Strategies for the Promotion of Household Water Treatment in Ica, Peru

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Abstract

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Boiling is the most common method of household water treatment in low- and middle-income countries, however, it is not always effectively practiced. We examined factors associated with *Escherichia coli* contamination of improved water supplies among 207 households in a rural population in Peru that practiced boiling. We subsequently conducted a randomized controlled trial among these households to assess the effectiveness of water pasteurization and safe storage interventions in reducing *Escherichia coli* contamination of household drinking water. Households were randomized to three study groups: two intervention groups that received either a safe storage container or a safe storage container plus water pasteurization indicator and a control group that continued usual practices. Although over 90% of households used an improved water source at baseline, 47% of source and 43% of stored water samples were contaminated with *E. coli*. Pouring or using a spigot to obtain water from the storage container instead of dipping a hand or object was associated with decreased risk of contamination of stored water (adjusted prevalence ratio (aPR)=0.58, 95% CI=0.42, 0.80). Container cleanliness (aPR=0.67, 95% CI= 0.45, 1.00) and correct handwashing technique (aPR=0.62, 95% CI=0.42, 0.90) were also associated with decreased contamination risk. During a 13-week follow-up period, households that received a safe storage container and water pasteurization indicator had a higher prevalence of stored drinking water contamination relative to the control group, although the difference was not statistically significant (Prevalence Ratio (PR): 1.20, 95% CI: 0.94, 1.52). Receipt of only a safe storage container had no effect on the contamination of stored drinking water (PR: 1.02, 95% CI: 0.79, 1.31). Although use of low-cost water pasteurization indicators and locally available storage containers did not significantly increase the safety of household drinking water in this study, future research could help illuminate factors that facilitate the use of these interventions to effectively improve water quality and reduce the risk of waterborne disease in populations that boil drinking water.

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CHAPTER I: ‘Improved’ but not necessarily safe: an assessment of fecal contamination of household drinking water in rural Peru

INTRODUCTION

Approximately 1.8 billion people use fecally contaminated water sources globally, with the majority living in low- and middle-income countries.[1] Ingestion of pathogens in water contaminated with feces represents the greatest water-related health risk and is a major cause of diarrheal disease.[2, 3] The Millennium Development Goal (MDG) Target 7c of halving the number of people without access to safe drinking water has already been reached, but it is widely recognized that this achievement overstates access because the indicator of progress—use of an ‘improved’ source—is not an adequate proxy for safety.[4, 5, 6] There is evidence of substantial between- and within-country variation in the fecal contamination of improved drinking water supplies,[5, 7, 8] and it is important to accurately assess this risk in order to improve the precision of global estimates of the burden of disease attributable to unsafe drinking water, which currently assume that the use of an improved source implies no health risk.[9]

The WHO/UNICEF Joint Monitoring Programme for Water and Sanitation (JMP), which monitors progress toward this MDG target, classifies a water source as ‘improved’ if it has some measure of protection from outside fecal contamination such as a piped supply, boreholes, protected dug wells, protected springs, and rainwater.[10] In contrast, an ‘unimproved’ source includes unprotected dug wells, unprotected springs, water from tanker trucks or carts with small tanks or drums, bottled water, and surface water.[10] In preparation for the end of the MDG monitoring period in 2015, the JMP has proposed new targets and indicators for measuring the expansion of access to safe drinking water.[11] The proposed indicator of ‘basic’ drinking water service is identical to the MDG indicator, except for the additional requirement that the total collection time of the water source does not exceed 30 minutes.[11] The proposed indicator of ‘intermediate’ drinking water service requires access to an on-premises improved water supply that meets WHO guideline values for *E. coli*, which implies that routine microbial testing will be added to all national household surveys.[11]

In Peru, as in other low- and middle-income countries, rural areas are at particularly high risk of having fecally contaminated drinking water, even when restricting to piped supplies.[5, 12] In coastal areas of Peru outside of metropolitan Lima (the capital), an estimated 68% of households with children under 5 years use microbiologically contaminated water[12] despite 81% access to a piped supply.[13] National data indicate that the majority of piped water systems are at risk of contamination because of inadequate chlorination[12, 13] and intermittent service,[14] which results in negative pressure in poorly maintained water pipes, permitting the entry of contaminants from the surrounding soil. The Peruvian coast is at particular risk of water infrastructure damage due to its high frequency of seismic activity. Intermittent supply also necessitates water storage in the home, where contamination can occur through unsafe storage and handling.[15, 16, 17] To better understand household access to safe drinking water in a rural area on the southern coast of Peru, we evaluated source and stored water quality and examined factors associated with stored drinking water safety.

MATERIALS AND METHODS

Study site. In January 2014, we conducted a cross-sectional survey of households in Humay District of Pisco Province, which includes 33 communities and a population of approximately 5,800 people. The district is located in the agricultural Pisco River Valley, where the annual rainfall is approximately 2 mm. Sixteen percent of the population of rural areas of Ica

Department (to which Pisco Province belongs) are in the poorest national wealth index quintile, while almost half (49%) are in the second poorest quintile.[13] The water in piped distribution systems comes either directly from the Pisco River, from irrigation channels (fed by the river) or, for one community, from a well. Water piped directly from the river runs through a sand or gravel filtration gallery before distribution; this is also the case for the water in some piped systems supplied by irrigation channels. Water piped from the well is unfiltered. The irrigation channels typically run through agricultural fields where animals are also pastured. In the two largest communities, chlorination of piped water is intermittent due to inconsistent treatment by the water authorities. The majority of water service is intermittent due to lack of supply, with daily service ranging from 2 to 20 hours. Typically there are 1-2 standpipes per block in larger communities and at least one centrally located standpipe in smaller communities.

Enrollment. A district-wide census was conducted before the study start to identify households meeting the eligibility criteria for study participation, which included a child under the age of 5 years, an adult female member $\geq$18 years, and ability to heat water in the home. A randomly ordered list of eligible households was created using a pseudorandom number generator in STATA 13.1 (StataCorp, College Station, TX). Eligible houses from the list were approached in order and enrolled until a total of 210 participants was reached. If the female head of household was unavailable at the first visit to the home, one additional attempt was made to enroll the household.

Sample size. On the basis of previous water monitoring data from this area, we estimated that 88% of stored water samples would be contaminated with *E. coli*. A sample size of 210 was chosen to measure the prevalence of *E. coli* contamination with a precision of ±4%, assuming a population size of 450 eligible households estimated from census data.

