

# Diarrhea prevention in Bolivia through point-of-use disinfection and safe storage: a promising new strategy

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## Summary

A novel water quality intervention that consists of point-of-use water disinfection, safe storage and community education was field tested in Bolivia. A total of 127 households in two periurban communities were randomized into intervention and control groups, surveyed and the intervention was distributed. Monthly water quality testing and weekly diarrhoea surveillance were conducted. Over a 5-month period, intervention households had 44% fewer diarrhoea episodes than control households ( $P = 0.002$ ). Infants < 1 year old ( $P = 0.05$ ) and children 5-14 years old ( $P = 0.01$ ) in intervention households had significantly less diarrhoea than control children. *Campylobacter* was less commonly isolated from intervention than control patients ( $P = 0.02$ ). Stored water in intervention households was less contaminated with *Escherichia coli* than stored water in control households ( $P < 0.0001$ ). Intervention households exhibited less *E. coli* contamination of stored water and less diarrhoea than control households. This promising new strategy may have broad applicability for waterborne disease prevention.

## Introduction

Diarrheal diseases, which are frequently transmitted by faecally-contaminated water, continue to be a leading cause of morbidity and mortality among children in developing countries [1,2]. The optimal approach to preventing waterborne diseases, which includes the construction of water disinfection and delivery systems and sewage treatment facilities, is very expensive and time consuming [3].

The Centers for Disease Control and Prevention (CDC) and the Pan American Health Organization (PAHO) have developed an inexpensive, rapidly implementable alternative for water quality improvement [4]. This intervention consists of three elements: (1) point-of-use treatment of contaminated source water with disinfectant produced locally using appropriate technology; (2) safe storage of treated water; (3) community education. In a pilot trial, an Aymara Indian community in El Alto, Bolivia, used this intervention on water from contaminated surface sources to produce drinking water that met World Health Organization guidelines for microbiologic water quality [5]. To determine the efficacy of this intervention in preventing diarrhoeal diseases, we conducted an intervention trial in two periurban communities of Montero, Bolivia, a city located in the subtropical eastern lowlands.

## Methods

All households of the two study communities were invited to participate in the study. In July 1994, we interviewed the person responsible for handling water in the household, usually the female head of household or oldest daughter, about family

socioeconomic and demographic characteristics, hygienic habits and water handling practices.

In August 1994, we collected baseline water samples from household wells and drinking water storage containers and observed sanitary conditions of the household and surrounding yard. We determined free and total chlorine levels using the N,N-diethylphenylenediamine colorimetric method (Hach Co., Loveland, CO). Water samples were tested for *Escherichia coli* contamination with the membrane filtration technique [6], using selective m-TEC agar (Difco Laboratories, Detroit, MI).

Throughout the study, water disinfectant was produced by a local community health worker using a MIOX unit (LATA, Inc., Los Alamos, NM). From a 3% brine solution, the MIOX unit electrolytically produces disinfectant with hypochlorite, chlorine dioxide, ozone, peroxide and other oxidants. The disinfectant was packaged in 500-ml reusable containers with caps that were used to measure and dispense disinfectant into drinking water.

For household water storage, 20-litre polyethylene water vessels (referred to hereafter as the special vessel) with a narrow mouth, a spigot and a comfortable handle were used (Toico, Inc., Toledo, OH). Labels illustrating appropriate use of the vessels and disinfectant were affixed to the vessels. The labels also illustrated suggested applications of treated water, which included drinking, handwashing, cleaning utensils and washing produce. Visiting community health volunteers were already promoting all of these suggested hygienic measures, including the importance of water treatment, on a regular basis to all households, including controls, in both study communities.

Households were randomized by a simple public lottery into two groups; one to receive the intervention, the other to serve as a control group. From 22 to 25 August 1994, community health volunteers distributed one container of disinfectant and two special vessels to each intervention household and explained how to treat and store water with these products. Once a week, community health volunteers distributed containers with freshly prepared disinfectant to each intervention household, removed old containers, and used the labels on the special vessels to reinforce messages about proper use of the disinfectant and vessels and remind participants of different applications for treated water.

Six visits at monthly intervals were made to all participating households from September 1994 to February 1995 to survey water-handling practices and to test stored and source water quality as described above.

