Ceramic water filters impregnated with silver nanoparticles as a point-of-use water-treatment intervention for HIV-positive individuals in Limpopo Province, South Africa: a pilot study of technological performance and human health benefits

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ABSTRACT

Waterborne pathogens present a significant threat to people living with the human immunodeficiency virus (PLWH). This study presents a randomized, controlled trial that evaluates whether a household-level ceramic water filter (CWF) intervention can improve drinking water quality and decrease days of diarrhea in PLWH in rural South Africa. Seventy-four participants were randomized in an intervention group with CWFs and a control group without filters. Participants in the CWF arm received CWFs impregnated with silver nanoparticles and associated safe-storage containers. Water and stool samples were collected at baseline and 12 months. Diarrhea incidence was self-reported weekly for 12 months. The average diarrhea rate in the control group was 0.064 days/week compared to 0.015 days/week in the intervention group ($p < 0.001$, Mann–Whitney). Median reduction of total coliform bacteria was 100% at enrollment and final collection. CWFs are an acceptable technology that can significantly improve the quality of household water and decrease days of diarrhea for PLWH in rural South Africa.

Key words | ceramic water filters, *Cryptosporidium parvum*, HIV, point-of-use, South Africa

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INTRODUCTION

Poor sanitation leads to contamination of drinking water sources by pathogenic microorganisms (United Nations Human Settlements Programme 2003; Morones *et al.* 2005). Enteric pathogens in untreated water are particularly problematic for people living with the human immunodeficiency virus (PLWH) (Frisby *et al.* 1997; Hoxie *et al.* 1997; Dwivedi *et al.* 2007; Kulkarni *et al.* 2009). Enteric infections may increase mortality even in those who are treated with anti-retroviral therapy (ART) (Dillingham *et al.* 2009). In rural South Africa, there is an unfortunate confluence of acquired immune deficiency syndrome (AIDS) and untreated drinking water (Obi *et al.* 2007). In Limpopo

Province, the site of this study, a South African National Department of Health Survey reports 13.8% human immuno-deficiency virus (HIV) prevalence in the general population and 21.4% HIV prevalence among antenatal women (Makubalo *et al.* 2003). Seroprevalence estimates for rural Limpopo Province suggest even higher rates of HIV infection (Makubalo *et al.* 2003). A recent study of opportunistic infections in HIV-infected patients in South Africa demonstrated high prevalence of *Cryptosporidium* infection in the Venda region of Limpopo Province (Samie *et al.* 2006).

A recent review of the literature sponsored by the World Health Organization (WHO) concludes that simple, socially acceptable, and low-cost interventions at the household (point-of-use (POU)) and community level have the potential to significantly improve the microbial quality of household water and reduce the risk of diarrheal disease and death, particularly among children (Clasen *et al.* 2006). A recent meta-analysis of water-quality interventions aimed at reducing diarrheal disease, reported that household water interventions are more effective at improving water quality than interventions at the source, and that household water treatment (HWT) can be more cost-effective in the long run compared to centralized water treatment and distribution systems (Clasen *et al.* 2007). Although studies have demonstrated numerous advantages to treating water at the household level, there are some concerns that exist regarding the acceptability and scalability of HWT systems (Schmidt & Cairncross 2009).

A large cohort study in HIV-affected households in Uganda demonstrated that a POU water system that employed chlorine disinfection and small-mouthed container storage decreased the number of episodes of diarrheal illness in HIV-infected household members by 25% (Lule *et al.* 2005). This system also significantly reduced diarrheal episodes in non-HIV-infected children aged 3–12, but it did not significantly reduce the rates for other non-HIV-infected household members. In a recent study in Nigeria utilizing a similar technology and population, diarrhea rates were reduced by 46% among users. This change was significant in the group that did not receive prophylactic antibiotics (Barzilay *et al.* 2011). These findings highlight the importance of targeting the most vulnerable populations, PLWH and families with young children, with improved, sustainable technologies for household treatment of water (Lule *et al.* 2005; Barzilay *et al.* 2011).