Data collection. After obtaining informed consent, study workers administered a survey to ascertain participant demographic information; socioeconomic status; water supply; behaviors related to water boiling and other household water treatment methods; sanitation and hygiene behaviors; presence of a handwashing station (defined as a designated place for handwashing with a water source and soap present); and ability to demonstrate correct handwashing technique (lathering all surfaces of the hands with soap). Household source and stored drinking water were tested for the presence of total chlorine using an orthotolidine (OTO) pool test kit (Pentair, Minneapolis, MN). Samples of source and household stored drinking water were collected in sterile 100mL bottles and transported on ice to the study center, where they were tested for *Escherichia coli* (*E. coli*) using the Compartment Bag Test (Aquagenx, Chapel Hill, NC). Samples were processed within 6 hours of collection and incubated for at least 20 hours at 35-44.5^{0}C.[18] Positive and negative controls were processed and incubated on each day of water sample collection. The Compartment Bag Test is a validated measure of the most probable number (MPN) of *E. coli* ranging from undetectable to >100 *E. coli* per 100mL.[19]

Data Analysis. All data were entered into a Microsoft Access 2010 (Redmond, WA) database and analyzed using STATA 13.1 (StataCorp, College Station, TX). We considered drinking water to be safe if <1 MPN *E. coli* was detected in a 100mL sample, per the WHO standard.[3] Descriptive statistics were generated using cross-tabulations. Chi-square or Fisher's exact tests were used to determine associations between stored household water quality and demographic and socioeconomic characteristics, and water, sanitation, and hygiene knowledge and practices. For continuous variables with non-normal distributions, equality-of-medians tests were used to compare median values by stored drinking water contamination. The distributions of source and stored drinking water samples were stratified by the WHO *E. coli* risk categories:

low risk/safe (<1 *E. coli*/100 mL), intermediate to high risk (1-100 *E. coli*/100 mL), and very high risk (>100 *E. coli*/100mL) for human consumption.[3] The WHO intermediate and high risk categories were combined in this analysis in order to reduce the probability of misclassification, given the lack of precision of individual MPN values.[18] For participants who reported boiling, a McNemar's test was used to compare the prevalence of *E. coli* contamination between source and stored water samples in order to evaluate the degree to which this practice was associated with an improvement in water quality.

We used log-binomial models to estimate the prevalence ratios (PRs) for the association of household characteristics with the contamination of stored household drinking water. The WHO/UNICEF Joint Monitoring Programme for Water and Sanitation definitions were used to classify water sources as 'piped on premises' (piped into the dwelling, plot, or yard), 'other improved' (i.e. public tap), and 'unimproved' (i.e. uncovered well or irrigation channel) while sanitation infrastructure was categorized as household ownership of a toilet or latrine or other (shared facilities/open defecation).[10] Principal component analysis of household assets, building materials, and home ownership (by self or a family member) was used to calculate a socioeconomic index for each household.[20] The first principal component of each variable was used to generate the index[21] and natural breakpoints in the data were used to group households into terciles for analysis. Water source, treatment, storage and handling variables, demographic variables, and socioeconomic status were considered as potential confounders and were included in adjusted models if they altered the prevalence ratio by 10% or more and cell sizes allowed for adequate adjustment. A forward stepwise approach was used for the inclusion of confounders in adjusted models and a significance level of 0.05 used for all hypothesis testing.

Ethical considerations. The study protocol was reviewed and approved by the Institutional Review Boards of the University of Washington, the U.S. Naval Medical Research Unit No. 6, and the Ica regional Ministry of Health. Written informed consent was obtained from all study participants. Personal identifying information was irreversibly removed from databases at the end of the study.

RESULTS

Demographic characteristics of the study population. Of 210 women enrolled, 3 did not meet the study eligibility criteria and were excluded from analysis. The median age of the 207 remaining participants was 31 years (range 18-64) and the median number of household members was 4 (range 2-15). Fifty-seven percent of the participants had completed secondary school or more education.

Water sources, treatment, and storage. The primary drinking water source of 93% of participants was improved, including piped water inside the home (59%), piped water outside the home (33%), and a covered well (1%). Unimproved primary water sources reported by participants included surface water (5%) and an uncovered well (1%). Household piped supplies were fed directly by the river (86%), by irrigation channels (9%), or by a well (5%). Forty percent of participants believed their water was safe to drink.

Of 207 participants, 203 (98%) reported treating their drinking water, 201 by boiling and 2 by chlorinating and boiling. Although 97% of those who treated reported having treated water currently stored in their home, 32% admitted to sometimes not treating their drinking water. Definitions of boiling provided by 194 participants included bubbles rising from the bottom to the top of the container (82%), steam rising from the surface of the water (13%), the teapot whistling (4%), and bubbles breaking on the surface (1%). Water treatment was reported to be expensive by 46% and easy by 92% of respondents. When asked why they boiled their drinking water, 49% cited health, 18% said to make it clean, 13% to kill microbes or parasites, and 11% to make the water safe for drinking. The two most common reasons for non-treatment were that it took too much time (45%) and a lack of fuel (20%). Among 203 participants who reported treating their water, 77% primarily used a gas stove for cooking, 9% an open fire, 7% a 'plancha' stove (which has a griddle and combustion chamber), and 7% used a gas stove and a wood-burning stove with equal frequency.

Water storage containers reported by participants included plastic beverage container (51%), cooking pot (17%), teapot (11%), barrel (9%), and bucket (9%). At the time of the visit, drinking water was observed to be stored in a covered container in 94% of households; 95% appeared to be clean (free of dirt, debris, garbage, fecal matter, etc.). Chlorine was detected in 9 (5%) source water samples and no stored water samples.

Sanitation and Hygiene. Of 207 participating households, 81% used a household toilet or latrine, 14% practiced open defecation, and 5% used the bathroom of a neighbor or relative. A handwashing station was observed in 64% of participant homes; 91% of participants were able to demonstrate correct handwashing technique.

Microbiological water quality and boiling. *E. coli* was detected in 47% of source and 43% of stored water samples (p for McNemar's test= 0.14). Of 200 total stored water samples, 58% had no detectable *E. coli* and 21% were highly contaminated (>100 MPN per 100ml) (Figure 1). *E. coli* contamination was detected in a lower percentage of water samples collected from sources piped on premises (35%) than other improved water sources (57%, p=0.007) and unimproved sources (93%, p<0.001; Figure 2). All water samples from improved sources fed by irrigation channels were contaminated with *E. coli*; contamination of improved sources was less frequent when supplied by a well (57%, p=0.02) or directly by the river (35%, p<0.001). The type of water supply distributed by on-premises piped sources was similar to that of other improved sources (p=0.26).

Among participants who reported boiling their currently stored drinking water, *E. coli* was detected with similar frequency in paired source and stored samples (p=0.13). Among the 78 (38%) participants with contaminated source water who reported boiling, 38 (49%) had paired stored water samples with undetectable *E. coli* (p<0.0001).

Correlates of microbiologically safe stored water. In multivariate analysis, participants who poured or used a spigot to obtain water from their storage container were 42% less likely to have contaminated stored drinking water than those who dipped an object or a hand to extract water from the container (aPR=0.58, 95% CI=0.42, 0.80). Container cleanliness was associated with a reduced risk of contamination (aPR=0.67, 95% CI=0.45, 1.00), as was correct handwashing technique (aPR=0.62, 95% CI=0.42, 0.90). Households that primarily used water from on-premises piped sources were less likely to have detectable *E. coli* in stored water than households using unimproved sources (aPR=0.61, 95% CI=0.37, 1.03); however, this association did not reach statistical significance. Reported water treatment was associated with a nearly 50% reduction in risk of having contaminated stored water in the unadjusted model, but the adjusted model did not converge due to small numbers (Table 2). No other factors were significantly associated with stored drinking water contamination in multivariable models.

