WATER STORAGE IN RURAL HOUSEHOLDS: INTERVENTION STRATEGIES TO PREVENT WATERBORNE DISEASES

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WATER STORAGE IN RURAL HOUSEHOLDS: INTERVENTION STRATEGIES TO PREVENT WATERBORNE DISEASES

by

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I, the undersigned, declare that the thesis hereby submitted to the University of Pretoria for the degree PhD (Medical Virology) and the work contained therein is my own original work and has not previously, in its entirely or in part, been submitted to any university for a degree.

Signed _____, this the _____ day of _____ 2007.

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DEDICATION

Just enough light

Sometimes only the step I'm on,
or the very next one ahead,
is all that is illuminated for me.

God gives just the amount of light I need
for the exact moment I need it.

At those times I walk in surrender to faith,
unable to see the future,
and not fully comprehending the past.

And because it is God who has given me
what light I have,
I know I must reject the fear and doubt
that threaten to overtake me.

I must determine to be content where I am,
and allow God to get me where I need to go.

I walk forward,
one step at a time,
fully trusting that the light God sheds,
is absolutely sufficient.

(Stormie Omartian, 1999)

I dedicate this work to my Lord and Saviour, Jesus Christ
He is shaping and building my character each second of my life.

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SUMMARY

Poor sanitation, unhygienic practices and close living associations between people and animals in rural communities increase the risk of zoonoses and add to faecal contamination of stored drinking water. Point-of-use interventions can improve the microbiological quality of household drinking water and a combination of microbial and chemical indicator tests could identify the origin of faecal pollution. The improvement of the microbiological quality of drinking water in rural households by the implementation of intervention strategies which included the use of traditional storage containers as well as an improved safe storage container (CDC, USA), with or without the addition of a sodium hypochlorite solution were determined. The origin of faecal contamination in the water sources and household stored water were determined using male specific F-RNA subgroup genotyping. This study attempted to assess the survival of indicator microorganisms and selected bacterial pathogens and viruses in the improved safe storage container in borehole and river water samples.

An intervention study was conducted in two rural villages utilising different source water. Results indicated that the improved safe storage container without the addition of a stabilized sodium hypochlorite solution did not improve the microbiological quality of the stored drinking water and had counts of indicator microorganisms similar to that found in the traditional storage containers. However, the households using the 1% and the 3.5% sodium hypochlorite solutions have shown an effective reduction in the counts of indicator microorganisms in both the traditional and the improved safe storage containers. The compliance with the use of the sodium hypochlorite interventions ranged between 60% and 100%, which was in agreement with similar studies carried

out in other developing countries. One village complied with the intervention while the other village did not. Reasons for this included financial factors, an unsupportive infrastructures and lack of education and knowledge on health risks by the households.

Male specific F-RNA bacteriophage genotyping showed that faecal contamination in the water source samples and both the traditional and improved safe storage containers at the point-of-use were primarily of animal origin (Subgroup I). Households using river water had subgroup II F-RNA bacteriophages present in the stored household water, which was associated with human faecal pollution. However, subgroup II F-RNA bacteriophages has been isolated from faeces of cattle and poultry, which indicated that F-RNA subgroup typing might not be a specific tool to determine the origin of faecal pollution in water sources.

Laboratory seeding experiments indicated that 1% sodium hypochlorite solution were less effective in reducing heterotrophic bacteria, *Escherichia coli*, *Salmonella typhimurium*, *Clostridium perfringens*, F-RNA bacteriophages and coxsackie B1 virus counts in the improved safe storage containers filled with river water with a high turbidity. However, the 1% sodium hypochlorite solution did reduce the indicator and seeded microorganisms within 60 min in containers filled with borehole water with a low turbidity. The 3.5% sodium hypochlorite solution effectively decreased the numbers of microorganisms to undetectable limits within 60 min in both the borehole and river filled storage containers irrespective of the turbidity values. This study has showed that a combination of intervention strategies can provide rural communities with microbiologically safe drinking water.

Keywords: improved safe storage container, F-RNA genotyping, intervention strategies, microbiological quality; compliance, sustainability, sodium hypochlorite solution, waterborne diseases.

