Silver-Impregnated Ceramic Water Filters to Improve Water Quality and Health

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Dedication

To my parents, Abeba and Shawel, and my brother, Ermias. I sincerely appreciate your continued prayers, love, and support. Thank you, Dad, for always having a verse of encouragement. Thank you, Mom, for being an unwavering source of love, encouragement, and support. And finally, thank you, Erm, for putting up with my often senseless monologues and coaching me every step of the way. You are my heroes. I know that my journey does not end here. I hope to continue to make you proud and pass forward the compassion for others, love for learning, and humility you have impressed upon me.

Abstract

The World Health Organization (WHO) estimates 884 million people are without improved sources of drinking water. Unsafe water can lead to gastrointestinal infections. The WHO estimates that 94% of diarrheal diseases are preventable and suggests the use of cost-effective means of water disinfection at the household level, or point-of-use (POU). Unlike a number of POU systems, silver-impregnated ceramic-water-filter units provide safe water storage and demonstrate effective microbial disinfection and physical filtration capabilities without residual odor or taste. To date, little is known about the health benefits associated with the use of these filters. Also, while various applications of silver nanoparticles are being explored, studies have been limited to bacterial disinfection and have not extended to other harmful classes of pathogenic microorganisms such as parasitic protozoans. Finally, despite a growth in the number of the filter factories worldwide, there is no documentation on the process of establishing a ceramic water filter factory.

A field study was conducted to evaluate whether a household-level ceramic-water-filter (CWF) intervention can improve drinking water quality and decrease days of diarrhea in human-immunodeficiency-virus-(HIV-)positive individuals in rural South Africa. 74 participants were randomized in an intervention group with CWF and a control group without filters. Participants in the CWF arm received ceramic water filters 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. It was determined that CWFs are a socially acceptable technology that can significantly improve the quality of household water and decrease days of diarrhea for people living with HIV (PLWH) in rural South Africa.

Cryptosporidium parvum is a recalcitrant parasitic protozoa which causes cryptosporidiosis. Cryptosporidiosis disproportionately affects people in developing countries. The significant decrease in *C. parvum* infection among filter users was analyzed by examining the improvement attributable to disinfection versus physical removal. The results herein report the first documentation of the effects of silver salt and nanoparticles on *C. parvum*. Using a murine (mouse) model, we observed silver-nitrate- and proteinate-coated-silver-nanoparticle-treated oocysts resulted in decreased infection relative to untreated oocysts. Microscopy and excystation experiments support the mouse model results. However, while a reduction in infection was achieved, silver did not demonstrate a reduction sufficient for recommendation for use in drinking water treatment alone nor suggest disinfection was the primary mechanism of reduction of *C. parvum.* Subsequently, ceramic disks were produced to investigate the transport of *C. parvum*. Two factors were varied: sawdust size and clay-to-sawdust ratio. All five disk types examined in this study had a removal efficiencies ranging between 80% and 99% and log removal ranged from 0.7 and 2.4. The most effective combination was the 9:1 clay-to-sawdust ratio disk with 16-mesh sawdust, which had the greatest mean removal, 99.21%. These results, in combination with silver disinfection experiments, indicate that physical filtration is the primary removal mechanism of *C. parvum* for silver impregnated ceramic water filters.

Finally, based on the performance of CWFs in the clinic-based study, we decided to establish a factory that would produce filters in Limpopo Province, South Africa. To date, there is little documentation of the process of establishing ceramic water filter factories. The process was divided into three phases: feasibility study, implementation, and training and operation. Herein, we describe our process, lessons learned, and make recommendations for guidelines for establishing future filter factories.

Acknowledgements

I would like to begin with thanking the greatest advisors a graduate student could ever ask for, Jim Smith and Becca Dillingham. I sincerely appreciate your support and encouragement. I am very humbled by your confidence in me and grateful for your mentorship.

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Clinic study

This project was completed through collaboration between University of Virginia (UVA), University of Rhode Island (URI), and University of Venda (Univen). It would not have come to be without the guidance and support of Drs. James A. Smith (UVA), Rebecca Dillingham (UVA), Vinka Oyanedel-Craver (URI), and Samie Amidou (Univen). I would like to express my gratitude towards the Water and Health in Limpopo (WHIL) faculty and administrative staff at UVA, especially April Ballard, and Univen for facilitating international student research.

The research team consisted of students from the US and South Africa. I would personally like to thank Julia Brant (UVA), Sophia Narkeiwizc (URI) from the US, and Alukhethi Singo, Beauty Mashau, Beauty Selima, Caroline Dhavhana, Dr. Paul Mojapelo from the University of Venda in South Africa for their contributions towards the completion of the study.

Above all, I am sincerely grateful to St. Joseph's Clinic for welcoming the team, and I would like to thank the participants of our study for opening their homes.

Laboratory study

Thank you, Dr. Richard Guerrant and the Center for Global Health for welcoming me into your lab. Many thanks to Dr. James Roche, Jones Barbosa, Orleancio Azevedo, Francisco Noronha, Dr. Luther Bartelt, and David Bolick for your kindness and patient instruction in laboratory techniques, experiment design and setup, and analysis during the animal studies.

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Factory case study

I would like to thank the Jefferson Public Citizens Program, Center for Global, and WHIL for coordinating efforts of the undergraduate and graduate students in the Ceramic Water Filter Team. Through your support and guidance, we were able to conduct research in Tshapasha, Tshibvumo, Thohoyandou, and Louis Trichardt. Most importantly, we were able to build partnerships with the Mukondeni Women's Pottery Cooperative to work together to establish a filter factory. Also, to Rachel Schmidt for her contributions to Chapter 5 regarding training and operation. Rachel was a Fulbright Scholar who was in South Africa during the training phase of the study. Her insights were invaluable toward the completion of Chapter 5.

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List of Acronyms

AgNPs	Silver nanoparticle
$AgNO_3$	Silver nitrate
AIDS	Acquired Immunodeficiency Syndrome
ART	Anti-retroviral therapy
C. parvum	Cryptosporidium parvum
CFU	Colony Forming Unit
CWF	Ceramic Water Filter
DI	Deionized
DNA	Deoxyribonucleic acid
E. coli	Escherichia coli
HIV	Human Immunodeficiency Virus
HOCl	Hypochlorous acid
HWT	Household Water Treatment
HWTSS	Household Water Treatment and Safe Storage
HSW	Hygiene, Sanitation, and Water
IACUC	Institutional Animal Care and Use Committee
JMP	Joint Monitoring Program
NGO	Non-governmental organization
NTU	Nephelometric turbidity unit
PCR	Polymerase chain reaction
PFP	Potters for Peace
PLWH	People living with the human immunodeficiency virus
POU	Point of use
PVP	Polyvinylpyrrolidone
T&T	Tshapasha and Tshibvumo
TCFU	Total Colony Forming Unit
WHO	World Health Organization

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Chapter 1

Introduction

Water and Global Health

Global water problem

The 1980s marked the start of global efforts of governmental and international agencies to respond to the growing global disparity in water and sanitation needs (1). This decade-long initiatory period called the International Drinking Water Supply and Sanitation Decade from1981 to 1990, aimed not only to assess the gap, but also implement projects to meet the needs of those without access (2). The objectives proved to be ambitious within the timeframe; however, lessons learned led to understanding the need for proper assessment tools for monitoring, development of appropriate technology, and improvement of water health and hygiene education (3). Most recently, tangible measures were established by the United Nations in the Millennium Development Goals (MGDs) to 'halve the proportion of people who are unable to reach or to afford safe drinking water by 2015" (4).

The Joint Monitoring Program (JMP), in its Progress on Sanitation and Drinking Water 2010 Update, estimates 884 million people are without improved sources of drinking water (5). "Improved sources of drinking water" is defined as access to safe drinking water from improved sources, which consist of house connections, protected springs and wells, and boreholes. However, not all "improved sources guarantee safe water as they may contain turbidity or pathogenic microorganisms".

The most common and widespread waterborne diseases caused by unsafe drinking water are diarrheal diseases caused by gastrointestinal infections. In developing areas, there are 4 billion cases of diarrhea and an estimated 1.8-2.2 million deaths annually, of which the majority are children under 5 (6, 7). Consequently, on a global scale, diarrheal diseases place as the sixth highest burden of disease (7, 8). Multiple studies have found correlation between diarrheal disease/enteric infections and growth stunting and cognitive impairment in children (9-12). In addition to contributing to morbidity and mortality rates globally, diarrheal diseases contribute to loss of workdays, missed school days, and increased health expenses, which ultimately results in adverse impacts to family resources (13).

Point-of-Use Technology

The World Health Organization (WHO) estimates 94% of diarrheal cases are preventable through providing access or improvements to hygiene, sanitation, and water (HSW) at the household level (6). Interventions targeted towards improving access to water, providing clean drinking water at the household level, hand washing, or sanitation have demonstrated evidence of reduction in the relative risk of diarrheal illness (14-17). Fewtrell et al. observed relative risk estimates that ranged between 0.63 and 0.75(14). Additionally, meta-analyses conducted by Clasen et al., and Fewtrell et al. reported drinking water quality improvements yielded the most significant risk reductions (14, 18). Clasen et al. also reported health basis alone, water quality interventions, particularly at the point-of-use (POU), result in the most significant positive outcome (18).

Recent meta-analyses have shown POU to effectively prevent diarrheal disease in all ages, including children under the age of 5 (14, 16). Criteria that have shaped the development of technologies are technical effectiveness, consumer acceptance, and scalability. The following is a list of existing low-cost technologies that meet these criteria: chlorination, solar disinfection, filtration, combined flocculation and disinfection, boiling, and safe water storage (6, 19) (refer to Appendix A.1 for descriptions). Studies have demonstrated ceramic water filters (CWFs) are a recommended type of household water treatment and safe storage (HWTSS) intervention, based on their sustainability, quantity of water treated per unit time, ease of use, and cost (19-21).

Ceramic Water Filters

The primary components of ceramic water filters are clay, sawdust, and water. The mixture is formed into a filter by a press and then fired in a kiln wherein the temperature is gradually increased to a range between 830 to 860 °C over an 8–9-hr period. The high temperature causes sawdust to combust and the clay to harden, thus creating a porous matrix wherein water can percolate through the medium. The filters look similar to clay pots, with flat or rounded bottom designs exposed to the lower reservoir. The rim of the filter protrudes allowing the ceramic pots to be suspended in 5-gallon plastic containers, which in turn serve as safe-storage reservoirs. Spigots are attached to the lower reservoir, where filtered water is accessed after passing through the filter (Figure 1.1). Water can filter through at rates between 1.0-3.0-L/hr and the unit can filter up to 25 liters per day. The price of the filter unit

Figure 1.1 An advertisement for ceramic filters developed by Potters for Peace

costs between 15 and 30 USD. Silver is applied to the filter in one of three different methods: coating the inside of the ceramic after firing, dipping in silver after firing, or including in the mixture before firing. Silver, in the form of colloidal silver, or an aqueous suspension of the silver nanoparticles are applied onto the filters (20). Silver nanoparticles (AgNPs) are effective microbial disinfectants (21-23). The combination of the porous ceramic and silver provide clean drinking water through physical filtration and the deactivation of pathogenic microorganisms (21).

The leading non-governmental organization that has promoted the distribution of ceramic water filters is Potters for Peace (PFP). While PFP was established in 1986, the organization did not get involved in water treatment until Hurricane Mitch devastated Nicaragua in 1998(20). It was then and under the guidance of Ron Rivera, former in-country supervisor, that ceramic water filters were introduced as a means of water treatment and establishing micro-enterprises of ceramicists who produce, assemble, and distribute the units (20). The micro-enterprises are established under the guidance of non-governmental organizations, such as PFP, partner with local community members to train and provide education on the CWF technology. Since then, PFP and other similar organizations have established over 35 filter factories in 18 countries around the world including Cambodia, Cuba, Ghana, Guatemala, Honduras, Indonesia, Kenya, Mexico, Sudan, and Yemen(24).

Multiple prior studies have been conducted on ceramic water filters in the laboratory and field settings. Laboratory studies of bacterial transport demonstrated 97.8 to 100% removal efficiency of microorganisms, and efficiency improved with the application of colloidal-silver (21). Subsequent laboratory experiments have investigated the bacterial treatment efficiency and removal of virus-to-protozoan-sized particles (25, 26). Field studies were conducted in communities in Bolivia, Cambodia, Guatemala, South Africa, and Zimbabwe, wherein the technological performance of filters was assessed (18, 27-29). These studies followed cohorts recruited from communities and varied in length and intervals of household visits. To our knowledge, these studies utilized silver nitrate on the CWFs, with the exception of Kallman et al. (29), and have largely focused on the technical performance and not human health benefits of the filters.

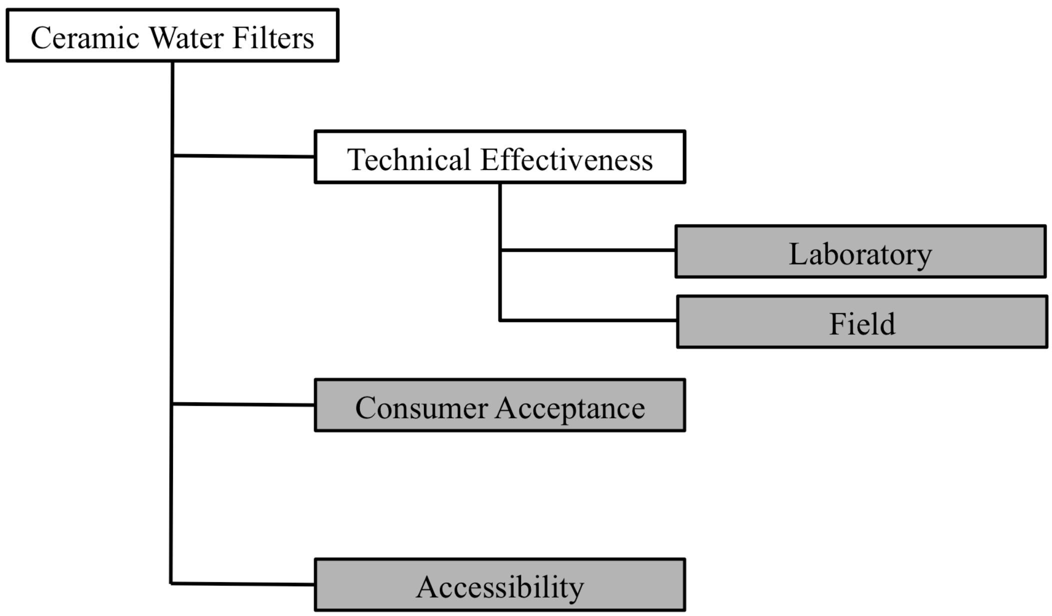


Figure 1.2 Diagram of areas of research on ceramic water filters

Research objectives

Overall, research surrounding ceramic water filters has focused on three major areas: technical effectiveness, consumer acceptance, and accessibility (Figure 1.2). While a popular technology and subject of research, critical knowledge gaps exist in each area. To date, little is known about the health benefits associated with the use of these filters. Also, while various applications of silver nanoparticles are being explored, studies have been limited to bacterial disinfection and have not extended to other harmful classes of pathogenic microorganisms such as parasitic protozoa. Finally, there is a dearth of information on critical factors related to scalability of initiating, constructing, and operating a sustainable filter factory. Therefore, the research objectives of this dissertation project was to address gaps through the following projects: investigating the health impact of ceramic water filters on human immunodeficiency virus (HIV)-positive individuals; analyzing the disinfection capacity and physical removal of *C. parvum*; and documenting the process of establishing a filter factory to increase accessibility of CWFs.

The first research aim was to investigate the health impact of ceramic water filters on HIV-positive individuals in a clinic based randomized controlled trial in Limpopo Province, South Africa. In many developing-world regions, such as Southern Africa, there is a confluence of poor access to water and sanitation, waterborne diseases, and (HIV). In Limpopo Province, in particular, a recent South African National Department of Health Survey reports 13.8% HIV prevalence in the general population and 21.4% HIV prevalence among antenatal women; seroprevalence estimates for rural Limpopo province suggest even higher rates of HIV infection (30, 31). Immuno-compromised individuals, such as people infected with the HIV, are particularly susceptible to infection by waterborne pathogens(31-34). Several studies have recorded the heavy burden of diarrhea morbidity in South African patients living with HIV (31).

The second aim was to determine the capacity of AgNPs to disinfect *Cryptosporidium parvum (C. parvum).* As aforementioned, CWFs provide two mechanisms of water purification: disinfection through silver and physical removal through retention in pore spaces. Thus, a laboratory investigation was conducted to determine the effects of silver on *C. parvum* and removal capacity of CWFs. *C. parvum* is the cause of Cryptosporidiosis, which is one of the leading causes of diarrhea associated with malnutrition in the developing world (35, 36). Its persistence is attributed to the nature of the oocysts, resistance to chlorine disinfection, low infectious dose, and transmission modes (35). As described in Dillingham et al., once ingested the oocysts adhere to the walls of the small intestines (35). Oocysts subsequently invade the epithelium, thus initiating excystation of sporozoites, which are followed by various successive forms of the parasite. Fecal-to-oral route of waterborne transmission is of the most common pathway in drinking water treatment.

The final aim was to document the process of establishing filter factory to serve as a model for future filter factories. Filters have typically been manufactured in factories established by non-governmental organizations, such as PFP, with locally sourced materials, local potters and entrepreneurs. While the PFP model has replicated in numerous factories around the world, there is little documentation offering insights on the process of establishing a factory. Therefore, this study aims to document the process of establishing a ceramic filter factory in order to serve as a model for future ceramic water filter factories.

Summary of objectives:

1. Investigate human health benefits associated with the filters.
2. Determine disinfection effects of silver nanoparticles on Cryptosporidium parvum
3. Investigate removal of Cryptosporidium parvum oocysts by physical filtration
4. Document the process establishing ceramic water filter technology in a resource limited setting

Dissertation Outline

This report is divided into 7 sections. Chapter 1, Introduction, presents the global water problem and the importance point of use water treatment technology, and introduces ceramic water filters. The main points discussed in the introduction are restated and further elaborated on in introductory sections of Chapters 2 through 5. Chapter 2 presents a field study using the ceramic water filters as a form of intervention. The field study was conducted in Limpopo Province, South Africa to determine the impact of a clinic-based filter intervention on the health outcomes of people living with HIV (PLWH). The site was selected due to the prevalence of HIV and rates of waterborne diarrheal diseases. Chapters 3 and 4 present laboratory experiments investigating the capacity of silver-impregnated ceramic water filters to disinfect and physically remove Cryptosporidium parvum. Silver salt and nanoparticles were tested to determine effects on the deactivation of C. parvum. Porous-media was prepared to simulate the transport of *C. parvum* and determine filtration/sorption removal. Chapter 5 presents a case study documenting the process of establishing a filter factory in Limpopo Province, South Africa. The process includes a feasibility study, implementation phase, and training and operation. Finally, Chapter 6 provides a summary of conclusions and recommendation for future research. Appendices follow with original surveys, data from surveys, and results from preliminary experiments.

Chapter 2

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

Introduction

Poor sanitation leads to contamination of drinking water sources by pathogenic microorganisms (22, 37). Enteric pathogens in untreated water are particularly problematic for people living with the Human Immunodeficiency Virus (PLWH) (32-34, 38). Enteric infections may increase mortality even in those who are treated with anti-retroviral therapy (ART) (39). In rural South Africa, there is an unfortunate confluence of AIDS and untreated drinking water (31). In Limpopo Province, the site of the this study, a South African National Department of Health Survey reports 13.8% HIV prevalence in the general population and 21.4% HIV prevalence among antenatal women (30). Seroprevalence estimates for rural Limpopo Province suggest even higher rates of HIV infection (30). 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 (40).

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) 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 (41). 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 can be more cost-effective in the long run compared to centralized water treatment and distribution systems (16). 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 household water treatment (HWT) systems (42).

A large cohort study in HIV-affected households in Uganda demonstrated that a point-of-use 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% (43). 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 using a similar technology and population, diarrhea rates were reduced by 46% among users. This change was significant in the arm that did not receive prophylactic antibiotics (44). 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 (43, 44).

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) (27, 29). 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 (21, 22, 45). Water passing through the filters is purified by the combined effects of physical filtration and chemical disinfection (21). The filters are suspended in 5-gallon plastic containers with spigots, which in turn serve as safe-storage reservoirs. Filtration rates range from 1 to 4 L/hr and can purify up to 30 liters of water per day. The price of the filter unit ranges from 5 – 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 ceramic water filter 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 Cryptosporidia sp., 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 PLWH as a result of introduction of POU water filtration (43). 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 reason for participation in the study, frequency of use and maintenance of the filter, taste and smell of treated water, and cost of 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 headquarters 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 antiretroviral 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 10.

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 (20, 29).

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 ® bags in intervention households. Water samples from households in the control group were collected from their household drinking water source. During the final collection period, some households' water samples had levels of coliform in the effluent that 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 (46). 100 mL 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 (46). The turbidimeter measures the light scattering of a water sample caused by suspended particulates in sample cells of 10 mL through a nephelometric principle (range 0-1100NTU). 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.

*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 Nelseen method. Analysis of Cryptosporidium sp. was repeated with real-time PCR, or RT-PCR, to quantify the number of oocysts present in the sample (40).

Data analysis

Data analyses were performed using SA 9.2, Minitab 16 and GraphPad Prism 5. Diarrhea rate was determined by calculating the proportion of episodes over the number of observation days and compared 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 2.1. 93 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.

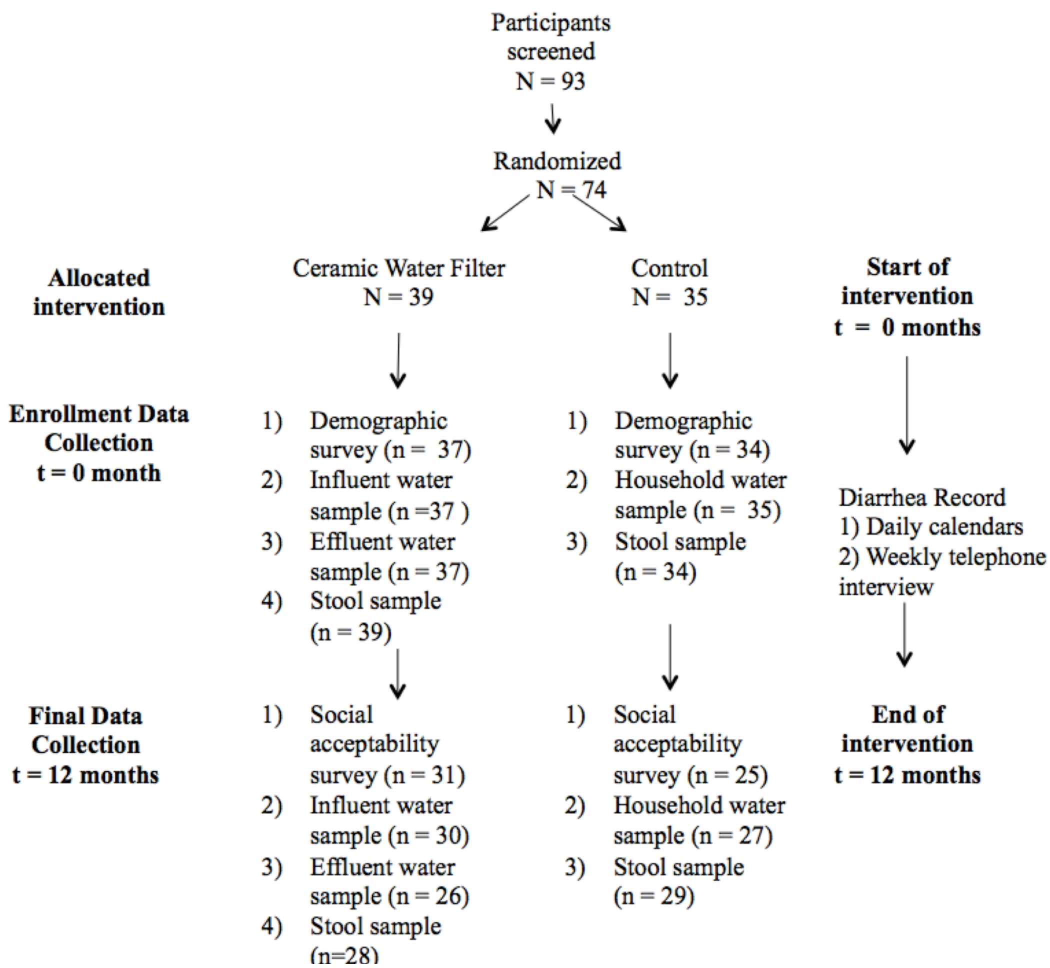


Figure 2.1 Schematic of Study Design

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, *E. coli* removal, and silver release were assessed for each of 80 newly-manufactured filters. The average flow rate of all eighty CWFs was 3.94 L/h with a standard deviation of 1.10 L/h (refer to Appendix B.1). Replicate laboratory microbial removal tests showed all filters reduced *E. coli* concentration by 3 logs (refer to Appendix B.2). A subset of 6 filters was tested for the removal of 10^6 cfu/100 mL and exhibited a 6-log removal of *E. coli*.

Demographic

71 participants completed a demographic survey (refer to Appendix B.3) at baseline (Intervention N= 37, Control N = 34). Table 2.1 summarizes participant characteristics at baseline. The overall group median age of participants was 40, ranging from 21 to 64 years of age. 90% 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.

TABLE 2.1. Selected Summary of Demographic Data from Study Groups at Baseline

	Intervention (N=37)		Control (N=34)	
	No.	%	No.	%
Personal				
Age, mean (SD)	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*				
Less than R250 (USD30)	9	24	15	44
R250-R1000 (USD30-130)	17	46	11	32
R1000-R1500 (USD130 - 200)	9	24	8	24
R1500-R3500 (USD200 - 450)	1	3	0	0
Health				
Diarrhea in the past month				
Yes	11	30	7	21
No	26	70	27	79
Water Supply				
Primay water source*				
Personal tap in home	15	41	15	44
Community tap	18	49	15	44
River	2	5	1	3
Storage*				
Plastic buckets	22	59	20	50
Plastic Bottles	11	30	9	26
Other	2	5	0	0
Current water treatment practice*				
Boil water	4	11	2	6
Tablets or liquid chemical	1	3	0	0
Do not treat water	31	84	32	94

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

Water supply and health

Primary water sources were personal taps in the home or community taps. Water was primarily 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. 21% 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”. 59% 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 have been summarized in Table 2.2.

TABLE 2.2 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?*				
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?*				
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?*				
Yes	14	38	18	53
No	22	59	16	47
What kind of sickness can you get from water?*				
Fever	2	5	2	6
Stomach ache	1	3	2	6
Vomiting	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

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

Diarrhea recall

The diarrhea rate is defined as the total number of days of reported diarrhea divided by the number of days of observation (refer to Appendix B.4). Figure 2.2 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 for the control and intervention groups; the error bars represent ±1 standard error of the mean. The two treatment groups have statistically different rates of diarrhea ($p < 0.001$, Mann-Whitney test).

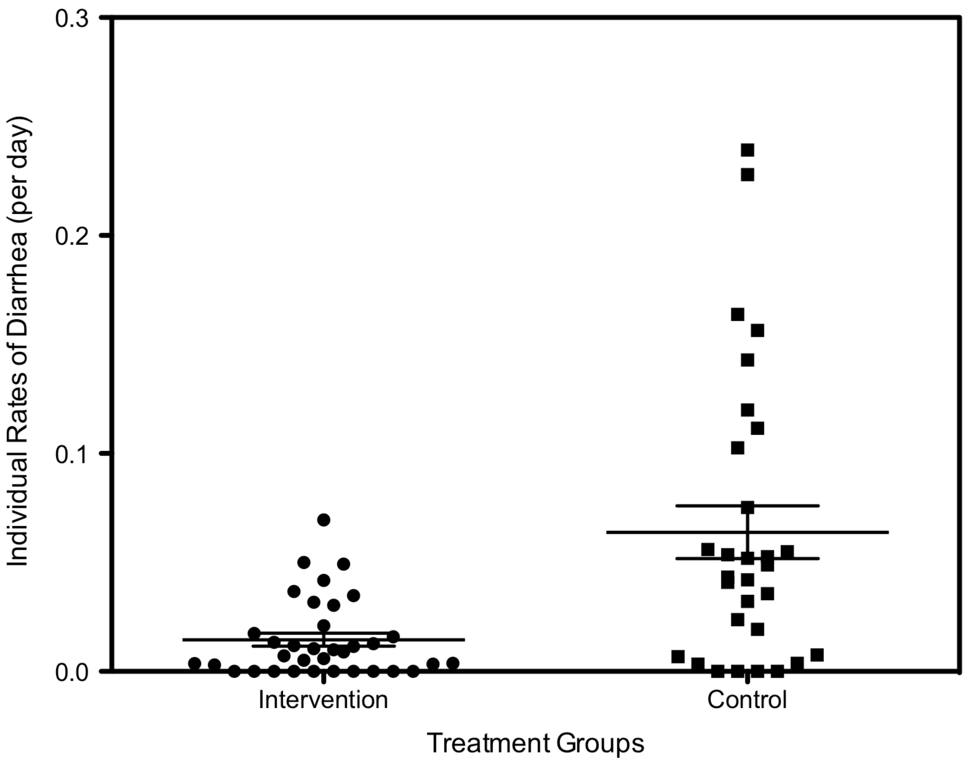


Figure 2.2 Plot of individual rates of diarrhea incidents per days (observed number of episodes / total follow-up time) by group. The groups represented in this figure are the intervention and control groups. The horizontal lines mark the group medians (Control rate median = 0.046, Intervention rate 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.

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 2.3. The outcome variable was the total number of events, with the total number of observed days used as an offset.