One POU water treatment technology that has demonstrated sustainability and social acceptance in various parts of the world is silver-impregnated ceramic water filters (CWFs) (Brown *et al.* 2008; Kallman *et al.* 2011). Unlike a number of POU systems, silver-impregnated CWF units provide safe water storage and demonstrate effective microbial disinfection and physical filtration capabilities without residual odor or taste (Lantagne 2001). These filters are manufactured by combining clay, water, and sawdust in appropriate proportions, pressing the mixture into the shape of a pot, and firing the pot in a kiln. During firing, the clay hardens into a ceramic, and the sawdust combusts, leaving behind pores for water flow. After cooling, the filters are painted with an aqueous suspension of silver nanoparticles, which presumably lodge in the pore space of the filters. The silver nanoparticles are effective microbial disinfectants (Morones *et al.* 2005; Oyanedel-Craver & Smith 2008; Zhang *et al.* 2012). Water passing through the filters is purified by the combined effects of physical filtration and chemical disinfection (Oyanedel-Craver & Smith 2008). The filters are placed in a 5-gallon plastic container, which allow the filter to be suspended on the rim. The containers have a spigot attached at the bottom and serve as safe-storage reservoirs. Filtration rates range from 1 to 4 L/hr and can purify up to 30 L of water per day. The price of the filter unit ranges from 5 to 30 USD. With the exception of the silver, the filters can be manufactured with local materials and labor. Funds from filter sales can therefore remain primarily in the local community, creating a sustainable business model.

The primary hypothesis of this investigation was that household-level CWF interventions would decrease diarrhea rates in PLWH in rural South Africa. In addition, we hypothesized that the filters would significantly improve the microbiological quality of household water. Finally, we evaluated whether the filters would be a socially acceptable POU technology. For this study, we recruited PLWH from a clinic delivering ART into a randomized trial comparing a CWF intervention to usual clinical care. We collected data over 12 months on episodes of diarrhea, CWF performance,

rates of fecal positivity for *Cryptosporidium*, and CWF acceptability.

METHODS

Ethical clearance

This study was approved by Institutional Review Board of the University of Virginia and University of Venda as well as the participating clinic before the commencement of the study.

Study design

This pilot study is a randomized, controlled trial carried out from June 2009 through August 2010. Participants were approached at St Joseph's Clinic over the course of a two-month period with the intention of recruiting 100 participants. The sample size was calculated based on published data about expected reduction in diarrhea rates in PLWH as a result of introduction of POU water filtration (Lule *et al.* 2005). At baseline, demographic data, water quality data, and stool samples were collected. Additional information on episodes of diarrhea was recorded on a weekly basis over the entire 12 months. Finally, stool and water samples were collected at the end of the period of observation. A social acceptability survey was administered to participants with and without filters. This survey addressed the reason for participation in the study, frequency of use and maintenance of the filter, taste and smell of treated water, and cost of the filter. We compared diarrhea rates, water quality data, and stool pathogen rates over the period of observation between participants in the CWF arm and the control arm.

Study setting

Thohoyandou is the administrative center of the Vhembe District, known as Venda. During apartheid South Africa, Venda was a self-governing homeland. St Joseph's is a free clinic supported by Catholic charities located in Thohoyandou. Patients cared for at this clinic received ART at no cost based on WHO and South African Ministry of Health guidelines.

Study site and conditions

Community health workers (CHW) employed by the clinic, research group members from the University of Venda (Univen) fluent in the local dialect, and University of Virginia (UVA) researchers were involved in recruitment. CHW identified clinic patients who met the selection criteria. Participants were 18 years or older and had been receiving anti-retroviral therapy for at least 6 months prior to enrollment. Identified patients were then approached by Univen researchers to explain the purpose of the study and were consented individually. Upon consenting, participants were randomized into the intervention group or the control group using a permuted block randomization system with block sizes of ten maximum (Matts & Lachin 1988).

Participants randomized to the control arm received usual clinical care including education about safe water and hygiene at the clinic. Participants randomized to the intervention arm received the same education about water and hygiene, a CWF, and education about how to use and care for the CWF. CWF arm households were visited upon recruitment to deliver the CWF and explain how to use and maintain it. Participants were instructed not to remove the filters from the lower storage reservoir to avoid potential contamination of the filter and reservoir. Participants in the control arm received filters at the conclusion of the study. CWFs used in this study were produced according to the methods recommended by Potters for Peace and previously described (Lantagne 2001; Kallman *et al.* 2011).

Collection of diarrhea data

Diarrhea was defined as the passage of three or more soft stools in a 24-hr period. Diarrhea recall records were obtained using two separate methods of collection. A pictographic diarrhea record was distributed to each participant. Enough records for 4–5 weeks of data collection were provided at each clinic visit. Each participant obtained new forms and submitted records for the prior month during regularly scheduled clinic visits. Participants were also called on

a weekly basis to obtain total days of diarrhea in the previous week to cross-check the written records returned to the clinic. In the event that the phone record did not match the written record, the phone records were used.