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DIE STOOR VAN WATER IN PLATTELANDSE HUISHOUDINGS: INTERVENSIE STRATEGIEË OM WATEROORDRAAGBARE SIEKTES TE VOORKOM

deur

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OPSOMMING

Swak sanitasie, higiene en ‘n noue verblyf verhouding tussen mense en diere in plattelandse gemeenskappe verhoog die oordrag van soonosis en dra by tot die fekale besoedeling van gestoorde drinkwater. Intervensies in die huishoudings en ‘n kombinasie van chemiese en mikrobiologiese indikatore toetse kan moontlik ‘n aanduiding gee van die oorsprong van fekale besoedeling. Verbeteringe in die mikrobiologiese kwaliteit van die huishoudelike drinkwater met die instelling van intervensies soos ‘n verbeterde huishoudelike stoorhouer (CDC, VSA) en die gebruik van ‘n natrium hipochloriet oplossing was ondersoek. Die oorsprong van die fekale besoedeling van die water was bepaal deur gebruik te maak van molekulêre hibridisasie van die F-RNA bakteriofaag isolate. Hierdie studie het ook die oorlewing van indikator en geselekteerde patogene mikroorganismes in die verbeterde huishoudelike stoorhouer gevul met boorgat- en rivierwatermonsters bepaal.

‘n Intervensie studie in twee plattelandse dorpies met verskillende waterbronne was onderneem. Die resultate het gewys dat die verbeterde huishoudelike stoorhouers sonder die gestabiliseerde natrium hipochloriet oplossing het nie die mikrobiologiese kwaliteit van die gestoorde water in die huishoudings verbeter nie en het dieselfde mikrobiologiese tellings getoon as die traditionele stoorhouers. Desnieteenstaande het die houers waarby die 1% en die 3.5% natrium hipochloriet oplossings gevoeg is, bewys dat die mikrobiologiese tellings van indikator organismes afgeneem het in beide die verbeterde huishoudelike en die traditionele stoorhouers. Die gebruik van die

natrium hipochloriet oplossings in die huishoudings het gewissel tussen 60% en 100% wat in ooreenstemming was met soortgelyke studies in ander ontwikkelende gemeenskappe. Die intervensie was volhoubaar met een van die studiegroepe maar nie met die ander studiegroep nie. Redes hiervoor het faktore soos onvoldoende finansies, swak infrastrukture en onvoldoende kennis aangaande gesondheids risikos in die huishoudings ingesluit.

Die manlik spesifieke F-RNA bakteriofaag geentipering het bewys dat fekale besoedeling hoofsaaklik van dierlike oorsprong (supgroep I) was in die waterbronne en ook in beide die verbeterde huishoudelike en die traditionele stoorhouers. Huishoudings wat water vanaf die rivier gebruik het, het ook supgroep II faag isolate gehad wat gassososieer word met menslike fekale oorsprong. Nie te wel, supgroep II faag isolate is al geïsoleer uit beeste en pluimvee se mis monsters en dit bewys dat F-RNA bakteriofaag molekulêre hibridisasie nie sodanig ‘n spesifiek genoeg metode is om te gebruik om die oorsprong van fekale besoedeling in watermonsters te bepaal nie.

Oorlewings studies in die laboratorium het bewys dat 1% natrium hipochloriet oplossing nie effektief was om *Escherichia coli, Salmonella typhimurium, Clostridium perfringens,* F-RNA bakteriofage en coxsackie B1 virus tellingsin die verbeterde huishoudelike stoorhouers wat gevul was met rivierwater met ‘n hoë turbiditeit, te verminder nie. Die 1% natrium hipochloriet oplossing het wel die tellings van indikatore en geselekteerde patogene in boorgatwater met ‘n lae turbiditeit binne 60 min verminder. Die 3.5% natrium hipochloriet oplossing het suksesvol die tellings van indikatore en geselekteerde patogene in beide rivier- en boorgatwater binne 60 min verminder ongeag die turbiditeits waardes van die waterbronne. Hierdie studie het bewys dat ‘n kombinasie van intervensie strategiëe wel mikrobiologies veilige drinkwater kan verskaf aan plattelandse gemeenskappe.