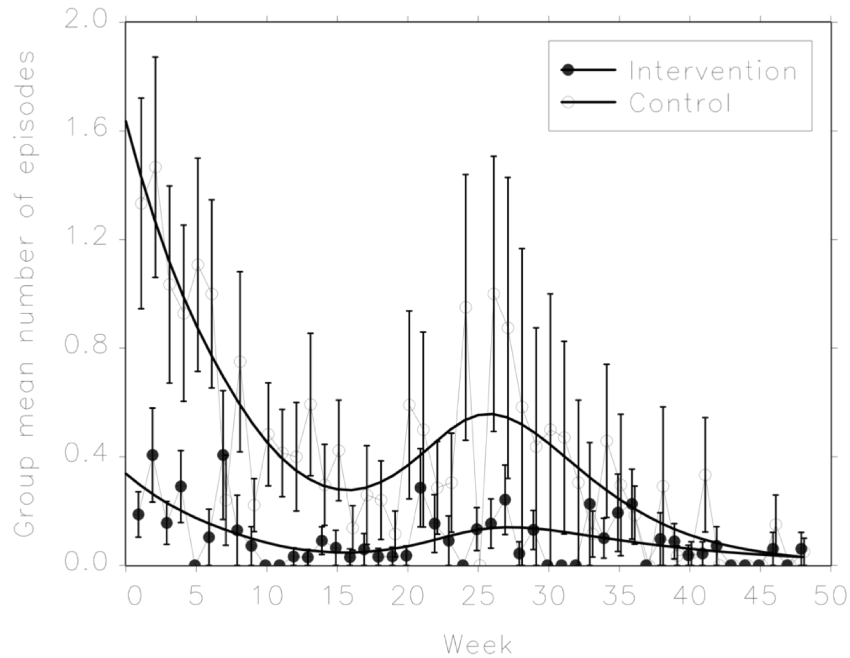


Figure 2.3 The plot shows the average number of episodes of diarrhea per week in the intervention and control groups. The error bars are plus or minus one standard error. The fitted lines are from a Poisson regression using restricted cubic splines with knots at 4,8,16,24,32 and 40 weeks.

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), $p < 0.0001$.

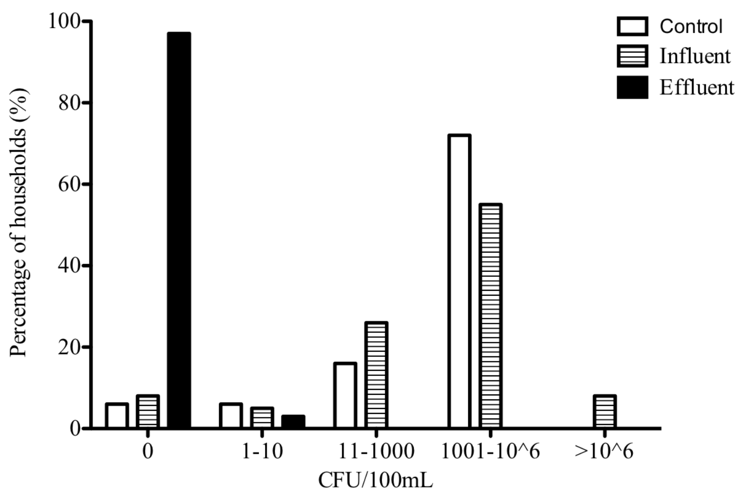
Water quality

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, summarized in Figure 2.4. Approximately 80% of households within the control group at enrollment and final collection periods measured coliform bacteria between $10^1 – 10^5$ cfu/100 mL. A significant majority of these houses had coliform levels between $10^3 – 10^5$ 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 $10^1 – 10^5$ cfu/100 mL, with most falling between $10^3 – 10^5$ 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 TMTC (too many to count).

A summary of water quality measurements in the intervention group represented in Table 2.3 shows median influent 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 below 2 NTU. The median turbidity of effluent samples was 17 NTU at enrollment. Median total silver in influent samples was less than 1μg/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.

A



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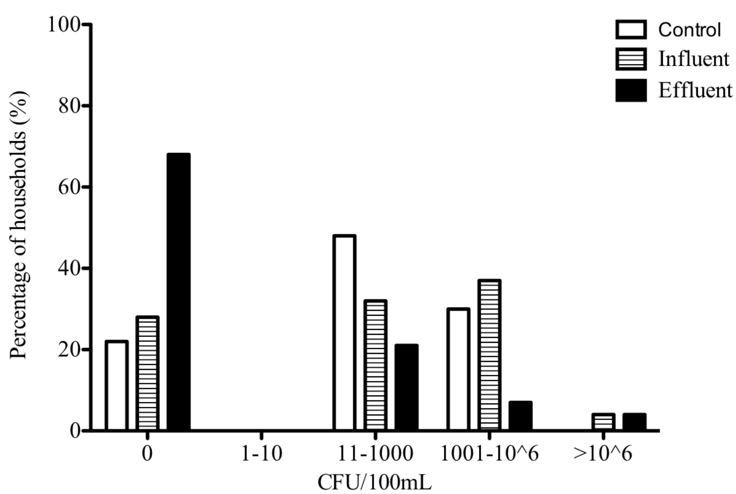

Figure 2.4 Summary of water quality data for total coliform bacteria in household water samples at enrollment and final sampling events. Tabulated values are the number of samples within the specified range of coliform bacteria concentrations or the percent of the total number of samples in that range. A) Enrollment collection and B) Final collection.

TABLE 2.3. Summary of Water Quality Measurements in Household during Enrollment and Final Data Collection

Collection Period	Sample Type	Median Total Coliform (cfu/100mL)	Percent Reduction (%)	Median Total Silver (ug/L)	Median Turbidity (NTU)
Enrollment	Influent	930	-	1.9	0
	Effluent	0	100	11.7	17
Final	Influent	416	-	1.28	0
	Effluent	0	100	1.89	0.89

Stool sample analysis

Presence of Cryptosporidium sp. was determined using RT-PCR at enrollment and final collection, summarized in Table 2.4. 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-percentage point reduction in 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).

TABLE 2.4. Summary of *Cryptosporidium parvum* data at Enrollment and Final Data Stool Collection

Intervention				
		Final Assessment		
		Absent	Present	Total [missing]
Enrollment *C. parvum*	Absent	18	1	19 (68%) [2]
	Present	8	1	9 (32%) [1]
	Total	26	2 (7%)	28 (100%) [3]
Control				
		Final Assessment *C. parvum*		
		Absent	Present	Total [missing]
Enrollment *C. parvum*	Absent	16	4	20 (74%) [3]
	Present	5	2	7 (26%) [3]
	Total	21	6 (22%)	27 (100%) [6]

Social Acceptability Survey

Results in Table 2.5 are from the social acceptability survey administered at the conclusion of the study indicated 94% of users said the CWF was easy to use. 81% of participants indicated they experienced a reduction in diarrhea (refer to Appendix B.5 for Exit Survey). A significant majority indicated that they would tell 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.

TABLE 2.5. Selected Summary of Social Acceptability Survey for Participants with Ceramic Water Filters 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
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

Discussion

The results of this pilot study demonstrate household-level ceramic water filters 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 ceramic water filters that were produced using the methods developed by Potters for Peace. Results from this study are especially important since gastrointestinal infections caused by waterborne pathogens are particularly harmful to immuno-compromised persons (32-35, 38, 39).

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 immuno-compromised individuals (35).

Filters exhibited good technological performance and effectively reduced fecal 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 (47). Similarly, 4 (11%) at baseline and 9 (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 deactivated in households with CWFs. 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 (47). 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(47). 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 (47).

Finally, household surveys conducted at the end of the study demonstrated the social acceptability 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 result in 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 for participant dropout over the course of the study, as shown in Figure 1.1. In some cases, diarrheal records had missing data, despite records being collected though 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 (48). 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 deactivation of Cryptosporidium sp. Presently, there is scarce quantitative data on the removal or deactivation of *Cryptosporidium* sp. by CWFs and the antimicrobial effects of silver nanoparticles on this pathogen are unknown (26). 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.

Chapter 3

Disinfection of *Cryptosporidium parvum* using silver salt and nanoparticles

Introduction

One of the most problematic waterborne diseases is Cryptosporidiosis. It is believed to be responsible for approximately 50% of waterborne diseases worldwide that are attributed to parasites (49). Cases have been reported in six continents in both developed and developing areas (35). Cryptosporidiosis is caused by *Cryptosporidium,* a protozoan parasite that infects the mammalian gastrointestinal epithelium (49). Genetic studies have identified 20 species of *Cryptosporidium*, of which C. *parvum* is attributed to the majority of human infections (35).

The infection is transmitted through the fecal-oral route, typically by consumption of contaminated water. *C. parvum* are shed by an infected mammalian host as thick-walled oocysts that can survive in natural waters for weeks or even months. If consumed in untreated or partially treated drinking water, sporozoites excysting from the oocyst in the small intestine lead to gastrointestinal infection and the production of additional oocysts by the host. Symptoms of infection can include watery diarrhea, nausea, vomiting, abdominal pain, low-grade fever, weight loss, loss of appetite, and general weakness (49). A relatively small numbers of oocysts can result in symptomatic infection of the human gastrointestinal system.

The deactivation and removal of *C. parvum* from water supplies has proven to be a particularly difficult problem, both in the developed world (50) and within communities of the developing world (51). The general guiding principle to the provision of safe drinking water worldwide involves protection of the water source, optimization of the treatment system, and proper maintenance of the distribution and/or storage system (52). In areas where centralized treatment systems exist, water treatment typically involves coagulation-flocculation, clarification, filtration, and disinfection. However, in resource-limited areas, filtration, disinfection, or a combination the two are applied where available (14, 16). In cases where only one is available, filtration is preferable, as filtration technologies have demonstrated the greatest potential to improve drinking water quality and reduce gastrointestinal illness (19). Relative to other waterborne pathogens, oocyst-forming *C. parvum* is resistant to disinfection by hypochlorous acid, chlorine dioxide, and chloramine (53). Therefore, due to the recalcitrant nature of C. *parvum*, its treatment is typically addressed through filtration and pretreatment with coagulation-flocculation to optimize its physical removal (52). Additionally, researchers continue to investigate alternative *C. parvum* treatment methods, particularly for use of developing countries.

Currently, several alternative disinfection technologies have been shown to be effective. Rochelle et al. (54) have shown that an average ultraviolet radiation dose of 7.6 mJ/cm^2 resulted in 99.9% inactivation of *Cryptosporidium sp.* and the irradiated oocysts were unable to regain pre-irradiation levels of infectivity (54). Exposure to 1mg/L ozone has been shown to result in greater than 90% oocyst inactivation (53). *Cryptosporidium sp.* are also susceptible to heat. For example, when water containing *C. parvum* oocysts reach temperatures of 72.4℃ or higher for one minute, infectivity is lost (55).

Silver has been used for centuries to treat burns and treat drinking water (56, 57). Silver salts and nanoparticles (AgNPs), which are a type of nanomaterial, exhibit antimicrobial activity and have widespread applications in various industries, such as textile, dentistry, and medicine. Their antimicrobial capacity has been attributed to their unique chemical and physical properties (23, 58). Early studies in the 1960s and 70s investigated the efficacy of silver salts to treat bacterial infections, specifically burns (59, 60). Since then, numerous studies have followed, documenting the antibacterial behavior of silver compounds or nanoparticles on multiple types of gram-negative bacteria, such as: *E.coli, V. cholera, P. aeruginosa,* and *S. typhus* (23, 61, 62), and gram-positive *S. aureus* (61). In 2010, De Gusseme et al. (62) were the first to report the antiviral effects of silver-based nanotechnology. Additionally, Duran et al. (63) and Gade et al. (61) have demonstrated the use of fungi, *A. niger and F. oxysporum,* to biosynthesize silver nanoparticles to optimize the bactericidal effects. To date, one study has investigated the use of silver as an anti-protozoan agent, however, the study was to determine the effect of silver as a form of *in vivo* therapy against giardia infection (64). Despite the advances in understanding the effectiveness of silver as an anti-microbial agent, little is known about its anti-protozoal capacity.

There are a number of detection methods that are commonly used to detect *C. parvum* (65, 66). The conventional methods for determining oocyst infectivity and viability are vital dyes (67), *in vitro* excystation (68), or *in vivo* studies (65). A comparison of assays was completed by Black et al. to determine viability of *C. parvum* where *in vitro* excystation, vital dyes, and an *in vivo*, or infectivity model, using mice (refereed to as a murine model) were compared(65). The comparison yielded a significant overestimation of viability when using in vitro excystation and vital dyes in comparison to infectivity ($p \leq 0.01$). Their findings suggest the use of methods other than animal infectivity model to determine changes in infectivity result in overestimation and unreliable reporting of viability.

Murine models were developed to mimic Cryptosporidial infections in human beings. These models combine cryptosporidial infection with malnutrition based on trends from longitudinal cohort studies, which have demonstrated short and long term impacts of cryptosporidiosis on growth and development of children (9-12). Studies have shown malnutrition greatly increases the risk for developing severe cryptosporidial infections (69, 70) Thus, murine models were developed with malnourished weaned mice, which have demonstrated severely impaired growth leading to substantially heavier infections (70-72). Therefore, the proposed research is to use a murine model to assess the *in vivo* infectivity in malnourished mice in order to determine the changes in infectivity of silver salt and silver nanoparticle treated oocysts.

The purpose of this study is to investigate the disinfection of *Cryptosporidium parvum* in water by heat, hypochlorous acid (HOCl), proteinate-capped silver nanoparticles (Pro-capped AgNPs), polyvinylpyrrolidone-coated silver nanoparticles (PVP-capped AgNPs), and silver nitrate ($AgNO_3$). Heat treatment is used primarily as point-of-use water treatment in resources limited settings. Hypochlorous acid is used in water treatment as a form of disinfection post-filtration (52). Finally, silver compounds and nanoparticles have a wide array of applications (22, 23, 73). Disinfection effects will be evaluated using a murine model.

Materials and Methods

Murine Model

Animals and Malnutrition. The protocol described herein is in accordance with the Institutional Animal Care and Use Committee (IACUC) policies of the University of Virginia. Weaned 21-day-old female C57BL/6 mice were purchased from Charles River Laboratories, Inc. Upon arrival, mice were acclimated, weighed and distributed in groups. On day 28, of life mice received chow containing 2% protein (Harlan Laboratories, Madison, WI). The animals remained on this diet for 7 days to establish malnutrition before infection and for the rest of the experiment post-infection. Diet and water were given ad libitum. Each mouse was weighed on a daily basis throughout the length of the experiment. Stool samples were obtained through the gentle stroking on the abdomen and collected on a daily basis from the start of the infection until the end of the experiment. The first day of infection is recorded as day 0. The mice were observed for 7 days post-infection.

Preparation of oocysts. *C. parvum* oocysts were purchased from Waterborne, Inc. Oocysts arrived in a stock solution of 1 X 10^9 per 50 ml phosphate buffered saline (PBS) solution. A hemocytometer was used to quantify the number of oocysts for each infection. Preparation of oocysts required washing and suspension in deionized water. Each infected mouse received freshly prepared 1 X 10^7 oocysts in 100 µL of PBS via oral gavage. Mice receiving treated *C. parvum* were administered inoculums from the same freshly prepared batch. Inoculums with no treatment were refrigerated along with inoculums with treatment for the duration of the treatment contact time. All groups were gavaged on the same day.

Preparation of treatments. Deionized (DI) water was used to prepare HOCl and aqueous silver solutions for treatment of *C. parvum* in water. HOCl and silver solutions were prepared at concentration higher than the target final concentration 100 mg/L of the treatments to account for the dilution once added to *C. parvum* in water. Therefore, we doubled the concentration of the treatments and used 100 µL of 200 mg/L. These treatments where then mixed with 100 µL of 1 X 10^7 of *C. parvum* resulting in a final volume of 200 µL and diluting the treatments to the desired 100 mg/L. Proteinate-capped silver was purchased from Laboratorios Argenol. Argenol silver-nanoparticles have a mean diameter of 15 nm in size (74) and arrive as dry solid power with 7.5 % silver content. 200 mg/L of Pro-capped AgNPs were prepared based on the silver content. 1000 mg/L of 10 nm PVP-capped AgNPs were purchased from nanoComposix. The aqueous solution was diluted using DI to prepare 100 µL of 200 mg/L. Finally, 100 µL of 200 mg/L $AgNO_3$ solution was prepared with silver nitrate (crystalline) from Fisher Scientific.

AgNP toxicity. 100 mg/L of Pro-capped and PVP-capped AgNPs were orally fed to mice to determine whether AgNPs have an adverse effect on mice. Each mouse was given 100 µL of 100 mg/L AgNP.

AgNP Characterization. Proteinate capping agent consists of bovine serum albumin, which constitutes of a single polypeptide chain of 583 amino acid residues (74-76) . Proteinate capping agent improves particle stability (74) . Studies have observed PVP sterically stabilizes AgNPs (77, 78)

Preparation of treatments groups. Mice were separated into the following groups: (I) Phosphate-buffered saline (PBS); (II) Heat-treated *C. parvum*; (III) HOCl-treated *C. parvum*; (IV) Pro-capped-AgNP-treated C. *parvum;* (V) PVP-capped-AgNP-treated *C. parvum*; (VI) Silver-nitrate-treated *C. parvum*; and (VII) *C. parvum.* Treatment groups are summarized in Table 3.1.

Group I Mice (n=3) were gavaged with 200 µL of PBS. This group was not infected.

Group II Mice (n=5) were each gavaged with 1 X 10^7 oocysts in 100 µL of DI water treated in 85° heat for 5 minutes.

Group III Mice (n= 5) were gavaged with 1 X 10^7 oocysts treated with 100 mg/L of HOCL. Inoculum was refrigerated for 4 hr before gavaving mice.

Group IV Mice (n=5) were gavaged with 1 X 10^7 oocysts treated with 100 mg/L Pro-capped AgNP. Inoculum was refrigerated for 4 hr prior to gavaging.

Group V Mice (n=5) were gavaged with 1 X 10^7 oocysts treated with 100 mg/L PVP-capped AgNP. Inoculum was refrigerated for 4 hr prior to gavaging.

Group VI Mice (n= 6) were gavaged with 1 X 10^7 oocysts treated with100 mg/L solution of $AgNO_3$. Inoculum was refrigerated for 4 hr prior to gavaging.

Group VII Mice (n=3) were gavaged with 1 X 10^7 oocysts in 100 µL of DI. This group received untreated oocysts.

Table 3.1: Summary of Mouse Model Treatment Groups

Group	Type of Treatment	Final Treatment Concentration (mg/L)	Final Inoculum Volume (µl)	No. of mice
I	No treatment	--	--	3
II	Heat			6
III	HOCL	100	100	6
IV	Pro-capped	100	100	6
V	PVP-capped	100	100	6
VI	$AgNO_3$	100	100	5
VII	No treatment	--	--	3

DNA extraction for parasite detection. Stools collected from the mice were stored at -20°C until extraction. DNA was extracted from stool samples using reagents from Qiagen QIAamp DNA Stool Kit (Qiagen Inc., Germantown, Maryland) and QIAcube, which automates the extraction process. Minor modifications were made to the traditional extraction process for the extraction of DNA from mouse stool. The modifications used as described in Costa et al. (71, 79) .

Quantitative PCR for C. parvum. Detection of *C. parvum* oocysts was performed using an iCycler iQ Multicolor quantitative PCR detection system (BioRad) with the use of known primers. A master mix consisting of 12.5 µL of Bio-Rad iQ SYBR Green Supermix (Bio-Rad

Laboratories, Hercules, California), 5.5 μL DEPC-treated nuclease free sterile water (Fisher Scientific, Pittsburgh, Pennsylvania) and 1.0 μL of forward and reverse primers (Invitrogen, Carlsbad, California) was prepared for qPCR. The primers target the 18s rRNA gene of the parasite (forward: 59-CTGCGAATGGCTCATTATAACA-39; reverse: 59-AGGCCAATACCCTACCG-TCT-39; GenBank no. AF164102). Bio-Rad iCycler multicolor PCR Detection System using iCycler softwater (version 3.0) were used to perform detection of oocysts. The amplification progression used as described by Costa et al. (71, 79) . Threshold cycle (C_t) values were obtained from each run and were transformed into the number of organisms per sample of stool. Finally, results from *C. parvum* detection were expressed in log counts per 10 mg of stool (average weight of stool is 10 mg).

Statistical analyses. Mouse weight was expressed in percent change based on body weight on day 0. Weight and shedding analysis were performed using GraphPad Prism Version 5.0c using Bonferroni post-tests (Two-way ANOVA).

Results

*Murine Model Experiments***.** A murine model was used to investigate the effect of silver salts and nanoparticles on *C. parvum* to determine whether oocysts treated with different disinfection technologies will result in reduced weight decrement and number of parasites shed in stool relative to experiments with untreated oocysts. The results shown in Figure 3.1 are mean ± standard error of the mean (SEM). Weight at day 0 is considered 100% and changes after day 0 reflected increases or decreases relative to day 0. The "infected" group demonstrated a mean loss of 12% body weight by day 3 post-infection, where *C. parvum* infection was at its peak. Mice that consumed heat-treated oocysts maintained the growth pattern of the "uninfected" group and 102.8% (~3% gain) of body weight at day 3 ($P<0.001$, day 3). HOCl and $AgNO_3$ treated oocysts groups exhibited ~97% (3% loss) of their weight, respectively ($p < 0.001$, day 3). Mice administered Pro- and PVP-capped silver nanoparticles did not exhibit severe weight loss, 92 and 93.9% (~8 and 6% loss), respectively.

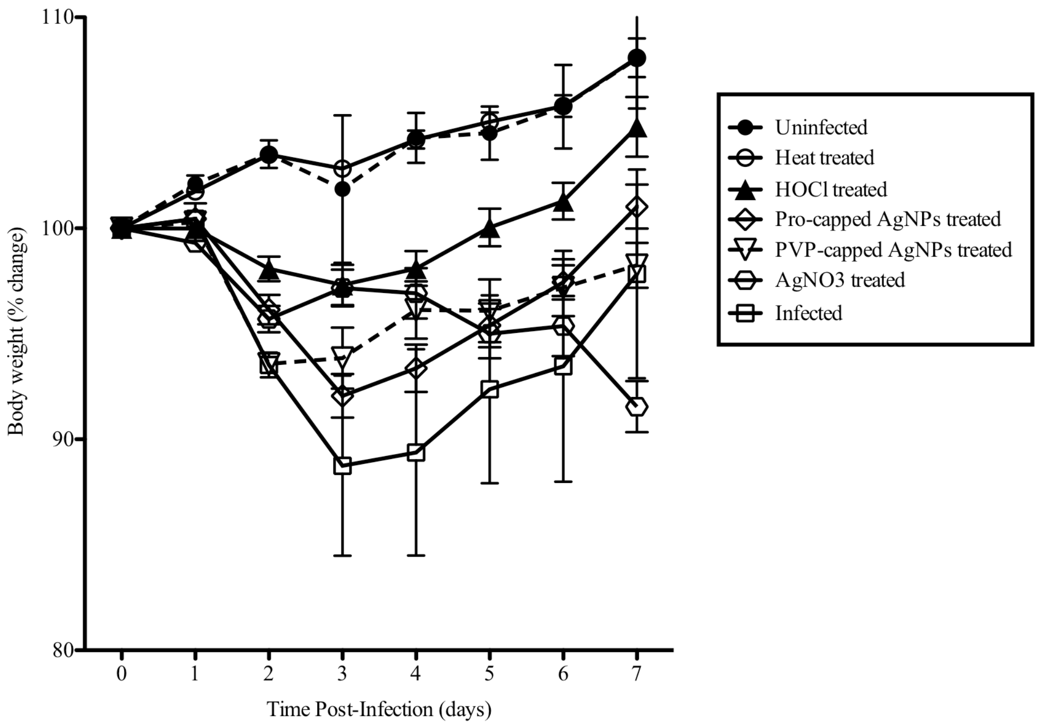

Figure 3.1 Plots of percent body weight change of mice fed *Cryptosporidium parvum* oocysts for a 7-d post-infection period. Each data set represents a different oocyst disinfection method as well as positive and negative controls. Error bars represent standard error for each measurement.

Figure 3.2 represents growth patterns for both types of AgNPs to determine whether AgNPs have an adverse effect on mice weight. The results shown in Figure 3.2 are mean ± standard error of the mean (SEM). . The "uninfected" group demonstrated a mean gain of ~1.8% body weight by day 3 post-infection. Mice that consumed Pro-capped AgNPs and PVP-capped maintained the growth pattern similar to the "uninfected" group. Pro-capped AgNPs maintained

106.8% (~7% gain) of body weight and PVP-capped AgNPs gained 105.5% (~6%) of body weight at day 3.

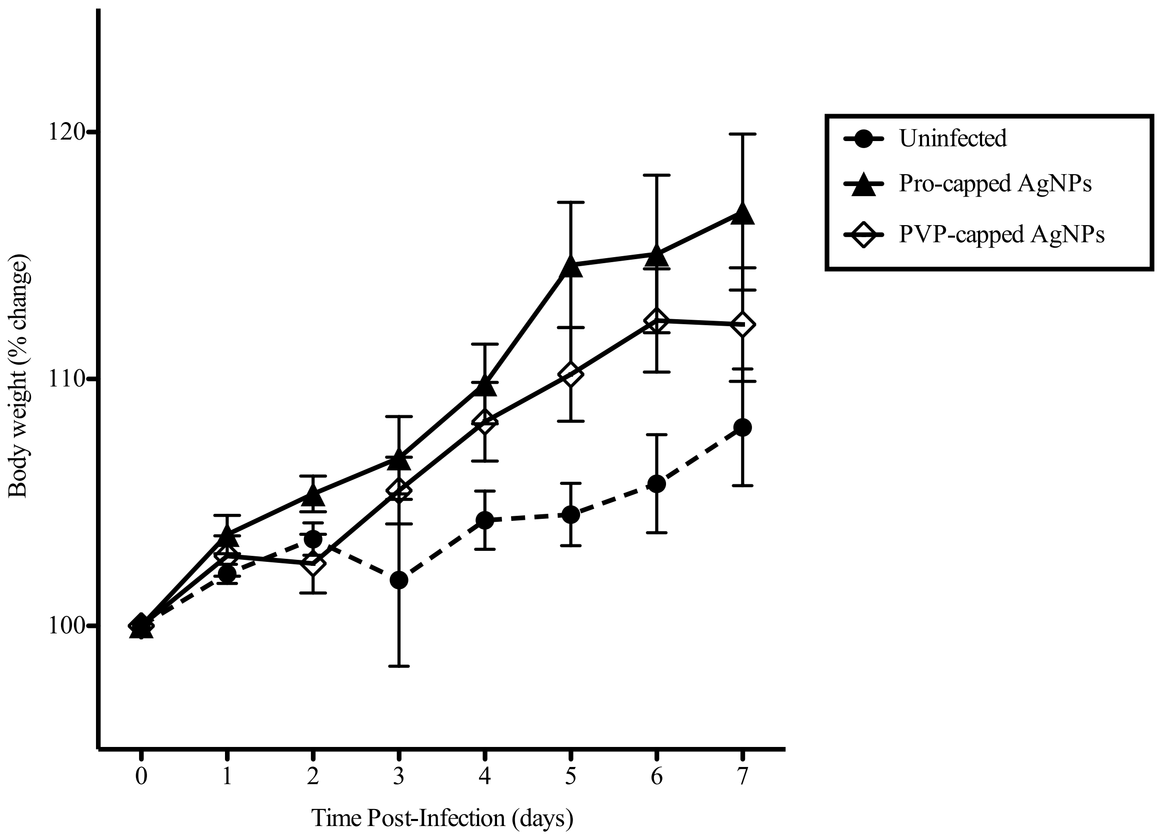

Figure 3.2 Plots of percent body weight change of mice fed Pro-capped and PVP-capped AgNPs to determine whether AgNPs have adverse effects on mice. Each data set represents a different oocyst disinfection method as well as positive and negative controls. Error bars represent standard error for each measurement.

Figure 3.3 gives the oocyst concentration in stool samples collected from the mice for 3d post-infection. These data were collected to determine whether the weight change pattern would be reflected in oocyst concentrations in stool samples. The oocysts stool concentration for mice in the group fed untreated oocysts was $10^{4.6}$ oocysts per 10 mg stool on day 3. Parasites were not detected in stool in stool samples in the heat-treated group. The HOCl-treated group shed $10^{2.1}$

oocysts per 10 mg stool ($P< 0.001$, day 3). The $AgNO_3$-treated group shed $10^{1.9}$ per 10 mg stool ($P< 0.001$, day 3). Among the silver nanoparticle groups, proteinate-capped treated group shed $10^{2.6}$ oocysts and PVP-capped silver nanoparticle group shed $10^{4.4}$ per 10 mg stool. Further, proteinate-capped and PVP-capped AgNPs alone were given to mice to determine toxicity of silver to mice.

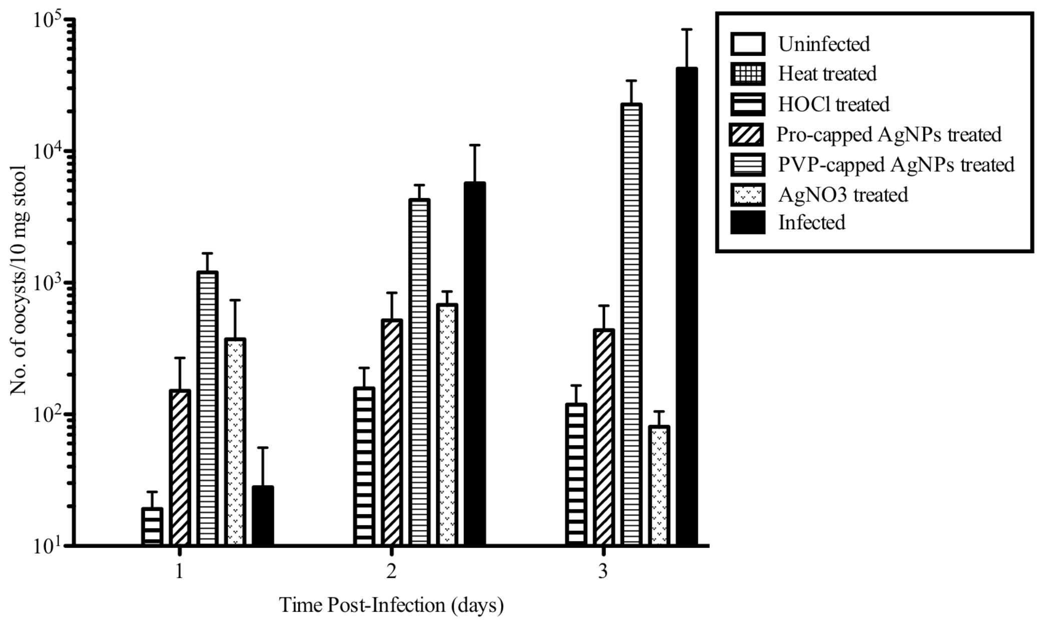

Figure 3.3 *Cryptosporidium parvum* oocyst concentration in stool shed from mice as a function of post-infection time for different oocyst disinfection treatments. Error bars represent standard error for each measurement.