Laboratory assays

Water collection and analysis

Influent and effluent 100-mL water samples for the CWFs were collected in sterilized Whirlpak® Thio-bags in intervention households. The water sample collection bags were lined with thiosulfate, which neutralizes disinfectants, thus preventing persistent disinfection at water sample collection. Water samples from households in the control group were collected from their household drinking water source. During the final collection period, nine households' water samples had levels of coliform in the effluent, which exceeded the influent coliform level. The study team returned to those homes. Members of the research team cleaned the lower reservoirs in these households and a second water sample was obtained.

Water samples were analyzed for three water-quality parameters. Detection and enumeration of total coliform bacteria was accomplished through the membrane filtration technique in accordance with Standard Method 9222 (APHA *et al.* 1998). One hundred milliliters of each sample was passed through a 0.45 μm membrane and placed in a culture dish with m-Endo medium containing lactose and incubated for 24 hr at 35 °C. The medium causes members of the coliform group to develop distinguishable colonies with a metallic sheen. Membrane filtration was conducted to quantify total coliform in influent and effluent water samples and household samples in the control group. Turbidity of samples was determined using a TB200TM Portable Turbidimeter from Orbeco-Hellige, according to Standard Method 2130 (APHA *et al.* 1998). The turbidimeter measures the light-scattering of a water sample caused by suspended particulates in sample cells of 10 mL through a nephlometric principle (range 0–1,100 NTU). A subset of effluent water samples selected at random was tested for the presence of total silver using an atomic absorption spectrophotometer from SpectraAA Varian 220 (APHA *et al.* 1998).

Stool collection and analysis

Leak-proof, sterile, and labeled plastic containers were distributed at the clinic at each stool sample collection cross-section. Stool samples were collected in stool collection cups and stored at −20 °C until analysis. The presence of *Cryptosporidium* sp. was examined through staining with the modified Ziehl Neelsen method. Analysis of *Cryptosporidium* sp. was assessed using real-time polymerase chain reaction (PCR), or RT-PCR (Samie *et al.* 2006).

Data analysis

Data analyses were performed using SAS 9.2, GAUSS 9.0, Minitab 16, and GraphPad Prism 5. Diarrhea rate was determined by calculating the proportion of episodes over the number of observation days and comparing the two using the Mann–Whitney test. Nearly identical results were obtained when the groups were compared using a two-sample t-test with unequal variances. A Poisson regression model was also used to compare the intervention and control groups with respect to diarrhea rate in the follow-up period.

RESULTS

Recruitment

The flow of participants throughout the study is represented in Figure 1. Ninety-three patients were screened at St Joseph's Clinic. Of the 93 patients, 19 were not included in the study. Reasons for non-participation included: decided not to participate after being discouraged by their partner, not feeling comfortable with providing a stool sample, or not providing us with proper contact information.