Kern woorde: verbeterde huishoudelike stoorhouer, F-RNA molekulêre hibridisasie, intervensie strategiëe, gebruike; volhoubaarheid; mikrobiologiese kwalitiet; natrium hipochloriet oplossing, wateroordraagbare siektes.

LIST OF ABBREVIATIONS

AFLP	-	Amplified Fragment Length Polymorphism
AMV	-	Avian Myeloblastosis Virus
AOC	-	Assimilable Organic Carbon
ARDRA	-	Amplified Ribosomal DNA Restriction Analysis
ATCC	-	American Type Culture Collection
BGM	-	Buffalo Green Monkey
ºC	-	degrees Celcius
C. perfringens	-	*Clostridium perfringens*
$CaCl_2.2H_2O$	-	Calsium Chloride
CaCo-2	-	colonic epithelial carcinoma continuous cell line
CDC	-	Centre for Disease Control
CDP	-	disodium 2-chloro-5-4 (methoxyspiro{1,2-dioxetane-3,2'-5'-chloro) tricycle [3.3.1.1.3.7] decan}-4-yl)-1-phenyl phosphate
cfu	-	colony forming unit (s)
$CH_3COOHNa$	-	Sodium Acetate
cm	-	centimeter
CO_2	-	Carbon Dioxide
DIG	-	Digoxigenin
dNTP	-	dideoxy Nucleotide Tri-Phosphate
DNA	-	Deoxy Ribonucleic Acid
DOH	-	Department of Health
DPD	-	N, N-diethyl-phenylenediamine
DWAF	-	Department of Water Affairs and Forestry
E. coli	-	*Escherichia coli*
EMEM	-	Eagle's Minimum Essential Media
ERIC-PCR	-	Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction
FRhK-4R	-	Foetal Rhesus Monkey Kidney continuous cell line
FWA	-	Fluorescent Whitening Agents
g	-	gram
$g.cm^{-3}$	-	gram per cubic square meter
GPS	-	Global Positioning Satelite
h	-	hour
HAV	-	Hepatitis A Virus
HCl	-	Hydrochloric Acid
HH	-	Household
ISO	-	International Standardization Organization

ITS-PCR	-	Internal Transcribed Spacer Polymerase Chain Reaction
KCl	-	potassium chloride
km	-	kilometer
l	-	litre
LAB	-	Long Chain Alkylbenzenes
mg	-	milligram
$MgCl_2$	-	Magnesium Chloride
min	-	min
ml	-	millilitre
mm	-	millimeter
mM	-	milli Molar
MUG	-	4-methyl-umbelliferyl-β-D-glucuronidase
ng	-	nanogram
NaCl	-	Sodium Chloride
NaOH	-	Sodium Hydroxide
NCTC	-	National Culture Typing Collection
NGO	-	Non Govermental Organisation
nm	-	nanometer
NTU	-	Nephelometric Turbidity Units
PAHO	-	Pan American Health Organization
PBS	-	Phosphate Buffered Saline
PCA	-	Plate Count Agar
PCR	-	Polymerase Chain Reaction
PEG	-	Polyethylene Glycoll
PFGE	-	Pulsed Field Gel Electrophoresis
pfu	-	plaque forming unit (s)
PLC/PRF/5	-	Primary Liver Carcinoma continuous cell line
pmol	-	picomol
%	-	percentage
RFLP	-	Restriction Fragment Length Polymorphism
RNA	-	Ribonucleic Acid
rpm	-	revolutions per minute
RSA	-	Republic of South Africa
RT-PCR	-	Reverse Transcriptase Polymerase Chain Reaction
s	-	second
SABS	-	South African Bureau of Standards
SDS	-	Sodium Dodecyl Sulfate
SSC	-	Saline Sodium Citrate
S. typhimurium	-	*Salmonella typhimurium*

STP	-	Sodium Tri Polyphosphate
Temp	-	Temperature
Turb	-	Turbidity
U	-	Unit (s)
µg	-	microgram
µl	-	microlitre
µm	-	micrometer
UN	-	United Nations
UK	-	United Kingdom
USA	-	United States of America
WHO	-	World Health Organization

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PUBLISHED REPORTS:

Grabow WOK, Taylor MB, Viviers JC, **Potgieter N** and Gaobepe MG (2003) The health impact of waterborne viruses and methods of control in high risk communities. WRC report 743/1/02. Water Research Commission, Pretoria, South Africa.