DISCUSSION

In a rural population on the southern coast of Peru with 93% coverage by improved water sources, we found that 47% of source water samples were contaminated with *E. coli*. The contamination of improved water supplies observed in this study can be explained by the use of inadequately treated water from a river and irrigation channels, both unimproved sources, in the piped water systems. These results are consistent with the findings of other studies that demonstrated that improved sources are not necessarily safe.[5, 6, 8, 22, 23, 24, 25]

Although transporting water to the home from an off-premises source has been associated with contamination in other studies,[15, 17] in this population, microbiologic quality of stored water tended to be similar to, or better than, source water. This finding likely resulted from the high percentage of reported water treatment in the population, which may have been a reliable indicator of treatment because of the use of gas stoves, which are more efficient and therefore boil water faster than open fires (Do Hoang T and others, unpublished data; McLaughlin C and others, unpublished data).[26] A study conducted in Vietnam also found off-premises piped sources to contain more fecal contamination than on-premises piped sources, with evidence of similar stored water quality for both source types.[27]

Taken together, these findings highlight the need for interventions at the household level, including water treatment, safe water handling, improved storage, and hand hygiene to ensure safe drinking water, even in populations using improved supplies. In this study, reported boiling was associated with a significant reduction in contamination of stored water among households with contaminated source water, a finding that is consistent with other research.[27, 28, 29, 30, 31,32] In some studies, however, reported boiling has been associated with no effect[33, 34] or even an increase in contamination.[35] Because there is no objective measure of boiling, assessment relies on self-report, which may substantially overestimate actual practice.[36] In addition, boiling may be ineffective if water is subsequently recontaminated via unsafe storage and handling, as was suggested in this study by the decreased risk of contamination associated with pouring or using a spigot to extract water from the storage container, rather than dipping a hand or object. This was also suggested by the association between container cleanliness and decreased contamination risk. The association between the ability of a participant to demonstrate correct handwashing procedure and having stored water with no detectable contamination highlights the importance of proper hygiene for water handling. The finding in this study that people with less than a secondary education were more likely to have contaminated stored water underscores the importance of using targeted hygiene education interventions for high-risk populations.

This study had two important limitations. First, because this assessment was limited to rural households in a single coastal department, these results may not generalize to the rest of Peru. Second, the Compartment Bag Test (CBT) serves primarily as a qualitative measure of *E. coli* contamination because the *E. coli* MPNs have wide, overlapping confidence intervals[18] and the upper limit of detection of the test of 100 *E. coli* per 100mL does not permit an accurate determination of the amount of contamination in highly contaminated samples. However, a qualitative measure of contamination may be a sufficient indicator of risk since it corresponds with the WHO standard for drinking water safety.[3] The use of the CBT as a qualitative measure of contamination has three distinct advantages: no need for highly trained laboratory staff, a minimal need for laboratory equipment, and high sensitivity and specificity.[19] An additional justification for using a qualitative measure is that the association between the level of *E. coli* contamination in drinking water and diarrheal disease risk is unclear. Some research has shown an association between the consumption of highly contaminated water and diarrhea,[37,38] but other investigations have found no significant association between the level of fecal contamination and disease risk.[39, 40, 41,42] A recent systematic review and meta-analysis demonstrated an association between the detection—but not the amount—of *E. coli* contamination and diarrhea.[43]

The considerable contamination of improved water sources observed in this and other studies demonstrates that the current MDG indicator for water quality should not be equated with safety. The indicator of basic water service proposed for the post-2015 monitoring period will have similar limitations. Because implementation of global microbiological testing is unlikely to be immediate, research is needed to develop more accurate indicators of safe drinking water access. Our findings underscore the importance of household water treatment, safe storage and handling, and information, education, and communication materials appropriate for all educational levels to ensure the safety of household drinking water. Because boiling is the only household water treatment method that is used at meaningful scale,[44] future studies should investigate ways to improve the objective measurement and effectiveness of this method. Although there has been minimal research to investigate the determinants of boiling behavior, existing evidence suggests that technological interventions as simple as water pasteurization indicators can increase the effectiveness of heat treatment of household drinking water.[45] A better understanding of the effectiveness of behavioral and technological approaches to the promotion of boiling will determine the extent to which expansion of this method can increase global access to safe drinking water.

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TABLES

Table 1. Participant characteristics by *Escherichia coli* contamination of stored household drinking water in Pisco, Peru, January 2014*

	Uncontaminated (N=115)		Contaminated (N=85)		p
	n	(%)	n	(%)	
Median age (range)	30 (18-62)		35 (19-64)		0.02
Median household size (range)	4 (2-15)		5 (2-15)		0.01
Education					<0.001
Less than complete secondary	35	(40.7)	51	(59.3)	
Secondary complete or above	80	(70.2)	34	(29.8)	
Socioeconomic Index[a]					0.34
Poorest Tercile	32	(51.6)	30	(48.4)	
Middle Tercile	39	(60.9)	25	(39.1)	
Wealthiest Tercile	39	(60.0)	26	(40.0)	
Primary water source					0.01
Unimproved source	6	(42.9)	8	(57.1)	
Other improved source	31	(44.9)	38	(55.1)	
Piped source on-premises	77	(67.5)	37	(32.5)	
Storage container type					0.054
Teapot	17	(77.3)	5	(22.7)	
Wide-mouthed container	97	(55.8)	77	(44.3)	

Covered storage container[b]					0.13
Yes	111	(59.4)	76	(40.6)	
No	4	(33.3)	8	(66.7)	
Clean storage container[b]					0.06
Yes	112	(59.6)	76	(40.4)	
No	3	(27.3)	8	(73.7)	
Method of water extraction					0.002
Poured/dispensed with a spigot	88	(65.7)	46	(34.3)	
Dipped with a cup, other utensil, or hands	23	(41.1)	33	(58.9)	
Boiled currently stored water[b]					0.02
Yes	112	(59.6)	76	(40.4)	
No	2	(20.0)	8	(80.0)	
Toilet/latrine					0.85
Yes	93	(58.1)	67	(41.9)	
No	22	(56.4)	17	(43.6)	
Presence of a handwashing station					0.048
Yes	80	(63.0)	47	(37.0)	
No	35	(48.6)	37	(51.4)	
Correct handwashing[b]					0.02
Yes	101	(62.4)	61	(37.7)	
No	4	(28.6)	10	(71.4)	

*Seven subjects did not have stored water for collection at the time of the survey and were therefore excluded from this analysis. Numbers in the table may not sum to total due to missing values. [a] p-value for trend; [b] p-value for Fisher's Exact test

Table 2. Prevalence ratio (PR) estimates of the association of *Escherichia coli* contamination of stored drinking water with water, sanitation, and hygiene practices in Pisco, Peru, 2014