From 1 October 1994 to 28 February 1995, a specially-trained health worker made weekly visits to all households to obtain information about all household cases of diarrhoea, defined as equal to or greater than 3 loose or watery stools in 24h, with onset in the preceding 7 days. At each visit, the health worker attempted to obtain from every person with diarrhoea two rectal swabs that were placed in Cary-Blair media for transport to a local laboratory for bacterial culturing, and a stool sample in a plastic container for microscopic analysis and enzyme-linked immuno-sorbent assay (ELISA) testing for rotavirus (Cambridge Biotech, Boston, MA). Swabs were tested for *salmonella*, *shigella*, *Campylobacter*, and *Vibrio cholerae* using standard techniques. Five lactose-positive and two lactose-negative colonies were selected from MacConkey agar plates and sent to CDC to test for enterotoxigenic *E. coli*

(ETEC) using previously described techniques [7]. Fresh stool specimens were examined microscopically for parasites.

### **Data analysis**

Epi Info, Version 6.02 (USD, Inc., Stone Mountain, GA) software was used for descriptive and univariate data analysis. The Kruskal-Wallis test was used to analyse data that were not normally distributed. SAS software (SAS Institute, Gary, NC) was used for univariate and multivariate analyses of data on microbiologic water quality and diarrhoea. Generalized estimating equations were used to analyse repeated observations of diarrhoea episodes in individuals over time in intervention and control groups, controlling for clustering within households [8].

### **Informed consent**

The study protocol was approved by the CDC Institutional Review Board and informed consent was obtained from all subjects or their guardians.

## **Results**

A total of 127 households with 791 persons participated in the study (mean, 6.2 persons per household), representing 91 % of households in the study area. The median age of the study population was 14 years (range, 1 month-83 years): women made up 51.6%. Of 362 persons over age 14 years for whom there were data, 50 (14 %) were illiterate and 182 (50 %) reported having < 6 years of schooling. Mean per capita annual income was \$230. Twelve households from the two communities were excluded from the study: 10 families moved during the study and two refused to participate. There were no statistically significant demographic differences between excluded and participant households.

Shallow (<5m deep), uncovered household wells were the primary drinking water source for 111 (87%) of 127 households. A covered, 50-m-deep well with a handpump was the source for 14 (11 %) households: 2(16 %) households used water from a household tap in a neighbouring community. Water was stored in 98 households (77%); it was fetched for immediate use as needed for the other 29. Of 98 households storing water, 94 (96%) used wide-mouth containers, which would permit hand contact with stored water: in 64 (68%) of 94 homes where direct observations were made, at least one water storage container was uncovered. Respondents from 48 (51 %) of these 94 households acknowledged that hands sometimes touched the stored water while water was being obtained from the container.

Only 48 (38%) of 127 respondents reported ever treating their drinking water: 16 (33%) boiled it, 15 (31%) added bleach, 11 (23%) did both, 4 (8-3%) filtered it through a cloth, and 2 (4%) added lemon juice. During baseline water sampling, 80 (63%) of 127 households had stored water, but only 17 (21 %) of these 80 respondents claimed it had been treated. No stored water samples had detectable chlorine. The median *E. coli* colony count was 57050/100 ml (range, 1-8000000) for well water samples, and 46950/100 ml (range, 1-8000000) for samples of stored water.

Ninety-eight (78%) of 126 respondents claimed always to wash their hands after defecation, while 28 (22%) individuals reported engaging in this practice almost always or sometimes: handwashing before food preparation was reported to be

performed always by 96 (76%) of these respondents and almost always or sometimes by 30 (24%) individuals. Handwashing before eating was reported to be practised always by 88 (70%) respondents, almost always or sometimes by 36 (29%) individuals, and never by only 2 (2%) respondents. Raw fruits and vegetables were claimed to be washed always by 82 (65 %) respondents, almost always or sometimes by 41 (33 %) persons, and never by 3 (2%) respondents. Of 126 respondents, 101 (80%) claimed to wash cooking and eating utensils always, while 24 (19%) indicated almost always or sometimes, and only 1 (1 %) never washed utensils.

Seventy (55%) of 127 households had a latrine that was in good or fair condition, but only 60 (47%) respondents reported using it: the remainder disposed of human waste on the open ground or in a hole in the ground. One hundred and twelve (88%) households possessed animals, including dogs (77%), chickens (71%), and ducks (67%). Human or animal faeces were observed in the yard surrounding 97 (76%) of 127 households.

### Intervention-phase results

The intervention group consisted of 64 households with 400 individuals (mean, 6-3 persons per household: range, 2-13). The control group had 63 household with 391 individuals (mean, 6-2 persons per household: range, 1-16). There were no statistically significant differences between intervention and control households in demographic characteristics, sanitary conditions, water handling practices, hygienic practices, or baseline *E. coli* colony counts in either well or stored water.