SUBMITTED PUBLICATIONS:

Potgieter N and Ehlers MM (2007) Inactivation of pathogens and indicator organisms in drinking water stored in an improved household storage container. To be submitted for publication to: *Journal of Population, Health and Nutrition.*

Potgieter N, Vrey A and Ehlers MM (2007) Determination of origin of faecal polution in stored drinking water from rural households in South Africa using male specific F-RNA bacteriophage subgroup typing. To be submitted for publication to: *Water SA.*

Potgieter N, Becker PJ and Ehlers MM (2007) Evaluation of intervention strategies to improve the microbiological quality of stored drinking water in rural communities in South Africa. To be submitted for publication to: *Journal of Water and Health.*

Potgieter N, Becker PJ and Ehlers MM (2007) Sustainability of a water quality intervention in rural communities in South Africa. To be submitted for publication to: *Journal of Water and Health.*

CONFERENCE CONTRIBUTIONS:

Potgieter N, Very A, Mavhungu NJ, Mushau FMG, Musie E, Du Toit PJ and Grabow WOK (2000) The quality of water supply, handling and usage in Venda, South Africa. Oral presentation at the WISA Biennial Conference, 28 May-1 June, Sun City, South Africa.

Potgieter N, Musie E, Obi CL and Du Toit PJ (2000) Evaluation of different growth media for the recovery of sulfide reducing anaerobic *Clostridium perfringens* from the environment. Poster presentation at the WISA Biennial Conference, 28 May-1 June, Sun City, South Africa.

Potgieter N, Mamathunstha LP, Very A, Obi CL and Grabow WOK (2002) The microbiological quality of water at the source point and the point-of-use in rural households of South Africa. Oral presentation at the WISA Bienniel Conference, 19-23 May, Durban, South Africa.

Potgieter N, Obi CL, Mushau FMG, Bessong PO, Igumbor EO and Grabow WOK (2003) The use of F-RNA coliphages serotyping to determine the origin of faecal contamination in drinking water stored in rural household containers. Poster presentation at the International Symposium on Health-Related Water Microbiology, 14-19 September, Cape Town, South Africa.

Potgieter N, Ehlers MM and Grabow WOK (2003) Inactivation of pathogens and indicator organisms in drinking water stored in rural household containers. Oral presentation at the International Symposium on Health-Related Water Microbiology, 14-19 September, Cape Town, South Africa.

Potgieter N, Becker PJ and Ehlers MM (2005) Evaluation of intervention strategies to improve the microbiological quality of stored drinking water in rural communities in South Africa. Oral presentation at the 26th African Health Sciences Congress, 28 November-01 December, Ain Soukhia, Egypt.

Potgieter N and Ehlers MM (2006) Inactivation of pathogens and indicator microorganisms in drinking water stored in an improved household storage container. 20-22 February 2006. EnviroWater Conference, Stellenbosch, South Africa.

Chapter 1

INTRODUCTION

Waterborne diseases due to faecal pollution of human and animal origin, are responsible for approximately 2.2 million deaths annually in children under the age of five years in developing countries (WHO, 2002a; WHO, 2002b). Most of these deaths are due to inadequate potable water supplies, poor hygiene practices and insufficient sanitation infrastructures (Sobsey, 2002; WHO, 2002a; WHO 2002b). The World Health Organization (WHO) estimated that 1.2 billion of the world's population lack access to safe drinking water and these people use any source of water, usually the most convenient source, regardless of its quality (WHO, 2002a).

In many developing communities it is impossible to supply every household with an in-house tap due to economical reasons. A standpipe on the dwelling or a tap inside the house will reduce the need for storing water supplies and therefore decrease the risk of infections associated with stored water supplies (Jagals *et al.*, 1999). However, the provision of treated drinking water from standpipes is not sufficient to ensure safe drinking water, since water storage containers are often not cleaned properly or protected from contamination such as dirty hands, improper handling practices, dirty utensils, dust, animals, birds or insects (Esrey and Habicht, 1986; Daniels *et al.*, 1990; Mintz *et al.*, 1995; Reiff *et al.*, 1996; Genthe *et al.*, 1997; CDC, 2001; White *et al.*, 2002; WHO, 2002a; WHO, 2002b).