	Unadjusted PR	(95% CI)	Adjusted PR[a]	(95% CI)
Primary water source				
Unimproved source	1.00	(Ref)	1.00	(Ref)
Other improved source	0.96	(0.58, 1.59)	0.99	(0.62, 1.58)
Piped source on premises	0.57*	(0.34, 0.96)	0.61	(0.37, 1.03)
Storage container type				
Wide-mouthed container	1.00	(Ref)	1.00	(Ref)
Teapot	0.51	(0.23, 1.13)	0.63	(0.28, 1.40)
Container covered	0.61*	(0.39, 0.94)	0.70	(0.46, 1.06)
Container cleanliness	0.56**	(0.37, 0.83)	0.67*	(0.45, 1.00)
Water treatment	0.51***	(0.35, 0.72)	NC	--------
Method of water extraction				
Dipped with an object or hands	1.00	(Ref)	1.00	(Ref)
Poured or used a spigot	0.58***	(0.42, 0.80)	0.58**	(0.42, 0.80)
Toilet/latrine	0.96	(0.64, 1.43)	0.99	(0.66, 1.49)
Correct handwashing demonstration	0.53**	(0.36, 0.78)	0.62*	(0.42, 0.90)

[a] Models adjusted for the dipping of a cup/other utensil/hands.
Significance codes: $p < 0.001$ '***', $p < 0.01$ '**', $p < 0.05$ '*'.
NC= model does not converge on 200 iterations.

FIGURES

Figure 1. *Escherichia coli* contamination of water samples by WHO health risk category

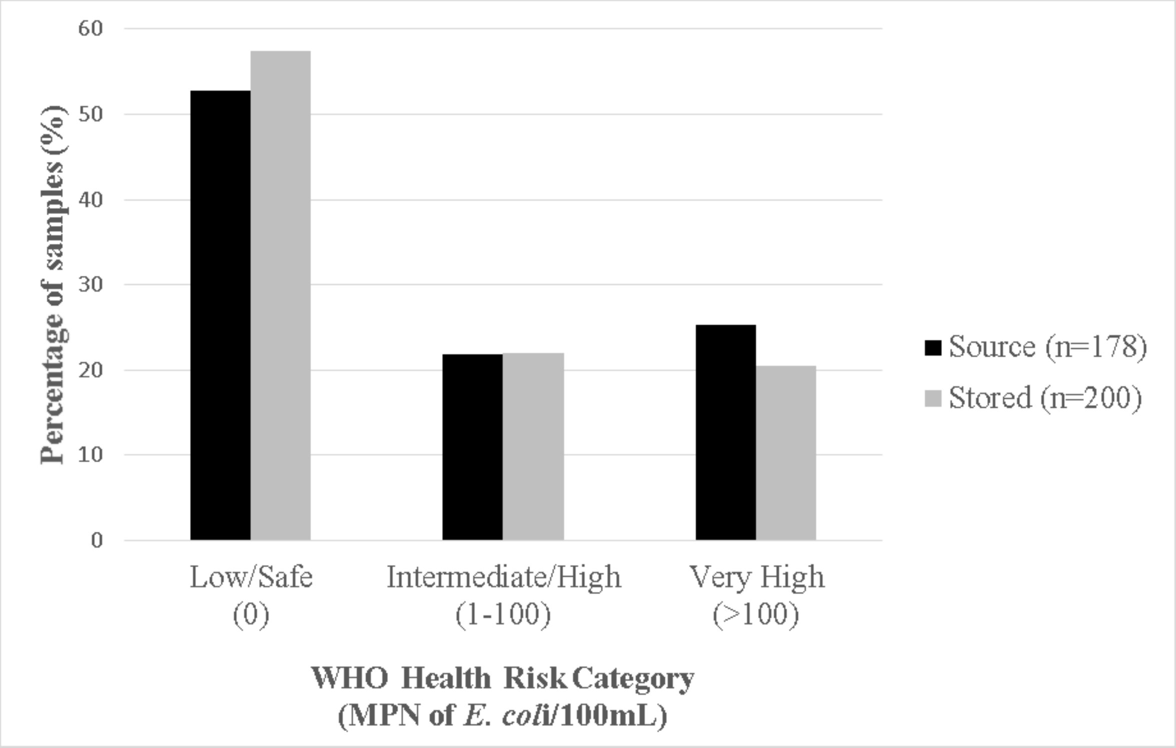
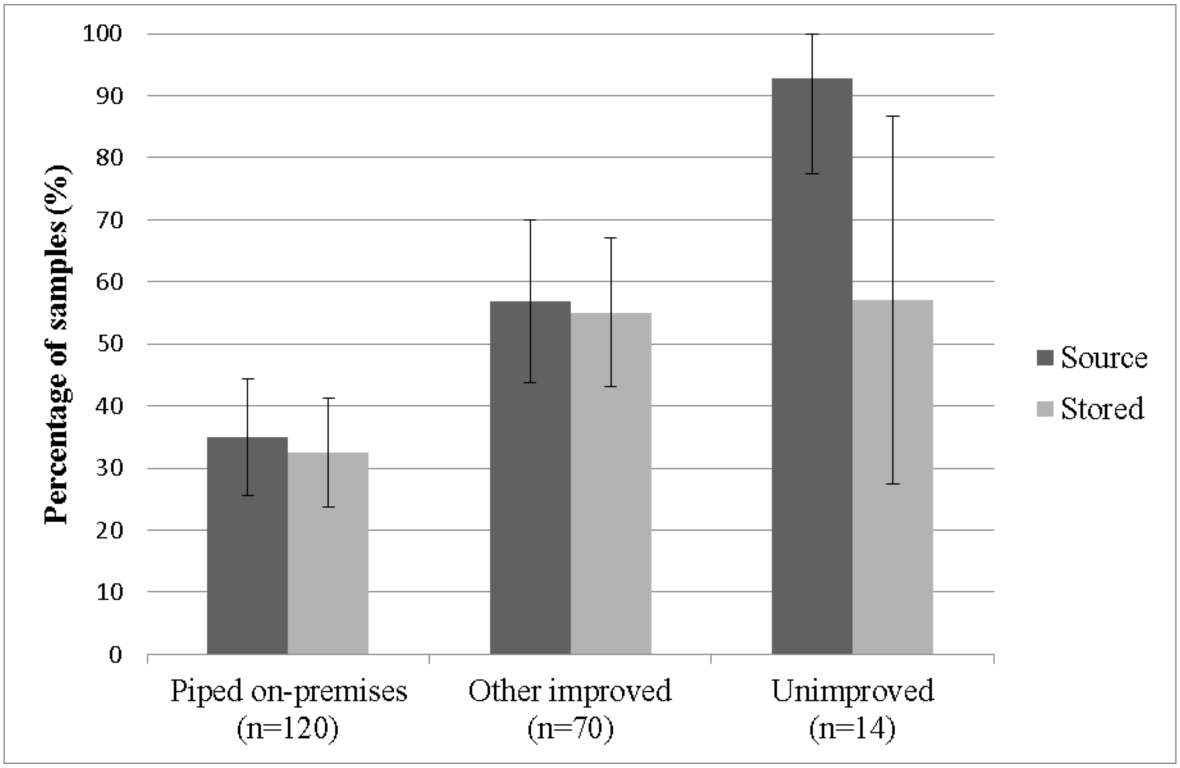