Discussion

To determine the disinfection effects of silver salt and AgNPs on *C. parvum* we examined the response in weight change and oocyst shedding in mice. We observed silver nitrate and proteinate-capped AgNPs demonstrated the greatest effect on treating *C. parvum*.

Our results show that mice that consumed $AgNO_3$-treated oocysts lost 3% of their weight (Figure 3.1) ($p < 0.001$, day 3), which was a statistically less than the 12% weight loss experienced in mice that consumed untreated oocysts. Mice that consumed proteinate-capped- and PVP-capped-AgNP-treated *C. parvum* did not achieve a statistically significant difference in weight loss when compared to untreated, but weight loss was not as pronounced as mice that consumed untreated oocysts (Figure 3.1) (6-8% loss). The oocyst concentration for mice that were fed silver-nitrate-treated- and proteinate-capped-AgNP-treated oocysts were $10^{1.9}$ and $10^{2.6}$, respectively, and significantly less than mice fed untreated oocysts, which was $10^{4.6}$ at peak infection (Figure 3.3) ($P<0.0001$, day3).

The literature on the mechanism of disinfection of silver is only partially understood and attributed to antimicrobial capacity to silver ions (Ag^+) and reactive oxygen species (ROS) (23, 80) . Rai et al. (23) provide summaries of three hypotheses to explain silver nanoparticle toxicity: 1) AgNPs adhere to and accumulate on to the surface of the cell membrane eventually causing degradation and permeability of the structure; 2) AgNPs penetrate the cell wall and cause damage to DNA; 3) the release of Ag^+ increases bactericidal activity. Silver salts release ionic silver rapidly release silvers ions at the binding site (81) . In general, the presence of a capping agent affects the nanoparticle's physiochemical properties and as a result modifies the release of silver ions and ROS (77, 81) . Morones et al. determined AgNPs with sizes ranging from 1 to 10 nm demonstrated the greatest antibacterial effect (22). Additionally, shape and size affects the release of ions and ROS, therefore silver nanoparticles have an advantage over ions alone due to their increased surface area, which allows for greater contact area to the binding site and a slow release of Ag^+ and ROS (23).

While infectivity was decreased with the use of silver nitrate, mice that consumed silver-nitrate-treated oocysts exhibited continued weight loss. The continued weight loss pattern is not common after peak infection and continued weight loss was not exhibited in other groups. One explanation for continued weight loss due to silver nitrate is gastrointestinal colitis induced by direct exposure to nitrate (82, 83) .

Results suggest AgNPs capping agents have differing effects on oocysts as demonstrated in the mouse model and microscopy results. When we compared proteinate-capped AgNPs versus PVP-capped AgNP, we observed mice that consumed PVP-capped-AgNP-treated oocysts behaved similarly to the group that received oocysts treated with proteinate-capped AgNPs in terms of weight reduction (~2% difference). However, oocyst shedding in the group that received PVP-capped AgNPs was similar to mice that consumed untreated oocysts, whereas the group that received oocysts treated with proteinate-capped AgNPs experienced significantly less shedding than ones that received untreated oocysts. In general, the capping on AgNPs affects the physiochemical properties of the nanoparticles and modulates the release of silver ions and ROS(77) . Unfortunately, little is known about how the capping affects the silver ion release rate; to date, most studies have focused on aggregation and dissolution (77, 84-87) . Further, Tejamaya et al. (77) demonstrated surface chemistry, shape and size are also affected by AgNP capping agent.

Further, we examined heat and HOCl treatment on *C. parvum* to compare with silver salt and AgNP results. Heat and HOCl are commonly used water purification methods. Mice that consumed heat-treated oocysts exhibited a weight trend similar to uninfected mice and oocysts were not detected in mouse stool samples. Heat-treated oocysts results are consistent with literature and other non-chemical methods of disinfection such as ozone and ultraviolet light (52, 53). Hypochlorous acid treatment also presented a significant reduction in weight loss and shedding at a concentration of 100 mg/L and 4 hr of exposure time. For treated water in the US, HOCl levels after the initial chlorine demand is met are around 0.2-0.8 mg/L (88) : the concentration of HOCl used exceeded conventional water treatment levels safe for consumption.

This study has several limitations. Silver salts and nanoparticles did result in significantly reducing infectivity of *C. parvum* but did not fully prevent infection. Furthermore, the results reported in mice may not be the same for human populations; however, weight and shedding are a commonly used method to assess effectiveness of treatments and therapeutics.

In summary, silver nitrate prevented significant weight loss at peak infection, but while decreasing infectivity of *C. parvum*, it may have caused adverse health effects in mice. Two types of AgNPs were examined in this study: proteinate-capped and PVP-capped. Proteinate-capped AgNPs outperformed PVP-capped AgNPs, suggesting proteinate is more effective against *C. parvum* than PVP-capped AgNPs. Results from this study have direct implications in commercial water treatment technologies, such as Silverdyne and silver-impregnated ceramic water filters. In the following chapter we will investigate the effects of physical filtration on *C. parvum* removal in ceramic filters. The study will involve investigation of ceramic water filters to determine their effectiveness for the physical removal *C. parvum*.

Chapter 4

Ceramic water filtration as a means of point of use water treatment of *Cryptosporidium parvum*

Introduction

Cryptosporidium is a recalcitrant parasitic protozoan that causes cryptosporidiosis. Cases of cryptosporidiosis have been reported in over 40 countries around the world in both healthy and immunocompromised patients (35, 89) . Although cases of cryptosporidiosis have been reported in industrialized nations, the burden of disease has been largely in developing countries (89) . As summarized in Dillingham et al., over 20 species of *Cryptosporidium* have been identified, however, human infections have been attributed to *Cryptosporidium parvum* (*C. parvum*). The threat to humans from exposure *C. parvum* is attributed to four characteristics: resistance to conventional disinfection; its small size, which ranges from 4 to 6 μm, rendering it elusive to physical filtration; low infectious dose of 1 to 10 oocysts; and its persistence when shed (35). *C. parvum* exposure occurs through the fecal-oral route or through water transmission (35).

Conventional methods of drinking water treatment to address *C. parvum* in industrialized nations involve filtration and, when available, coagulation-flocculation to optimize physical removal (52). However, in resource-limited settings in developing countries, centralized treatment facilities are often scarce and point-of-use (POU) household treatment methods are the primary alternative (6). It is estimated that 94% of all diarrheal cases in those setting are preventable through the use of POU technology (90) .

Ceramic water filters (CWFs) were developed in the 1980s in Guatemala out of growing demand for decentralized, household water treatment systems. Since their creation, Potters for Peace have distributed them all around the world. Potters for Peace is a non-profit organization that has established over 30 filter factories that produced CWF in countries in Africa, Asia, and Central and South America (20, 24). CWFs gained popularity because materials to produce the filters are locally sourced, cost-effective, easy to use and have demonstrated laboratory and field effectiveness (19).

The filters resemble a flowerpot shaped ceramic media. They are porous, and rounded at the base. The ceramic media is suspended in a 5-gallon plastic bucket with a lid. Water is poured into the filter and percolates through into the lower reservoir. Water in this reservoir is then accessed through a spigot attached at the bottom. The ceramic media are either impregnated or coated with silver nanoparticles (AgNPs). Silver nanoparticles have demonstrated antimicrobial properties(22, 23, 80) . As a result of percolation and silver, the water is purified through physical filtration and chemical disinfection (caused by the silver impregnated into the ceramic matrix).

Few studies have evaluated the transport of *C. parvum* through ceramic water filters, one of which used a proxy for *C. parvum* (20, 26). Lantagne (24) conducted *C. parvum* removal experiments using a filter with no duplicates and reported 4.6-log removal (99.997%). The study expected significant reduction of *C. parvum* in effluent as a result of size exclusion due to the pore size of the filters, which ranged between 0.6 and 3 μm. Bielefeldt et al. (26) conducted experiments using microspheres with similar size, surface charge, and density as *C. parvum*, and filters with and without silver. Researchers observed >99.6% removal efficiencies, and found coating with silver did not improve removal of spheres greater than 0.02 μm. The use of microspheres to model oocyst transport is not without debate. Laboratory and field comparisons of microsphere transport have reported microsphere transport either over- or under-predict oocyst transport (91, 92) ; therefore, microspheres should be used with caution.

Two studies used ceramic disks to mimic ceramic water filters in order to investigate the transport of water-borne pathogens (21, 29). Oyanedel-Craver and Smith (21) investigated removal of *E. coli* through ceramic disks with and without silver. They measured removal efficiency ranging from 97.8% and 99.97% using disks without silver, and 100% using disks with silver. Kallman et al. (29) used ceramic disks with varied sawdust percentage and found a positive correlation between sawdust and pore size. Kallman et al. (29) also studied *E. coli* transport through ceramic disks and observed removal efficiencies decreased with increased pore size. The observations from these studies suggest the main mechanism of treatment of bacteria was size exclusion: bacteria were retained in small pores. No further investigations have been conducted to examine the removal efficiency of ceramic filters for POU treatment.

The purpose of this investigation is to conduct transport experiments using *C. parvum* oocysts with filters fabricated using multiple ratios of clay to sawdust and sawdust particle sizes. To investigate this objective, ceramic filter disks made to mimic ceramic filters were produced and tested in flexible-wall permeameters (93) . Transport of a conservative tracer and *C. parvum* oocysts were quantified. The physical properties of the porous media flow were parameterized through tracer breakthrough tests. *C. parvum* oocysts were passed through disks and effluent samples were collected and analyzed for presence of oocysts to investigate the transport of *C. parvum* through disks.

Materials and Methods

Ceramic Disk Synthesis

Ceramic disks were manufactured to mimic transport through ceramic water filters (21). The disks were manufactured using clay, sawdust, and water. The clay was used is from the Mukondeni Pottery Cooperative in Mashamba in Limpopo Province, South Africa. We chose to test clay from Mukondeni because of a newly established filter factory created by the non-profit organization, PureMadi, the University of Virginia, the University of Venda, and Rotary International in the region. As aforementioned, *C. parvum* is problematic in developing countries, particularly where there is a confluence of poor quality water and HIV prevalence. This unfortunate confluence exists in Limpopo and further motivated testing the clay from this region. The sawdust was acquired from a lumber mill in Ruckersville, Virginia.

Table 4.1 summarizes the five clay and sawdust combinations investigated in this study. The clay and sawdust were mixed, and combined with water. The mixture was divided into four equal portions. Each portion was then placed in a 6.5-cm-diameter polyvinylchloride cylindrical mold, and compressed for 1 min at 1000 psi. The compacted mixture yielded a disk that was approximately 1.5 cm thick. The disks were air dried at room temperature for 3 d. The disks were then placed into an electric kiln and were subjected to the following temperature program: from room temperature to 600 C, the temperature increased at a rate of 150 ˚C/h; from 600 to 900 C, the rate increased at 300 ˚C/h. The temperature was held at 900 ˚C for 3 hr.

TABLE 4.1 Combinations of clay vs. sawdust for ceramic disk preparation (formula for 4 disks)

No.	Mesh Size	Clay to Sawdust Ratio	Clay (g)	Sawdust (g)	Water (mL)
1	10	9:1	225	25	75
2		8:1	222.2	27.8	75
3	16	9:1	225	25	75
4		10:1	227.3	22.7	75
5	20	9:1	225	25	75

Tracer and C. parvum transport

Tracer transport was performed once on each ceramic disk. Tritiated water ($[^3H]H_2O$) was used as a conservative tracer that can be applied to solute transport experiments to determine advection and dispersion parameters. The experiments were performed using a flexible-wall permeameter, a high performance liquid chromatography (HPLC) pump, and a three-way stopcock connected to the inflow and a 1 mL syringe. A 0.005 N $CaSO_4$ solution was prepared and used as the inflow solution. The HPLC pump maintained a 0.6 mL/min inflow rate of the PBS solution. The inflow rate was calculated in Oyanedel-Craver and Smith (21) to correspond to a whole filter flow rate of 1.5 L/h.

Each disk was saturated for 12 h with the PBS solution prior to each transport test. After the saturation period, a 1.0 mL syringe was used to inject a pulse of 4.3 μCi $[^3H]H_2O$ into the ceramic disk. 1 mL effluent samples were collected every 5 min over 90 min. The conservative tracer was quantified using a Packard 1900 CA Liquid Scintillation Analyzer.

C. parvum transport experiments were performed in duplicate on each ceramic disk. Each disk was re-fired in the kiln in between the first and second run. *C. parvum* transport experiments were conducted using oocysts purchased from Waterborne, Inc., New Orleans, Louisiana. Oocysts arrived in a stock solution of 1 X 10^9/ 50 mL phosphate buffered saline (PBS). A 25 mL solution of 1 X 10^7 oocysts per 0.6 mL was prepared from the stock solution using PBS. A 0.6 mL pulse of 1 x 10^7 *C. parvum* oocysts was injected into the filter and 1 ml of effluent was collected every 5 min for 90 min.

Quantification of C. parvum

Effluent samples were stored at 4 ˚C until extraction. Extraction from effluents samples was conducted by centrifuging at 5000 rpm for 5 minutes in 2 mL Qiagen sample collection tubes to spin down oocysts. The supernatant was discarded and the remaining sample was resuspended in 400 µL of ASL buffer and heated at 95 ˚C for 5 min before loading into the QiaCube. DNA was extracted using reagents from Qiagen QIAamp DNA Stool Kit (Qiagen Inc., Germantown, Maryland) in the QIAcube, which automates the extraction process. Minor modifications were made to the traditional extraction process for the extraction of DNA. The modifications made to the protocol are described in Costa et al. (71, 79) .

Detection of *C. parvum* oocysts was performed using an iCycler iQ Multicolor quantitative PCR detection system (BioRad) with the use of known primers. A master mix consisting of 12.5 µL of Bio-Rad iQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, California), 5.5 µL DEPC-treated nuclease free sterile water (Fisher Scientific, Pittsburgh, Pennsylvania) and 1.0 µL of forward and reverse primers (Invitrogen, Carlsbad, California) was prepared for qPCR. The primers target the 18s rRNA gene of the parasite (forward: 59-CTGCGAATGGCTCATTATAACA-39; reverse: 59-AGGCCAATACCCTACCG-TCT-39; GenBank no. AF164102). Bio-Rad iCycler multicolor PCR Detection System using iCycler softwater (version 3.0) was used to perform detection of oocysts. The amplification progression used as described by Costa et al. (75, 97). Threshold cycle (C_t) values were obtained from each run and were transformed into the number of organisms in sample.

Transport simulations

Effluent [^{3}H]H_2O water concentrations from the ceramic disks were simulated using the following transient one-dimensional form of the advection-dispersion equation:

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} - v\frac{\partial c}{\partial x}$$

with the following initial and boundary conditions:

$$c(x,0) = 0$$

$$c(0,t) = c_0 \; for \; t < t_0$$

$$c(0,t) = 0 \; for \; t > t_0$$

$$\frac{\partial c(L,t)}{\partial x} = 0$$

wherein c is concentration of [^{3}H]H_2O (counts per minute/ 0.6 mL), t is time (min), t_0 is the tracer or *C. parvum* pulse injection time, D is the dispersion coefficient (cm^2 min^{-1}), x is distance into the ceramic disk, v is the linear velocity (cm min^{-1}), and L is the thickness of the disk. CXTFIT was used to determine v and D for each disk from the [^{3}H]H_2O transport experiments (94) .

Results

Table 4.2 lists the combinations of mesh size and clay-to-sawdust ratios of all five disks. Pore volume is defined as the volume of void space in the ceramic disk. Pore volume for each disk was determined based on the difference in weight of the saturated disk and dry disk and the density of water (1 g/mL). Porosity was determined by dividing pore volume by the total volume of the disk. The pore volume of the disks range between 16.06 and 17.9 mL and porosity ranges between 32.09 and 35% (Table 4.2). Disks manufactured with 10-mesh sawdust had the greatest linear velocity among all mesh sizes. However, the 16-mesh/8:1 clay-to-sawdust ratio disk had the greatest pore volume and porosity, whereas 10-mesh/9:1 clay-to-sawdust ratio disk had the lowest pore volume and porosity.

Table 4.2 Disk properties for ceramic disks using Mukondeni clay

disk no.	1	2	3	4	5
mesh size	10	16	16	16	20
clay to sawdust ratio	9:1	8:1	9:1	10:1	9:1
sawdust percentage (%)	10.00	11.11	10.00	9.09	10.00
pore volume (mL)	16.06	17.9	17.20	16.88	16.66
porosity (%)	32.09	35.00	34.22	33.42	33.84

Tracer and *C.* parvum transport experiments were performed on each disk. Figure 4.1 presents results from tracer experiments. Effluent tracer concentration was normalized to the influent pulse concentration and plotted as a function of pore volumes of flow. This figure presents results from tracer transport experiments for all five disks. Simulated concentrations of $[^3H]H_2O$ agree with experimentally observed data. Over 96% over the conservative tracer was recovered with the exception of the 10-mesh sawdust.

Figure 4.2 represents *C. parvum* experimental data with results. The results are expressed in mean ± standard error mean. The dashed lines in each figure indicate the tracer breakthrough peak for each disk. *C. parvum* peak preceded tracer breakthrough for all disk with the except of 16-mesh/9:1 clay-to-sawdust ratio disk where tracer and *C. parvum* peak occurs at the same time. Large error bars are a result of variability between the first and second run.

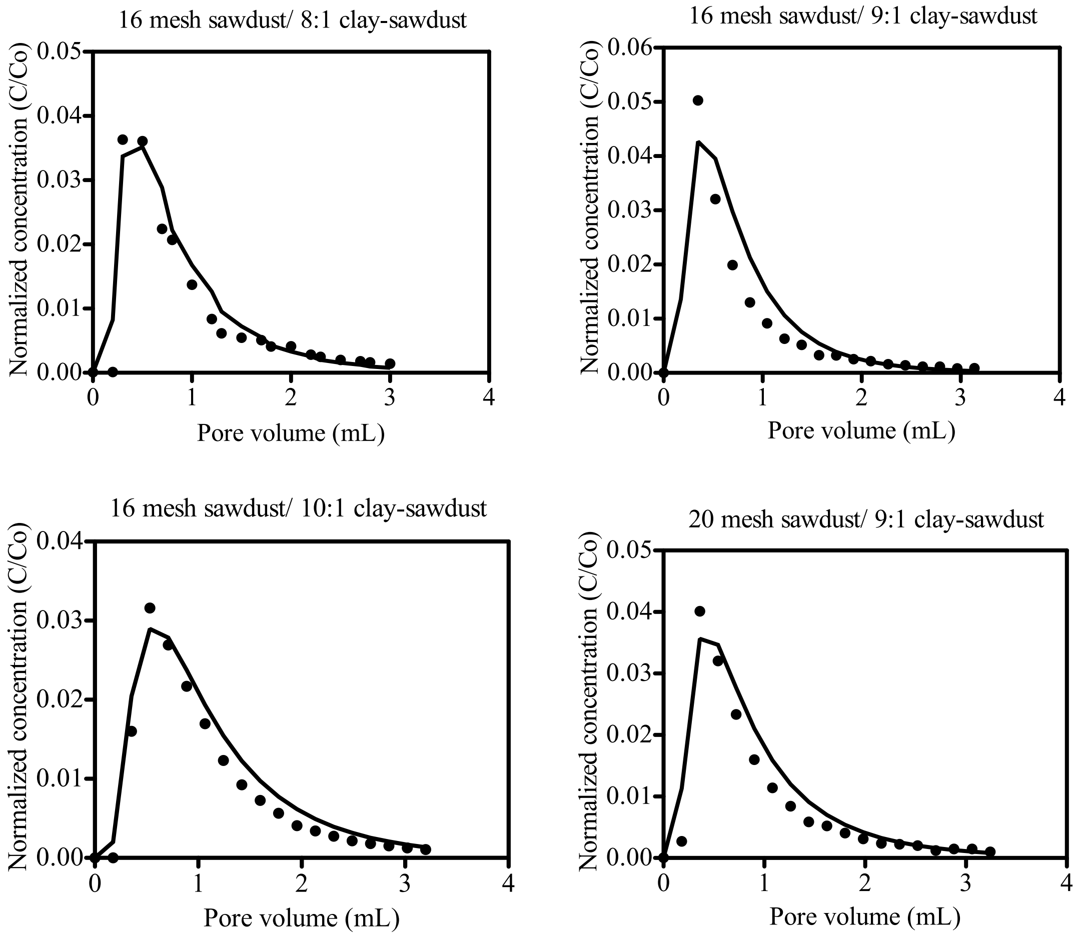

Figure 4.1 Effluent concentrations of $[H^3]H_2O$ normalized by influent pulse concentration as a function of pore volumes of flow for ceramic disks fabricated with Mukondeni clay. Dots represent observed data and solid line represent optimized solute-transport model fits.

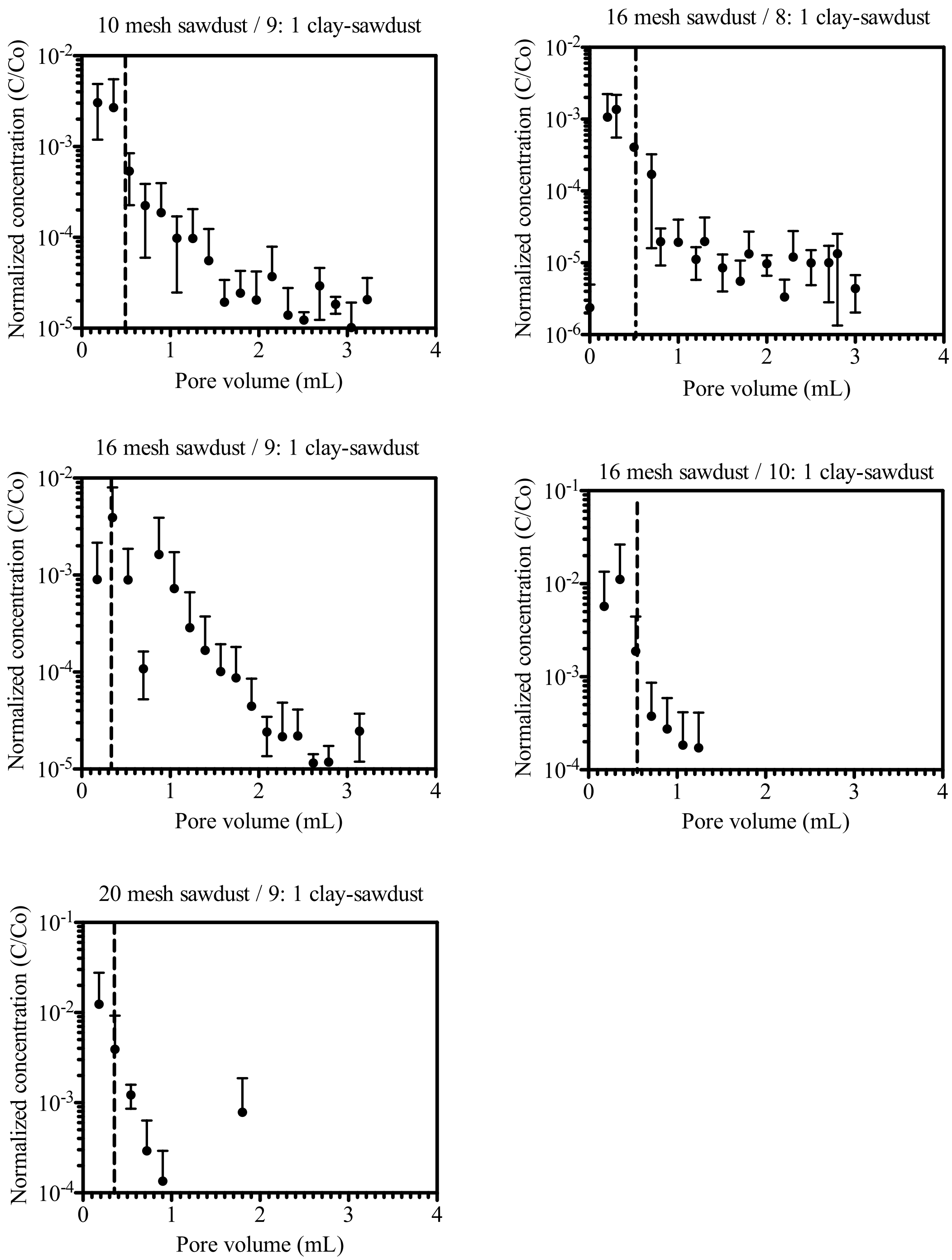

Figure 4.2 Effluent concentrations of *C. parvum* concentrations normalized by influent pulse concentration as a function of pore volumes of flow for ceramic disks fabricated with Mukondeni clay. Results are displayed as mean ± standard error mean. Dashed line indicates the peak breakthrough from tracer experiment.

Table 4.3 presents optimized values of the linear velocity and dispersion coefficient for the five filters based on analysis of the tracer transport experiments. CXTFIT was unable to fit values for the disk made from 10-mesh sawdust and 9:1 clay-to-sawdust ratio. Therefore, the dispersion coefficient and linear velocity are not listed for this disk. Additionally, Table 4.3 shows percent removal, which was determined based on *C. parvum* collected in the effluent and *C. parvum* injected into the disk. Percent removal ranged between approximately 80 to 99.63%. Also, in 3 out of 5 disks, percent removal decreased during the second test (recall disks were fired between *C. parvum* transport tests). The mean percentage removal in the first test across five disks was 98.65% with a standard deviation of 0.77%, whereas the second test was 91.19% with a standard deviation of 9.04%.

Table 4.3 Tracer and *C.parvum* transport properties for ceramic disks using Mukondeni clay

disk no.	1	2	3	4	5
	tracer transport				
linear velocity, v (cm/min)	*	0.0561	0.0676	0.0453	0.0565
coefficient of hydrodynamic dispersion, D (cm2/min)	*	0.0243	0.0264	0.0182	0.0271
	C. parvum transport				
percent removal, (%)					
run 1	98.46	97.64	99.178	99.63	98.34
run 2	94.40	99.19	99.23	80.49	82.65

*not available

The mean log removal of *C. parvum* for all five disks is presented in **Figure 4.3.** Error bars on columns are expressed ± standard error of the mean. The log removal across five disks ranged between 0.7 and 2.4-log with a mean of 1.67-log and a standard deviation of 0.58-log.

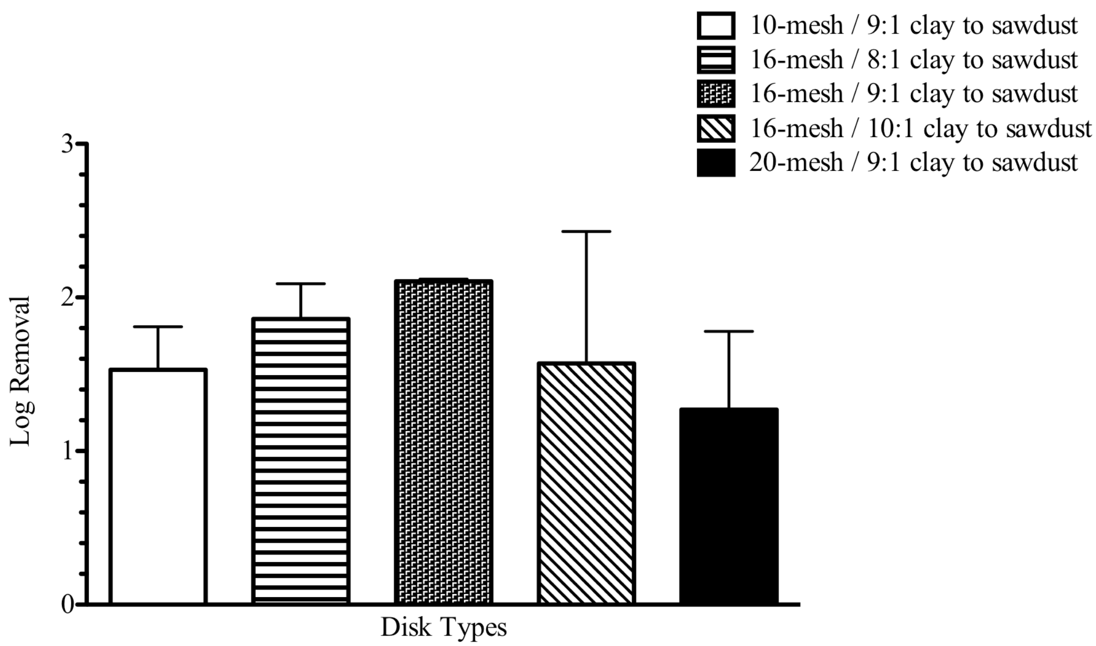

Figure 4.3 Average log removal of *C. parvum* by ceramic disks. The error bar represents the standard error of the mean.

Discussion

C. parvum transport

The experimental data demonstrates ceramic disks physically remove *C. parvum* in water. All five disk types examined in this study had removal efficiencies ranging between 80% and 99% (refer to Table 4.2) and log removal ranged from 0.7 and 2.4 (refer to Figure 4.3). The most effective combination was the 9:1 clay-to-sawdust ratio disk with 16-mesh sawdust, which had the greatest mean removal, 99.21% (refer to Table 4.2**)**. . The log removal across five disks ranged between 0.7 and 2.4, of which 16-mesh 10:1 clay-to-sawdust combination produced the greatest log reduction.