Laboratory testing

All filters were tested for their technological performance in the laboratory prior to being shipped to the University of Venda in Limpopo Province, South Africa. Flow rate, *Escherichia coli* removal, and silver release were assessed for each of 80 newly manufactured filters. The average flow rate of all 80 CWFs was 3.94 L/hr with a standard
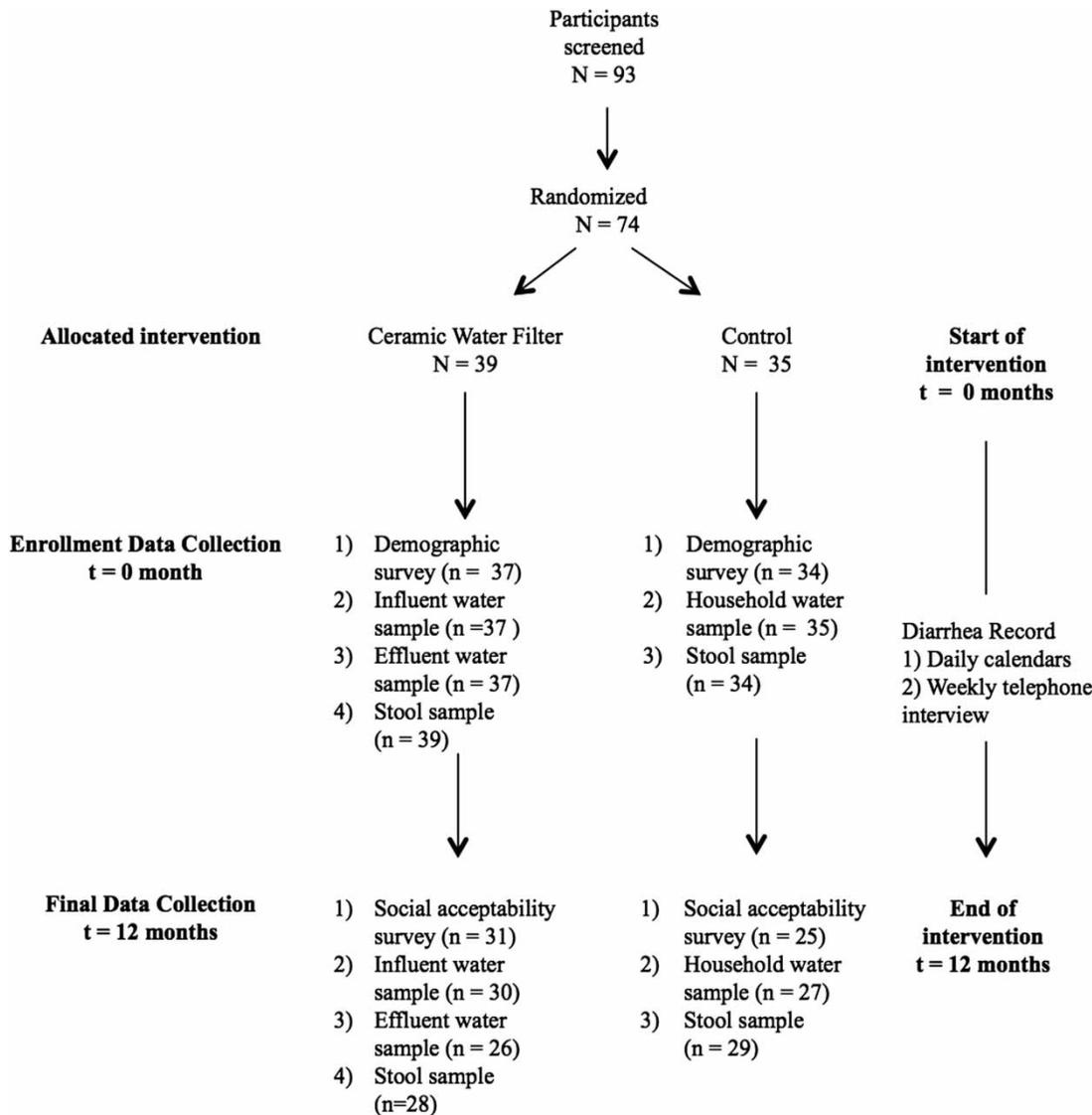
Figure 1 │ Schematic of study design.

deviation of 1.10 L/hr. Replicate laboratory microbial removal tests showed all filters reduced *E. coli* concentration by 3-logs. A subset of six filters was tested for the removal of 10^6 cfu/100 mL and exhibited a 6-log removal of *E. coli*.

Demographic

Seventy-one participants completed a demographic survey at baseline (intervention $N = 37$, control $N = 34$). Table 1 summarizes participant characteristics at baseline. The overall

group median age of participants was 40, ranging from 21 to 64 years of age. Ninety percent of our participants were women who completed at most some secondary school education and earned US$30–130 on a monthly basis. There was no significant difference at baseline between the control and intervention groups based on survey responses.

Water supply and health

Primary water sources were personal taps in the home or community taps from a treated source. Water was primarily

Table 1 | Selected summary of demographic data from study groups at baseline

	Intervention (N = 37)		Control (N = 34)	
	No.	%	No.	%
Personal				
Age, mean (SD[a])	40	(8.96)	41	(10.52)
Sex				
Female	32	86	32	94
Male	5	14	2	6
Family status				
Single	10	27	4	12
Married	9	24	13	38
Divorced/separated	9	24	7	21
Widow/widower	9	24	10	29
Education level				
Some primary	6	17	11	33
Completed primary	0	0	0	0
Some secondary	26	72	19	58
Completed secondary	4	11	3	9
Monthly income[b]				
Less than R250 (USD30)	9	24	15	44
R250–R1,000 (USD30–130)	17	46	11	32
R1,000–R1,500 (USD130–200)	9	24	8	24
R1,500–R3,500 (USD200–450)	1	3	0	0
Health				
Diarrhea in the past month				
Yes	11	30	7	21
No	26	70	27	79
Water supply				
Primary water source[b]				
Personal tap in home	15	41	15	44
Community tap	18	49	15	44
River	2	5	1	3
Storage[a]				
Plastic buckets	22	59	20	50
Plastic bottles	11	30	9	26
Other	2	5	0	0
Current water treatment practice[b]				
Boil water	4	11	2	6
Tablets or liquid chemical	1	3	0	0
Do not treat water	31	84	32	94

[a]SD, standard deviation.
[b]The percentages do not add up to 100%, as some respondents did not answer the question.
Note: R, South African rand; USD, United States dollar.

stored in plastic buckets; however, plastic bottles served as a common secondary storage container. Over 70% of participants in both groups reported their storage container was covered and they used a cup with a handle. Twenty-one percent of the control group and 30% of the intervention group reported diarrhea in the past month and over 80% in both groups reported that they were not treating their water at the commencement of the study.