Figure 2. Prevalence of *Escherichia coli* contamination of water by source type*



*Error bars represent 95% Confidence Intervals

CHAPTER II: The challenge of improving boiling: lessons learned from a randomized controlled trial of water pasteurization and safe storage in Peru

INTRODUCTION

In low- and middle-income countries (LMICs), diarrhea is a leading cause of morbidity and mortality among children under five years of age.[1, 2] Household water treatment and safe storage are recommended as part of the WHO strategy to reduce diarrhea risk,[3] yet only 10% of people living in LMICs report using an effective method to treat their household drinking water.[4] Boiling is the sole method of household treatment that has reached scale in any country, and is over three times more frequently practiced than any other method.[4] On the basis of the demonstrated effectiveness and scalability of boiling, its improvement and expansion has been proposed as a potentially effective means of increasing household access to safe drinking water. [5]

Despite the relatively high acceptability of boiling, the practice has a number of potential shortcomings that may limit its effectiveness and use. In the absence of safe storage and handling in the home, boiled water supplies may be recontaminated;[6, 7, 8] other drawbacks include collection time and cost of fuel,[9, 10] risk of scalding,[11, 12, 13, 14] and, among populations that rely on biomass fuel, indoor air pollution[15] and a detrimental effect on the environment.[16, 17, 18, 19]

There is evidence that some of the obstacles to effective boiling can be addressed by simple technological interventions. The results of randomized trials indicate that the risk of recontamination of boiled water supplies can be reduced by the use of storage containers that prevent the introduction of hands or dipping objects,[20, 21] although one study found no effect.[22] A study conducted in Kenya demonstrated the feasibility and effectiveness of teaching a low-income population to pasteurize their water—that is, heat water to sub-boiling temperatures to inactivate pathogens—using a thermosensitive indicator.[23] Among reported users, pasteurization reduced the prevalence of microbial contamination of drinking water by over 30% and the incidence of severe diarrhea by 45% relative to individuals who drank untreated water. No other research has been conducted to assess the effect of pasteurization indicator use on household drinking water safety.

In rural Peru, 70% of households report boiling,[24] yet one investigation documented that 95% of household drinking water samples contained microbiological contamination,[25] which suggests that the effectiveness of the practice is sub-optimal. We evaluated the effectiveness of two simple technological interventions—an improved storage container and a water pasteurization indicator—in reducing fecal contamination of household drinking water in a rural Peruvian population that boils drinking water.

MATERIALS AND METHODS

Study design. We conducted a randomized controlled trial, assigning households in a 1:1:1 ratio to one of two intervention groups or to a control group. The trial was conducted between January and April 2014.

Study setting. The study was conducted in Humay district, Pisco province, on Peru's southern coast. The district is located in the Pisco River Valley and has an estimated population of 5,800 inhabitants. It receives approximately 2 mm of rainfall annually. The majority of households use piped drinking water sources. Water distributed by piped systems either flows directly from the Pisco River, or indirectly through irrigation channels. The piped system of one community distributes water from a borehole. In some communities, the water from piped water systems passes through a sand or gravel filter prior to distribution. Piped water is intermittently manually chlorinated in elevated tanks before distribution in the two largest communities. Because piped water is available between 2 and 20 hours a day, a proportion of households pump water into elevated tanks above their homes in order to be able to use piped water outside of the normal hours of service.

Sample size. The sample size was calculated to detect a 15% difference in the prevalence of *Escherichia coli* contamination in household drinking water between the intervention and control arms with 95% confidence and 80% power, assuming up to 6 follow-up microbiologic tests of stored drinking water per household, and an estimated 88% prevalence of *E. coli* contamination in stored drinking water, 20% loss to follow up, and a design effect of 2. We aimed to enroll 70 subject households per arm to ensure more than sufficient power to detect this difference based on these assumptions.

Participants and enrollment. Households were eligible to participate in the study if they contained a female member ≥18 years old, a child <5 years old, and were able to heat water in their home. Field teams conducted a census of the district before the study to identify eligible households. A computerized pseudorandom number generator was used to create a randomly ordered list of eligible houses for recruitment. Using a geographically organized list of eligible households, enumerators enrolled households to participate until a total of 210 was reached; households were randomly assigned to one of the 3 study groups. If a female head of household was not available at the initial visit, two additional attempts were made to enroll the household. All participants provided written informed consent prior to the initiation of any study procedures.

At the enrollment visit, a baseline survey was administered to assess participant demographic characteristics; socioeconomic status; household water supply, treatment, storage, and handling behaviors; sanitation and hygiene; presence of a handwashing station (defined as a designated place for handwashing with a water supply and soap present); and ability to demonstrate correct handwashing technique (lathering all surfaces of the hands with soap). A socioeconomic index was generated using principal component analysis of household assets, building materials, and home ownership.[26] The index was comprised of the first principal component of each variable[27] and households were grouped into index terciles for analysis. Field workers used an orthotolidine (OTO) pool test kit (Pentair, Minneapolis, MN) to test household source and stored drinking water for the presence of total chlorine. Source and stored household water samples were collected in sterile bottles and analyzed for *E. coli* using the Compartment Bag Test (Aquagenx, Chapel Hill, NC); this test is a highly sensitive and specific qualitative measure[28] and has a minimum threshold of detection of 1 *E. coli*/100mL.[29] Samples were transported to the study center and processed within 6 hours of collection. All samples were incubated for a minimum of 20 hours at 35-44.5˚C.[29] Positive and negative controls were incubated daily with the samples to verify the correct functioning of the tests.

Randomization and study interventions. The randomization list of eligible houses was computer-generated by the principal investigator. Opaque, sealed envelopes were used to conceal the group assignments from field workers until completion of the baseline survey. At the end of the enrollment visit, all participants were informed of the dangers of drinking untreated water. Participants were randomly assigned to one of three groups. Study group A received a locally available 20L storage container with a cover and spigot (improved container); study group B received an improved container and a water pasteurization indicator (Solar Cookers International, Sacramento, CA; Figure 1) with written and pictorial instructions for its use. The indicator consists of a wax that melts at the pasteurization temperature of 65˚C in a polycarbonate tube connected to a stainless steel cable that is used to dip the indicator tube into the container in which water is being heated. Two key advantages of indicator use described in the manufacturer's instructions—fuel and time savings—were highlighted in the instructions distributed to participants. Field workers additionally performed a short demonstration of how to use the indicator to pasteurize water. Study group C received no additional intervention and served as a control group. Participants who received an intervention were asked to use it during the course of the study for their household drinking water.

Outcome assessment. The primary outcome was the presence of detectable *E. coli* contamination in stored drinking water. Follow-up home visits began 3 weeks following enrollment and were conducted every two weeks over a 10 week period, yielding a total of 6 possible follow up visits and 13 weeks follow-up. At each visit, source and stored household water samples were collected and analyzed for *E. coli* using the Compartment Bag Test following the same procedures as described for the enrollment visit.