The percentage removal of oocysts using each disk combination was upwards of 94%, with the exception of two data points from the second transport experiment. Findings from a similar study reported a higher removal efficiency using a ceramic water filter which yielded a 4-log reduction (20). In contrast, Bielefeldt reported similar removal efficiencies of approximately 99.6% using protozoan sized microspheres (26).

Experiments were conducted to determine the effect of sawdust size and percentage of sawdust. Five combinations of disks were tested wherein mesh size and clay-to-sawdust ratio were varied to investigate their effects on removal efficiencies. 9:1 clay-sawdust ratio (10% sawdust) disks were prepared using 10, 16, and 20 mesh screened sawdust, and 8:1 (11%) and 10:1 (9%) clay-sawdust ratio disks with 16-mesh screened sawdust. Results indicate there is no correlation between mesh size and sawdust content, and removal efficiencies. In contrast, Kallman et al. (29) examined a wide range of sawdust percentages, which were 4%, 9%, and 17% sawdust, and found pore size and porosity increased with sawdust percentage. Subsequently, Kallman et al. observed 99.997% (4.56 log) reduction with 4% sawdust and 99.97% (2.55 log) with 17% sawdust use *E. coli*: as sawdust percentage was increased and removal efficiency decreased. Results from our experiment and previous studies confirm the mechanism for microbial removal in ceramic media was size exclusion (20, 21, 26, 29).

Mechanism of C. parvum removal

Tracer transport data show relatively small differences between the disk types with the exception of the 10-mesh sawdust with 9:1 clay to sawdust ratio. Our results indicate the *C. parvum* peaks occur before breakthrough (refer Figure 4. 2**)**. Our observations were similar to

Oyanedel-Craver and Smith (21) and Kallman et al. (29). The observations are likely a result of retardation of oocysts transport due to size. Since most of the oocysts are retained in the ceramic media, the oocysts that do exit the filter likely traveled through some of the largest pores that had faster velocities compared to the average velocity. Therefore, these oocysts break through slightly earlier than the tracer. This has been observed in previous studies with smaller sized bacteria (21, 95, 96) . Additional, Oyanedel-Craver and Smith suggest microorganisms encounter pores that do not extend through the thickness of the filter, and therefore cannot pass through. It is clear that substantial oocysts are retained in the pores, but 10-mesh disk and 16 mesh 8:1 and 9:1 clay-to-sawdust ratio disks exhibit tailing behavior. This suggests some oocysts detach and eventually exit the ceramic filter media. Two studies confirm that pore size was the variable which significantly affected oocysts in the effluent samples, however, they disagree on the role of variables such as turbidity and pH (91, 97). Tufenkji et al. (98) observed straining played a role in low ionic-strength conditions, and suggested a combination of straining and physiochemical filtration occurs.

AgNPs on Ceramic Filters

As mentioned, findings from this study confirm that the primary removal mechanism is a result of size exclusion wherein oocysts get attached or retained in pores. However, questions still remain as to whether the presence of AgNPs in ceramic filters enhances the treatment of *C. parvum* in water. One hypothesis is that the presence of silver in pore spaces will result in prolonged contact with the oocysts that have either attached or are trapped in pores. This could result in improve oocyst disinfection. This hypothesis has been examined by several studies with *E.coli* and microorganism-sized microspheres. Bielefeldt et al. (26) observed silver increased removal with virus-sized microspheres, but no difference was observed for *C. parvum*-sized microspheres, which are larger. Oyanedel-Craver and Smith (21) observed the use of AgNPs significantly improved *E. coli* removal, wherein no bacteria were detected in effluent samples, resulting in100% removal.

Limitations

There are limitations in this study regarding over predicting oocysts present in effluent water. The detection limit for quantitative PCR for *C. parvum* was 100 oocysts. Therefore, measurements 100 and under were below the detection limit. However, for this analysis, all oocysts were included in the calculation of percent removal and log reduction, which may result in over-estimation oocysts.

Conclusion

The results herein demonstrate the use of ceramic water filters results in significant removal of *C. parvum* in water. WHO health-based water-technology recommendations for household water treatment technology suggest protozoal log reductions of 2 are protective, and 4 are highly protective (99) . Therefore, based on their recommendations, our results demonstrate of the ratios and sawdust used, ceramic filters manufactured using a mesh size of 16 with a 9:1 clay-to-sawdust ratio yields the most favorable and consistent reduction. Future work should investigate pore-size distribution to determine the relation between mesh sizes and pore size to determine implications on *C. parvum* transport.

Chapter 5

Case Study of a Ceramic Water Filter Factory in Limpopo Province, South Africa

Background

Water and health

Resource limited communities in the developing world are disproportionately affected by poor water quality, which leads to gastrointestinal infection (6, 100) . Globally, there are an estimated four billion cases of diarrhea each year (6). Diarrheal diseases cause nearly two million deaths annually, of which the majority are children under age five (6). As a result of high diarrheal morbidity and mortality rates, diarrheal diseases place as the sixth highest global burden of disease (7, 8). Finally, diarrheal diseases often contribute to lost workdays, missed school days, and increased health expenses, which ultimately result in adverse impacts to family resources (13). The World Health Organization (WHO) estimates 94% of diarrheal diseases are preventable through providing safe water, adequate sanitation and hygiene (6).

Meta-analyses have been conducted to compare the relative effectiveness to reduce gastrointestinal infection through improvements in access to water, drinking water quality, hand washing, and sanitation (14-17). Findings indicated interventions significantly reduce infection, with relative risk estimates that ranged between 0.63 and 0.75 (14). Interventions exhibited a similar degree of effectiveness, with the exception of drinking water improvements, specifically point-of-use (POU), which yielded the greatest reductions in diarrheal diseases (14). Furthermore, research suggests POU treatment significantly reduces microbial contamination and diarrhea, continue to be the most effective among water, sanitation, and hygiene improvements, remain affordable, and have proven to socially acceptable (6, 15-17).

POU technologies have become increasingly recognized and incorporated into strategies to reduce gastrointestinal infection in rural and resource-limited settings. Ceramic water filters (CWFs) are a type of POU water purification technology that has demonstrated laboratory and field effectiveness. Laboratory testing has determined CWFs could remove and disinfect bacteria, retain particles the size of viruses and protozoan, and have a lifespan of up to 5 years (20, 21, 25, 26, 29, 101). Additionally, household interventions in Bolivia, Cambodia, Guatemala, South Africa, and Zimbabwe, demonstrated technical effectiveness, reduced rates of diarrhea, affordability, and social acceptability (18, 27-29).

Ceramic water filters

History of CWFs

Ceramic filtration was originally developed in Guatemala through a study commissioned by the World Bank Group in 1980 (20, 24). The original intention was for the technology to be produced by local artisans from locally sourced material to improve water quality and as a means of gaining income for locals producing filters (20, 24). The most common form of ceramic filtration was a ceramic pot with a flat-bottom, typically referred to as CWFs; other forms include candle and disk filters (24). In 1999, Potters for Peace (PFP), a non-profit organization, developed a streamlined manufacturing process as a response to Hurricane Mitch in Nicaragua to provide safe drinking water (20, 24). Their process transformed earlier methods of producing filters, which were handmade or involved a potter's wheel, and mechanized the process in order to create reproducible and good quality CWFs (20, 24). Their model is been to establish micro-enterprises or factories through partnerships with local NGOs and artisans to manufacture and distribute filters. PFP has since replicated their process in over 30 factories across Africa, Asia, the Caribbean, and Central and South America (24).

Manufacturing process

CWFs are made of clay, water, and combustible material, such as sawdust, rice husk, or other forms of agricultural waste. The filter is produced through a series of steps that involve a sieve, hammer mill, mixer, press, and kiln. The sieve is used to refine sawdust down to the desired size and the hammer mill grinds the clay into fine particles. Once the clay and sawdust have been prepared, they are weighed and placed into an electric mixer with water. The mixture is portioned out to press into individual molds, which produces one filter per mold. Finally, the kiln is used to fire the filters at a gradually increasing temperature to 900°C. When fired, the combustible material burns out creating micrometer-size pore spaces within the ceramic structure, allowing water to percolate through.

An additional component in the production of CWFs is silver, which is added either during mixing wet and dry components, or is painted after firing. Silver is a critical component to the water purification process due to its antimicrobial properties demonstrated in prior studies (21, 23, 63, 102) . The combination of the porous structure in the ceramic media and the silver nanoparticles results in two methods of purification: physical removal of microbial contamination and turbid particles, and chemical disinfection as water passes through the filter and comes in contact with silver within the pore spaces.

The ceramic media rests on the inner rim of a five-gallon plastic bucket or a ceramic receptacle (Figure 5.1). The schematic in Figure 5.1 depicts a round-bottom filter. Water is poured into the filter media, percolates through, and rests in the lower reservoir. A spigot is attached at the bottom of the bucket, which allows access to the treated water and prevents potential recontamination. Typically, ceramic water filters purify water at a rate of 1.5 to 3 L/hour, and can filter up to 25 L/day depending on frequency of use.

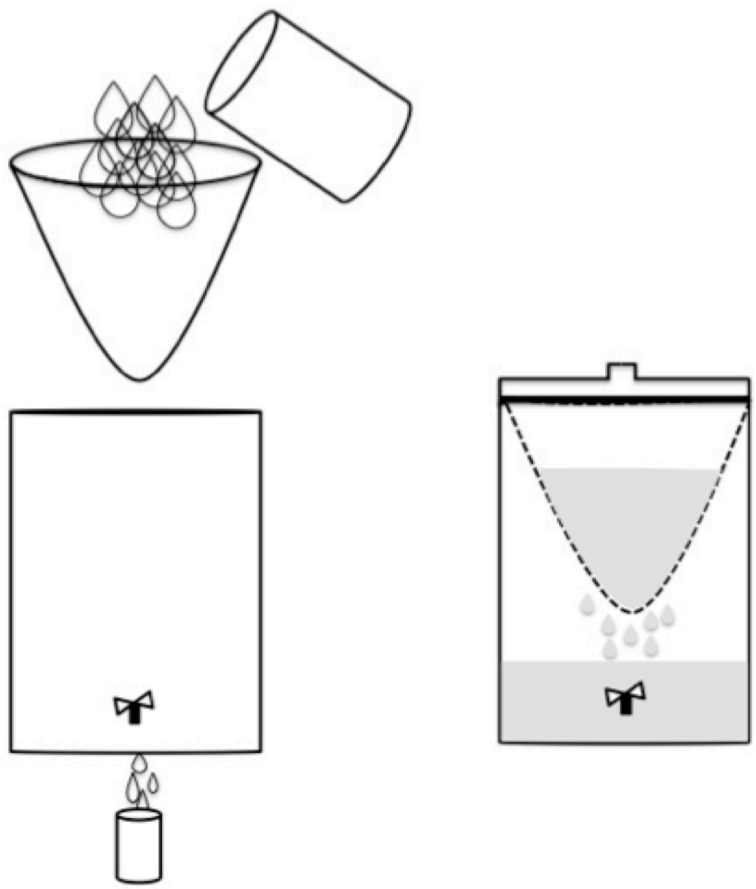

Figure 5.1: Schematic of ceramic water filter. (Left) Filtration process; (Right) Filter with safe water storage unit. (Image provided by Lydia Abebe)

Currently

According to a study in 2009, there were over 30 filter factories around the world, of which 25 reported production rates of 45 to 4480 filters per month, an average of 1500 filters per month (24). The study compared production procedures in order to standardize the production process and regulate quality control (24). While there is documentation of manufacturing processes and efforts to standardize production, there is little documentation offering insights on the process of establishing a ceramic filter factory.

Motivation

The motivation for this case study is to document the process of establishing a ceramic water filter factory in order to serve as a model for future factories. The study consists of three phases: 1) a feasibility study, 2) implementation, and 3) training and operation. The case study was conducted in the Venda region of Limpopo Province, South Africa. The research team involved students and faculty from the University of Virginia, in the United States, and University of Venda, in South Africa.

Structure of the report

The feasibility study section presents an assessment that was conducted to determine the need for CWFs in the study area, identify local sources for raw materials, and search for local partners in the community (for example, artisans, NGOs, local government, and etc.). The implementation portion describes the partnership with the artisans who were identified and the process of expanding their existing business. Also, we finalized raw material suppliers, began construction, installed a borehole, wired electricity, and implemented production equipment. We investigated marketing strategies and identified distribution channels based on marketing surveys and local partnerships. In the training and operations section, we describe our process of launching training, running operations, initiating marketing and distribution, creating an extension to an existing business, and obstacles we encountered. Finally, we conclude with lessons learned and recommendations for future steps for the factory.

Study Site

The study site was located in the Vhembe District of Limpopo Province, South Africa and is commonly known as Venda (Figure 5.2). The population of the Venda region is approximately 1.2 million people (40). Venda was selected for this case study due to prior studies conducted by researchers at the University of Venda that found evidence of microbial contamination in stored drinking water, weaning food, and a high prevalence of HIV (40, 103) . The confluence of poor water quality and HIV leads immuno-compromised individuals to be susceptible to chronic and prolonged diarrheal infections, lower CD4 counts, and death (31, 33, 34, 38). In addition to prior studies, the investigation in Chapter 2 of this dissertation demonstrated a significant reduction in diarrhea and a significant decrease in *Cryptosporidium* in HIV-positive individuals in Venda using CWFs leading to the desire to establish a local factory.

Figure 5.2 Map of South Africa. Limpopo Province (A). (Image provided via Google Maps)

Feasibility study

The purpose of the feasibility study was to assess the need for improved water quality and ceramic filters in the region, and determine availability of resources for starting a filter factory. The first objective was to establish need, which was determined through surveys regarding the existing relationship between the local population and water purification, and by collecting and testing water samples from households within the representative communities. We searched for potential partnerships with local potters, entrepreneurs, clinics, non-governmental organizations, government agencies, and businesses. Finally, we identified sources to supply the raw materials required for production.

Part of the feasibility study was conducted in the villages of Tshapasha and Tshibvumo (T&T), approximately 30 km from Thohoyandou (refer to Figure 5.3). Thohoyandou is the headquarters of the Vhembe district. These communities were identified through a larger international collaboration between the University of Virginia and University of Venda called Water and Health in Limpopo (WHIL).

Figure 5.3 Map of Tshapasha and Tshibvumo. Households that participated in the feasibility study were located in the two adjacent villages. (Images provided via Google Maps)

Water Quality

The first element of our feasibility study was to determine if water quality was substandard and potentially a threat to human health in these two rural communities. To address this issue, we collected 100 mL water samples from storage containers from 205 households in Tshapasha and Tshibvumo. The samples were collected in sterilized Whirlpak ® bags. Total coliform (TC) and *Escherichia coli* (*E. coli*) were enumerated through membrane filtration in accordance with Standard Method 9222 (46). TC and *E. coli* are commonly used bacterial indicators for fecal contamination of water (104, 105) . The water samples were passed through 0.45 μm membrane filters. The filters were subsequently placed in a culture dish with m-Coliblue24 or Eosin Methylene Blue (EMB) and incubated for 24 hr at 35˚C. Results were reported in number of *E. coli* per 100 mL or total colony-forming units (TCFU) per 100 mL. Some samples had colonies that were too numerous and were considered too many to count (TMTC).

The water quality results are summarized in Figure 5.4. We grouped the *E. coli* and TCFU results into the WHO risk categories developed for *E. coli.* Based on WHO categorization standards, 68% of the household water samples tested were zero risk in terms of *E. coli*/100 mL, however, 5% of households were considered “low” risk, 13% “medium” risk, and 15% “high” risk. Although these WHO risk categories do not apply to TCFU, TCFU results were also grouped into the same risk categories. It was determined that only 4% of households were zero risk, and TCFU was detected in approximately 96%, of which 76% were in the “high” risk and TMTC

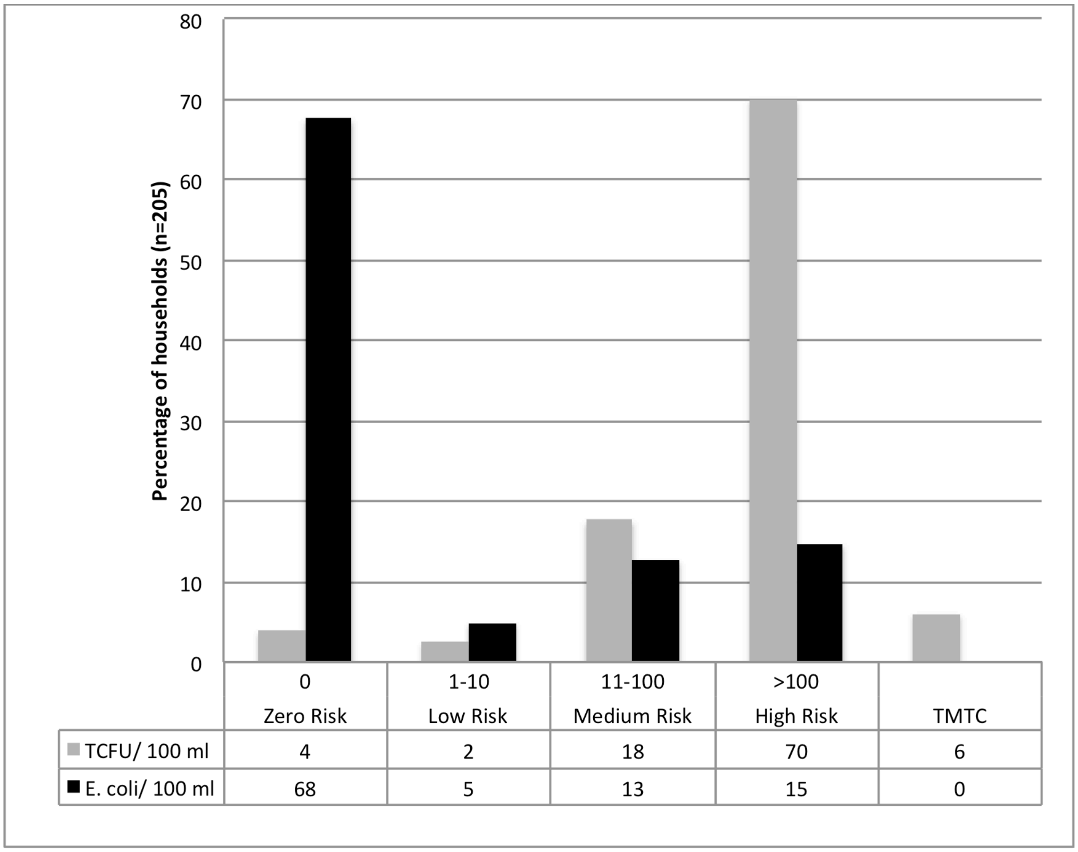

Figure 5.4 Percentage of households (n=205) in Tshapasha and Tshibvumo with TCFU and *E. coli* (per 100 mL) concentrations for household water samples collected according to World Health Organization Risk Categories. Percentages do not sum, not all households provided samples for testing.

Table 5.1 Summary of water, health, and hygiene practices		
Water Practices and Storage		
Primary source of drinking water		
	Piped into yard/plot	64%
	Public tap/standpipe	11%
Type of frinking water storage container		
	Plastic buckets	20%
	Jerrycan	44%
Are the storage containers covered?		
	All covered	67%
	Some covered	13%
What do you use to get water?		
	Pour directly	19%
	Use cup with handle	63%
Water and Health		
Have you had diarrhea in the last week?	Yes	2%
	No	81%
	No answer	17%
What do you think causes diarrhea?		
	Food	12%
	Water	15%
	No answer	67%
Do you think you can get sick from you water?		
	Yes	40%
	No	41%
Health Practices		
Do you clean your storage vessel?		
	Water from tap	87%
	With Soap	2%
Do you use soap for handwashing in the home		
	Yes	64%
	No	4%
Do you use soap before cooking?		
	Yes	73%
	No	19%
Do you use soap before eating?		
	Yes	69%
	No	21%

Survey results

The research team administered a 50-question in-home survey to 218 households in Tshapasha and Tshibvumo (refer to Appendix E.1). The purpose of the survey was to examine water practices and storage, water and health understanding, water beliefs, health practices, water purification beliefs, preferred purification technology characteristics, interest in CWFs, and filter market potential. Tables 5.1 through 5.4 present an abridged summary of results from the survey. The top two to three responses are listed in the tables along with percentages of participants who provided those answers. The percentage of participants who did not respond is provided for questions that had low response rates.

Table 5.1 consists of results from water practices and storage, water and health, and health practices. The purpose of these questions was to determine their primary source of drinking water, understand their storage practices, and examine their water, health, and hygiene practices. The questions determined need for CWFs and identified knowledge gaps for future development of educational material. 64% of households who participated had water piped into their yard/plot, 11% relied on community standpipes or taps, and the rest either did not answer or relied on surface water. 67% covered all of their storage water, and primarily stored water in plastic buckets (20%) or Jerry cans (44%). 63% accessed their stored water with a cup, whereas 13% acquired water through pouring from storage container. 81% of participants said they had experienced diarrhea in the last week. Of those who responded to “what causes diarrhea,” 12% said food, 15 % said water, and the rest did not respond. When directly asked if they thought water caused diarrhea 40% answered “yes,” 41% answered “no,” and the rest did not respond.

Table 5.2 summarizes water purification beliefs. 83% of respondents indicated they have heard of water treatment technologies from clinics. 66% indicated that believe their water needs treatment. The majority indicated their preferred treatment methods were boiling (56%), tablets or liquid chemicals were the next preferred method (15%), and the rest did not answer. 86% believed that purifying or treating their water would result in "better health."

Table 5.2 Summary of water purification beliefs		
Water Purification Beliefs		
Have you heard of water treatment technologies?		
	Clinic	83%
	Hospital	9%
Do you think the water you use for drinking needs treatment?	Yes	66%
	No	25%
What do you think is the best way to treat water in your home?	Boil water	56%
	Tablets or liquid chemical	15%
	No answer	21%
What would be the benefit of purifying/treating your drinking water?		
	Better health	86%

Table 5.3 summarizes results from the survey section focused on examining characteristics of water purification technologies that were important to users. This part of the survey was developed based on criteria from the WHO (6). Characteristics included the quantity of water that the technology can purify, taste, rate of purification, coloration, ease of use, lifespan, cost, local availability, and ease of maintenance. Respondents were given four options to choose from: not important, a little important, important, very important. Responses under 10% were not listed in the table. Most responses ranged from a little important to very imporant for each characteristic and ranged between 15% and 35%. The characteristics that had percentages from 10 to 15% as not imporant were taste, rate, and coloration. Participants were approximately evenly divided with regard to choosing between a cheap technology they had to replaceed often versus an expensive technology with a longer lifespan.

Table 5.3 Characteristics important in a water purification technology

Quantity it can purify		
	A little important	25%
	Important	29%
	Very important	22%
Taste of water after purifiation		
	Not imporant	13%
	A little important	17%
	Important	27%
	Very important	22%
Rate of purification/time		
	Not important	11%
	A little important	18%
	Important	26%
	Very important	24%
Coloration/odor after purification		
	Not important	12%
	A little important	18%
	Important	20%
	Very important	28%
Ease of use for purifying water		
	A little important	17%
	Important	34%
	Very important	22%
Replacement time/how long the product lasts	A little important	14%
	Important	34%
	Very important	20%

Table 5.3 (continued)		
Cost		
	A little important	19%
	Important	28%
	Very important	24%
Locally availability		
	A little important	15%
	Important	32%
	Very important	28%
Ease of maintenance		
	A little important	17%
	Important	33%
	Very important	23%
Would you rather use technology that is cheaper, but has to be replaced more often, or one that is more expensive		
	heaper, replace more often	45%
	ensive, replaced less often	41%

Table 5.4 summarizes interest in ceramic water filters and potential market for filters. The research team also demonstrated examples of treatment technolgies for this part of the survey including chlorine tablets and ceramic water filters. 83% of partcipants indicated interest in buying filters and 80% asked to be contacted when CWFs become available locally. 28% indicated they would purchase filters immediately, and 51% said would purchase them within the next 6 months. Participants also indicated they would purchase them if they were made available through NGOs or health care providers.

Table 5.4 Ceramic Water Filter Market		
Ceramic Water Filter		
Would you be interested in buying a filter?		
	Yes	83%
Filter Market		
If a local potter were to sell CWFs, how likely is it that you would buy a filter from him/her?		
	Yes	78%
How likely is it that you would buy a filter if a NGO sold them?		
	Yes	80%
How likely is it that you would buy a filter if a health care provider recommended them or sold them?		
	Yes	78%
If filters were to become available how soon would you purchase one?	Immediately	28%
	Within 6 months	51%
How long would you be willing to wait for the water to filter before drinking it?		
	≤ 1 hour	67%
Would you like to be contacted if someone starts selling a purification technology nearby		
	Yes	80%

Interpretation of survey results

A feasibility study was conducted in two rural communities in Venda region of Limpopo Province of South Africa: Tshapasha and Tshibvumo. A total of 218 households participated and 205 of those households provided water samples. Water testing was performed with two bacterial indicators, total coliform and *E.coli*. A significant percentage of households were exposed to high levels of coliforms putting people who consume the water at risk for gastrointestinal infections.

Survey responses indicated water storage and handling practices further complicate existing conditions as participants stored water and would dip cups into their storage containers, which increases risk of contamination. A study in similar rural communities in South Africa demonstrated evidence of survival and growth of TC consistently, and *E. coli* from time to time, in both polyethylene and galvanized steel containers over a 48-hr period (106) . In addition, direct hand contact or utensils can introduce contamination to water, so even if drinking water was safe for consumption, in-home practices can re-introduce contamination (107, 108) . A significant percentage of participants indicated they had not experience diarrhea in the last week. Additionally, 17% of respondents chose not to respond. However, studies have shown that shorter recall periods, such as 3 or 4 days, increase accuracy. Additionally, stigma associated with diarrheal diseases results in under-reporting (48). Overall, despite that not many respondents reported diarrhea, levels of water contamination and existing water practices indicate need for water treatment and improved safe water storage practices.

Multiple survey questions were aimed at identifying a ceramic water filter market. Water treatment methods currently in use by communities primarily include boiling, chlorine tablets, and liquid chemicals. Ceramic water filters have a competitive advantage when compared to boiling in areas where fuel is scarce, and therefore food preparation takes priority for using available fuel sources. Additionally, another competitive advantage CWFs possess is that tablets and liquid chemicals alter the taste of treated water, whereas CWFs treat water without affecting its taste.

The survey also highlighted the importance of clinics. Respondents cited clinics as a source of where they learned about the necessity for purifying water or methods of treating water. Surveys also revealed many participants misunderstand the causes of diarrheal diseases. Therefore, our findings emphasize the need for water, health, and hygiene education for distribution with ceramic water filters and suggest pursuing partnerships with local clinics.

Partnerships

Our team also searched for local partners to assist with manufacturing and testing filters, and distributing them once they are produced. For the purpose of establishing a factory, we worked with the University of Venda to find local potters and/or entrepreneurs. We identified candidates and evaluated potters based on the following criteria: level of interest in technology; location of existing business; knowledge and understanding of ceramics; business skills; relationship with the community; labor availability; and capacity to expand existing business. Based on these criteria, we identified a women's cooperative in Mashamba known as the Mukondeni Pottery Cooperative. We approached the Department of Health, multiple local clinics, various local non-profit organizations, the Small Enterprise Development Agency (SEDA), Rotary International, and the South African consulate to join in the evolving partnership.

Material Source

The team searched for sources of raw materials for the production of filters including clay, combustible, and firewood. The Mukondeni Pottery Cooperative had direct access to a clay source where they could collect the clay at zero cost. Examples of combustible material typically used are sawdust, rice husk, or agricultural waste (24). We identified various sources of combustible material, primarily sawdust, which is available for free through a number of local lumber mills.

Mukondeni Potters

The Mukondeni Pottery Cooperative consists of 45 female potters who produce handmade pottery fired in an open pit (Figure 5.5). The potters make handmade pottery that ranges from 7 cm to 1.5 m tall. The ceramics are fired in an open pit and decorated with designs.

The Cooperative is located in the village of Mashamba, about 80 km from Thohoyandou, which is home to the University of Venda and the Vhembe District Government Offices. Mashamba is approximately 60 km from Louis Trichardt and a major interstate road, allowing for direct access to diverse demographics (Figure 5.6). Thohoyandou is predominantly populated with small-scale subsistence farmers and low-income families, while Louis Trichardt is home to the low-income and middle-income populations.