Hygiene survey

When asked how often participants wash their hands after bathroom use, over 80% in both groups responded 'always'. The remaining participants responded 'sometimes'. Similarly, a significant majority, more than 90% in both groups, reported that they 'always' wash their hands before eating. However, 35% of the intervention group reported 'always' using soap while washing hands, whereas 62% of the control group reported 'always'. Fifty-nine percent of the intervention group did not attribute getting sick to water, while 53% in the control group did. About 50% of participants in both groups declined to answer questions regarding types of sicknesses that can be attributed to water. However, of those who did, the primary answer was diarrhea. The participants were approximately equally divided in regard to whether they believed that their water quality was poor. Results are summarized in Table 2.

Diarrhea recall

The diarrhea rate is defined as the total number of days of reported diarrhea divided by the number of days of observation. Figure 2 shows the diarrhea rates for subjects in the intervention and control groups. The horizontal lines represent the group medians, which are 0.046 for the control group and 0.009 for the intervention group. The means for the control and intervention groups are 0.064 and 0.015, respectively. The two treatment groups have statistically different rates of diarrhea ($p < 0.001$, Mann–Whitney test).

A Poisson regression model was used to compare the intervention and control groups with respect to the diarrhea rate in the follow-up period (Figure 3). The outcome variable was the total number of events, with the total number of observed days used as an offset.

The data were not collected as day-to-day reporting of diarrhea, 'yes' or 'no'. It was collected as a weekly report of number of episodes. In this case, the Poisson model (appropriate for count data) is preferable.

With these models, the estimated effect of the intervention is the ratio of diarrhea rates: intervention to control. The estimated ratio is 0.23, with 95% CI: (0.19, 0.27), $p < 0.0001$. Adjusting for reported diarrhea at baseline, the estimates ratio of rates is 0.212, 95% CI: (0.18, 0.26), $p < 0.0001$. Adjusting for reported diarrhea at baseline, age, and sex, the estimated ratio of rates is 0.213, 95% CI: (0.18, 0.26), $p < 0.0001$. Adjusting for reported diarrhea at baseline, age, sex, number of children in household, the estimated ratio of rates is 0.214, 95% CI: (0.18, 0.26), $p < 0.0001$.

In order to assess the effect of the Poisson assumption on the estimates, we also did these analyses assuming a negative binomial model for the number of diarrhea events. The results are nearly identical with this model, yielding an estimated ratio of rates equal to 0.228, with 95% CI: (0.12, 0.42), $p < 0.0001$.

Water quality

Water samples from 72 households were collected upon enrollment (water was collected from one household that had not completed a demographic survey) and at the final data collection, and summarized in Figure 4. Approximately 80% of households within the control group at enrollment and final collection periods measured coliform bacteria between 10^1 and 10^5 cfu/100 mL. A significant majority of these houses had coliform levels between 10^3 and 10^5 cfu/100 mL at both collection periods. Similarly, over 70% of the influent water samples from the intervention group households had total coliform levels ranging from 10^1 to 10^5 cfu/100 mL, with most falling between 10^3 and 10^5 cfu/100 mL. By contrast, filter effluent water samples from 97% of intervention households during enrollment and 81% of households during the final collection period measured 0 cfu/100 mL. Five untreated samples had too many coliform forming units on the membrane filter to count and are labeled as TNTC (too numerous to count).