Adherence and Other Measures. Field workers completed a short survey at all follow-up visits to assess cooking practices, chlorination of source and stored water, and a proxy measure of boiling behavior (observation of water in a pot on a stove or open fire). In the control group, water storage practices were observed. In both intervention groups, field workers evaluated adherence to use of the storage container (defined as observation of water in the container), and the condition of the container. Participants in study group B were asked to demonstrate and explain how they use their indicator. Correct indicator use was defined as proper indicator tube placement (wax end of tube pointed upward) in a container of water being heated, along with knowledge to stop heating water when the wax falls to the bottom of the tube. Use of the indicator could not be assessed directly because we lacked the resources to do structured observations and, because the indicator tubes were reusable, no used or discarded tubes could be observed.

Statistical Analysis. All data were entered into a Microsoft Access 2010 (Redmond, WA) database and analyzed using STATA 13.1 (College Station, TX). Cross-tabulations were used to examine the distribution of baseline characteristics by randomization assignment. We used generalized estimating equations (GEE) with a log link function, binomial distribution, working exchangeable correlation structure to account for clustering by household, and robust standard errors to compare the prevalence of *E. coli* contamination between paired source and stored water samples. As our primary analysis, we used an intention-to-treat approach to assess the effect of the interventions on *E. coli* contamination. The prevalence ratios (PRs) of *E. coli* contamination were estimated using a GEE model with the same specifications as above. We assessed the sensitivity of these results to possible confounding caused by imbalances in the baseline demographic and socioeconomic characteristics of the randomization groups and or by imbalances in potentially confounding follow-up variables (*E. coli* contamination of source water, source water chlorination, and type of cooking stove). Substantial confounders were defined as variables that altered the PR estimates by 10% or more in adjusted models. The characteristics of participants who adhered to improved container use (had water in the container at 4 or more follow up visits) were evaluated using Chi-squared and Fisher's Exact tests. The prevalence of *E. coli* contamination in stored water was compared between participants who were and were not adherent to container use within each intervention arm using GEE to adjust for household-level clustering.

Ethical considerations. This study protocol was approved by the Institutional Review Boards of the University of Washington, the U.S. Naval Medical Research Unit No. 6 (NAMRU-

6), and the Ica regional Ministry of Health. Written informed consent was obtained from all subjects and all subject personal identifiers were irreversibly removed from electronic databases following the end of data collection.

RESULTS

Participants. Field teams invited a total of 333 households to participate and 210 were enrolled and randomized (Figure 2). Of the 123 households that were not enrolled, the most common reasons for non-participation were not being available at two attempts to enroll (34%), not having a child under age 5 (28%), and refusal to participate (18%). The geographic distribution of households did not differ by enrollment status (p=0.65). The baseline characteristics of eligible participants randomized to each of the three study groups were similar (Table 1). No chlorine was detected in stored water samples at baseline. Three participants were excluded from analysis post-randomization due to failure to meet study eligibility criteria. Two participants moved following the enrollment visit, resulting in a total of 205 participants who provided follow-up data for analysis.

Overall, 59% of households used a water source that was piped to the home, 34% used an improved source not piped to the home (primarily community standpipes), and 7% used an unimproved source. Nearly all participants (98%) reported treating their household drinking water, with 99% of those doing so by boiling and 1% using both boiling and chlorination. When asked an open-ended question regarding the definition of boiling, 194 (94%) participants provided a response consistent with adequate heating for water disinfection. Eighty-nine percent of participants stored their drinking water in a wide-mouthed container; 11% used a teapot.

Storage containers were observed to be covered at 94% of enrollment visits. Thirty percent of households dipped a hand or an object to extract water from the container.

***E. coli* contamination of stored drinking water.** During the follow up period, the percentages of paired stored and source water samples contaminated by *E. coli* were similar in groups A (storage container only) and C (the control group; p-values from GEE models>0.13). In group B (storage container plus indicator), stored water samples were less likely to be contaminated than their paired source water samples, but this association did not reach statistical significance (p=0.053). In the control group, 36.7% of stored water samples contained detectable *E. coli*, as compared to 37.9% in the group that received only a storage container, and 44.2% in the group that received a storage container and an indicator; these differences were not statistically significant (p-values for pairwise comparisons in GEE models>0.15). The prevalence ratio of *E. coli* contamination relative to the control group was 1.02 (95% CI: 0.79, 1.32) for group A (storage container only) and 1.20 (95% CI: 0.93, 1.54) for group B (storage container plus indicator) (Table 2). There was no evidence of confounding of these estimates by demographic or socioeconomic factors or potentially confounding follow-up variables.

There was no trend in the prevalence of contamination of stored water over time in any of the study groups (all p-values>0.15; Figure 3). *E. coli* was detected at a subset of follow-up visits for 194 of 205 (95%) households; one household had contaminated drinking water at all follow up visits; ten households had no *E. coli* detected in stored drinking water during the follow-up period, and these households were roughly equally distributed among the three study groups.

Adherence to interventions and control group practices. In the intervention groups, improved containers were observed at 97% of follow-up visits and were observed to have water inside at 54% and 57% of the follow up visits in study groups A and B, respectively. Container usage decreased over time in both intervention groups (p-values of GEE models<0.01); approximately 50% of households in both intervention arms were using their storage container at the final visit (Figure 4). The indicator was observed at 90% of visits; four participants lost their indicator during the follow up period. Knowledge of correct indicator use was demonstrated by over 90% of participants during each biweekly follow-up period (Figure 4).

About half (48%) of participants in both intervention groups adhered to use of the improved storage container (defined as use at four or more visits). Adherent participants were more likely to have an off-premises water source (54 vs. 27%; p=0.002), tended to cook using a stove rather than an open fire (94 vs. 82%; p=0.06), and tended to have completed secondary school (65 vs. 49%; p=0.06). No other variables were associated with adherence. In both intervention groups, participants who adhered to improved container use had similar stored water quality as participants who used their containers less frequently (Table 3).

In group C (the control group), the storage container was covered at 86% of the visits. Water was observed in a pot on the stove or open fire at 36% of visits; this indicator of boiling was observed with similar frequency in groups A (36%) and B (32%).

Perceptions of the interventions. When asked to cite the advantages and disadvantages, if any, of the safe storage container, participants in Groups A and B most frequently stated that it was easy to use (53%), it made the water safer for drinking (42%), and allowed them to store water in larger quantities (31%). The most frequently cited disadvantage was that the container was too large (3%). Group B participants reported that the primary advantages of using the indicator were saving fuel (83%), saving time (29%), and ease of use (28%). Disadvantages included difficulty of use (3%) and needing to keep an eye on the indicator to use it correctly (3%).