Figure 5.5: Street view of Mukondeni Pottery Cooperative located in Mashamba in Limpopo Province, South Africa (Image provided by Caroline Hackett)

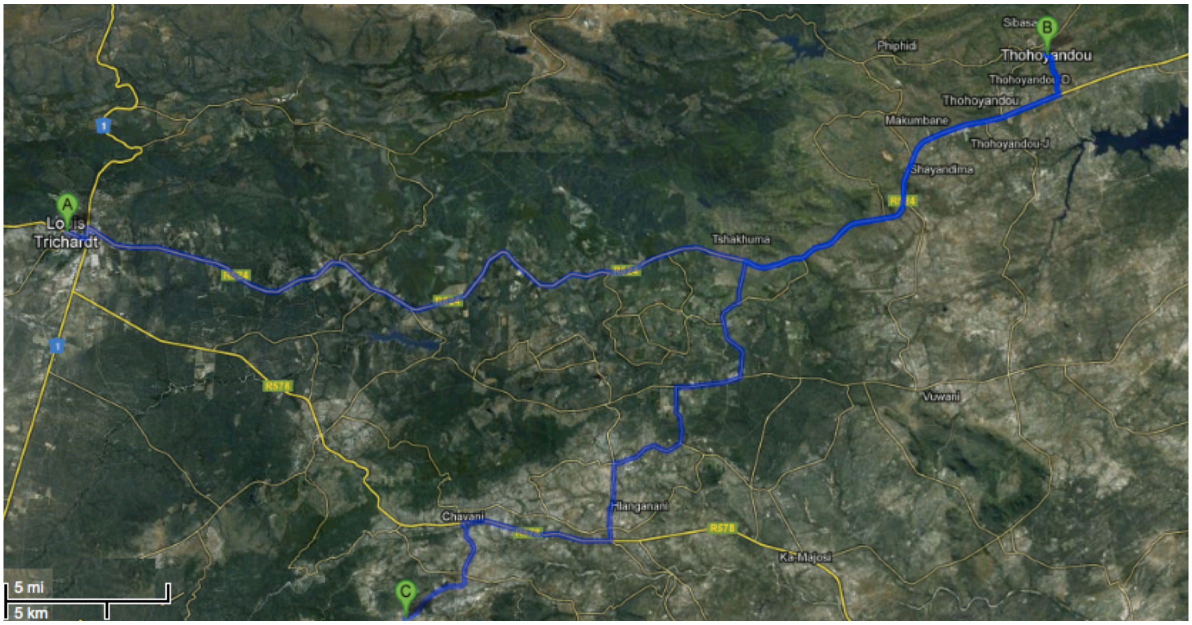

Figure 5.6 Map of Louis Trichardt (A), Thohoyandou (B), and Mashamba (C) in Limpopo Province, South Africa

Implementation

Starting the factory

The Cooperative originally had a 22 m by 18 m building consisting of two large rooms and two small offices. The property has two sets of gates and an outdoor area where pottery is typically displayed in front of the building. Immediately to the right of the building was unoccupied space covered with small trees and shrubs. Behind the building were outdoor toilets, and an open pit used for firing pottery. The clay deposit is located approximately 75 m northeast of the Cooperative. The deposit is approximately 3 to 5 m deep, 50 m wide, and 1 km long. The Cooperative has unlimited access to the clay deposit at no cost, but limited access to water and electricity.

Figure 5.7 provides a schematic diagram of the existing building and the CWF factory layout. The area to the right of the existing building was cleared and the ground was leveled for the construction. We constructed a 9 m by 22 m wood pole structure with a metal corrugated roof, dirt floor, and no walls. A sawdust-screening table was constructed with 8, 16, and 20 mesh screens available. We purchased a hammer mill from Hippo Mills. Annik Engineering in Louis Trichardt manufactured both the mixer and filter press to our specifications. The drying area for the clay was covered with a tarp. The soaking basin was constructed with bricks and lined with a waterproof sealant. A press with three male molds and one female mold with hydraulic jack was installed. FilterPure Inc. originally developed the design of the press. Figure 5.8 provides images of the press, hammer mill, sieve, drying rack, soaking basin, and mixer. A borehole was installed to improve access to water. Three-phase electricity was extended to the factory to power the hammer mill and mixer (refer to Figure 5.9).

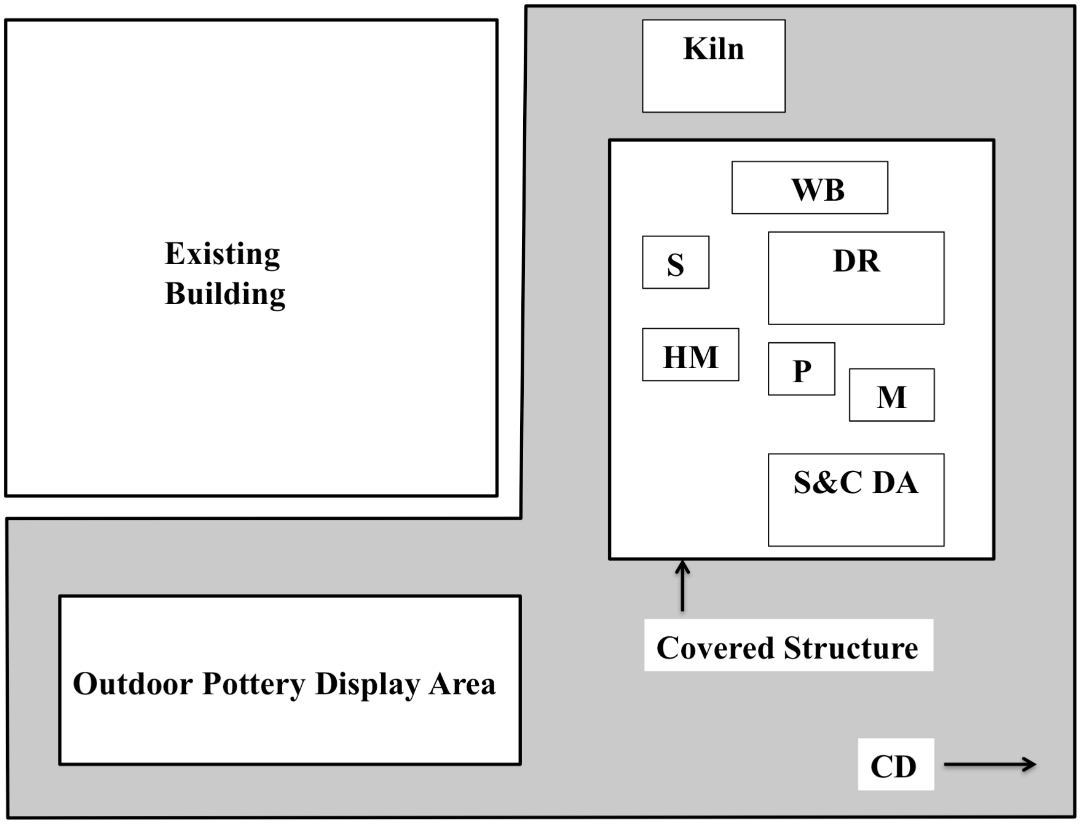

Figure 5.7 Diagram of factory layout provided by Lydia Abebe. Key: sawdust sieve (S), water basin (WB), drying racks (DR), hammer mill (HM), mixer (M), press (P), sawdust & clay drying area (S&C DA), and clay deposit (CD).

Figure 5.8 The following components used during CWFs manufacturing process were installed during the implementation phase (left to right). Row 1: filter press and hammer mill. Row 2: wire sieve and drying rack. Row 3: soaking basin and mixer.

Figure 5.9 The existing factory had only intermittent access to water and no electricity. Upon expanding the factory we installed a borehole and three-phase electricity (left to right). Images provided by James Smith.

The final step of setting up the factory was constructing a kiln. Figure 5.10 includes images of the brick kiln that was constructed, pyrometric cones, and a pyrometer. We constructed a Mani-kiln (24). The kiln is a large crossdraft kiln. The interior dimensions of the kiln are 1.75 m wide, 1.4 m deep, with 0.9 m walls, and 1.2 m tall at the center of the arch (can fit approximately 65-70 filters). The kiln has two fireboxes with moveable doors over the upper half, one opening that is sealed once the kiln has been filled, and a 4-m tall chimney. Pyrometric cones are distributed throughout the kiln and are used as a means of gauging temperatures within the kiln and even distribution of heat. Finally, dual pyrometer probes are placed at the top and bottom of the kiln to measure temperature during firing.

Figure 5.10 Images of ceramic water filter factory kiln, pyrometer, and pyrometric cones. Pictured here: Kiln (Left). Pyrometric cone (Top Right) and Pyrometer (Bottom Right). Images provided by James Smith.

Marketing

A 60-question survey was administered in marketplaces in Thohoyandou and Louis Trichardt, previously mentioned cities (refer to Appendix E.2). 187 people participated in the survey. Table 5.5 displays an abridged summary of questions from the survey. The top two to three responses are listed in the tables along with percentages of participants who provided those answers.

Table 5.5 indicates clinics (61%) and schools (37%) were the primary sources of education on water treatment. 94% of those who responded preferred to learn through educational pamphlets. Only 18% reported that they traveled in personal vehicles and 67% used public transportation in the form of buses and taxis. Finally, churches and citizen associations were reported as the top-most community gathering where participants acquire new information.

Table 5.5 Marketing and Distribution Survey

Where did you learn about water treatment?		
	Clinic	61%
	School	37%
How would you like to receive this information?		
	Pamphlet	96%
	Live demonstration	4%
How do you transport goods home?		
	Bus	36%
	Taxi	31%
	Personal vehicle	18%
Where do you learn about new products?		
	Advertisements	25%
	Television	16%
What type of community gatherings?		
	Church	35%
	Citizen's associations	10%

Distribution Channels

Potential distribution channels were enumerated based on responses from the feasibility and marketing surveys (refer to Figure 5.11). Six major avenues were considered for marketing and distribution purposes to reach consumers: non-profit organizations, wholesalers, retailers, door-to-door sales model, community gatherings, and health clinics.

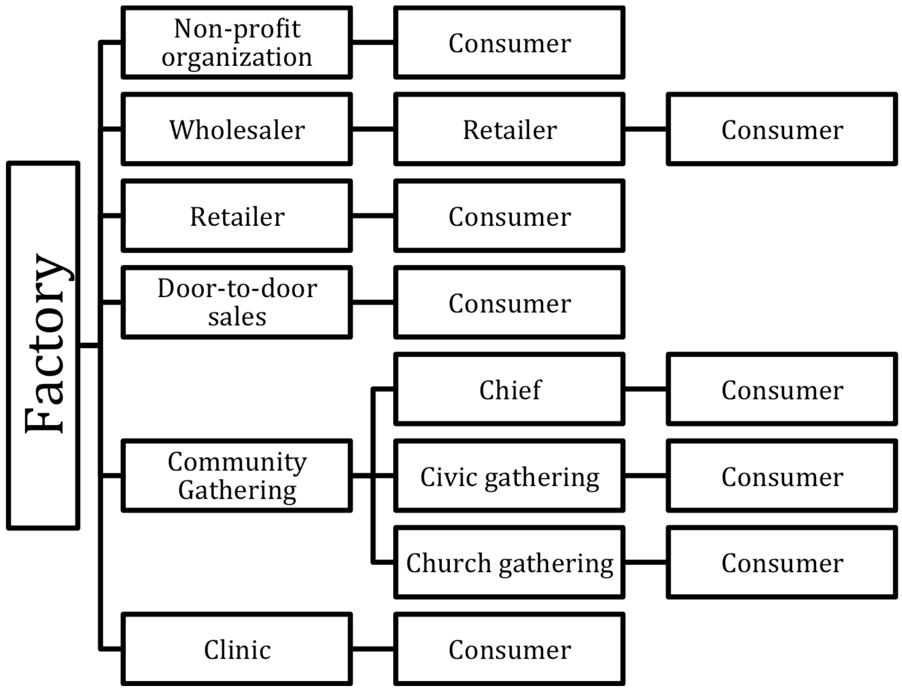

Figure 5.11 Distribution channels for marketing and distribution of ceramic water filters in Limpopo Province, South Africa

Training and Operation

Initial stages

The first stage in implementing training and operation for Mukondeni was to teach the potters how to produce the CWFs. An important aspect in training is gaining high initial participation, and keeping employees motivated throughout the process (109) . At Mukondeni, we kept the potters motivated by presenting them with physical certificates recognizing their progress and stating their various accomplishments with the CWFs. It is also helpful to gain the support of a representative member of the group who is highly respected by the group (110) . At Mukondeni, the representative was the already established Cooperative Manager. The representative was elected and appointed by the potters to become the Factory Manager. The employees trust that her decisions are in their best interest. Another large advantage is working with local partners since they can assist in translating when necessary, explain traditions and customs, and forming a bridge for the growing relationship. Finally, utilizing local knowledge is beneficial, as it provides for a co-learning platform, and helps the community gain confidence and increase empowerment (109) . At Mukondeni, we depended heavily on the potters to share their ceramic artistry knowledge to enhance the production process.

Before we began producing filters we had to determine the optimal proportions of sawdust, clay, and water. Currently, there is no standard ratio that applies to all factory sites; therefore, a trial and error method is required to achieve a flow rate between 1.5 and 3 L/hr. To achieve the desired flow rate, sawdust percentage ranging between 5 and 20% on a mass basis were tested with either 16 or 20 mesh-screened sawdust. It was determined ~10% sawdust percentage with 16-mesh sieve sawdust consistently yielded the desired flow rate. The final formula was 68 kg of clay, 8 kg of sawdust, and 28 L of water. This formula produces 20 filters.

During the process of developing this formula filters were cracked to determine the presence of a black layer in the walls of the filter, commonly referred to as a carbon layer (Figure 5.12). The carbon layer is caused by pyrolysis (111) . Pyrolysis occurs when organic matter is exposed to high temperatures through combustion with low oxygen levels (111) . The advantages and disadvantages of the carbon are a debated subject (24). According to Rayner, some factories claim the carbon assists with the removal of turbidity and microorganism, while some assert reduces the flow rate to unacceptable slow levels. The claim that the carbon layer assists with treatment has not been tested (24). Therefore, we aim to produce filters without the carbon layer.

The final critical step in training for filter production was teaching the potters how to test filters for proper performance, and how to apply silver to the filters. We trained the potters to conduct the following steps to test the filter performance: pressure test and flow rate test. The pressure test involves submerging the rounded end of the filter in water up to the rim for 10 s to determine if there are cracks. . If wet spots become visible on the inside of the filter within the 10-s period, the filter is deemed unacceptable. Flow-rate testing requires soaking the filters overnight (in the water basin shown in Figure 5.7) to fully saturate pore spaces and then filling filters with water and recording the change in volume over the span of an hour. Filters with flow rates not within the range of 1.5 to 3 L/hr were discarded. Filters that pass the test are subsequently coated with silver. Silver was purchased from Ames Goldsmith Corp in New York State (S2-80W). The quantity that was purchased was1.8 kg silver for approximately USD 1,500. The silver was in a water solution and had 50% silver content by mass. We diluted the silver to 0.3% solution (by mass) and applied it on the inside and outside walls of the filters with a paintbrush.

Figure 5.12 Fired ceramic water filters were purposely cracked to determine the presence of sawdust that was not fully combusted. The black colored layer in the walls of filters at the top of the picture indicates partial firing, whereas the filter at the bottom of the picture shows a fully fired filter with fully combusted sawdust. The gray layer is commonly referred to as a carbon layer. (Image provided by James Smith)

Developing a Small Business

Ultimately, the success of a small business such as a filter factory is based on finding and attracting the target market. To assist the potters, the researchers developed relationships with various local stakeholders in the Venda region. These partners have helped to identify a market, determine advertising campaigns, and develop distribution methods.

Business Management

The business management strategy at Mukondeni has changed drastically in the last few years. Before their involvement in this partnership, the potters shared an informal understanding of how the business was operated. Through this collaboration, the women have received formal training in business management, which taught them how to run a simple business, the necessity of marketing their products, and the value of attempting additional business ventures. The potters also participated in cooperative governance training, which focused on how to register as an official cooperative, gain the government's acknowledgment and assistance, write a new constitution, and develop an internal organization structure.

With regards to CWF production, the researchers and Factory Manager split the women into different teams, to be rotated every few weeks. The goal of this strategy was to keep a majority of the women working each day on different steps of the filter creation process. The potters insisted that the teams rotate through the different tasks, although it would have been more effective to have each potter fully understand and perfect a single step in the process. The teams are as follows: the Clay Team works with the clay and sawdust preparation; the Pressing Team operates the filter press; the Firing Team handles the firewood and firing; the Testing Team completes the quality assurance tests; the Packaging Team applies silver and prepares buckets; and remaining members work on other business ventures within the Cooperative. Images of the Cooperative performing the steps listed above are depicted in Figure 5.10.

Another concern that emerged during training was the need to develop skills necessary to perform tasks within each step. In order for the Clay Team to prepare clay and sawdust the use of a hanging scale is required to measure the weight of the clay and sawdust for each filter batch. A batch of filters has a total weight, and of that weight a percentage is sawdust and the remainder is clay. Therefore, basic levels of arithmetic were necessary to add the components of each batch. This posed as a problem for some of the potters since the level of education varied for the members of the Cooperative. Additionally, the some of the potters did not know how to use a scale to measure clay and sawdust. In order to address these issues, we spent time teaching the potters arithmetic and how to use a scale. The Pressing Team had to learn how to operate a manually operated jack. We spent time with the potters to teach how to operate the jack and improve their understanding of the tension and slack in the supporting cables. The Firing Team had difficulty reading the pyrometer and did understand the necessity of gradually increasing temperature through slowly feeding the kiln firewood. To address this issue, we taught the potters how to use the pyrometer and the importance of timing in feeding the kiln firewood. Finally, the Packaging Team required safety training with the handling of silver to prevent direct skin contact with silver. They also required training to measure and prepare the diluted silver solutions. Lastly, the Packaging Team was in charge of preparing the buckets with spigots. So, the team had to be trained to use a drill in order to drill a hole for the spigot.

Figure 5.13 Images of the potters operating various steps of the process (Left to Right). Row 1: Clay Team and Pressing Team, Row 2: Firing Team and Testing, and Row 3: Packaging Team (silver application and spigot installment. (Images provided by James Smith and Rachel Schmidt)

Advertising & Marketing

The first step to appropriately marketing CWFs to the surrounding communities is new advertising. The potters and researchers have discussed hanging a large sign at Mukondeni to attract local customers. Additionally, they have decided to buy four small billboards to place along different roads leading to the site, each marketing the services and products available. Finally, the women want to create brochures, business cards, and a website. Each item would be geared toward slightly different audiences or selling points.

The Cooperative will sell the filters at a wholesale price from the factory to the community for 190 South African Rand, about $20 US dollars. The potters will negotiate with local hardware shops, grocery stores, and small businesses and ask them to sell the filters from their shelves. Local partners will speak with the Regional Municipality to discuss donating a selling stand to the Cooperative in Thohoyandou or Louis Trichardt from which to sell their products. The hope is that these measures will help the filters reach a wider market, and gain interest on a larger scale. Also, the South Africa Bureau of Standards (SABS) is currently testing the filters for certification. With this certification, the filters will display a sticker letting consumers know that the South African Government approves and endorses the filters for household water purification needs.

Distribution

Safe, reliable distribution of the filters remains a problem for the Cooperative. Local partners are working to help the Cooperative apply for government funding, specifically to invest in a car. This car will provide the women with more freedom and the ability to further advertise their revived business, but it also poses problems. None of the women have a driver's license, and it would cost additional money to train them or to hire a driver. Fuel and maintenance will add an additional financial burden, and will take away from the profits after selling their products.

Obstacles

Thus far, we have encountered four major problems in establishing the ceramic water filter factory: lack of ownership, communication, stakeholder management, and sustainability.

I. Ownership

At Mukondeni Pottery Cooperative, the potters have not yet taken ownership over the ceramic water filter portion of their business. Over two years after joining the partnership, the women are familiar with all of the filter-based infrastructure and procedures, yet they do not display ownership over the production process or filters. When the research team partners are not at the site, the women rarely work with the filter machinery, indicating that they are not comfortable, do not understand, or are not committed. While the timeline must be considered, the potters knew the basic steps to making a filter just two months into the training and operation phase. Part of this ownership dilemma is an extension of having such high membership numbers at the Cooperative. Since the Cooperative currently employs 45 women, it is easy to avoid or not commit to responsibility on the job. As a result, very few potters take control over the production process.

This lack of ownership may also be attributed to forced development and differing priorities. "Forced development" is labeled as the outside-in approach to development, and is described as one that "does not build on a country's unique strengths, does not respect its social traditions, does not allow the autonomy to grow indigenous leaders… [and is] against the natural inclinations and even the will of the people" (112) . Current community-based research practices strictly rebuke this method, opting for 'indigenous development' or an inside-up method. The partners involved with Mukondeni Pottery Cooperative explicitly tried to avoid forced development, as is recommended. However, it is important to consider what the women were being offered by joining into the partnership with the researchers: the chance to enhance and revive their struggling business, at no financial cost of their own, with the physical labor of producing filters split between 45 women. Although they did not take the initiative and seek out the new business venture, there is no clear incentive why the women would decline joining the collaboration. Researchers such as Minkler (109) argue that had the question or research statement originated within the local community, the citizens would have displayed honest interest and ownership over the project. Note that the women did not explicitly express an unprompted desire for filtration units, or overt concern for their overall water quality. Understandably, the potters want to enhance their business and bring home a higher salary. However, they lack a passion for the filter product or the health concerns revolving around their new business. Without these inherent values, they do not feel the importance of selling their products, besides the immediate financial benefit. The approach of implementing this project at the Cooperative could help explain why the women have not yet claimed ownership over the project.

While the over-arching goals for all partners involved in the collaboration is to create a sustainable business for the Cooperative by selling water filters, the independent goals of the individuals involved are drastically different. The potters want money to provide for their families, but the time and labor required to make water filters and successfully sell them at a profit is a far-removed solution to their immediate income need.

II. Communication

Communication is a frequent problem, especially when working across international and cultural boundaries. Only a handful of the 45 women at Mukondeni speak enough English to communicate with a non-Tshivenda speaker. This leads to complications when trying to explain complex concepts such as scientific testing, business organization, financial management, or marketing strategies. Additionally, there are many aspects of daily life in the Venda culture that an outsider simply cannot understand, e.g. the complex hierarchy and ways of paying respect. Israel et al. (110) cites problems often associated with differences in cultural norms, decision-making protocol, values, principles, assumptions and beliefs. There also may be differences linked to gender, race, ethnicity, class, and age diversity (110) . While every partner has made a strong effort at cultural awareness, this does not translate to an inherent understanding and often requires extra effort and patience from all partners.

III. Stakeholder Management

Direct stakeholders in the Mukondeni Ceramic Water Filter Business include the researchers as well as multiple local government, the Rotary club, and small-business players. Within this partnership, problems have come up with consistency of individual partners representing each stakeholder. In addition, rapid turnover makes it very difficult to understand the dynamics and relationships.

The benefit of working with so many stakeholders is the co-learning platform that allows partners to share ideas, promote teamwork, expand local involvement, and apply for funding opportunities. However, the more complicated aspect to this approach is the constant shifting of authority. With so many stakeholders, it is difficult to know who should be held responsible for different action items at Mukondeni. For example, when the roof blew off of the factory structure, there were many stakeholders trying to make important decisions. One partner managed the funds required, another held future architectural plans and knowledge, a different partner wanted to approve the service provider, but could not provide any to choose from, and still another had resources to rebuild but needed to work on a short timeline. Almost unavoidably, some partners will be unhappy with the outcome. Managing each of the stakeholders and their individual motivations requires patience, understanding, trust, and compromise.

IV. Sustainability

Sustainability is a key objective for the filter factory. Through training workshops, hands-on assistance, and mentoring opportunities, the partners plan to assist the Cooperative until they can produce and sell filters independently. At this stage, the structural sustainability and current age range are two major concerns that could threaten the sustainability of the Business.

One strong indicator of the worrisome structural sustainability was a large windstorm, which blew the roof off of the factory building in October of 2012. No one was injured in the destruction, although the standing structure was badly damaged. With daily temperatures rising and the rainy season approaching, it was difficult to work outdoors without a protective cover. It took one month to find a reliable carpenter, buy materials, and build a new roof. In early January, two of the bottom molds of the filter press became extensively damaged while pressing. For at least four weeks, the women pressed filters with only one mold, causing large delays in production. These events, among others, have caused concerns over the reasons behind the damage, and the sustainability of the equipment being installed and utilized. While the partners have done their best to make appropriate decisions, it is important to invest time and money to initially get things right, helping to motivate the potters, and maintain their confidence.

The women at Mukondeni range in age from around 35 to nearly 80 years old. However, there are no younger members joining the cooperative, mainly due to the Cooperative's lack of financial success in recent history. If this trend continues, the Cooperative will struggle to hold up its membership numbers in the next ten to twenty years. The stakeholders are trying to tackle this problem by starting a program with the nearby schools in which the potters invite local students to the Cooperative to teach them traditional ceramic artistry. They hope this will spark interest in their business, recruit young members, and spread the indigenous ceramic knowledge.

Discussion

In summary, based on the performance of ceramic water filters describe in Chapter 2, we decided to establish a filter factory as a means of providing affordable and cost-effective drinking water treatment locally. The process of establishing the filter factory was divided into three phases: feasibility study, implementation, and training and operation. A timeline of our process has been summarized in Figure 5.14. The process began 2009, when our initial results from the HIV study in Chapter 2 demonstrated decreases in diarrheal infection, which led to the initial plans to design the case study. The research team started to form and WHIL assisted in identifying representative villages for the purpose of conducting the feasibility study. Results from the feasibility study confirmed the need for improved water quality. We identified the Mukondeni Pottery Cooperative and initiated partnership building with the Cooperative. We jointly began construction, implemented training, and expanded the existing business to incorporate ceramic water filters.

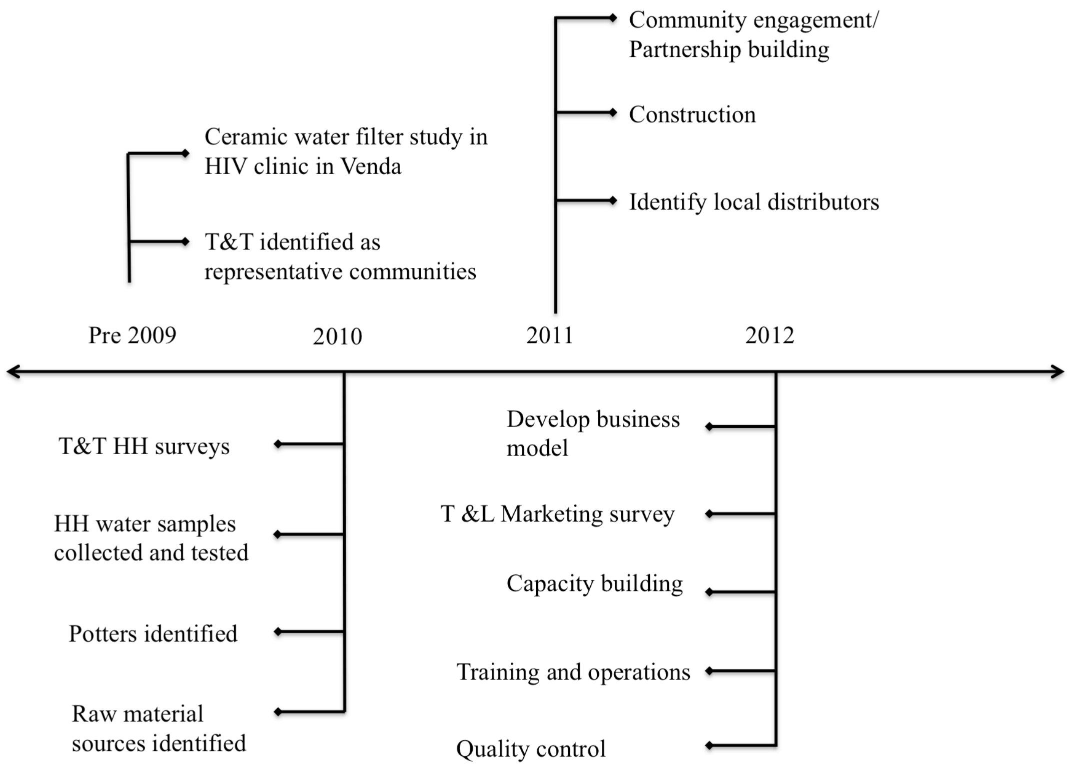

Figure 5.14 Timeline of activities starting from the conclusion of the ceramic water filter in 2009 study through the end of 2012. (T&T: Tshapasha and Tshibvumo, representative communities identify through prior work from the Water and Health in Limpopo, a collaboration between the University of Virginia and the University of Venda; T&L: Thohoyandou and Louis Trichardt, two cities in the region).

Lessons learned

The process of establishing the filter factory taught us many lessons. Among the lessons learned were key variables that affect the sustainability of the factory, such as funding, factors affecting training, the importance of safety education, and factors outside of our control, such as weather. Funding is a critical aspect necessary for the establishment and maintenance of a factory until production commences. It is important to have a vetted budget to purchase equipment and extra funds available for unexpected expenses that may arise. Another aspect was the level of education within the Cooperative, which varied. Initially, this was a barrier in measuring and adding components to weigh mixes, measuring flow rate, operating equipment, using a pyrometer, and preparing silver dilutions. Additionally, we originally intended to create a safety manual. The process of training the potters allowed us to better tailor our manual based on areas we observed were particularly relevant to the Cooperative. Finally, the incident with the structural stability of the factory heightened our understanding of the role of weather in the production of the filters, which included the potential destruction of operation and hindrance in filter production. The rainy season in the region can not only be destructive, but also obstruct filter preparation through delaying drying of materials and pressed filters (before firing). To address this barrier, we plan to build a walled structure solely used for drying raw materials and filters.

Additionally, one critical lesson learned that has been previously discussed by Rayner is the need to standardize production and the difficulties with training artisans to reduced variability in filter production (24). This criticism has been echoed in a number of filter factories around the world (24) and similarly at the Cooperative where ceramic artist learned their craft through intuition and not careful measuring. This is problematic with ceramic water filter production as a distinction is required between ceramic production as an art and standardized production of ceramics for health improvement (24). The creation of CWFs requires rigid reproducibility with minimal variability in the end products.

Next Steps

We have come a long way, developed a recipe, trained potters, and distribute filters to the potters (refer to Figure 5.15). However, a number of steps are required before the factory begins selling and distributing filters. The first critical step is to finalize production and testing wherein filters are being produced with minimum variability. The production manual and safety manual should be finalized as well. Second, in order to confirm that filters are being reproduced with minimal variability a quality control process that involves on-site flow rate and microbial removal testing needs to be developed and finalized. Also, packaging, storage, and transportation of filters require finalization. Lastly, it is important to follow-up with SABS for certification along with developing and testing a sales and marketing plan.

Figure 5.15 Image of potters at Mukondeni Pottery Cooperative with filters produced by the potters at the factory. (Image provided by Rachel Schmidt)

Other worthwhile considerations are the development of alternative receptacles. The receptacles currently in use were purchased in local stores at a reasonable cost. However, if receptacles were made of ceramic, they would significantly lower the cost of inputs and possibly display the potters talents and artistry. These receptacles could also be designed specifically to improve the filtration process; for example, the receptacles could have a large inner rim to ensure good balance, and the lower reservoir can be taller to safely store larger quantities of water. Additionally, it would be wise to consider alternative fuel sources. We are currently using firewood, which contributes to the cost of inputs and is not environmentally friendly. We are also considering using an electric kiln. The use of an electric kiln is often prohibitive due to the high demand of electricity to operate an electric kiln. Other forms of agricultural or milling waste should be explored to lower cost of inputs and decrease environmental degradation.