A summary of water quality measurements in the intervention group represented in Table 3 shows median influent

Table 2 │ Summary of hygiene survey from study groups at baseline

|	Intervention (*N* = 37)		Control (*N* = 34)	
	No.	%	No.	%
How often do you wash hands after the bathroom?				
Always	33	89	29	85
Sometimes	4	11	5	15
Rarely	0	0	0	0
Never	0	0	0	0
How often do you wash your hands before eating?				
Always	35	95	33	97
Sometimes	2	5	1	3
Rarely	0	0	0	0
Never	0	0	0	0
How often do you wash your hands before cooking?[a]				
Always	20	54	23	68
Sometimes	11	30	8	24
Rarely	2	5	1	3
Never	1	3	1	3
How often do you use soap while washing hands?[a]				
Always	13	35	21	62
Sometimes	21	57	8	24
Rarely	0	0	2	6
Never	2	5	3	9
Do you think you can get sick from water?[a]				
Yes	14	38	18	53
No	22	59	16	47
What kind of sickness can you get from water?[a]				
Fever	2	5	2	6
Stomach ache	1	3	2	6
Vomitting	1	3	0	0
Diarrhea	10	27	12	35
Weight loss	0	0	0	0
Malnutrition	0	0	0	0
Other	1	3	2	6
Do not get sick from water	4	11	1	3

[a]The percentages do not add up to 100%, as some respondents did not answer the question.

coliform levels at baseline were 930 and 416 cfu/100 mL at final collection. Median turbidity levels in influent samples at both collection periods and effluent samples at the final collection period were <5 NTU (Yamamura *et al.* 2000). The median turbidity of effluent samples was 17 NTU at enrollment.

Median total silver in influent samples was less than 2 μg/L at both collection points. Median total silver in effluent samples was 11.7 μg/L at baseline and 1.89 μg/L at final collection. Finally, median percent reduction of total coliform was 100% for both the enrollment and final collection periods.
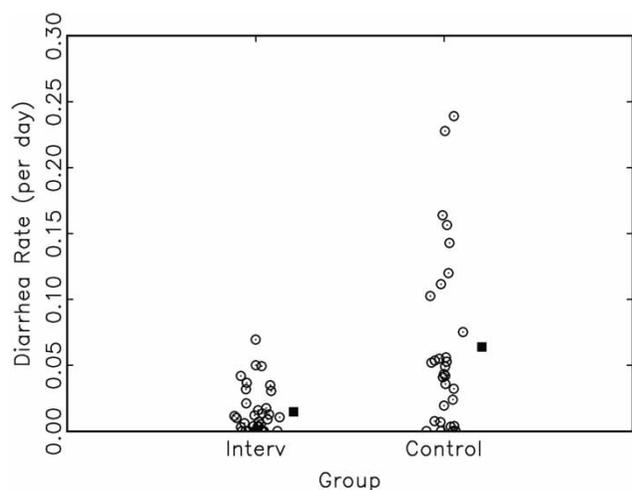
Figure 2 | Plot of individual rates of diarrhea incidents per days (observed number of episodes/total follow-up time) by group. The groups represented in this figure are the intervention and control groups. The solid blocks represent mean values. The horizontal lines mark the group medians (control rate median = 0.046, intervention rate median = 0.009). The means for the control and intervention groups are 0.064 and 0.015 for the control and intervention groups; the error bars represent ±1 standard error of the mean.
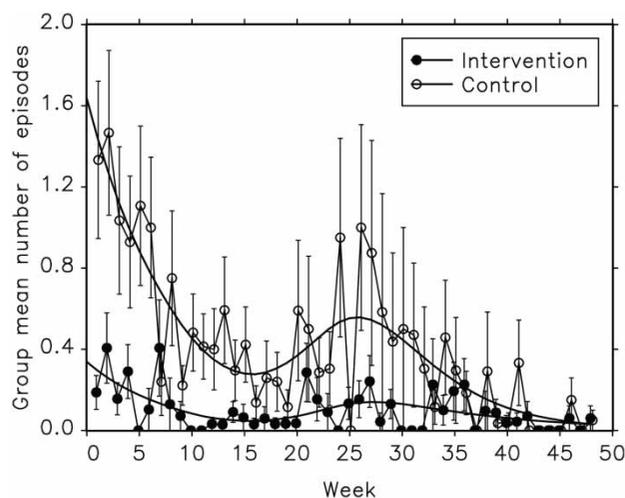
Figure 3 | The plot shows the average number of episodes of diarrhea per week in the intervention and control groups. The error bars are plus or minus 1 standard error. The fitted lines are from a Poisson regression using restricted cubic splines with knots at 4, 8, 16, 24, 32, and 40 weeks.