During the first month of the intervention phase, investigators observed stored water in special vessels in 92% of intervention households: over the subsequent 6 months, this proportion declined to 69% (Table 1). The most common reason cited for not having water in the special vessel at the time of the monthly-visit was that the vessels had not yet been filled that day. Over the course of the study, the proportion of households that reported using the special vessel declined from 100 to 98%. The proportion of stored water samples with detectable levels of total chlorine increased from 71 % at the time of the first observation to 95% at the final visit (Table 1).

**Table 1.** Percent of households with water observed in storage vessels and chlorine detected in stored water during monthly-field visits. Montero. Bolivia, July 1994-February 1995

Sampling round	% of intervention households with water in special vessels	% of intervention households with detectable total chlorine in vessel water	% of intervention households with water in special vessel and chlorine in vessel water	% of intervention households with detectable total chlorine in usual water storage vessel
Round 1	92	71	65	0
Round 2	89	76	69	0
Round 3	89	70	62	6
Round 4	80	71	57	3
Round 5	77	79	61	5
Round 6	69	95	64	6

In each of the six sampling rounds, stored water samples from intervention households had significantly lower median *E. coli* colony counts than samples from control households ( $P < 0.0001$ ) (Table 2). The proportion of water samples from special vessels that had no detectable *E. coli* colonies ranged from a low of 56% in the first sampling round to a high of 79% in the fourth round (Table 2).

**Table 2.** Median *Escherichia coli* colony counts per 100 ml in water stored in special vessels in intervention households and water stored in usual vessels in control households, and percent of water samples with no detectable *E. coli* colonies. Montero. Bolivia. July 1994-February 1995

Sampling round	Intervention households		Control households		<i>P</i> value for comparison of median <i>E. coli</i> colony counts between groups
	Median <i>E. coli</i> colony count per 100 ml (range)	% of household samples with no <i>E. coli</i> colonies	Median <i>E. coli</i> count per 100 ml (range)	% of household samples with no <i>E. coli</i> colonies	
Baseline	9200 (0-8000000)	5.1	80000 (0-8000000)	5.4	0.3
Round 1	0 (0-3100000)	55.9	84100 (1100-2200000)	0	< 0.0001
Round 2	0 (0-305000)	57.9	8400 (0-8000000)	10.3	< 0.0001
Round 3	0 (0-95500)	59.3	4950 (0-8000000)	3.3	< 0.0001
Round 4	0 (0-105000)	79.2	8800 (4008000000)	0	<0.0001
Round 5	0 (0-800)	74.5	8200 (0-8000000)	10.5	< 0.0001
Round 6	0 (0-8000000)	71.1	6400 (0-2050000)	13.3	<0.0001

**Table 3.** Age-specific diarrhoea episodes and mean episodes per person in intervention and control groups. Montero. Bolivia. October 1994-February 1995

Age-group (years)	Number of persons	Intervention group			Control group		<i>P</i> value for comparison of mean episodes between groups
		Number of diarrhoea episodes	Mean diarrhoeal episodes per person	Number of persons	Number of diarrhoea episodes	Mean diarrhoeal episodes per person	
< 1	16	11	0.69	27	40	1.48	0.02
1-4	53	41	0.77	64	52	0.81	0.69
5-14	130	15	0.12	113	33	0.29	0.01
15-44	153	11	0.07	146	12	0.08	0.91
45+	49	5	0.10	40	11	0.28	0.16
Total	401	83	0.21	390	148	0.38	0.002

**Table 4.** Univariate analysis by GEE\* of risk factors for diarrhoea among individuals living in intervention and control households. Montero. Bolivia. October 1994-February 1995

Risk factor	Estimated odds ratio	P value	95%CI
Vessel /disinfectant	0.57	0.004	(0.39, 0.84)
Age†	0.95	< 0.001	(0.93, 0.98)
Male sex	1.59	0.02	(1.09, 2.27)
Annual per capital income†	1.00	n.s.	(0.99, 1.00)
No. of persons/room†	0.98	U.S.	(0.92, 1.04)
Household latrine	0.65	0.08	(0.40, 1.05)
Absence of stools in yard	0.74	0.48	(0.49, 1.5)
Touch water with hand	1.12	n.s.	(0.78, 1.83)
Own animals	0.91	n.s.	(0.54, 1.52)
Animals allowed in house	0.96	n.s.	(0.61, 1.53)

\* Generalized estimating equations.

† Continuous variables.