In terms of long-term plans, the potters have been trained to produce filters and are currently undergoing certification process to improve their business management practices. However, for the business to grow, it would be advantageous for the business to hire a member who knows how to use a computer and has internet access to eventually market the filters in other provinces in South Africa and eventually to countries beyond South Africa. Also, the structure of the factory is flimsy and is susceptible to future damage, so we recommend improving the structure to make it more durable.

Recommendations

The aim of this project was to document the process of establishing a ceramic water filter factory to provide a model that will help future factories. Listed below are guidelines for the phases discussed in this study:

Feasibility Study

- Conduct a feasibility study to establish need through:
    - Examine local water, health and hygiene knowledge
    - Determine interest in ceramic water filter technology
    - Evaluate local drinking water quality
        - Use *E. coli* as indicator
        - Classify risk using WHO standards
- Identify an entrepreneur to run the business with the following criteria:

    - Leadership, computer, internet, and language skills
    - Presentation and organization skills
    - Can interact and negotiate with various stakeholders, such as, non-profit organizations, local businesses, wholesalers, and local community.
- Identify potters or skilled laborers to produce filters
    - On the one hand, potters have the advantage of knowing how to produce ceramics; however, on the other hand, they also have a tolerance for variability in the end product. Therefore, it is not a necessity for potters are involved in the creation of filters for the economic sustainability of a filter factory.
- Identify sources for the following inputs at a cost-effective price:
    - Clay
    - Organic material
    - Water
    - Fuel/Energy source

Implementation

- Build partnership and assign responsibility
- Design factory based on:
    - Stakeholder feedback
    - Size/capacity of production
    - Local environment and weather
    - Safety regulations
- Assess and fundraise for construction before building for:
    - Construction
        - Material
        - Labor
    - Equipment
        - Purchase
        - Installment
    - Travel costs to bring experts
        - Oversee construction
        - Conduct training

    - Payment of employees during training
- Involve local government, NGOs, clinics, etc.
- Identify marketing and distribution channels

Training and Operation

- Reassess partnership and strength relationship between stakeholders
- Develop formula for production of filters
- Train production of filters
- Develop training and safety manual

Continuing research needs to be done include marketing, production, and sales of filters. Further research investigation is necessary to identify factors that contribute to the economic sustainability of a ceramic water filters factory.

Chapter 6

Conclusion

As we approach the end of 2012, Millennium Development Goals (MDGs) to “halve the proportion of people without sustainable access to safe drinking-water and basic sanitation” seem to be an ambitious target to meet by 2015. In fact, a recent report is projecting water goals will be met by 2035, and sanitation by 2108 (113) . Difficulties remain in the broader context to bridge inequalities in Water, Sanitation and Hygiene (WASH). However, a growing body of evidence has shown that POU methods improve water quality, which is a critical aspect of WASH. CWFs are an example of a POU method that has demonstrated great promise in improving water quality at the household level (6, 14, 16). In the literature, CWFs reduce diarrheal disease, and provide cost-effective, and socially acceptable solutions (6). However, challenges and questions still remain in the advancement of CWFs as a means of POU water treatment

To date, field studies have focused on microbial-effectiveness and social acceptability in field interventions, but have not measured the impact on human health (18, 27-29). Disinfection studies regarding silver salts and nanoparticles have largely focused on bacterial removal and disinfection. Thus, little is known about the antimicrobial effects of silver and the retention capacity of the pore spaces with respect to reduction of harmful pathogens other than bacteria. Finally, there is a dearth of documentation on critical steps in initiating, constructing, and operating a sustainable filter factory in order to promote accessibility. Therefore, the aim of this dissertation project was to address gaps through the following projects: investigating the health impact of ceramic water filters on HIV-positive individuals; analyzing the disinfection capacity and physical removal of *C. parvum*; and documenting the process of establishing a filter factory to increase accessibility of CWFs.

The human health impact study examined the effect of CWFs on HIV-positive individuals in a clinic-based randomized controlled trial, which yielded two major findings. First, the study observed ceramic water filters significantly reduced diarrheal disease, and, second, *C. parvum* infection can be greatly reduced through the use of ceramic water filters. These findings support continued use of ceramic water filters, particularly among vulnerable populations such as people living with HIV. It also generates questions regarding the mechanism that caused the reduction of *C. parvum.*

As aforementioned, CWFs provide two mechanisms of water purification: disinfection through silver and physical removal through retention in pore spaces. Therefore, the significant decrease in *C. parvum* infection among filter users was analyzed by examining the improvement attributable to disinfection versus physical removal. Thus, a laboratory investigation was conducted to determine the effects of silver on *C. parvum* and removal capacity of CWFs. Results indicated silver decreases the infectivity of *Cryptosporidium parvum*. Oocysts exposed to silver nitrate salt and proteinate-coated silver nanoparticles demonstrated the greatest reduction in infectivity to mice when compared to mice exposed to untreated oocysts. However, while a reduction in infection was achieved, neither silver salts nor nanoparticles demonstrated a reduction sufficient for recommendation for use in drinking water treatment nor suggest disinfection was the primary mechanism of reduction of *C. parvum* consistent with the clinic-based study. Following the disinfection study, laboratory transport experiments were conducted to test the physical removal of *C. parvum* using ceramic disks to mimic transport through CWFs.

Observations from this study indicated significant reduction in *C. parvum* through physical removal of oocysts and determined that physical removal was the primary mechanism of treatment of *Cryptosporidium* in contaminated water.

Finally, the results herein further support the use and positive impact of ceramic water filters on users' health. However, a challenge that remains is the accessibility of the technology. Thanks to Potters for Peace, the filters are already in use in over 30 countries around the world (24). However, prior to this study there was little documentation of the process of establishing ceramic water filter factories. Herein, the process of establishing a filter factory in Limpopo Province, South Africa was presented. The study was divided into three phases: feasibility, implementation, and training and operation. We describe each phase of the process; including obstacles encountered and lessons learned and make recommendations for the factory as a means of providing a model for future factories.

Recommendations for future work

The reduction in diarrhea observed in the clinic-based study involving HIV-positive individuals was the first reported study investigating health impacts associated with the technology targeted at vulnerable populations. More research needs to be conducted to determine the health impact of CWFs on other vulnerable populations such as children under 5, as they are especially susceptible to chronic and prolonged infection, which has long-term health implications such as cognitive impairment (9, 10, 114)

Despite the health benefits HIV-positive filter users experienced, the intervention detected re-contamination of the lower reservoir during use (Chapter 2). The issue of recontamination is commonly addressed through guidance on how to maintain the filters. More attention and research needs to be focused on the causes of re-contamination, developing methods of prevention, and improving the technology to prevent it.

C. parvum findings were critical to understanding the mechanism that caused the reduction in *C. parvum* infection during the filter intervention. However, more research needs to be conducted to investigate the treatment capacity of filters vis-à-vis other harmful parasitic protozoa, such as Giardia, and viruses, as they are other common causes of gastrointestinal infection contributing to morbidity and mortality. More laboratory and field research needs to be conducted to investigate the microbial-effectiveness of the filters regarding harmful pathogens and associated health impact.

Finally, additional research is needed to identify critical factors in establishing a filter factory to ensure economic sustainability of the factory and increase accessibility through demystifying the process of establishing a filter factory. The case study on the filter factory documented in this dissertation was only the beginning step in understanding the process of establishing a filter factory for use as a model for future factories. Among other things the report discusses the role of local stakeholders, the importance of building partnerships, the process of capacity building, and local buy-in, which have been suggested as building blocks for the sustainability of international development projects (115) . Future work should be considered to better understand these factors and their implications on economic sustainability of the factory and increased accessibility of the filters. Further, research should be done to validate the role of these factors through surveying existing factories.

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Appendix

Appendix A. 1 Point-of-Use Technologies

Table 1 Summary of Point-of-Use technologies (Sobsey, 2008) (19)

Technique	*Description*	*Studies*
Free Chlorine with storage	Free chlorine or hypochlorite water disinfection with safe storage unit.	(116, 117)
Combined coagulation and chlorination	Commercially produced tablets of sachets combining dried coagulant-flocculant and chlorine which are added to water	(118, 119)
Solar Disinfection	Solar disinfection of aerated source water stored in transparent polythethylene terephthalate bottles and exposed to solar UV during sunlight hours	(120-124)
Ceramic filters	Porous ceramic made from clay, sawdust and water and coated with silver nanoparticles used to filter microbes and turbidity.	(18, 27-29)
Biosand filters	Adapted from large-scale slow sand filter	(125-127)

Appendix B. 1 Laboratory Flow Rate Results

Filter	Participant ID #	Flow Rate (L/hr)		
		First	Second	Third
1	001	2.695	2.725	2.6
2	003	4.375	4.425	4.34
3	005	3.55	3.54	3.55
4	007	4.7	4.85	4.77
5	009	3.63	3.6	3.625
6	011	4.3	4.48	4.45
7	013	3.225	3.24	3.375
8	015	2.845	2.7	2.75
9	017	2.611	2.97	2.82
10	019	2.96	2.87	2.89
11	021	3.5	3.6	3.7
12	023	0.81	0.865	0.86
13	025	3.66	3.755	3.75
14	027	2.35	2.325	2.175
15	029	3.2	2.155	3.31
16	031	3.77	3.91	3.86
17	033	4.425	4.075	5.27
18	035	3.38	3.68	3.71
19	037	3.915	4.075	3.625
20	039	3.77	3.825	4.27
21	041	3.65	4.2	3.725
22	043	3.18	3.495	3.45
23	045	3.97	3.96	4.05
24	047	3.43	3.8	3.98
25	049	3.175	3.9	4.05
26	051	3.94	4.075	4.125
27	053	2.125	2.16	2.18
28	055	4.285	4.3	4.445
29	057	3.9	3.98	3.95
30	059	2.62	2.81	2.725
31	061	3.345	3.275	3.35
32	063	3.2	3.465	3.38
33	065	3.55	3.48	3.575
34	067	2.92	2.915	2.96
35	069	3.825	4.05	4.135
36	071	2.75	2.135	2.12
37	073	3.84	4.375	3.975
38	075	2.925	3.05	3.125
39	077	3.775	3.75	3.815
40	079	4.25	3.755	4.05

Appendix B. 2 Laboratory Microbial Removal Results

Filter	Participant ID #	Sample	First Flush			
			Weight (g)		Volume	#cfu/ 100mL
			w/o Sample	w/ Sample	Sample (mL)	
1	001	I1-1F	39.9514	146.2163	106.2649	0
		E1-1F	39.2196	177.2068	137.9872	965.9104
		E2-1F	40.4369	176.548	136.1111	952.7777
2	003	I1-1F	39.5303	144.1627	104.6324	732.4268
		E1-1F	39.5261	154.3421	114.816	803.712
		E2-1F	39.4932	158.8874	119.3942	835.7594
3	005	I1-1F	40.491	142.31	101.819	712.733
		E1-1F	39.4705	181.1507	141.6802	991.7614
		E2-1F	39.59	172.1155	132.5255	927.6785
4	007	I1-1F	38.7655	159.0916	120.3261	842.2827
		E1-1F	39.1317	168.4598	129.3281	905.2967
		E2-1F	39.399	170.4002	131.0012	917.0084
5	009	I1-1F	38.9982	144.827	105.8288	740.8016
		ID-1F	40.2555	141.4332	101.1777	708.2439
		E1-1F	40.5494	180.9734	140.424	982.968
		E2-1F	39.5545	182.6994	143.1449	1002.0143
		ED-1F	39.3907	193.3359	153.9452	1077.6164
6	011	I1-1F	39.5858	170.3967	130.8109	915.6763
		E1-1F	39.7199	169.8309	130.111	910.777
		E2-1F	38.8297	164.6603	125.8306	880.8142
7	013	I1-1F	39.2624	140.0024	100.74	705.18
		E1-1F	39.5904	198.2671	158.6767	0
		E2-1F	24.7131	159.7845	135.0714	0
8	015	I1-1F	39.1234	137.7766	98.6532	838.5522
		E1-1F	40.3542	196.0165	155.6623	0
		E2-1F	39.1813	188.2631	149.0818	0
9	017	I1-1F	38.9375	137.8501	98.9126	692.3882
		E1-1F	24.4538	162.2848	137.831	0
		E2-1F	39.4324	192.6519	153.2195	0
10	019	I1-1F	39.1845	142.2458	103.0613	824.4904
		E1-1F	39.5532	190.1932	150.64	0
		E2-1F	39.0947	191.7065	152.6118	0
11	021	I1-1F	39.5552	138.1537	98.5985	345.09475
		ID-1F	39.6175	140.8499	101.2324	506.162
		E1-1F	39.0912	200.4974	161.4062	0
		E2-1F	39.3099	202.4484	163.1385	0
		ED-1F	39.4156	191.0531	151.6375	0
12	023	I1-1F	39.5769	140.3806	100.8037	1310.4481

		E1-1F	19.8898	154.8415	134.9517	0
		E2-1F	40.0555	201.7035	161.648	0
13	025	I1-1F	2.0895	110.3568	108.2673	1136.80665
		E1-1F	2.0875	109.7663	107.6788	0
		E2-1F	2.044	119.9432	117.8992	0
14	027	I1-1F	2.0946	105.9558	103.8612	1246.3344
		E1-1F	2.1501	110.2642	108.1141	0
		E2-1F	2.1458	114.9155	112.7697	0
15	029	I1-1F	2.09	108.2931	106.2031	1433.74185
		E1-1F	2.1458	110.6464	108.5006	0
		E2-1F	2.1251	114.4189	112.2938	0
16	031	I1-1F	2.0943	106.1074	104.0131	1508.18995
		E1-1F	2.1309	111.7536	109.6227	0
		E2-1F	2.1123	109.175	107.0627	0
17	033	I1-1F	2.0887	104.9071	102.8184	1285.23
		ID-1F	2.0795	106.4069	104.3274	1095.4377
		E1-1F	2.1198	117.5616	115.4418	0
		E2-1F	2.0958	108.0701	105.9743	0
		ED-1F	2.0975	111.364	109.2665	0
18	035	I1-1F	2.0693	103.7205	101.6512	457.4304
		E1-1F	2.0965	110.5213	108.4248	0
		E2-1F	2.1095	113.4617	111.3522	0
19	037	I1-1F	2.0871	107.1248	105.0377	1102.89585
		E1-1F	2.0651	112.1808	110.1157	0
		E2-1F	2.0652	111.2034	109.1382	0
20	039	I1-1F	2.1006	101.3097	99.2091	744.06825
		E1-1F	2.1228	117.8268	115.704	0
		E2-1F	2.1098	117.0085	114.8987	0
21	041	I1-1F	2.1002	107.0941	104.9939	944.9451
		E1-1F	2.1395	118.4817	116.3422	0
		E2-1F	2.129	118.4619	116.3329	0
22	043	I1-1F	2.1042	106.3245	104.2203	885.87255
		E1-1F	2.132	109.5709	107.4389	0
		E2-1F	2.1318	111.8034	109.6716	0
23	045	I1-1F	2.1059	107.3833	105.2774	1000.1353
		ID-1F	2.1007	109.8398	107.7391	915.78235
		E1-1F	2.1239	115.3311	113.2072	0
		E2-1F	2.1209	111.5674	109.4465	0
		ED-1F	2.1308	115.8421	113.7113	0
24	047	I1-1F	2.1082	98.8556	96.7474	725.6055
		E1-1F	2.1537	115.8421	113.6884	0
		E2-1F	2.1497	113.3108	111.1611	0
25	049	I1-1F	2.1036	105.5328	103.4292	672.2898
		E1-1F	2.1215	112.2995	110.178	0
		E2-1F	2.1419	113.495	111.3531	0

26	051	I1-1F	2.1031	111.4276	109.3245	765.2715
		E1-1F	2.0406	119.6638	117.6232	0
		E2-1F	2.1132	111.7364	109.6232	0
27	053	I1-1F	2.0943	109.7939	107.6996	1507.7944
		E1-1F	2.1101	105.95	103.8399	0
		E2-1F	2.1071	117.6284	115.5213	0
28	055	I1-1F	2.1086	109.6465	107.5379	1720.6064
		E1-1F	2.1011	115.6241	113.523	0
		E2-1F	2.1081	115.7886	113.6805	0
29	057	I1-1F	2.0999	107.878	105.7781	1851.11675
		ID-1F	2.1104	103.5595	101.4491	0
		E1-1F	2.1031	114.125	112.0219	0
		E2-1F	2.026	111.517	109.491	0
		ED-1F	2.0331	113.477	111.4439	0
30	059	I1-1F	2.0545	106.6113	104.5568	1986.5792
		E1-1F	2.064	117.626	115.562	0
		E2-1F	2.0933	105.9312	103.8379	0
31	061	I1-1F	2.0873	113.0155	110.9282	887.4256
		E1-1F	2.0943	110.6467	108.5524	0
		E2-1F	2.0194	113.4399	111.4205	0
32	063	I1-1F	2.1318	105.3745	103.2427	1600.26185
		E1-1F	2.1483	112.9219	110.7736	0
		E2-1F	2.1282	119.6681	117.5399	0
33	065	I1-1F	2.1403	105.5726	103.4323	1189.47145
		E1-1F	2.1238	111.6956	109.5718	0
		E2-1F	2.1212	109.2244	107.1032	0
34	067	I1-1F	1.919	109.2024	107.2834	750.9838
		E1-1F	2.0319	112.2316	110.1997	771.3979
		E2-1F	2.0346	118.588	116.5534	815.8738
35	069	I1-1F	1.9321	111.9375	110.0054	770.0378
		ID-1F	2.0404	107.3625	105.3221	737.2547
		E1-1F	2.121	117.5149	115.3939	807.7573
		E2-1F	2.099	113.8996	111.8006	782.6042
		ED-1F	2.1083	111.9299	109.8216	768.7512
36	071	I1-1F	2.051	109.8344	107.7834	754.4838
		E1-1F	2.0378	121.2999	119.2621	834.8347
		E2-1F	2.099	113.8787	111.7797	782.4579
37	073	I1-1F	1.9936	114.0804	112.0868	784.6076
		E1-1F	2.1019	111.9831	109.8812	769.1684
		E2-1F	2.0932	109.8061	107.7129	753.9903
38	075	I1-1F	1.9972	109.666	107.6688	753.6816
		E1-1F	2.0831	112.82	110.7369	775.1583
		E2-1F	2.0972	117.5133	115.4161	807.9127
39	077	I1-1F	1.9971	105.2745	103.2774	722.9418
		E1-1F	2.1221	110.3708	108.2487	757.7409

		E2-1F	2.1244	115.001	112.8766	790.1362
40	079	I1-1F	2.0086	108.3369	106.3283	744.2981
		ID-1F	1.9991	104.1539	102.1548	715.0836
		E1-1F	2.1158	111.4237	109.3079	765.1553
		E2-1F	2.1312	114.9226	112.7914	789.5398
		ED-1F	2.1433	114.4659	112.3226	786.2582

Participant ID #	Sample	Second Flush			
		Weight (g)		Volume	#cfu/ 100mL
		w/o Sample	w/ Sample	Sample (mL)	
001	I1-1F	39.1198	137.9389	98.8191	0
	E1-1F	39.3278	189.0924	149.7646	1048.3522
	E2-1F	39.4637	173.041	133.5773	935.0411
003	I1-1F	39.2061	139.3607	100.1546	701.0822
	E1-1F	39.4407	169.1917	129.751	908.257
	E2-1F	39.1625	179.2235	140.061	980.427
005	I1-1F	39.0354	138.1254	99.09	693.63
	E1-1F	39.7855	171.0588	131.2733	918.9131
	E2-1F	34.5149	153.1171	118.6022	830.2154
007	I1-1F	38.8162	145.6946	106.8784	748.1488
	E1-1F	24.5384	164.9964	140.458	983.206
	E2-1F	24.5542	152.894	128.3398	898.3786
009	I1-1F	39.2114	141.1405	101.9291	713.5037
	ID-1F	38.0134	138.3505	100.3371	702.3597
	E1-1F	39.3407	180.4149	141.0742	987.5194
	E2-1F	39.1161	179.6476	140.5315	983.7205
	ED-1F	39.2424	189.3295	150.0871	1050.6097
011	I1-1F	39.2914	150.7301	111.4387	780.0709
	E1-1F	39.3639	173.6228	134.2589	939.8123
	E2-1F	39.3489	180.4686	141.1197	987.8379
013	I1-1F	20.1376	142.8996	122.762	859.334
	E1-1F	44.5461	191.0686	146.5225	0
	E2-1F	40.065	186.0618	145.9968	0
015	I1-1F	41.4425	143.2875	101.845	865.6825
	E1-1F	39.4876	184.1019	144.6143	0
	E2-1F	39.4452	184.5866	145.1414	0
017	I1-1F	39.1752	142.0014	102.8262	719.7834
	E1-1F	24.552	157.8012	133.2492	0
	E2-1F	25.5235	153.2384	127.7149	0
019	I1-1F	39.6036	147.8126	108.209	865.672
	E1-1F	39.9201	196.4131	156.493	0
	E2-1F	40.5443	182.0144	141.4701	0
021	I1-1F	39.6663	141.5003	101.834	356.419
	ID-1F	19.8354	149.8533	130.0179	650.0895

	E1-1F	39.5898	199.7538	160.164	0
	E2-1F	39.2464	197.9398	158.6934	0
	ED-1F	19.6403	152.87	133.2297	0
023	I1-1F	39.6445	135.8611	96.2166	1250.8158
	E1-1F	39.1828	200.803	161.6202	0
	E2-1F	39.4066	192.0065	152.5999	0
025	I1-1F	2.0633	113.0626	110.9993	1165.49265
	E1-1F	2.1322	109.0274	106.8952	0
	E2-1F	2.0801	112.8872	110.8071	0
027	I1-1F	2.1496	109.2938	107.1442	1285.7304
	E1-1F	2.0701	117.7253	115.6552	0
	E2-1F	2.0401	115.9448	113.9047	0
029	I1-1F	2.0601	108.9232	106.8631	1442.65185
	E1-1F	2.0531	112.5098	110.4567	0
	E2-1F	2.135	117.9576	115.8226	0
031	I1-1F	2.1364	107.013	104.8766	1520.7107
	E1-1F	2.078	109.7057	107.6277	0
	E2-1F	2.1404	116.794	114.6536	0
033	I1-1F	2.0565	105.6915	103.635	1295.4375
	ID-1F	2.0546	110.6195	108.5649	1139.93145
	E1-1F	2.1267	104.2283	102.1016	0
	E2-1F	2.1358	113.3321	111.1963	0
	ED-1F	2.1351	107.1	104.9649	0
035	I1-1F	2.0409	105.6022	103.5613	466.02585
	E1-1F	2.1314	111.9938	109.8624	0
	E2-1F	2.1458	114.1174	111.9716	0
037	I1-1F	2.0325	112.1636	110.1311	1156.37655
	E1-1F	2.1196	111.4806	109.361	0
	E2-1F	2.1274	117.2606	115.1332	0
039	I1-1F	2.1259	105.2674	103.1415	773.56125
	E1-1F	2.1519	111.0504	108.8985	0
	E2-1F	2.1373	117.0298	114.8925	0
041	I1-1F	2.1141	101.3927	99.2786	893.5074
	E1-1F	2.0942	119.4842	117.39	0
	E2-1F	2.0762	113.2218	111.1456	0
043	I1-1F	2.1103	105.3335	103.2232	877.3972
	E1-1F	2.1092	114.4136	112.3044	0
	E2-1F	2.1172	109.7016	107.5844	0
045	I1-1F	2.1172	108.2882	106.171	1008.6245
	ID-1F	2.108	103.8727	101.7647	864.99995
	E1-1F	2.1037	119.6834	117.5797	0
	E2-1F	2.136	112.0084	109.8724	0
	ED-1F	2.1155	115.9845	113.869	0
047	I1-1F	2.1229	108.213	106.0901	795.67575
	E1-1F	2.1072	115.0169	112.9097	0

	E2-1F	2.1114	113.17	111.0586	0
049	I1-1F	2.1133	107.5826	105.4693	685.55045
	E1-1F	2.1173	108.9395	106.8222	0
	E2-1F	2.1008	114.4492	112.3484	0
051	I1-1F	2.1048	108.5851	106.4803	745.3621
	E1-1F	2.0793	113.109	111.0297	0
	E2-1F	2.1268	113.0358	110.909	0
053	I1-1F	2.1116	110.6956	108.584	760.088
	E1-1F	2.0958	114.5384	112.4426	787.0982
	E2-1F	2.1178	114.1901	112.0723	784.5061
055	I1-1F	2.0926	111.7314	109.6388	767.4716
	E1-1F	2.0914	111.5863	109.4949	766.4643
	E2-1F	2.0977	118.2989	116.2012	813.4084
057	I1-1F	2.134	106.5285	104.3945	730.7615
	ID-1F	2.1187	102.9948	100.8761	706.1327
	E1-1F	2.09	113.4329	111.3429	779.4003
	E2-1F	2.1415	112.9776	110.8361	775.8527
	ED-1F	2.1194	113.5403	111.4209	779.9463
059	I1-1F	2.1201	112.8274	110.7073	774.9511
	E1-1F	2.128	108.8916	106.7636	747.3452
	E2-1F	2.1409	111.4479	109.307	765.149
061	I1-1F	2.1043	107.1024	104.9981	734.9867
	E1-1F	2.1143	111.4372	109.3229	765.2603
	E2-1F	2.1225	108.325	106.2025	743.4175
063	I1-1F	2.1086	102.1601	100.0515	700.3605
	E1-1F	2.1043	112.5034	110.3991	772.7937
	E2-1F	2.1078	115.0798	112.972	790.804
065	I1-1F	2.1101	105.9428	103.8327	726.8289
	E1-1F	2.1221	111.3407	109.2186	764.5302
	E2-1F	2.1318	112.2094	110.0776	770.5432
067	I1-1F	2.1133	102.9951	100.8818	706.1726
	E1-1F	2.0972	116.122	114.0248	798.1736
	E2-1F	2.0952	112.5985	110.5033	773.5231
069	I1-1F	2.1033	104.388	102.2847	715.9929
	ID-1F	2.0921	109.9327	107.8406	754.8842
	E1-1F	2.088	113.7793	111.6913	781.8391
	E2-1F	2.1026	116.3386	114.236	799.652
	ED-1F	2.0942	114.3986	112.3044	786.1308
071	I1-1F	2.115	102.8158	100.7008	704.9056
	E1-1F	2.1326	114.1366	112.004	784.028
	E2-1F	2.1231	106.7043	104.5812	732.0684
073	I1-1F	2.1075	102.4384	100.3309	702.3163
	E1-1F	2.1009	116.4528	114.3519	800.4633
	E2-1F	2.1301	114.5159	112.3858	786.7006
075	I1-1F	2.0916	103.1732	101.0816	707.5712

	E1-1F	2.1136	111.7587	109.6451	767.5157
	E2-1F	2.0964	109.3186	107.2222	750.5554
077	I1-1F	2.0988	101.6357	99.5369	696.7583
	E1-1F	2.1034	112.7801	110.6767	774.7369
	E2-1F	2.1031	112.6232	110.5201	773.6407
079	I1-1F	2.1001	102.631	100.5309	703.7163
	ID-1F	2.091	106.2368	104.1458	729.0206
	E1-1F	2.1199	113.1158	110.9959	776.9713
	E2-1F	2.0995	116.1354	114.0359	798.2513
	ED-1F	2.1111	115.2139	113.1028	791.7196

Appendix B. 3 Demographic Survey

Study Number:______
Study Group: ______
Cell Phone Number:________
GPS location/reading: ______
Date:__/___/___

Demographic data[i]

1. Age ________
2. Sex ________ F (1) M (2)

3. What is your family status?
   _____ single (1)
   _____ married (2)
   _____ divorced/separated (3)
   _____ widow/widower (4)

4. What is your education level?
   _____ primary (1)
   _____ some secondary (2)
   _____ completed secondary (3)
   _____ some college (4)
   _____ college graduate (5)
   _____ technical training (6)

5. Number of adults in household? (include you) _____
6. Number of children in household? ______

7. What are the ages of your children?

   Name________________________________ (1) Age _____

   Name________________________________ (2) Age _____

   Name________________________________ (3) Age _____

   Name________________________________ (4) Age _____

   Name________________________________ (5) Age _____

   Name________________________________ (6) Age _____

   Name________________________________ (7) Age _____

8. On a scale from 0 to 100 (100=perfect health), how would you rate your current health? ________

9. Have you had diarrhea in the past? Yes (1) or No (2) ____________
   If Yes, for how long? ______________ days/months (circle one)

10. What did you take to treat your diarrhea?
    _____ antibiotics (1)
    _____ other doctor prescribed medication (2)
    _____ homeopathic remedy (3)
    _____ no treatment (4)

11. Are you currently on antibiotics? Yes (1) or No (2) _______
12. Are you currently undergoing any medical treatment? Yes (1) or No (2) _________

    Explain:____

    ____

13. Is anyone in your family sick? Yes (1) or No (2) _________

14. What is your annual income?
    _____ Less than R 850 (1)
    _____ R 850–R 1,499 (2)
    _____ R 1,500–R 3,499 (3)
    _____ R 3,500– R7,499 (4)
    _____ R 7,500–R 9,999 (2)
    _____ R 10,000+ (3)

15. Do you rent _____ (1) or own _____ (2) your home?

16. Did you build your own home ______(1) or was it government-built ______(2)?

17. How do you get your information about where to get your water? (check your primary source)
    _____ Radio (1)
    _____ Market (2)
    _____ Television (3)
    _____ Newspaper (4)
    _____ Friends (5)
    _____ Family (6)
    _____ Meetings (7)
    _____ Other (8)

18. What type of toilet do you use?
    _____ Flush toiled (1)

_____ Pit latrine (2)
_____ Free range (3)
_____ Other (4)

19. How often do you wash your hands?
_____ Always (1)
_____ Sometimes (2)
_____ Rarely (3)
_____ Never (4)

When do you wash your hands?
20. After going to the bathroom? Yes (1) or No (2) _______
21. Before eating? Yes (1) or No (2) _______
22. Before cooking? Yes (1) or No (2) ___________

23. How often do you use soap when you wash your hands?
_____ Always (1)
_____ Sometimes (2)
_____ Rarely (3)
_____ Never (4)

24. Is your toilet indoor (1) or outdoor (2)? _______________________

25. Where do you get your drinking water?
_____ Personal tap in the home(1)
_____ Community tap (2)
_____ Pond (3)
_____ Well (4)
_____ River (5)
_____ Other (6)
Explanation if necessary: _________________________________

26. What do you use to store your drinking water?
_____ Ceramic vessels (1)
_____ Metal buckets (2)
_____ Plastic buckets (3)
_____ Jerrycan (4)
_____ Small pans (5)
_____ Cooking pots (6)
_____ Plastic Bottles (7)
_____ Other (8). Specify: _____________

27. Are your storage vessels covered? Yes (1) or No (2) ______
28. Do you use your drinking water for anything else?
_____ Everything (1)
_____ Cooking (2)

_____ Bathing (3)
_____ Cleaning (4)
_____ Washing (5)
_____ Other (6)
_____ Do not use drinking water for anything else (7)

29. What do you use to get the water from its storage?
_____ Pour directly (1)
_____ Use cup with handle (2)
_____ Use cup with hands (3)
_____ Use Spigot (4)
_____ Other (5)

30. How much water do you consume every day? _____________ liters
31. How much water overall do you have access to per month? _______liters
32. What if anything do you use to treat your water?
_____ Boil water (1)
_____ Tablets or liquid chemical (2)
_____ Filter (3)
_____ Other (4)
_____ Do not treat water (5)

33. Do you think that you can get sick from your water? Yes (1) or No (2) __________

34. What kind of sicknesses do you think you can get from your water? (check all that apply)
_____ Fever (1)
_____ Stomach ache (2)
_____ Vomiting (3)
_____ Diarrhea (4)
_____ Weight loss (5)
_____ Malnutrition (6)
_____ Other (7) ______________________
_____ Do not get sick from water (8)

35. Do you think your water is of poor quality? Yes (1) or No (2) ___________

36. What do you think makes your water poor?
_____ Limited access (1)
_____ Shared supply (2)
_____ Not treated (3)
_____ Not clean (4)
_____ Other (5) ______________________
_____ Water is not poor quality (6)

37. How far must you travel to get to this clinic? ____________
38. How much time does it take to get to this clinic? __________ minutes

39. How far must you travel for EMERGENCY medical care? In answering this question think about a potential emergency such as a broken leg. How far (ONE WAY) must you travel to get assistance such as stitches?
    _____ Approximate Travel Time (One way)

40. Please describe your source of emergency care (For example: nurse, hospital, clinic, community health worker etc.) ____
    ____

Appendix B. 4 Diarrhea Recall

Memory Aid to Record the Presence of Diarrhea
Participant ID # __________

Start Day: ___________
dd/mmm/yy

Please complete this form every day for each of the next 7 days.