Cryptosporidia data

Qualitative presence of *Cryptosporidium* sp. was determined using RT-PCR at enrollment and final collection. At the final collection, the prevalence of *Cryptosporidium* sp. was 7% in the intervention group and 22% in the control group ($p = 0.11$, chi-squared test). A 25% point reduction in
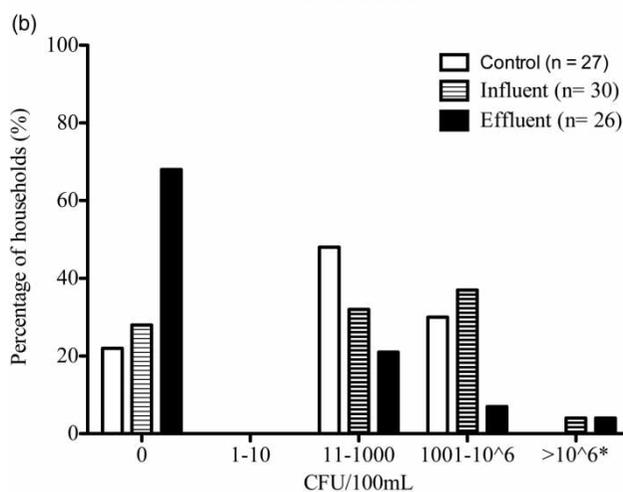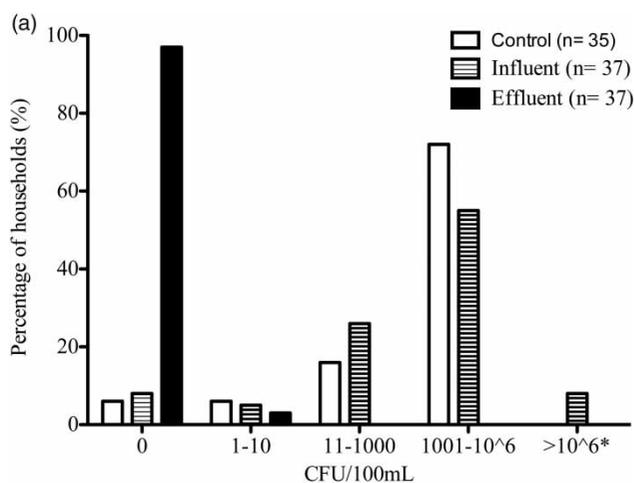
Figure 4 | Summary of water quality data for total coliform bacteria in household water samples at enrollment and final sampling events. Tabulated values are the number of samples within the specified range of coliform bacteria concentrations or the percent of the total number of samples in that range. (a) Enrollment collection and (b) final data collection. *Note:* >10^6* includes TNTC.

prevalence of *Cryptosporidium* sp. between enrollment and baseline in the intervention group was noted ($p = 0.020$, McNemar's test), whereas the control experienced a 4% reduction ($p = 0.74$, McNemar's test).

Social acceptability survey

Results in Table 4 from the social acceptability survey administered at the conclusion of the study indicated 94% of users said the CWF was easy to use. Eighty-one percent of participants indicated they experienced a reduction in diarrhea. A significant majority indicated that they would tell

Table 3 | Summary of water quality measurements in household during enrollment and final data collection

Collection period	Sample type	Median total coliform (cfu/100 mL)	Percent reduction (%)	Median total silver (μg/L)	Median turbidity (NTU)
Enrollment	Influent ($n = 37$)	930	–	1.9	< 1
	Effluent ($n = 37$)	0	100	11.7	17
Final	Influent ($n = 30$)	416	–	1.28	< 1
	Effluent ($n = 26$)	0	100	1.89	0.89

Table 4 | Selected summary of social acceptability survey for participants with CWFs at the conclusion of the study

	No.	%
Had you heard of a water filter like this one before this study?		
Yes	2	6
No	27	87
No response	2	6
Overall, how satisfied are you with the filter?		
Very satisfied	19	61
Satisfied	5	16
Neutral	0	0
Dissatisfied	0	0
Very dissatisfied	0	0
No response	2	6
Is the filter easy to use?		
Yes	29	94
No	0	0
No response	2	6
Did the filter help reduce diarrhea for you?		
Yes	25	81
No	4	13
No response	2	6
If the filter reduced diarrhea, when did you notice it?		
Immediately after using it	16	52
1 week	3	10
2 weeks	1	3
4 weeks	3	10
6 weeks	0	0
Greater than 6 weeks	2	6
No response	6	19
Did you talk to your family, friends, and/or neighbors about this filter?		
Yes	22	71
No	6	19
No response	3	10

(continued)

Table 4 | continued

	No.	%
Does the filtered water taste better or worse than other cleaning methods you have used?		
Much worse	0	0
Worse	0	0
About the same	0	0
Better	2	6
Much better	27	87
No response	2	6
Will you continue to use the filter?		
Yes	29	94
No	0	0
No response	2	6
Did you clean the filter?		
Yes	20	65
No	8	26
No response	3	10
If so, how often did you clean the filter?		
Daily	5	16
Weekly	11	35
Monthly	6	19
No response	9	29

family, friends, and neighbors about the filter. They plan to continue to use the filter beyond the conclusion of the study. Finally, 65% indicated that they cleaned the filters and of those, 16% said their filter was cleaned daily.