Over a 5-month period, the active surveillance system detected 231 cases of diarrhoea: 83 in intervention households versus 148 in control households (Table 3). This represented an overall reduction in diarrhoea incidence over the 5-month period of 44 %, from 0.38 episodes per person to 0.21 per person ( $P = 0.002$ ). The mean number of reported diarrhoea cases per household was 1.30 for intervention families and 2.35 for control families ( $P = 0-02$ ).

In all age-groups, intervention household members had fewer reported episodes of diarrhoea than did members of control households (Table 3). The protective effect was strongest for infants, among whom the reduction in incidence was 53 % ( $P = 0.02$ ), and for children 5-14 years old. among whom the reduction was 59% ( $P = 0.01$ ). Reductions in the mean number of diarrhoea episodes for persons in the age-groups 1-4 years and > 15 years did not reach statistical significance.

Univariate GEE analysis of potential risk factors for diarrhoea among individuals revealed that diarrhoea risk was less for older persons, and for individuals who belonged to intervention households (Table 4). Diarrhoea risk was greater for males. Diarrhoea risk tended to be less for individuals living in households with a latrine in active use, but this result did not reach statistical significance. We constructed a model that included the statistically significant and borderline significant risk factors from the univariate analysis. Multivariate GEE analysis of this model showed that belonging to an intervention household (OR 0.64,  $P = 0.02$ ) and older age (OR 0.95,  $P < 0.001$ ) were independently associated with having fewer episodes of diarrhoea. Male sex (OR 1.51,  $P = 0.02$ ) was independently associated with an increased risk for diarrhoea. Living in a household with no visible faeces in the yard (OR 0.73,  $P = 0.14$ ) tended toward an association with a lower risk for diarrhoea, but this result was not statistically significant. Interactions were tested between the independently-associated variables and none was found to be significant.

Rectal swabs were obtained from 36 (43%) of 83 diarrhoea patients in 22 household in the intervention group and from 60 (41%) of 148 patients in 28 control households. A bacterial pathogen was isolated from 27 (28 %) of the 96 swabs. *Campylobacter* was isolated from 20 (20%) swabs: 2 (6%) from intervention cases and 18 (30%) from control cases: all were < 5 years old. Because diarrhoea episodes within households are not independent of each other, we restricted univariate analysis of *Campylobacter* cases to the household, rather than to the individual level. *Campylobacter* was isolated from stool specimens from 2 (9%) of 22 intervention households and 12 (43%) of 28 control households (OR 0.2, 95% confidence interval 0.03-0.8). Stool specimens from 8 households yielded ETEC, 5 of which were obtained from patients in intervention households ( $P = 0.28$ ). Only one (1%) stool specimen yielded *salmonella* and one (1%) yielded *shigella*; both were from control group patients.

ELISA tests for rotavirus were conducted on 65 (68%) of 96 specimens; 4 (6-2 %) were positive, three of these from intervention group patients. Of 91 stool samples examined microscopically, *Ascaris lumbricoides* was identified in 38 (42%). *Giardia lamblia* in 21 (23%). *Enmrnoeba histolytka* in 1 (1%). and other parasites in 25 (27%). Thirty-three (35%) specimens were negative and 27 (30%) samples had multiple parasite species. There was no significant difference in the number of enteric parasitic infections in intervention and control group patients.

## Discussion

Households using a simple water storage and treatment intervention experienced substantially less diarrhoea during the summer diarrhoeal season in Bolivia than did households using traditional water-handling and storage practices. Participant households achieved this result despite living in a high-risk environment where drinking water sources were heavily contaminated with *E. coli* where only 53 % of households had access to a latrine, and where human and animal faeces were present on the ground around most homes. The intervention - an inexpensive combination of point-of-use water treatment, safe water storage vessels, and hygiene education - was readily adopted and applied.

The willingness of a population to adopt a novel health practice is an important determinant of an intervention's long term success. This study population demonstrated their acceptance of the intervention through consistently high compliance by several measures - reported use of the special vessel (98%), observed use of the special vessel (range 69-92 %), observed use of disinfectant in special vessel water samples (range 7&-95%), and observed concurrent use of the special vessels and disinfectant (57-69%) [Table 1]. The observed use of the special vessel declined from 92 to 69% during the course of the study. Although most respondents whose vessels were empty at the time of follow-up visits claimed that they had not yet filled their vessel that day, the decline in observed use could be indicative of a return to old habits and points to the importance of ongoing efforts to motivate a population to sustain changes in health behaviours. The best functional indicator of compliance in this study was observed concurrent use of the vessel and disinfectant, which remained remarkably consistent throughout the study.