1. Each morning when you wake up, record whether you had diarrhea during the previous day. Diarrhea means that you passed 3 or more loose or watery stools that were not normal on that day.

2. Go to the correct day. "☼" means today, "☼☼" means tomorrow, and so on. A day begins when you wake up in the morning and ends when you wake up the next morning.

3. If you had diarrhea that day, Mark "X" in the dark box for that day ☒ in the box for that day. If you did not have diarrhea, Mark "X" in the white box for that day ☒. **Each day, mark only on "X".**

4. If you forget for a few days, try to start again on the correct day.

5. Keep this form in a safe place. We will come to your house to collect the forms every two weeks.

DIARRHEA

NORMAL

(1)	☼ (today)		
(2)	☼☼		
(3)	☼☼☼		
(4)	☼☼☼☼		
(5)	☼☼☼☼☼		
(6)	☼☼☼☼☼☼		
(7)	☼☼☼☼☼☼☼		

Appendix B. 5 Exit Survey

WITH Ceramic Filter Technology

1) Why did you participate in this study?
   - o Better water quality for your family/household
   - o Improved health
   - o Free filter
   - o Other (please specify/explain):

2) Where do you keep your filter?
   - o Kitchen
   - o Living Room
   - o Other (please specify/explain):

3) How many days a week do you use it?
   - o Regular use (7 days/week)
   - o Irregular use (1-6 days/week
   - o Non-user (0 days/week)

4) Had you heard of a water filter before this study?
   Yes (1)
   5. No (2)

5) Overall, how happy/satisfied are you with the filter?
   - •Very happy/satisfied
   - •Happy/Satisfied
   - •Neutral
   - •Unhappy/Dissatisfied
   - •Very unhappy/dissatisfied

6) If you are not happy/satisfied, why not?
   The filter was too slow at filtering the water (1)
   The filter was too laborious to maintain and repair (2)
   The filter broke down or did not function properly too often (3)
   The filter left an after taste in the water (4)
   Other (please specify/explain):

7) Is the filter easy to use?
   - o Yes (1)
   - o No (2)

8) Do you treat all of the water the family uses for drinking in the house with the filter?

6. Yes (1)
7. No (2)

9) If not, how is the rest of the water treated?

Boiling water (1)
Tablets or liquid chemical, e.g. chlorination (2)
Different type of Filter (3)
Do not treat water (4)
Other (please specify/explain):

10) How much water is consumed without being treated at all? Approximately ____ Liters

11) Have you noticed any health improvement since you started using the ceramic filter?

8. Yes (1)
9. No (2)

12) Do you think the filter helped reduce diarrhea?

10. Yes (1)
11. No (2)

13) Do you think it helped reduce diarrhea for your children?

12. Yes (1)
13. No (2)

14) If you think that your health improved (measured as diarrheal incidence reduced) after using the filter, when did you notice it?

Immediately after using it
1 week
2 weeks
4 weeks
6 weeks
Greater than 6 weeks

15) Who is responsible for treating the water?

Father (1)
Mother (2)
Grandma (3)
Grandpa (4)
Male child/children (5)
Female child/children (6)

16) Who drinks the filtered water in the house?

Father (1)

Mother (2)
Grandma (3)
Grandpa (4)
Male child/children (5)
Female child/children (6)

17)Did you talk to your family, friends, and/or neighbors about this filter?

14. Yes (1)
15. No (2)

18) Did you recommend this filter to your:

Family (1)
Friends (2)
Neighbors (3)

19) Would you say this filter is better or worse at cleaning the water than other cleaning methods you use/have used (e.g. boiling water, chlorination, etc.)?

- Much worse
- Worse
- About the same
- Better
- Much better

20)Does the filtered water taste better or worse than other cleaning methods you use/have used?

- Much worse
- Worse
- About the same
- Better
- Much better

21) Does the filtered water smell better or worse than other cleaning methods you use/have used?

- Much worse
- Worse
- About the same
- Better
- Much better

22) Will you continue to use the filter?

16. Yes (1)
17. No (2)

23) Would you have bought a filter if it were not provided for free?

18. Yes (1)
19. No (2)

24) Would you buy it if it were sold in a local shop?

20. Yes (1)
21. No (2)

25) Would you buy it if someone in the village sold it door to door?

22. Yes (1)
23. No (2)

26)Would you consider buying a filter if a non-governmental organization (NGO) recommended it?

24. Yes (1)
25. No (2)

27) Would you consider buying a filter if your family, friends and/or neighbors recommended it?

26. Yes (1)
27. No (2)

28) Would you consider buying a filter if you heard about it in mass media (television, newspaper, etc.)?

28. Yes (1)
29. No (2)

29) Would you consider buying a filter if the doctors and nurses in the clinic recommended it?

30. Yes (1)
31. No (2)

30) Have you had any problems with the filter? If so, what types of problems have you had?

32. Yes (1); Brief description of problem:
33. No (2)

31) How often did you have problems with the filter?

- Daily
- Weekly
- Monthly

32) Do you think the filter can be improved? If so, how?

Ceramic Filter Operation and Maintenance

33) Did you clean the filter?

34. Yes (1)
35. No (2)

34) If so, how often did you clean the filter?
- Daily
- Weekly
- Monthly

35) Do you think you have enough financial resources to keep the filter running for your household?
- Yes (1)
- No (2)

36) If the filter broke or stopped working properly, would you buy a new one?
- Yes (1)
- No (2)

37) How much would you be willing to pay for this type of filter? R_____

38) Do you think your family, friends, and/or neighbors would buy one for this price?
- Yes (1)
- No (2)

WITHOUT Ceramic Filter Technology

1) Had you heard of a water filter before this study?

Yes (1)

36. No (2)

2) How did you treat your water during this study?

Boil water (1)
Tablets or liquid chemical, e.g. chlorination (2)
Different type of Filter (3)
Did not treat water (4)
Other (please specify/explain):

3) Would you have used the filter if it were provided for free?

37. Yes (1)
38. No (2)

4) Did you hear about the filter from someone? If so, was it:

Family (1)
Friends (2)
Neighbors (3)

5) Did you hear it could help reduce diarrhea?

39. Yes (1)
40. No (2)

6) Do you think it can help reduce diarrhea for adults?
41. Yes (1)
42. No (2)

7) Do you think it can help reduce diarrhea for children?
43. Yes (1)
44. No (2)

8) Would you buy it if it were sold in a local shop?
45. Yes (1)
46. No (2)

9) Would you buy it if someone in the village sold it door to door?
47. Yes (1)
48. No (2)

10) Would you consider buying a filter if a non-governmental organization (NGO) recommended it?
49. Yes (1)
50. No (2)

11) Would you consider buying a filter if your family, friends and/or neighbors recommended it?
51. Yes (1)
52. No (2)

12) Would you consider buying a filter if you hear it in mass media (television, newspaper, etc.)?
53. Yes (1)
54. No (2)

13) Would you consider buying a filter if the doctors and nurses in the clinic recommended it?
55. Yes (1)
56. No (2)

14) Would you be willing to pay for this type of filter for your house?
- Yes (1)
- No (2)

15) How much would you be willing to pay for this type of filter? R_____

16) Do you think your family, friends, and/or neighbors would buy one for this price?
- Yes (1)
- No (2)

Appendix C. 1 Exploratory Mouse Model Experiment Results

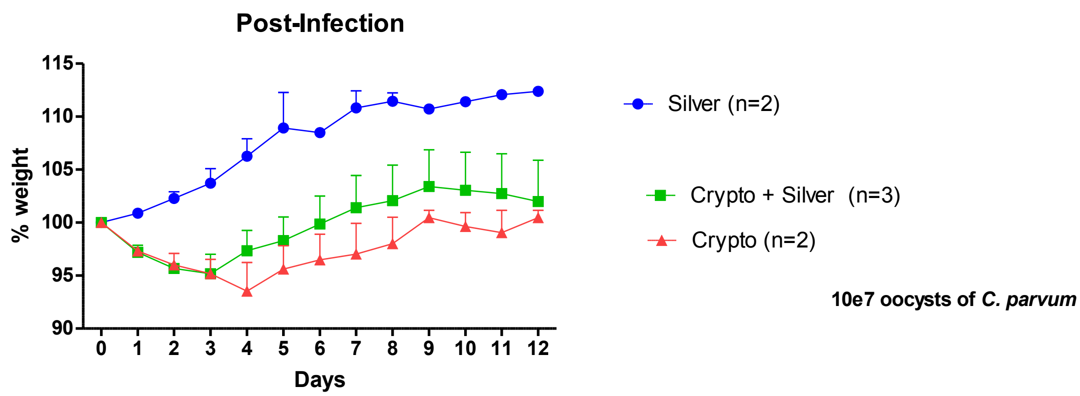


Figure 1: Weight loss post-infection. Silver used was proteinate-capped AgNPs.

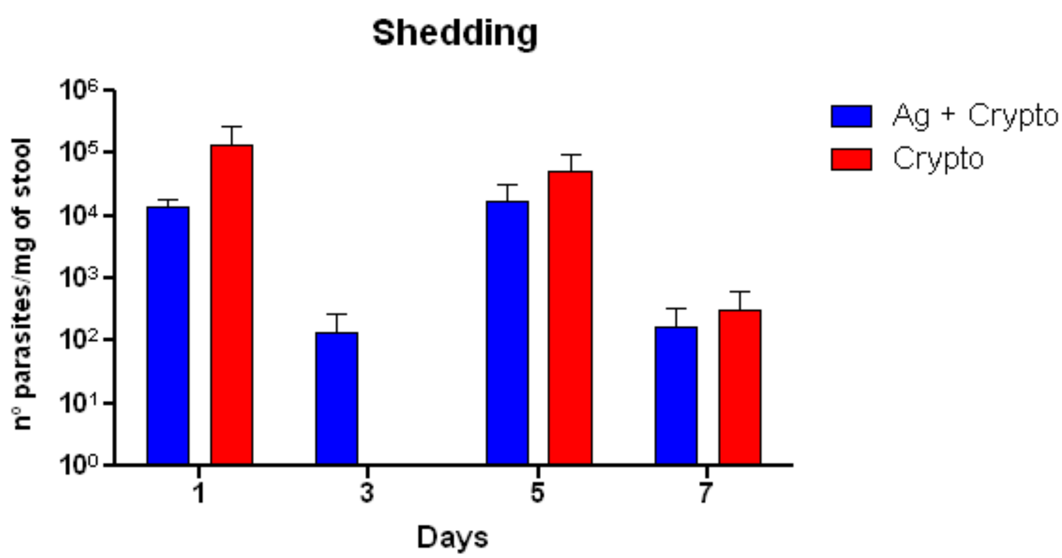


Figure 2: Shedding in mice post-infection. Silver used was proteinate-capped AgNPs.

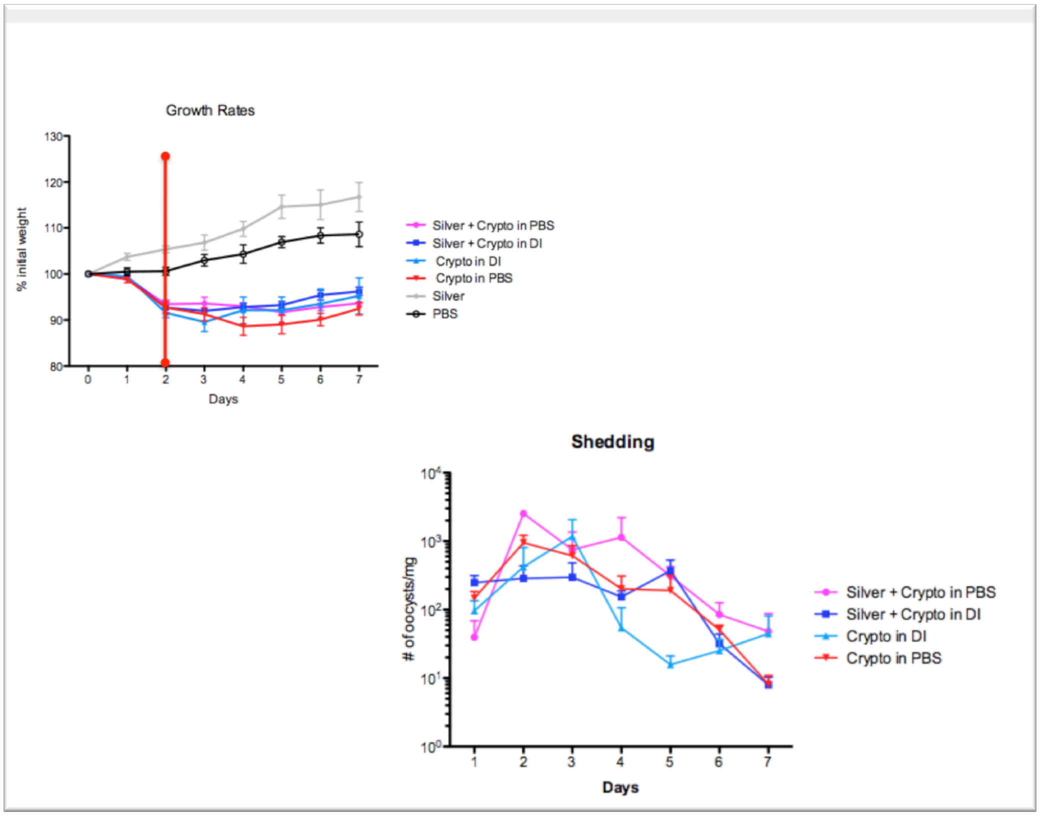
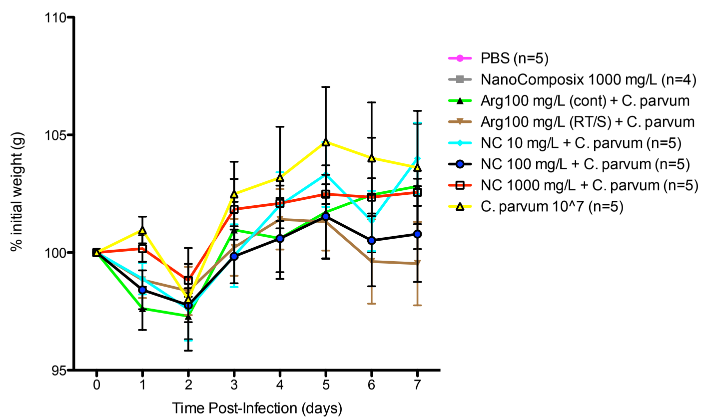
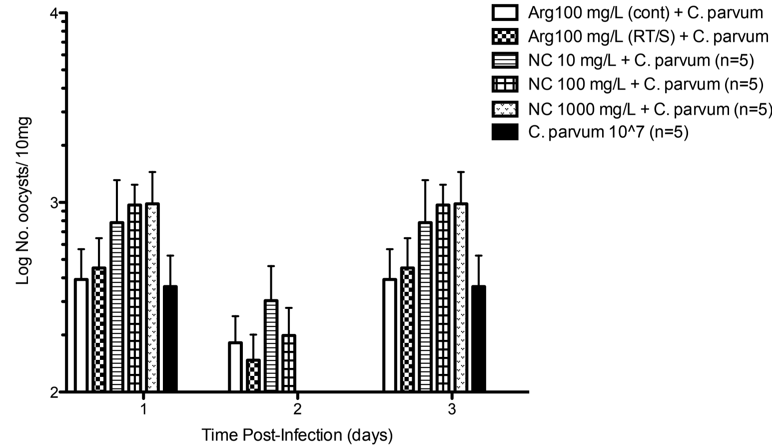


Figure 3: Weight loss post-infection. Silver used was proteinate-capped AgNPs. *C. parvum* was exposed to deionized (DI) to determine effects of DI on oocysts.



Body weight gain (%) from the experimental groups starting the day of inoculation until day 7 (7 days post-infection). All mice were malnourished for a period of 13 days.
Mean % initial weight at peak infection (d2):NC 1000 - **101%**, Arg 100 C + C – **97%**, Arg 100 RTS + C – **98%**, NC 10 + C - **97.6%**, NC 100 + C - **97.8%**, NC 1000 + C – **98.8%**, C.parvum - **98%**.

Figure 4: Weight loss post-infection. deionized (DI) to determine effects of DI on oocysts. Proteinate and Citrate-capped AgNPs were examined.



RT-PCR analysis on stool shed from mice starting the day after inoculation until day 7 (7 days post-infection). All mice were malnourished for a period of 13 days.
Oocysts were not detected in PBS and Boiled groups throughout infection. Mean log values at peak infection
(d2): Arg 100 C + C – **2.2** , Arg 100 RTS + C – **2.18**, NC 10 + C - **2.48**, NC 100 + C – **2.3**, NC 1000 + C – **1.8**, C.parvum – **1.9**.
(d3): Arg 100 C + C – **2.59**, Arg 100 RTS + C – **2.65**, NC 10 + C – **2.89**, NC 100 + C – **2.98**, NC 1000 + C – **2.99**, C.parvum – **2.56**.

Figure 5: Shedding post-infection. deionized (DI) to determine effects of DI on oocysts. Proteinate and Citrate-capped AgNPs were examined.

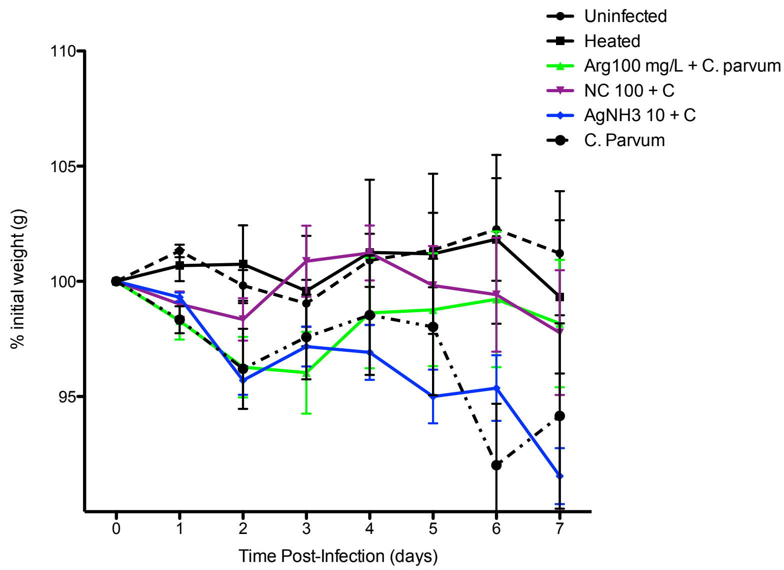


Figure 6: Weight loss post-infection. Proteinate-capped AgNPs, Citrate-capped AgNPs, anc
silver nitrate were examined.
*$AgNO_3$ not $AgNH_3$

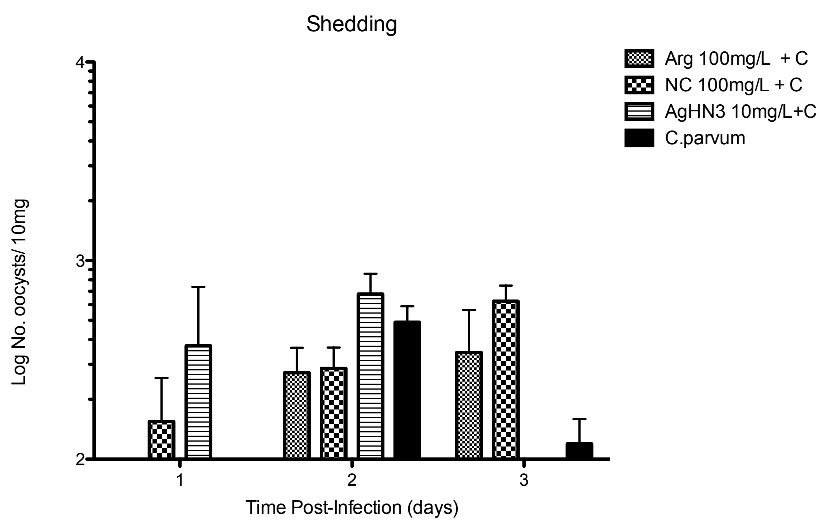


Figure 7: Shedding post-infection. Proteinate-capped AgNPs, Citrate-capped AgNPs, and s
nitrate were examined.
*$AgNO_3$ not $AgNH_3$

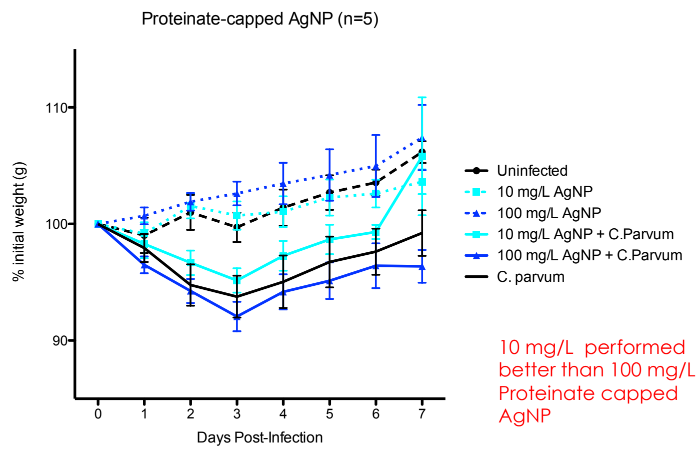


Figure 8: Weight loss post-infection. 10 mg/l and 100 mg/l Proteinate-capped AgNPs were examined.

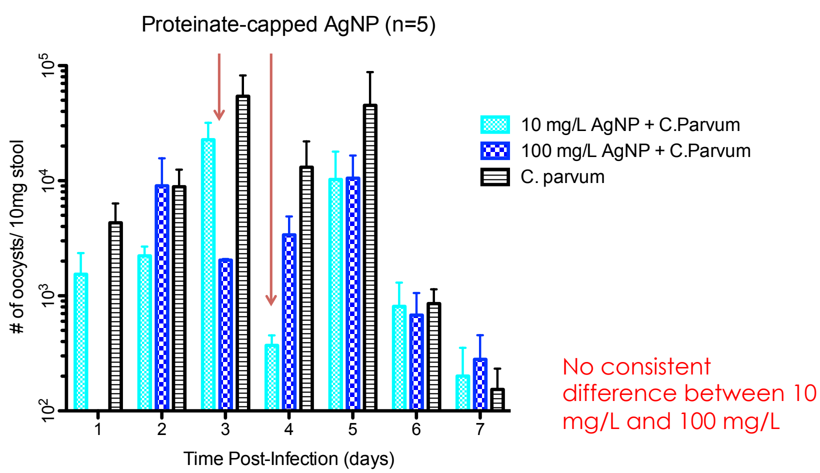
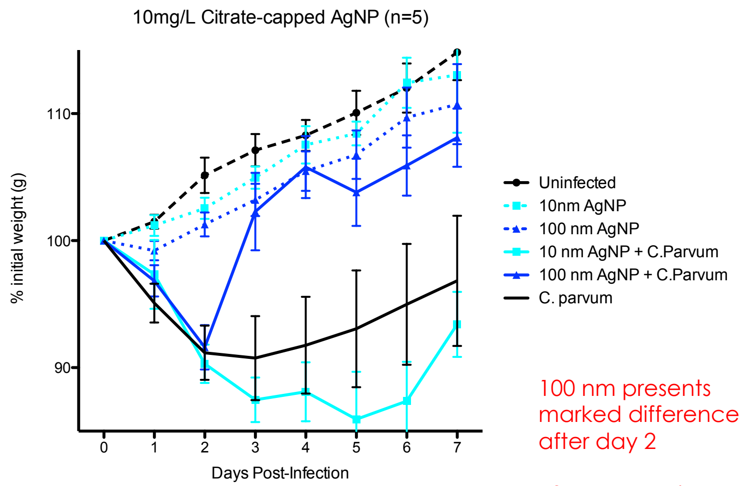


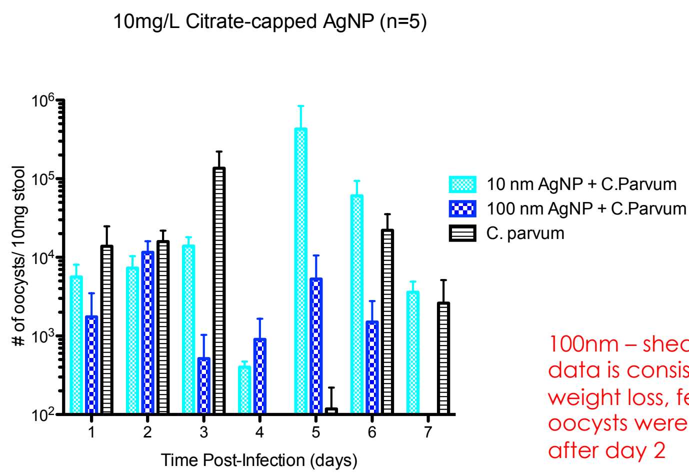
Figure 9: Shedding post-infection. 10 mg/l and 100 mg/l Proteinate-capped AgNPs were examined.



100 nm presents marked difference after day 2

10nm presents marked weight loss throughout infection

Figure 10: Weight loss post-infection. 10 mg/l of 10nm and 100nm citrate-capped AgNPs were examined.



100nm – shedding data is consistent with weight loss, fewer oocysts were shed after day 2

10 nm – shedding was greater than 100nm

Figure 11: Shedding post-infection. 10 mg/l of 10nm and 100nm citrate-capped AgNPs were examined.

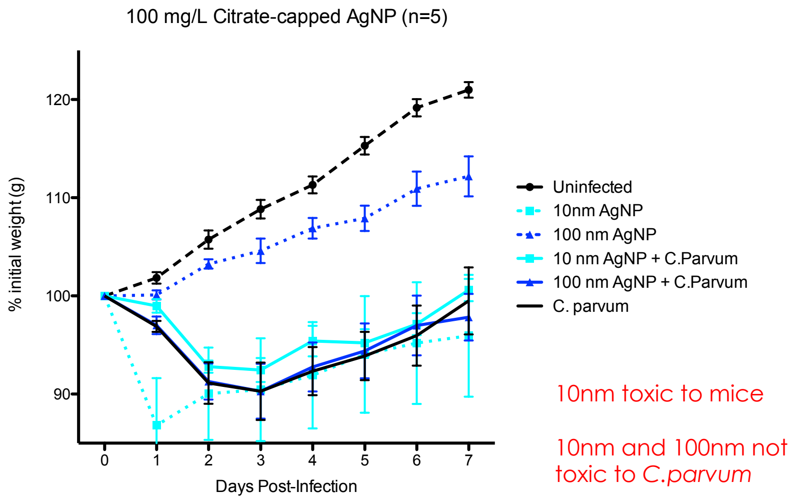


Figure 12: Weight loss post-infection. 100 mg/l of 10nm and 100nm citrate-capped AgNPs were examined.

