when no FS were found served as baseline risk in further analysis. The absolute risk is rather small; however, it is underestimated by a factor of 2.5 because only the cases occurring within the three-day period about the sampling day were considered. One can infer from Table 2 that the risk due to a given level of FS contamination is higher when FC are also present; the risk due to a given joint FC and FS exposure is higher in small villages than in larger villages; in larger villages, no risk seems associated with FS alone (i.e., when no FC are present), whereas they suffice to predict a significant risk (although weak) in small villages.

These data do not show a clear trend, but it is notable that the highest concentration of FS corresponds to the highest relative risk. The Mantel extension for the analysis of trend[10] was used on the crude data (i.e., unadjusted for the previous week experience) and yielded a significant positive trend only in small villages, in presence of FC [slope = 6.0×10^{-5} cases per person-week per unit of FS increase; χ_1^2 (slope) = 22.6; χ_2^2 = 4.6, testing departure from linearity]. This test for trend was done using the program written by Rothman and Boice for HP.41, applied to incidence rate data.[11]

In almost all strata, the lowest positive level of FS (1 to 5) is associated with a point estimate of the relative risk greater than 1. This class may be too large to assess whether there is a threshold. Another categorization can be adopted, where all concentrations greater than 5 bacteria/100 ml were merged (thus any inference from this highly heterogeneous class would be misleading). Table 3 shows how the relative risk changes with small increases of FS concentration, according to whether some cases had been reported the week before or not. The risk is systematically enhanced when cases had occurred previously. As to the threshold issue, no cut-off point other than 0/1 can be clearly identified.

TABLE 3—Relative Risk (and 95% CI) due to Increasing Concentrations of Fecal Streptococci with Special Focus on Low Levels (adjusting for the previous week experience)

	Fecal Streptococci Count/100 ml			
Cases Week Before	0	1–2	3–4	5+
None	1	.79 (.6–1.1)	1.56 (1.0–2.4)	1.40 (1.0–1.9)
1+	1	1.47 (1.0–2.1)	2.45 (1.1–5.5)	2.44 (1.8–3.3)

*Berlin J, Zmirou D: Poisson regression for the analysis of relative risk (Unpublished manuscript), Harvard School of Public Health, 1985.

Discussion

The categorization of villages into "small" (<400) and "large" (≥400) populations split the total set of villages into two groups of 24 villages each. Biologic and socioeconomic considerations may contribute to the understanding of the effect of the village size. In small villages, economic activity (agriculture is more oriented toward cattle breeding) and/or general hygiene and wealth may facilitate the spread of a contamination, and make more dramatic the effect of a given waterborne hazard, through direct interpersonal contamination, for example. This hypothesis is consistent with the multiplicative model of risk that underlies the analysis.[9] Although some work has been done on these issues, there is still no unequivocal evidence as to the relationship between indicator bacteria and pathogens, in drinking[12] or non-drinking[13,14] water. In 1981, 44 per cent of the water-related disease outbreaks reported to the Centers for Disease Control (CDC) remained of unknown origin.[15]

This study showed that the four classes of indicator bacteria had very different predictive value with respect to disease. Total bacteria and total coliforms had no predictive value; however, half of the cases of gastro-intestinal disease occurred when the water met the standards. Half of these were observed while only the total plate count was positive.

Those who are primarily interested in identifying which indicator germ best predicts the risk of disease should be concerned with FS and FC (as modifying the effect of FS). Geldreich claims that the FC/FS ratio provides a useful indicator as to the origin of the fecal pollution, human or animal.[16] In our study, the average FC/FS ratio was 4.0 and was different in large villages (5.2) and in small villages (1.3). This tends to confirm that the origin of the contamination in small villages was more related to animals than in larger villages. Those who want to set standards to decide whether a water is safe or not might also be interested in the total plate count. Finally the "background risk" (when all indicator germs are absent) amounts to about one-fourth of total cases; however, this figure may well depend on the specific conditions of this study such as the frequency of the water samples taken in the villages (one per week).

FS was the most predictive indicator and the validity of the standard threshold (0 bacteria per 100 ml) cannot be disputed with these data. We must recognize that our findings depend upon the ecological context and may not hold, for instance, in warm waters or treated waters. Surprisingly, most of the cases of gastro-intestinal disease were sporadic; throughout the 18 months of the survey, only one outbreak was observed (with about 50 cases), even though 42 per cent of the total water samples did not meet the bacteriology standards. Hence, this study shed special light on endemic water-related hazard, which may well be overlooked by health authorities. Although modest, this risk persists, and a lot remains to be done in order to provide a safe and wholesome drinking water to the consumers.

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REFERENCES

1. Hendricks CW (ed). Evaluation of the Microbiology Standards for Drinking Water. USEPA 570/9-78-00C; 1979.
2. Official Journal of the European Communities—No L229/11—Council Directive No 80/778/EEC 15 July 1980 relating to the quality of water intended for human consumption.
3. Federal Register: EPA-Water Programs. National Interim Primary Drinking Water Regulations, 1982.
4. Collin JF, Foliguet JM: A propos de quelques épidémies d'origine hydrique survenues en France. Microbiol Alim Nut 1983; 1:27–33.
5. Ferley JP, Zmirou D, Collin JF, Charrel M: Etude longitudinale des risques liés à la consommation d'eaux non conformes aux normes bactériologiques. Revue Epidém et Santé Publ 1986; 34:89–99.
6. Bishop YMM, Fienberg SE, Holland PW: Discrete Multivariate Analysis: theory and practice. Cambridge: MIT Press, 1975.
7. Laird N,Oliver D: Covariance analysis of censored survival data using log-linear analysis techniques. J Am Stat Assoc 1981; 76:374, 231–240.
8. Frome EL, Checkoway H: Use of Poisson regression models in estimating incidence rates and ratios. Am J Epidemiol 1985; 121:309–323.
9. Kleinbaum DG, Kupper LL, Morgenstern H: Epidemiologic Research. Principles and Quantitative Methods. Lifetime Learning Publications, 1982.
10. Mantel N: Chi-square tests with one degree of freedom; extensions of the Mantel-Haenszel procedure. J Am Stat Assoc 1963; 58:590–700.
11. Rothman KJ, Boice JD: Epidemiologic Analysis with a Programmable Calculator. Boston: Epidemiology Resources, Inc, 1982.
12. Petersen NJ, Hines VD: The relation of summertime gastro-intestinal illness to the sanitary quality of the water supplies in six rocky mountain communities. Am J Hyg 1960; 71:314–320.
13. Robertson WJ, Tobin AS: The relationship between three potential pathogens and pollution indicator organisms in Nova Scotian coastal waters. Can J of Microbiol 1983; 29:1261–1269.
14. Cabelli VJ: Health effects criteria for marine recreational waters, USEPA, 1983, 600/1-80-031.
15. Harris JR, Cohen ML, Lippy EC: Water-related disease outbreaks in the United States in 1981. J Infect Dis 1983; 148:759–762.
16. Geldreich EE: Fecal coliforms and fecal streptococcus density relationship in waste discharges and receiving wastes. Crit Rev Environ Control 1976; 6:349–369.