In order to improve the microbiological quality of water consumed by members of rural households, it is essential to address the quality of stored drinking water and the conditions under which the water supplies are stored. Several technologies for the treatment of household water in developing countries have been developed to improve the microbiological quality of the water and to reduce waterborne diseases (Mintz *et al.*, 1995; CDC, 2001; Sobsey, 2002). These technologies include physical methods such as boiling, heating, sedimentation, filtration, exposure to ultraviolet radiation from sunlight and chemical disinfection with agents such as sodium hypochlorite (Gilman and Skillicorn, 1985; Mintz *et al.*, 1995; Conroy *et al.*, 1996; CDC, 2001; Sobsey, 2002).

The Centers for Disease Control and Prevention (CDC) and the Pan American Health Organization (PAHO), have designed a 20 litre storage container to decrease the risk of contamination during storage (Mintz *et al*., 1995; Reiff *et al*., 1996; CDC, 2001; Sobsey, 2002). This container has been evaluated and implemented in various parts of the world including South America (Bolivia, Ecuador, Nicaragua, Guatamala and Peru), Eastern Europe (Uzbekistan), the Indian subcontinent (Pakistan and Bangladesh), and Africa (Kenya, Uganda, Madagascar, Malawi, Guinea-Bisseau and Zambia) (Quick *et al*., 1996; Luby *et al*., 1998; Macy and Quick, 1998; Semenza *et al*., 1998; Sobel *et al*., 1998; Daniels *et al*., 1999; Quick *et al*., 1999; Sobsey, 2002; Sobsey *et al*., 2003). In all of these studies it was found that the container together with a sodium hypochlorite solution improved the microbiological quality of the water (Quick *et al*., 1996; Luby *et al*., 1998; Macy and Quick, 1998; Semenza *et al*., 1998; Quick *et al*., 1999; Sobsey *et al*., 2003).

Previous studies to determine the microbiological quality of household stored water have mostly focused on the detection of indicator organisms such as heterotrophic plate counts, total coliforms, faecal/thermotolerant coliforms, *Escherichia coli* (*E. coli*) and faecal enterococci which indicated the presence of faecal pollution of water samples (Quick *et al*. 1996; Luby *et al*., 1998; Macy and Quick, 1998; Semenza *et al*., 1998; Quick *et al*., 1999; Momba and Mnqumevu, 2000; Momba and Kaleni, 2002; Sobsey, 2002; Momba and Notshe, 2003). However, these indicator organisms have shortcomings in assessing the microbiological safety of water, since some of the indicators concerned can multiply in stored water supplies while waterborne pathogens cannot (Goyal *et al.*, 1979; Echeverria *et al.*, 1987; Fujioka *et al.*, 1988; Pinfold, 1990; Grabow, 1996; Handzel, 1998). Furthermore, these indicators are not specific and sensitive enough to indicate the presence of certain pathogenic microorganisms such as viruses and protozoan parasites (Goyal *et al.*, 1979; Echeverria *et al.*, 1987; Fujioka *et al.*, 1988; Pinfold, 1990; Grabow, 1996; Handzel, 1998).

In addition, people in rural communities live in close contact with domestic animals and pets, which drink from and defecate in the same primary water sources used by these communities for drinking water. This increases the risk of faecal contamination of the water (Theron and Cloete, 2002; Hackett and Lappin, 2003). Although most microbial pathogens are species specific, a few animal pathogens have been associated with

zoonotic infections (Meslin, 1997; Sinton *et al*., 1998; Franzen and Muller, 1999; Slifko *et al*., 2000; Enriquez *et al*., 2001; Hoar *et al*., 2001; Leclerc *et al*., 2002; Theron and Cloete, 2002; Hackett and Lappin, 2003). However, faecal pollution from human origin constitutes a greater health threat to consumers compared to animal faecal pollution, due to the possible presence of pathogenic microorganisms (Sinton *et al*., 1998).