The 44% reduction in diarrhoeal disease episodes in intervention families was higher than that reported for most water quality interventions [9, 10], Most previous studies evaluated capital-, time-, and labour-intensive piped-water or well projects

that did not include chemical disinfection and safe water storage in the home, nor community education. In contrast, this intervention coupled initial water disinfection with barriers to recontamination. The first barrier was the water storage vessel itself. Clean water stored in the open buckets or barrels typically used in developing countries becomes contaminated [11,12]. Not surprisingly, water stored in narrow-mouth or covered water vessels is less likely to become contaminated [13-16]. The presence of residual hypochlorite in treated water provided a second barrier to recontamination [17,18]. Hygiene education was also an important component of the intervention. Alone, it can lead to a reduction in the incidence of diarrhoeal diseases in children [19,20], and this effect is multiplied when families are also given a safe storage vessel [10].

The observation of greatest benefit in two age-groups, infants < 1 year old and children 5-14 years old, may be due to behavioural characteristics of each group. Most infants are under the continual care of their mothers or older siblings, who control their drinking water source. Children 5-14 years old can be taught what to eat or drink and what to avoid, so the possibility of compliance with the intervention is improved. The lack of protective effect among children 1-4 years of age may reflect their ability to walk and explore their surroundings, and their inability to avoid exposure to potential pathogens in a faeces-laden environment. Because this age-group is most susceptible to diarrhoea morbidity and mortality, the ultimate impact of this intervention on mortality may be limited, although there is evidence that improved water storage protects against persistent diarrhoea [21], which has a much higher case fatality rate than acute diarrhoea. Reducing diarrhoea, and the consequent risk of mortality, in this age-group may require additional interventions that focus on reducing faecal contamination of the household environment.

The intervention was not the only factor associated with diarrhoeal incidence. In the univariate analysis, the presence of a functional latrine tended toward being protective, and in the multivariate analysis, the absence of observable faeces in the immediate household environment tended toward an independently protective effect. Although the intervention did not include a human waste disposal component, these findings, though not statistically significant, suggest the importance of waste disposal to diarrhoea prevention efforts and support the well-documented effectiveness of excreta disposal interventions for diarrhoea prevention [9, 10].

*Campylobacter* and ETEC were the only enteric bacterial pathogens detected frequently in this study. The high frequency of *Campylobacter* isolation may reflect the high percentage of families that possessed animals, particularly poultry, and that had inadequate environmental sanitation. Other studies of impoverished communities in Latin America have detected high rates of infection with *Campylobacter* [22]. The intervention specifically decreased *Campylobacter* infection rates, a plausible finding because water is a recognized vehicle of transmission of this microorganism [23-25]. ETEC was the second most commonly isolated bacterial pathogen. Although the numbers are small, the lack of an apparent protective effect for ETEC infections suggests that, in this population, water may not be the predominant mode of ETEC transmission. The rarity of positive rota virus ELISAs may reflect the seasonality of the infection. The study encompassed only the rainy summer season, while rotavirus may be more commonly isolated during drier winter months [26].



This intervention is a promising way of providing microbiologically safe water in developing countries. While supplying piped, treated water to all households remains elusive for many communities, this point-of-use disinfection and safe water storage intervention can be rapidly disseminated, is inexpensive, simple to use, and adaptable to a variety of conditions. A similar water vessel can be manufactured in Bolivia at a cost of under US \$4.00 each. The disinfectant can be produced in any community by inexpensive, solar-powered electrolysis of a salt water solution for as little as \$0.05 per family per year [27]. An earlier cost-effectiveness study estimated that this intervention would have no net cost to society if it decreased diarrhoea incidence by 20% or more [28]. Ultimately, the utility of this intervention will be determined by its acceptability and sustainability in diverse populations. Social marketing will be an important component of efforts to enhance the intervention's acceptability and to ensure its sustainability through commercialization. An attractive aspect of this intervention is that it yields a product, the disinfectant, which can be marketed beyond the community as an alternative to boiling, which is expensive and time-consuming, and to solar disinfection, which is time-consuming and does not prevent recontamination. However, to ensure success, formative research will be necessary to assess the need and demand for such an intervention in a given population, and provision must be made to produce all elements of the intervention in the implementing country. Start-up costs for the production of the special vessels, disinfectant, and promotional materials, and for the establishment of distribution networks, will be substantial. The prospect of local management of the project and either full or partial cost recovery enhances the potential for success. Further 'real life' investigations of this and similar strategies in other communities and at a larger implementation scale will define better the potential of this promising new strategy for waterborne disease prevention.

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