DISCUSSION

The results of this pilot study demonstrate household-level CWFs markedly reduce days of diarrhea of HIV-positive individuals. The difference indicates an 80% reduction in diarrhea in the intervention group in comparison to the control group. This is the first time that human health benefits have been reported for nanosilver-impregnated CWFs that were produced using the methods developed by Potters for Peace (Lantagne 2001). Results from this study are especially important since gastrointestinal infections caused by waterborne pathogens are particularly harmful to immunocompromised individuals (Frisby *et al.* 1997; Hoxie

et al. 1997; Dillingham *et al.* 2002, 2009; Dwivedi *et al.* 2007; Kulkarni *et al.* 2009).

Stool analysis of participants in the intervention group revealed a statistically significant decrease in the presence of *Cryptosporidium* sp. between the baseline and final sampling periods in the intervention group. While there is no statistically significant change in the prevalence of *Cryptosporidium* sp. between groups, it is important to note the statistically significant reduction of *Cryptosporidium* sp. within the intervention group. This reduction is notable due to the significant impact of Cryptosporidiosis on immunocompromised individuals (Dillingham *et al.* 2002).

Filters exhibited good technological performance and effectively reduced microbiological contamination. Coliform levels in over 80% of household water samples at baseline in the control group and in the influent water in the intervention group ranged from 10^3 to 10^5 cfu/100 mL. Two (6%) at baseline and six (22%) at final collection of water samples from households in the control group meet WHO guidelines

for drinking-water quality standards of total coliform in water (Yamamura *et al.* 2000). Similarly, four (11%) at baseline and nine (30%) at final collection of influent water samples in intervention households meet coliform level standards. Therefore, according to WHO standards, the majority of the household water samples demonstrated a considerably high-risk level of total coliform.

Coliform bacteria were effectively removed and/or inactivated in households with CWFs. The WHO standard for levels of total coliform bacteria is <1 cfu/100 mL. There was a median reduction of 100% in total coliform levels. This corresponds to a median 2-log removal of total coliform overall. While turbidity levels exceeded WHO standards, median turbidity was within an acceptable range (<5 NTU). The exception was the effluent samples at baseline (Yamamura *et al.* 2000). It is thought that particles were being released from the new filters and did not reflect ineffectual removal of turbidity from influent water. Silver levels did not exceed levels recommended by WHO (Yamamura *et al.* 2000). Silver is known to cause argyria, discoloration of the skin; however, WHO drinking water guidelines indicate that silver levels that do not exceed 0.1 mg/L are concluded safe (Yamamura *et al.* 2000).

Finally, household surveys conducted at the end of the study demonstrated the social acceptance of the CWFs. Overall, filters demonstrated ease of use and users experienced a reduction of incidents of diarrhea. Users also indicated that they clean the filters frequently, even though they were instructed not to. Participants were discouraged from cleaning the inside of the lower reservoir to prevent introducing a potential source of contamination. Therefore, removing the filters from the lower reservoir may lead to its contamination. When the filters were tested, often with cleaning by the research team, the filters removed 100% of total coliform bacteria.

LIMITATIONS

This study had significant attrition as a result of participant dropout over the course of the study, as shown in Figure 1. In some cases, diarrheal records had missing data, despite records being collected through two methods. However, missing data analysis through multiple imputations did not reveal any change in effect size. There was also no true placebo control in this study for our diarrhea data, or an unblinded group, which may bias HWT studies (Schmidt & Cairncross 2009). However, the microbial analysis of stool data provided an unbiased comparison of the ability of the filter to reduce infection of the participant's gastrointestinal tract with *Cryptosporidium* sp.

CONCLUSION

Findings from this pilot study confirm removal of coliform bacteria by the silver-impregnated CWFs and suggest effectiveness of removal or inactivation of *Cryptosporidium* sp. Currently, there is scarce quantitative data on the removal or inactivation of *Cryptosporidium* sp. by CWFs and the antimicrobial effects of silver nanoparticles on this pathogen are unknown (Bielefeldt *et al.* 2010). Future research should address these questions, as well as identifying more cost-effective ways to manufacture the CWFs while still maintaining their effective technological performance. Development of effective business models and marketing techniques will also be important to the sustainable dissemination of this technology through the developing world.

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