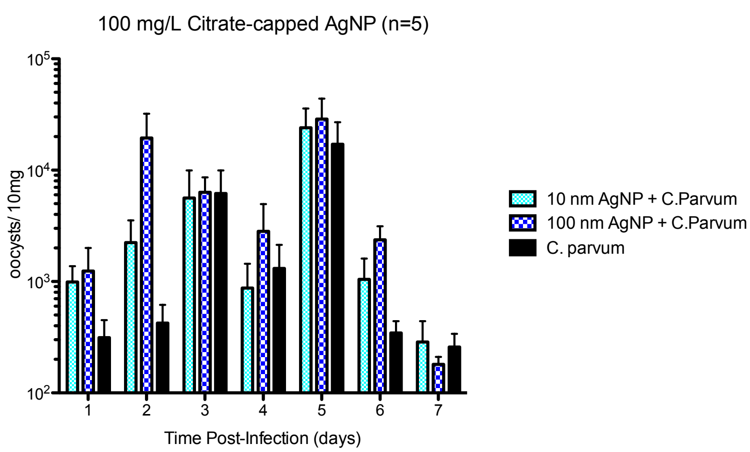
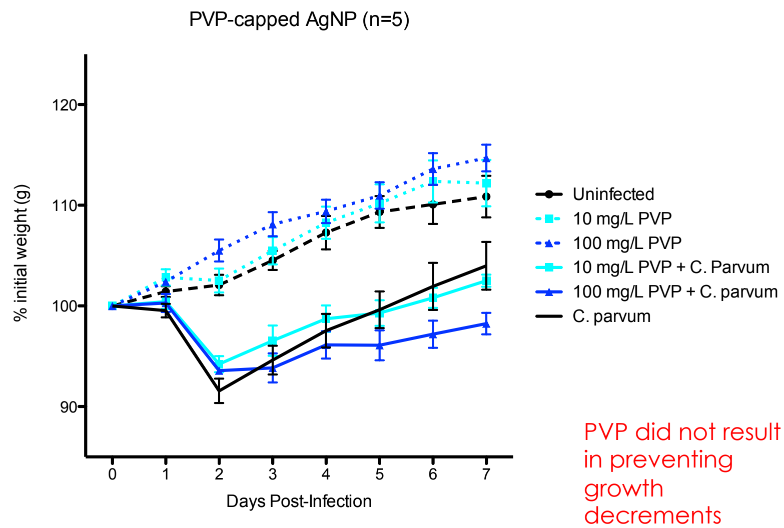
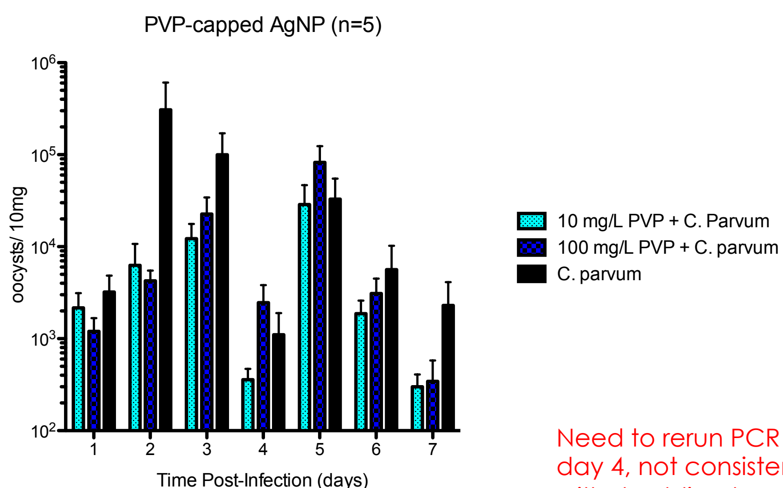


Figure 13: Shedding post-infection. 100 mg/l of 10nm and 100nm citrate-capped AgNPs were examined.



PVP did not result in preventing growth decrements

Figure 14: Weight loss post-infection. 10 and 100 mg/l PVP-capped AgNPs were examined.



Need to rerun PCR for day 4, not consistent with shedding trend

Figure 15: Weight loss post-infection. 10 and 100 mg/l PVP-capped AgNPs were examined.

Appendix D. 1 Exploratory Removal Experiments

No.	Sawdust Percentage	Sieve Size	Comments	No. of disk tested
1	20	16	Crumbled after saturation	2
2	15	16	Crumbled after saturation	2
3	10	16	Solid after saturation Tracer and Oocyst Transport Experiments Completed for solid disk	2

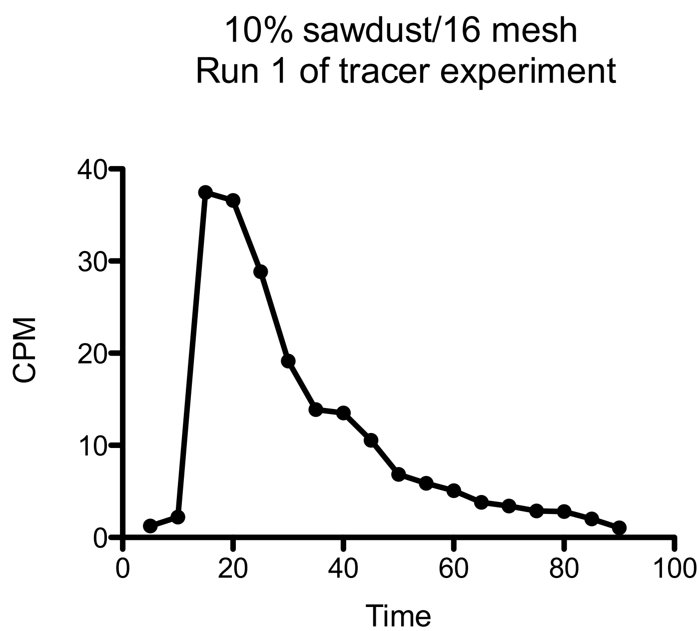


Figure 1 Tritiated water transport through ceramic disk with 10% sawdust/16 mesh seive

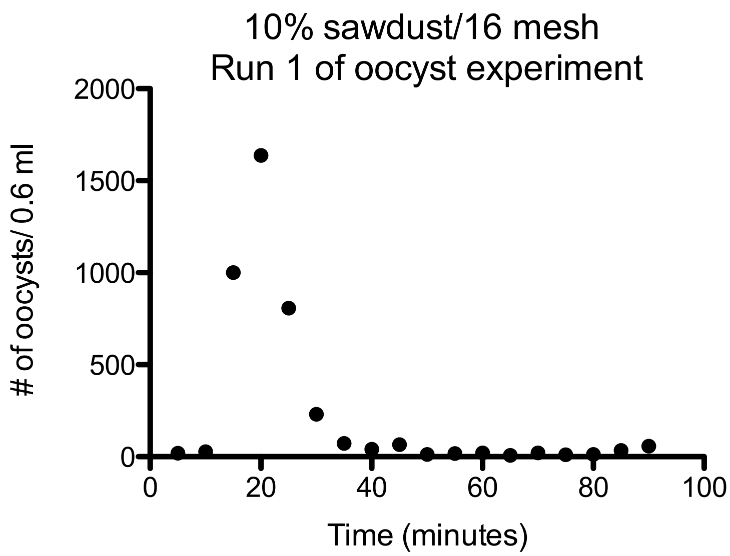


Figure 2 Oocysts transport through ceramic disk with 10% sawdust/16 mesh sieve (run #1)

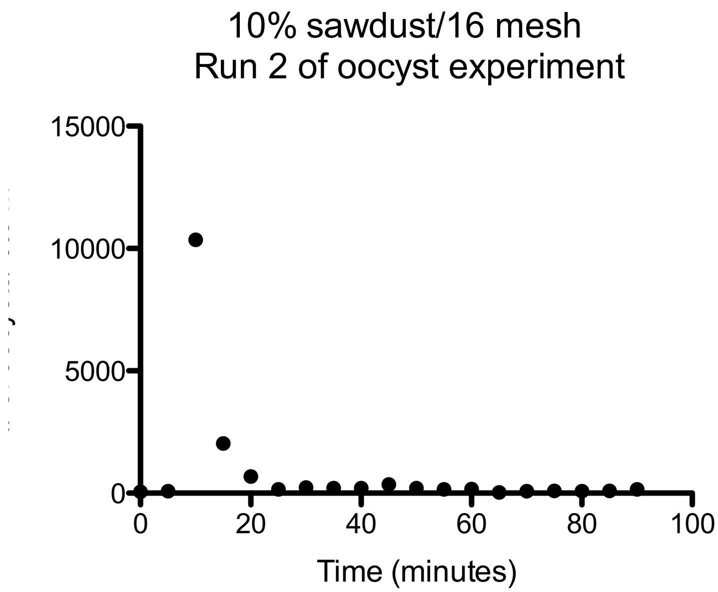


Figure 2 Oocyst transport through ceramic disk with 10% sawdust/16 mesh sieve (run #2)

Appendix E. 1 Feasibility Survey

Participant Number/ HHID______ Surveyor's Initials______ Date: ___ /___ /_____
dd /mm /yyyy

Ceramic Water Filter Factory Survey for Residents of Tshapasha and Tshibvumo

Demographics

1) Consent
   Yes
   No

2) Village

   □ Tshapasha (1)
   □ Tshibvumo (2)

3) GPS
   Latitude: ____ Longitude:____
4) Monthly Income

   _________ Less than R 850 (1)
   _________ R 850 –R 1,499 (2)
   _________ R 1,500–R 3,499 (3)
   _______ R 3,500– R7,499 (4)
   _________ R 7,500–R 9,999 (5)
   _________ R 10,000+(6)

Table 1:

Number	**Age**	**Currently in school (Y/N)- If yes, also enter number of days absent in last week**	**Number of years completed**	**Highest education level completed**	**If Adult, Occupation If child, does child receive grant money**	**What is the main cause of absenteeism from work or school**
1		Yes (1); # of days No (2)	*Open answer	Creche (1); Primary (2); Secondary (3); University (4); Other (5)	Adult: Not working (1); *Open answer Child: Yes (1); No (2)	*Open answer
2						
3						
4						
5						
6						

If there are no children 3 years of age or younger (see ages in table above), skip Table 2

Table 2: Children 3 years or younger.

Principal caregiver during the day	**Caregiver if principal caregiver is unable to care for child**	**If child is sick, who takes care of child**	**Does principal caregiver also work outside of the home (Y/N)**	**If the principal caregiver works, how many days of work were missed in the last week to care for a sick child**	**If someone other than the principal caregiver takes care of the sick child, how many days of work were missed in the past week to care for a sick child**
Mother (1) Father (2) Grandmother (3) Grandfather (4) Older sibling (5) Neighbor (6) Other (7)	Mother (1) Father (2) Grandmother (3) Grandfather (4) Older sibling (5) Neighbor (6) Other (7)	Mother (1) Father (2) Grandmother (3) Grandfather (4) Older sibling (5) Neighbor (6) Other (7)	Yes (1) No (2)	(1-7)	(1-7)

Table 3: Water Practices and Storage

Primary source for drinking water	**Secondary source for drinking water**	**What makes you change from using your primary source to your secondary source**	**Drinking water storage container (enter all container types)**	**Water storage location (Inside/ Outside) If inside, which room**	**Are the storage containers covered**	**What do you use to get the water from its storage**	**Who gets the water from its storage**	**Travel time to get water (minutes)**	**How often do you need to fetch water (days)**

Piped into house (1) Piped into yard/plot (2) Public tap/standpipe (3) Tube well/borehole (4) Protected well (5) Unprotected well (6) Protected spring (7) Unprotected spring (8) Rainwater (9) Tanker truck (10) Cart with small tank (11) Surface water (river/dam/lake/pond/stream/canal/irrigation canal) (12) Bottled water (13) Piped into neighbor's house (14) Piped into neighbor's yard/plot (15) Other (16)	Piped into house (1) Piped into yard/plot (2) Public tap/standpipe (3) Tube well/borehole (4) Protected well (5) Unprotected well (6) Protected spring (7) Unprotected spring (8) Rainwater (9) Tanker truck (10) Cart with small tank (11) Surface water (river/dam/lake/pond/stream/canal/irrigation canal) (12) Bottled water (13) Piped into neighbor's house (14) Piped into neighbor's yard/plot (15) Other (16)	*Open answer*	Ceramic vessels (1) Metal buckets (2) Plastic buckets (3) Jerrycan (4) Small pans (5) Cooking pots (6) Plastic Bottles (7) No Container (8) Other (9)	Inside (1) Outside (2) If inside, -Kitchen (1) -Dining room (2) -Bathroom (3) -Living room (4) -Other (5)	All covered (1) Some covered (2) None covered (3)	Pour directly (1) Use cup with handle (2) Use cup with hands (3) Use Spigot (4) Other (5)	Mother (1) Father (2) Grandmother (3) Grandfather (4) Child (5) Older sibling (6) Neighbor (7) Other (8)	# of minutes	# of days

Table 4: Water and Health

Have you had diarrhea (loose or watery stool) in the past week - If yes, for how long (days)	**Has anyone else in your family had diarrhea in the past week- If yes, which family members**	**What do you think caused the diarrhea**	**Do you think you can get sick from your water- If yes, what sicknesses do you think you can get**	**If you purified/treated water, would it affect frequency of diarrhea?**
Yes (1); for how long (# of days) No (2)	Yes (1); which family members No (2)	Food (1) Current medication (2) Water (3) Pathogen/Germ (4) Cold/Flu (5) Other (6)	Yes (1) No (2) If yes, check all that apply -Fever (1) -Stomach ache (2) -Vomiting (3) -Diarrhea (4) -Weight loss (5) -Malnutrition (6) -Other (7) -Do not get sick from water (8)	Diarrhea would increase (1) Diarrhea would not change (2) Diarrhea would decrease (3)

Table 5: Water Beliefs

Do you think your water is of poor quality- If yes what do you think makes the quality poor	**Do you think your water quantity is poor- If yes, what do you think makes the quantity poor**	**Have you ever discussed water quality or quantity with friends**	**Have you ever discussed water quality or quantity with friends who are not neighbors**	**Have you ever discussed water quality or quantity with** village officials**/ civic**	**Do you think that the amount of water or the quality of water is a bigger problem in your community?**	**If you have concerns about your water, who do you contact to get the problem resolved? (check all that apply)**
Yes (1); (check all that apply) -Not treated (1) -Not clean (2) - Taste (3) - Color (4) - Odor (5) - Water is not poor quality (6) No (2)	Yes (1); (check all that apply) -Limited access (1) -Intermittent Access (2) -Shared supply (3) -Other (4) -Water is not poor quantity (5) No (2)	Yes (1) No (2)	Yes (1) No (2)	Yes (1) No (2)	Amount of water (1) Quality of water (2)	Friend (1) Neighbor (2) Water Council (3) Chief (4) Municipality (5) Other (6)

Table 6: Health Practices

Do you clean your storage vessel?- If yes, how do you clean it and how often?	**Do you use soap after using the bathroom?**	**Do you use soap before cooking?**	**Do you use soap before eating?**	**Do other household members use the soap? –If yes, when?**
Yes (1) -Water from tap (1) -With soap (2) -Surface water (3) -With soap (4) -Boiled water (5) -With soap (6) -Other (7) No (2)	Yes (1) No (2)	Yes (1) No (2)	Yes (1) No (2)	Yes (1); (check all that apply) -After using the bathroom (1) -Before cooking (2) -Before eating (3) -Other (4) No (2)

Table 7: Surface Water

Have you recently bathed, waded, swum, or washed your clothes in the local freshwater source? –If yes, have you drunk straight from this source?	**If yes to either of the previous questions, have you experienced any of these symptoms after drinking or going in the water? Rash/Itchy Skin, Fever/Chills, Cough, Muscle Aches: Which ones?**	**Do you know about Bilharzia?- If yes, what do you think causes it?**
Yes (1); If yes, - Yes (1) -No (2) No (2)	Rash/Itchy Skin (1) Fever/Chills (2) Cough (3) Muscle Aches (4) Other (5) No Symptoms Experienced (6)	Yes (1); *open answer No (2)

Define water treatment technologies and give multiple examples

Table 8: Water Purification Beliefs

Have you heard of water treatment technologies- If yes, where from	**Do you think the water you use for drinking needs treatment**	**What do you think is the best way to treat water in your home**	**What, if anything, do you use to treat your water**	**If you purify your water, who drinks the purified water**	**What would be the benefit of purifying/ treating your drinking water?**	**Do you have any pets or animals around the home- If yes, what kind**
Yes (1); -Clinic (1) -Hospital (2) - Church (3) -Community Health Worker (4) -Other (5) No (2)	Yes (1) No (2)	Boil water (1) Tablets or liquid chemical (2) Filter (3) Other (4) Do not treat water (5)	Boil water (1) Tablets or liquid chemical (2) Filter (3) Other (4) Do not treat water (5)	Mother (1) Father (2) Grandmother (3) Grandfather (4) Child (5) Older sibling (6) Neighbor (7) Other (8)	Better tasting water (1) Better health (2) Other (3)	Yes (1); *open answer No (2)

Table 9: Technology Characteristics

How important are these characteristics in a product that would purify your water in your home: [Not important at all (1), A little important (2), Important (3), Very important (4), Extremely important (5)]

Quantity it can purify	**Taste of water after purification**	**Rate of purification/ Time**	**Coloration/Odor after purification**	**Ease of use for purifying water**	**Replacement time/ How long the product lasts**	**Cost**	**Locally available**	**Ease of maintenance**

Would you rather use a technology that is cheaper but has to be replaced more often or one that is more expensive but has to be replaced less often?

___Cheaper, replace more often (1)

___ More expensive, replaced less often (2)

How much would you be willing to pay, on a monthly basis, for water treatment technology? R_____

Introduce the filter and how it works. Explain the benefits and upkeep.

Table 10: Ceramic Water Filter

Would you be interested in buying a water filter?	**If you wanted to buy a filter, would you discuss it with neighbors/friends prior to purchase?**	**If your friends/neighbors used a filter, would they show you how it is used?**	**If your friends/neighbors used a filter, how likely would it be that you would use their filter as well?**	**If your friends/neighbors purchased a filter, how likely is it that would you also purchase one?**	**If you had the filter through some means (example, free distribution), how much would you like to sell it for?**
Yes (1) No (2)	Yes (1) No (2)	Yes (1) No (2)	Extremely Likely (1) Somewhat Likely (2) Neither Likely nor Unlikely (3) Somewhat Unlikely (4) Extremely Unlikely (5)	Extremely Likely (1) Somewhat Likely (2) Neither Likely nor Unlikely (3) Somewhat Unlikely (4) Extremely Unlikely (5)	Amount in Rands

Show and explain Willingness-to-Pay Payment Card

Table 11: Filter Market

How much would you be willing to pay, on a monthly basis, for water filter technology?	**If a local potter were to sell ceramic water filters, how likely is it that you would buy a filter from him/her?**	**How likely is it that you would buy a filter if a Non-Governmental Organization sold them?**	**How likely is it that you would buy a filter if a heath care provider recommended them or sold them?**	**If filters were to become available, how soon would you purchase one and why did you choose this time range?**	**How long would you be willing to wait for the water to filter before drinking it?**	**Would you like to be contacted if someone starts selling a purification technology nearby?**
Amount in Rands	Extremely Likely (1) Somewhat Likely (2) Neither Likely nor Unlikely (3) Somewhat Unlikely (4) Extremely Unlikely (5)	Extremely Likely (1) Somewhat Likely (2) Neither Likely nor Unlikely (3) Somewhat Unlikely (4) Extremely Unlikely (5)	Extremely Likely (1) Somewhat Likely (2) Neither Likely nor Unlikely (3) Somewhat Unlikely (4) Extremely Unlikely (5)	Immediately (1) Within the next 6 months (2) Within the next year (3) Within the next 2 years (4) Not in the foreseeable future (5); *Open answer	≤1 hour (1) >1-2 hours (2) >2-3 hours (3) >3-4 hours (4) >5 hours (5) I would not be willing to wait (6)	Yes (1) No (2)

For Water Collection

Consent to Take Sample:
___ Yes (1)
___ No (2)
Where did you get the water that is currently stored for drinking purposes?
___Piped into house (1)
___ Piped into yard/plot (2)
___ Public tap/standpipe (3)
___ Tube well/borehole (4)
___ Protected well (5)
___ Unprotected well (6)
___ Protected spring (7)
___ Unprotected spring (8)
___ Rainwater (9)
___ Tanker truck (10)
___ Cart with small tank (11)
___ Surface water (river/dam/lake/pond/stream/canal/irrigation canal) (12)
___ Bottled water (13)
___ Piped into neighbor's house (14)
___ Piped into neighbor's yard/plot (15)
___ Other (16)
Explanation if necessary: ______________________________

Notes about sample or source:

How long ago was this water obtained from the source? _____ days

For Households with a Child 20-28 Months old

Child Number ____
Birth Month and Year (mm/yy): ______
Height (cm): ________

Child Number ____
Birth Month and Year (mm/yy): ______
Height (cm): ________

Child Number ____
Birth Month and Year (mm/yy): ______
Height (cm): ________

Appendix E. 2 Construction

Figure 1: Street view of construction of factory (Image provided by Theresa Hackett)

Figure 2: Street view of construction of factory (Image provided by Theresa Hackett)

Figure 3: Street view of finalized factory (Image provided by Rachel Schmidt)

Figure 4: Street view of Cooperative building and filter factory (Image provided by Rachel Schmidt)

Figure 5: Foundation for original construction of Manny-kiln (Image provided by Theresa Hackett)

Figure 6: Original construction of Manny-kiln (Image provided by Theresa Hackett)

Figure 7: Original construction of Manny-kiln (Image provided by Theresa Hackett)

Figure 8: Mani-kiln modified by Peter Chartrand (Image provided by James Smith)

Appendix E. 3 Marketing Survey

Participant No.: ________________________________
UVA Student Name: ____________________________
Univen Student Name: __________________________
Date: __________________________

Personal Information

1) Age: ___________________

2) Gender:
   i) Male
   ii) Female

3) Area of Residence
   i) Thohoyandou: ________________
   ii) Outside of Thohoyandou: ___________________
   iii) Louis Trichardt: ___________________
   iv) Outside of Louis Trichardt: ___________________
   v) Other: ___________________

4) How many people live in your household? __________

5) What are their ages?
   i) Age ____
   ii) Age ____
   iii) Age ____
   iv) Age ____
   v) Age ____
   vi) Age ____

6) What is your highest level of education?
   i) Some primary
   ii) Completed primary
   iii) Some secondary
   iv) Completed secondary
   v) Some university
   vi) University degree or technical training

In-home Tap

7) Is it your primary or secondary water source?
   i) Primary
   ii) Secondary

8) How much do you pay for water per month?
   i) Less than R 25
   ii) R 25 – R 50
   iii) R 50 – R 100
   iv) Over R 100

9) How frequently is this source available?
   i) Once a week
   ii) 2 – 4 days a week
   iii) 5 – 7 days a week
   iv) Once a month

10) Do you treat your water?
   i) Yes
   ii) No

11) If yes, what is your primary method of water treatment?
   i) Boil water
   ii) Tablets or liquid chemical
   iii) Filter
   iv) Other ________________
   v) Do not treat water

12) Where do you obtain the materials, such as fuel or a purification technology, to treat your water?
   i) Tuck shop
   ii) Grocery store
   iii) Hardware store
   iv) Other ____________
   v) Do no treat water

13) Who in your household takes care of treating water?
   i) Mother
   ii) Father
   iii) Grandmother
   iv) Grandfather
   v) Children
   vi) Other Adult Household Members

Community tap

14) Is it your primary or secondary water source?
   i) Primary
   ii) Secondary

15) How much do you pay for water per month?

i) Less than R 25
ii) R 25 – R 50
iii) R 50 – R 100
iv) Over R 100

16) How frequently is this source available?
i) Once a week
ii) 2 – 4 days a week
iii) 5 – 7 days a week
iv) Once a month

17) How frequently do you collect water from the tap?
i) Once a week
ii) 2 – 4 days a week
iii) 5 – 7 days a week
iv) Once a month

18) How do you store the water?
i) Ceramic vessels
ii) Metal buckets
iii) Plastic buckets
iv) Jerrycan
v) Small pans
vi) Cooking pots
vii) Plastic Bottles
viii) Other, Specify: __________________

19) Do you treat your water?
i) Yes
ii) No

20) What is your primary method of water treatment?
i) Boil water
ii) Tablets or liquid chemical
iii) Filter
iv) Other ________________
v) Do not treat water

21) Where do you obtain the materials, such as fuel or a purification technology, to treat your water?
i) Tuck shop
ii) Grocery store
iii) Hardware store
iv) Other ____________
v) Do no treat water

22) Who in your household takes care of treating water?
    i) Mother
    ii) Father
    iii) Grandmother
    iv) Grandfather
    v) Children
    vi) Other Adult Household Members

If you use a well/borehole

23) Is it your primary or secondary water source?
    i) Primary
    ii) Secondary

24) Who owns the well/borehole?
    i) Municipality
    ii) Chief/Tribal council
    iii) I own it
    iv) Neighbor
    v) A collective group of neighbors

25) Do you pay a monthly fee?
    i) Yes
    ii) No

26) If yes, how much?
    i) Less than R 25
    ii) R 25 – R 50
    iii) R 50 – R 100
    iv) Over R 100

27) To whom do you pay?
    i) Municipality
    ii) Chief/Tribal council
    iii) I am paying it off
    iv) Neighbor
    v) A collective group of neighbors

28) How frequently is this source available?
    i) Once a week
    ii) 2 – 4 days a week
    iii) 5 – 7 days a week
    iv) Once a month

29) How frequently do you collect water from the tap?
    i) Once a week

ii) 2 – 4 days a week
iii) 5 – 7 days a week
iv) Once a month

30) How do you store the water?
i) Ceramic vessels
ii) Metal buckets
iii) Plastic buckets
iv) Jerrycan
v) Small pans
vi) Cooking pots
vii) Plastic Bottles
viii) Other, Specify: __________________

31) Do you treat your water?
i) Yes
ii) No

32) What is your primary method of water treatment?
i) Boil water
ii) Tablets or liquid chemical
iii) Filter
iv) Other _________________
v) Do not treat water

33) Where do you obtain the materials, such as fuel or a purification technology, to treat your water?
i) Tuck shop
ii) Grocery store
iii) Hardware store
iv) Other _____________
v) Do no treat water

34) Who in your household takes care of treating water?
i) Mother
ii) Father
iii) Grandmother
iv) Grandfather
v) Children
vi) Other Adult Household Members

If you use a surface water source

35) Is it your primary or secondary water source?
i) Primary
ii) Secondary

36) Do you pay a monthly fee?
   i) Yes
   ii) No

37) If yes, how much?
   i) Less than R 25
   ii) R 25 – R 50
   iii) R 50 – R 100
   iv) Over R 100

38) To whom?
   i) Municipality
   ii) Chief/Tribal council
   iii) I am paying it off
   iv) Neighbor
   v) A collective group of neighbors

39) How frequently is this source available?
   i) Once a week
   ii) 2 – 4 days a week
   iii) 5 – 7 days a week
   iv) Once a month

40) How frequently do you collect water?
   i) Once a week
   ii) 2 – 4 days a week
   iii) 5 – 7 days a week
   iv) Once a month

41) How do you store the water?
   i) Ceramic vessels
   ii) Metal buckets
   iii) Plastic buckets
   iv) Jerrycan
   v) Small pans
   vi) Cooking pots
   vii) Plastic Bottles
   viii) Other, Specify: __________________

42) Who in your household collects water from this source?
   i) Mother
   ii) Father
   iii) Grandmother
   iv) Grandfather
   v) Children

vi) Other Adult Household Members

43) Do you treat your water?
i) Yes
ii) No

44) What is your primary method of water treatment?
i) Boil water
ii) Tablets or liquid chemical
iii) Filter
iv) Other ________________
v) Do not treat water

45) Where do you obtain the materials, such as fuel or a purification technology, to treat your water?
i) Tuck shop
ii) Grocery store
iii) Hardware store
iv) Other ____________
v) Do no treat water

46) Who in your household takes care of treating water?
i) Mother
ii) Father
iii) Grandmother
iv) Grandfather
v) Children
vi) Other Adult Household Members

Water and Health

47) Do you think your water is poor quality?
i) Yes
ii) No
48) Where have you learned about water quality?
i) Clinic
ii) School
iii) Government program
iv) Government office
v) Chief
vi) Friend
vii) Advertisements
viii) Other: _________________________

49) Have you learned about water treatment?

i) Yes
ii) No

50) If yes, from where?
i) Clinic
ii) School
iii) Government program
iv) Government office
v) Chief
vi) Friend
vii) Advertisements
viii) Other: ________________________

51) If no, would you like to learn more about water treatment?
i) Yes
ii) No

52) How would you like to receive this information?
i) Pamphlet
ii) Live demonstration
iii) Video
iv) Advertisements
v) School
vi) Community assembly, etc.

53) Where do you learn about health issues/solutions?
i) Clinic
ii) Hospital
iii) Elders
iv) Friend
v) Advertisements
vi) Government program
vii) Other: ________________________

Community and Distribution

54) Where do you do your shopping?
i) Thohoyandou
ii) Louis Trichardt
iii) Polokwane

55) Type of store
i) Tuck shop
ii) Street market
iii) Outdoor market in Thohoyandou
iv) Complex in Thohoyandou
v) Louis Trichardt
vi) Other _______________

56) If you don't live in Thohoyandou or Louis Trichardt, how often do you come to the city to shop?
    i) Daily
    ii) Weekly
    iii) Bi-weekly
    iv) Monthly
    v) Other ____________

57) What is your primary reason for shopping in the city instead of closer to home?
    i) Groceries
    ii) Hardware
    iii) Electronics

58) How do you get your goods back home after shopping?
    i) Bus
    ii) Taxi
    iii) By foot
    iv) Personal vehicle
    v) By delivery

59) What sort of community gatherings do you attend?
    i) Church
    ii) Women's groups
    iii) Local government
    iv) Citizens' associations
    v) Child's school
    vi) Other: _________________________

60) Where do you learn about new products?
    i) When I see them at the store
    ii) Displays at the store
    iii) Advertisements
    iv) Magazine/ mailings
    v) Radio
    vi) TV
    vii) Friends or neighbors
    viii) Community events
    ix) Chief