The most commonly used faecal indicator microorganisms namely total coliform bacteria, thermotolerant coliform bacteria, *E. coli* and faecal enterococci, are found in both human and animal faeces, but do not allow to differentiate between human and animal faecal pollution (Sinton *et al*., 1998). However, studies have indicated that specific genotypes of male specific F-RNA bacteriophages are excreted by either humans or animals, and may be used to distinguish between faecal pollution of human and animal origin (Uys, 1999; Schaper *et al*., 2002a). Since male specific F-RNA genotyping may provide an indication of the origin of pathogens present, it could be used to determine the infection risk to the communities. This can assist in the implementation of preventative measures to control the transmission of waterborne diseases (Uys, 1999; Schaper *et al*., 2002b).

Currently, no meaningful information is available concerning the survival of waterborne pathogens such as bacterial pathogens, viruses and protozoan parasites during water storage practices in both traditional water storage containers and the CDC safe storage container in areas where communities have to use polluted water as their water source (Sobsey, 2002). A laboratory study by Momba and Kaleni (2002) have investigated the regrowth and survival of *Salmonella* spp, *Clostridium perfringens* (*C. perfringens*) bacteria, as well as somatic and male specific F-RNA bacteriophages on the surfaces of polyethylene and galvanized steel household storage containers used by rural communities in the Eastern Cape Province of South Africa. The results from this study have showed that both types of storage containers supported the growth and survival of these microorganisms for 48 h (Momba and Kaleni, 2002).

The present study focused on rural communities in the Vhembe region of the Limpopo Province, South Africa and investigated the microbiological quality of drinking water in rural households, evaluated the implementation, compliance and sustainability of intervention strategies such as the CDC safe storage container and chlorine practices,

assessed the survival of selected pathogens and investigated sources of faecal contamination in household stored water.

The objectives of this study were:

1. To assess an intervention strategy to improve the drinking water quality in rural households by:
   - Determining whether the household drinking water could be safely stored in the CDC safe storage container;
   - Determining the improvement of the microbiological quality of stored drinking water with the addition of a sodium hypochlorite solution;
   - Determining compliance of rural house households with the intervention strategy (improved storage container with addition of sodium hypochlorite solution);
   - Determining the sustainability of the intervention protocol.
2. To distinguish between faecal pollution of animal or human origin using molecular typing of male specific F-RNA bacteriophage subgroups isolated from water stored in the traditional household containers and the CDC safe storage container.
3. To determine the survival of selected indicator organisms (heterotrophic bacteria, total coliforms, faecal coliforms, faecal enterococci, *E. coli*, *C. perfringens*, somatic and male specific F-RNA bacteriophages) and selected waterborne pathogens (*Salmonella typhimurium*, vaccine strain of Poliovirus type 1 and Coxsackie B1 virus) in the CDC safe storage container using laboratory based seeding experiments. (Although a vaccine strain of Poliovirus was included in the original protocol, studies were excluded due to the global Poliovirus-containment).

Chapter 2

LITERATURE REVIEW

2.1 INTRODUCTION

The United Nations (UN) set a goal in their Millennium Declaration to reduce the amount of people without safe drinking water by half in the year 2015 (UN, 2000). Safe drinking water for human consumption should be free from pathogens such as bacteria, viruses and protozoan parasites, meet the standard guidelines for taste, odour, appearance and chemical concentrations, and must be available in adequate quantities for domestic purposes (Kirkwood, 1998). However, inadequate sanitation and persistent faecal contamination of water sources is responsible for a large percentage of people in both developed and developing countries not having access to microbiologically safe drinking water and suffering from diarrhoeal diseases (WHO, 2002a; WHO, 2002b). Diarrhoeal diseases are responsible for approximately 2.5 million deaths annually in developing countries, affecting children younger than five years, especially those in areas devoid of access to potable water supply and sanitation (Kosek *et al.*, 2003; Obi *et al*., 2003; Lin *et al*., 2004; Obi *et al*., 2004).