DISCUSSION

Although the results of this trial demonstrated the feasibility of teaching a low-income population to pasteurize their household drinking water via the use of a thermosensitive indicator, this intervention had no significant effect on the risk of *E. coli* contamination of household drinking water. This study was the first to test the effect of use of a water pasteurization indicator in a population in which water boiling was a culturally accepted practice; in the only previous study of a similar intervention, indicator users were compared to individuals who drank untreated water.[23] We found that the provision of an improved storage container had no effect on the prevalence of *E. coli* contamination of household drinking water, which was similar to the result of one previous trial of an improved container[22] but inconsistent with others that demonstrated reductions in fecal contamination.[20, 21]

The lack of effect of the pasteurization indicator intervention could be explained several ways. First, despite retaining knowledge of how to use the indicator, the participants may not have used them. Because the indicators are reusable, we could not verify this hypothesis. Second, participants may have used the indicators, but more consistent or effective treatment behavior did not result. The observation of the proxy for boiling—water in a pot on a stove or fire –at over 30% of home visits coupled with the lack of significant reductions in contamination from source to stored water across study arms supports this hypothesis, although we could not confirm the effectiveness of treatment. An alternative explanation for the null effect is that although use of the pasteurization indicator resulted in more effective treatment behavior, the receipt of an indicator led participants to reduce their investment in other protective behaviors such as handwashing, thus negating the impact of more effective treatment on water quality. The effect of the prevalence of an exposure such as a public health intervention on demand for disease prevention—termed *prevalence elasticity*—has been observed for other health-related outcomes,[30] but because we did not assess preventive behaviors including hygiene or safe water handling at follow up visits, we could not confirm whether this occurred. In the group that received only a water storage container, generally safe water storage and handling practices and good hygiene in the control group likely limited our ability to detect a benefit of the intervention.

The results of this study demonstrate the challenge of improving boiling and safe water storage in a population in which both practices have been accepted but perhaps inconsistently or ineffectively practiced. Although participants generally expressed satisfaction with the container, these stated perceptions likely overstated actual satisfaction due to courtesy bias. Moderate use of the storage container highlighted the importance of identifying interventions that the population perceives as offering a relative advantage over current storage practices to increase the probability of use.[31] Similarly, although most Group B participants exhibited knowledge of indicator use and perceived its advantages, we could not confirm indicator use. The lack of effect of the indicator may have reflected lack of use for similar reasons as for the storage container.

This study had two important limitations. First, we based our sample size calculation on the prevalence of *E. coli* contamination in drinking water samples collected from the same district in 2010, as this was the only relevant data available at the time of protocol development. Although the majority of drinking water from even piped sources in rural Peru is contaminated with *E. coli*,[25, 32] progressive expansion of access to piped water infrastructure in the area likely contributed to the unexpectedly low prevalence of contamination in the control group, which limited our ability to detect statistically significant differences. Second, we assessed our primary outcome, *E. coli* contamination, using the Compartment Bag Test. This test has the advantages of requiring minimal materials or technical skill to perform, but yields imprecise quantitative results.[29] It is, however, a highly sensitive and specific qualitative measure of contamination.[28] A qualitative assessment of *E. coli* contamination may be adequate given that it corresponds with the WHO standard of drinking water safety.[33] Moreover, the results of a recent systematic review and meta-analysis indicate that while the presence of *E. coli* contamination in drinking water is associated with the risk of diarrhea, increasing levels of *E. coli* contamination in drinking water beyond a detectable level did not demonstrate a dose-response relationship with risk.[34] This finding was based on a small number of studies, however, and more research is needed to determine whether a qualitative measure of *E. coli* contamination is sufficient indicator of health risk.

Our study findings indicate that the use of a water pasteurization indicator was not effective in reducing the fecal contamination of household drinking water in a population that had already adopted boiling as a method of treatment. However, this investigation and a previous one[23] provided evidence of the feasibility of teaching a low-income population to use a thermosensitive indicator to pasteurize their drinking water. Future research is needed on methods to improve objective measurement of indicator use and assess the impact of this intervention on drinking water safety and diarrheal disease in populations at high risk for waterborne disease. Formative research may additionally be useful to better understand the factors that influence use of water treatment and storage interventions designed to limit the contamination of drinking water in the home.

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TABLES

Table 1. Study population characteristics by study arm*

	Total (N=207)		Container Only (N=70)		Container + Indicator (N=70)		Control (N=67)	
Median age (range)	31 (18-64)		32 (19-62)		31 (19-56)		31 (18-64)	
Median household size (range)	4 (2-15)		4 (3-8)		4 (2-13)		5 (3-15)	
Completed secondary school or above	117	(56.5%)	37	(52.9%)	42	(60.0%)	38	(56.7%)
Socioeconomic Index								
Poorest Tercile	66	(33.3%)	24	(34.8%)	24	(37.5%)	18	(27.7%)
Middle Tercile	66	(33.3%)	24	(34.8%)	22	(34.4%)	20	(30.8%)
Wealthiest Tercile	66	(33.3%)	21	(30.4%)	18	(28.1%)	27	(41.5%)
Primary water source								
Piped to the home	120	(58.8%)	44	(63.8%)	40	(57.1%)	36	(55.4%)
Improved source outside the home	70	(34.3%)	18	(26.1%)	24	(34.3%)	28	(43.1%)
Unimproved source	14	(6.9%)	7	(10.1%)	6	(8.6%)	1	(1.5%)
Detectable chlorine in source water	9	(5.3%)	1	(1.6%)	3	(5.1%)	5	(10.0%)

Thinks water is safe to drink	82	(39.8%)	31	(44.3%)	23	(33.3%)	28	(41.8%)
Storage container type								
Teapot	22	(10.8%)	10	(14.9%)	7	(10.0%)	5	(7.6%)
Wide-mouthed Container	181	(89.2%)	57	(85.1%)	63	(90.0%)	61	(92.4%)
Covered storage container	194	(94.2%)	65	(92.9%)	66	(94.3%)	63	(95.5%)
Clean storage container	195	(94.7%)	67	(95.7%)	65	(92.9%)	63	(95.5%)
Method of water extraction								
Poured/used a spigot	138	(70.4%)	49	(71.0%)	48	(72.7%)	41	(67.2%)
Dipped with an object/ Hands	58	(29.6%)	20	(29.0%)	18	(27.3%)	20	(32.8%)
Boiled currently stored water	194	(94.6%)	66	(95.7%)	65	(92.9%)	63	(95.5%)
Toilet/latrine	167	(81.1%)	57	(82.6%)	57	(81.4%)	53	(79.1%)
Presence of a handwashing station	131	(63.6%)	47	(67.1%)	42	(60.0%)	42	(63.6%)
Correct handwashing	167	(91.3%)	58	(93.5%)	55	(90.2%)	54	(90.0%)

*Numbers may not sum to total due to missing values.