Political upheaval, high numbers of refugees in some developing countries, and the global appearances of squatter camps and shanty rural towns, which lack proper sanitation and water connections, have contributed to conditions under which disease causing microorganisms can replicate and thrive (Leclerc *et al*., 2002; Sobsey, 2002; Theron and Cloete, 2002). The people most susceptible to waterborne diseases include young children, the elderly, people suffering from malnutrition, pregnant woman, immunocompromised individuals, people suffering from chemical dependencies and persons predisposed to other illnesses like diabetes (Sobsey *et al*., 1993; Gerba *et al*., 1996; Grabow, 1996; Leclerc *et al*., 2002; Theron and Cloete, 2002). Furthermore, an increasing number of people are becoming susceptible to infections with specific pathogens due to the indiscriminate use of antimicrobial drugs, which have lead to the selection of antibiotic resistant bacteria and drug resistant protozoa (WHO, 2002c; NRC, 2004).

In developing countries, many people are living in rural communities and have to collect their drinking water some distances away from the household and transport it back in various types of containers (Sobsey, 2002). Microbiological contamination of the water may occur between the collection point and the point-of-use in the household due to unhygienic practices causing the water to become a health risk (Sobsey, 2002; Gundry *et al*., 2004; Moyo *et al*., 2004).

To improve and protect the microbiological quality and to reduce the potential health risk of water to these households, intervention strategies is needed that is easy to use, effective, affordable, functional and sustainable (CDC, 2001; Sobsey, 2002). Many different water collection and storage systems have been developed and evaluated in the laboratory and under field conditions (Sobsey, 2002). In addition, a variety of physical and chemical treatment methods to improve the microbiological quality of water are available (Sobsey, 2002). The aim of this study was to improve the microbiological quality of drinking water in rural households by the implementation of intervention strategies which include the use of traditional storage containers as well as the CDC safe storage container, with or without the addition of a sodium hypochlorite solution at the point-of-use.

2.2 WATERBORNE DISEASES

Many infectious diseases are associated with faecally contaminated water and are a major cause of morbidity and mortality worldwide (Leclerc *et al*., 2002; Theron and Cloete, 2002). Waterborne diseases are caused by enteric pathogens such as bacteria, viruses and parasites (Table 2.1) that are transmitted by the faecal oral route (Grabow, 1996; Leclerc *et al*., 2002; Theron and Cloete, 2002). Waterborne spread of infection by these pathogenic microorganisms depends on several factors such as: the survival of these microorganisms in the water environment, the infectious dose of the microorganisms required to cause a disease in susceptible individuals, the microbiological and physico-chemical quality of the water, the presence or absence of water treatment and the season of the year (Deetz *et al*., 1984; Leclerc *et al*., 2002; Theron and Cloete, 2002).

Table 2.1 **Waterborne pathogens and their associated diseases (Bifulco *et al.*, 1989; Grabow, 1996; WHO, 1996a; Guerrant, 1997; Leclerc *et al.*, 2002; Theron and Cloete, 2002; Yatsuyanagi *et al.*, 2003; NRC, 2004)**

Pathogen		Diseases
Bacteria	*Campylobacter* spp.	Diarrhoea and acute gastroenteritis
	Enteropathogenic *Escherichia coli*	Diarrhoea
	Escherichia coli O157:H7	Bloody diarrhoea and haemolytic uremic syndrome
	Salmonella spp.	Typhoid fever, diarrhoea
	Shigella spp.	Dysentery, diarrhoea
	Vibrio cholera	Cholera, diarrhoea
	Yersinia spp.	Diarrhoea, gastrointestinal infections
Viruses	Adenoviruses	Diarrhoea, respiratory disease, conjunctivitis
	Astroviruses	Diarrhoea
	Coxsackie viruses (Enterovirus)	Respiratory, meningitis, diabetes, diarrhoea, vomiting, skin rashes
	Echoviruses (Enterovirus)	Meningitis, diarrhoea, myocarditis
	Enteroviruses 68-71	Meningitis, diarrhoea, respiratory diseases, rash, acute enteroviral haemorrhagic conjunctivitis
	Hepatitis viruses (A, E)	Hepatitis (jaundice), gastroenteritis
	Caliciviruses	Diarrhoea, vomiting
	Poliovirus (Enterovirus)	Poliomyelitis
	Rotaviruses	Diarrhoea, vomiting
	Small Round Structured viruses	Diarrhoea, vomiting
Protozoan parasites	*Cryptosporidium parvum*	Cryptosporidiosis, diarrhoea
	Entamoeba hystolytica	Amoebic dysentery
	Giardia	Giardiasis, diarrhoea
Helminths	*Dracunalis medinensis*	Guinea worm (Dracunculiasis)
Emerging opportunistic pathogens	*Actinobacter* spp.	Septicemia, meningitis, endocarditis
	Aeromonas spp.	Diarrhoea, gastroenteritis
	Cyclospora spp.	Diarrhoea, abdominal cramping, fever
	Isospora spp.	Diarrhoea
	Legionella spp.	Legionnaires disease, Pontiac fever
	Microsporidia spp.	Gastrointestinal infections, diarrhoea
	Nontuberculosis *Mycobacteria*	Skin infections, cervical lymphadenitis, nontuberculosis mycobacterium disease
	Pseudomonas aeruginosa	Septicaemia, wound and eye infections