Table 2. Effect of interventions on the mean prevalence of *Escherichia coli* contamination of stored drinking water in Pisco, Peru, 2014

Study Arm	Observation-days	Observation-days with *E. coli* contamination (%)	Risk difference relative to control	Prevalence ratio	(95% CI)
Control	335	123 (36.7)	Ref	1.00	(Ref)
Container only	354	134 (37.9)	1.1	1.02	(0.79, 1.32)
Container and Indicator	346	153 (44.2)	7.5	1.20	(0.93, 1.54)

Table 3. Mean prevalence of *Escherichia coli* contamination of stored drinking water by adherence with improved storage container use

Study Arm	Adherent		Non-adherent		p
	% *E. coli* contamination	(95% CI)	% *E. coli* contamination	(95% CI)	
Container only	42.8	(35.5, 51.7)	33.5	(25.2, 44.4)	0.15
Container and indicator	47.9	(38.6, 59.5)	43.5	(34.7, 54.4)	0.54

FIGURES

Figure 1. Water pasteurization indicator

Source: http://shop.solarcookers.org/?pn=WAPI&cn=Water+Pasteurization&p=630&c=31
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Figure 2. Trial profile

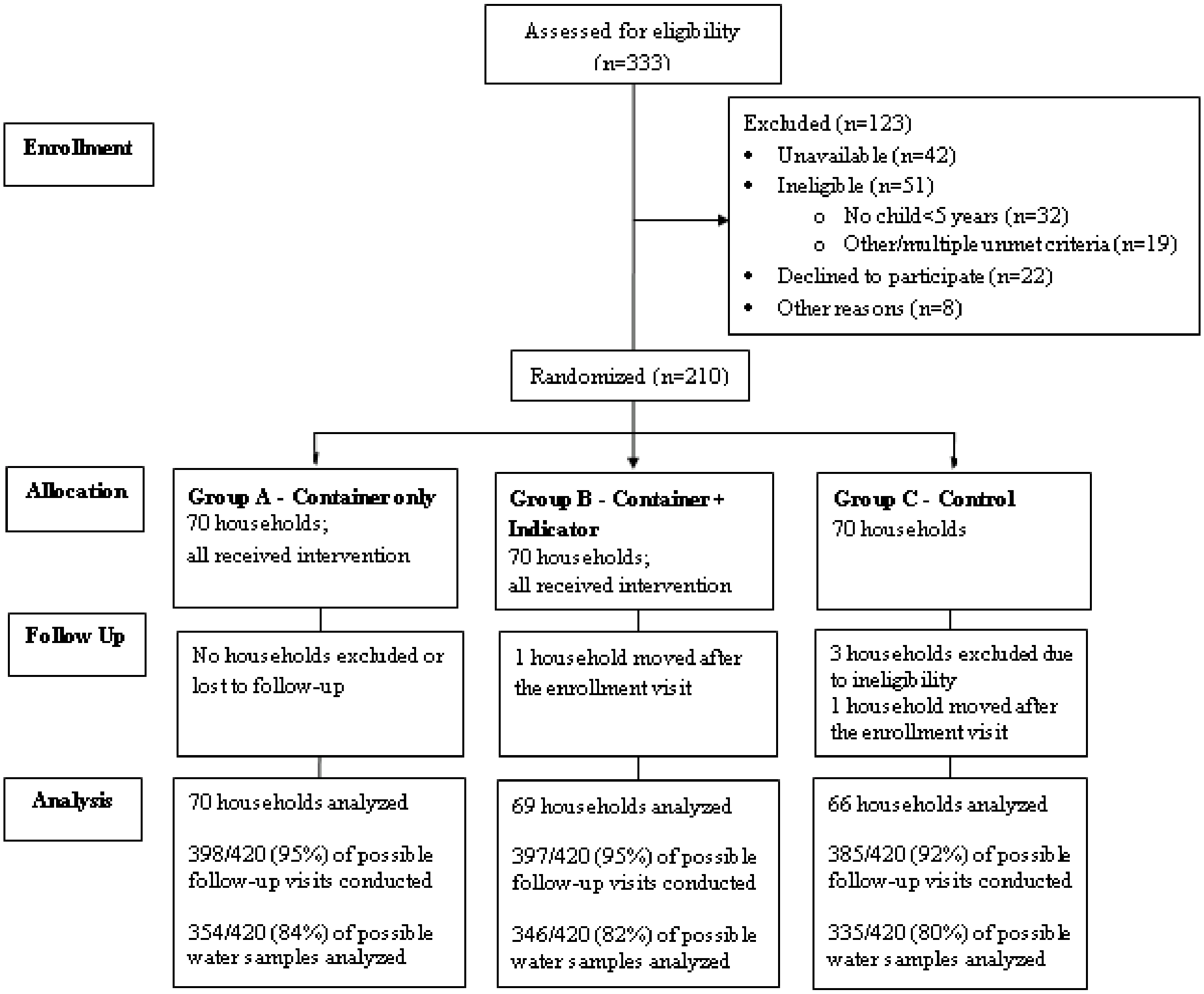

Figure 3. Percentage of stored drinking water samples contaminated by *Escherichia coli*, by study arm and follow-up visit round, Pisco, Peru, January-April 2014

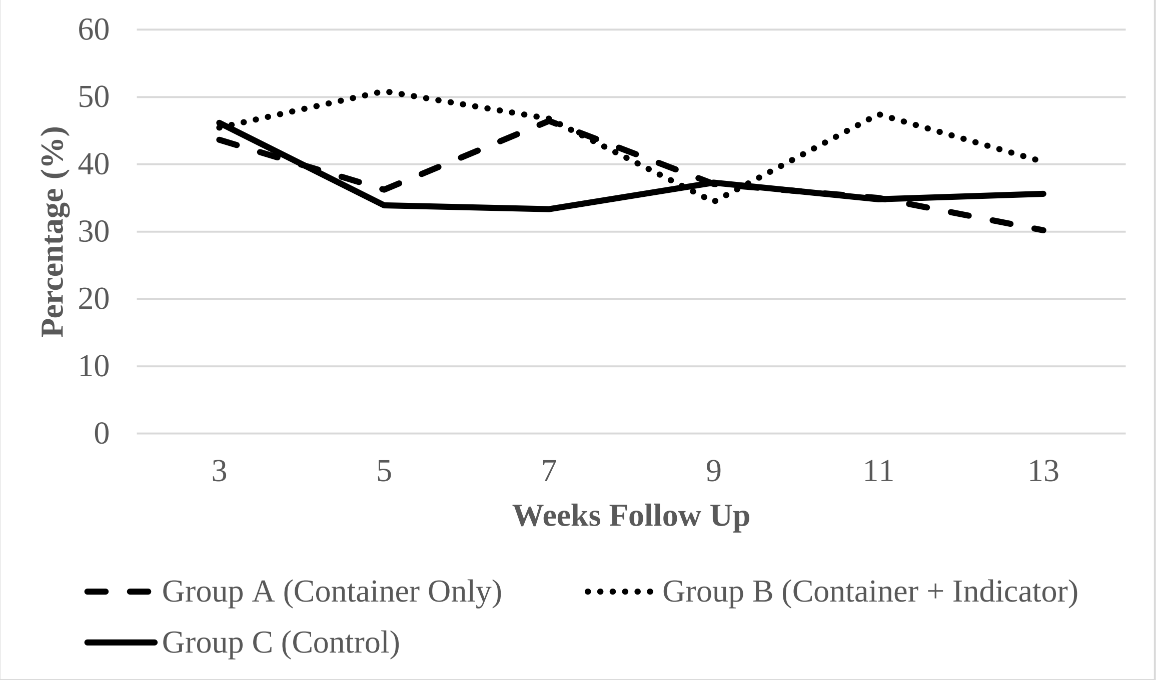

Figure 4. Percentage of intervention group A and B participants using improved storage container, and intervention group B participants with knowledge of proper indicator use, by follow-up visit round, Pisco, Peru, January-April 2014