The survival of microorganisms such as bacteria in water environments depends on the presence of nutrients and the water temperature (Edberg *et al*., 2000; Leclerc *et al*., 2002). The infectious dose of some bacteria range between 10^7 to 10^8 cells, with some enteric bacteria able to cause infections at doses as low as 10^1 cells (Edberg *et al*., 2000; Leclerc *et al*., 2002). Viruses cannot replicate outside living cells, but can survive for extended periods in the water (Raphael *et al*., 1985; Leclerc *et al*., 2002). The infectious dose of viruses has been established to be as low as 1 to 10 infectious particles (Raphael *et al*., 1985; Leclerc *et al*., 2002). Enteric protozoa such as *Giardia* and *Cryptosporidium* cannot replicate in water and are highly resistant to most disinfectants and antiseptics used for water treatment (Leclerc *et al*., 2002; Masago *et al*., 2002). The infectious dose for parasites depends on host susceptibility and strain virulence (Leclerc *et al*., 2002; Masago *et al*., 2002). The infectious dose for *Giardia* might be as low as 10 oocysts and for *Cryptosporidium* the presence of 30 oocysts might cause an infection (Leclerc *et al*., 2002; Masago *et al*., 2002; Carlsson, 2003).

Although waterborne pathogens are distributed worldwide, outbreaks of cholera, Hepatitis E and *Dracunculiasis* tend to be subjected to geographical factors (Sacks *et al*., 1986; Alarly and Nadeau, 1990; Kukula *et al*., 1997; Kukula *et al*., 1999; Hänninen *et al*., 2003; Hrudey *et al*., 2003). In the last number of years several outbreaks of pathogenic diseases have appeared that cannot be prevented by traditional water treatment. In 1981 a community waterborne outbreak in Colorado, USA, could be traced to Rotavirus (Hopkins *et al*., 1984). In 1983 and in 1987 two community outbreaks of waterborne *Campylobacter* spp were reported in the USA and Canada, respectively (Sacks *et al*., 1986; Alarly and Nadeau, 1990). In 1993 in Milwaukee, USA, 400 000 people fell ill with 54 deaths from using drinking water that was contaminated by *Cryptosporidium* cysts (Hoxie *et al*., 1997). In 1998, Calici-like viruses in municipal water were responsible for an acute gastroenteritis outbreak in Heinävesi, Finland, affecting approximately 3 000 people (Kukkula *et al*., 1997; Kukkula *et al*., 1999). In 2000, *E. coli* O157:H7 was responsible for 2 300 people falling ill in Walkerton, Canada (Hrudey *et al*., 2003). Recent flooding in Bangladesh has lead to 67 718 reported cases of diarrhoea and 9 people died due to waterborne diseases (International Water Association, 2004)

Consequently, during the past 5 years in rural communities in South Africa, severe outbreaks of cholera in the KwaZulu Natal, Limpopo, Eastern Cape and Mpumalanga have been reported with confirmed cases of mortality (DOH, 2000; DOH, 2002; DOH, 2003; NICD, 2004a; NICD, 2004b). In addition, typhoid cases have been reported in the Limpopo and the Mpumalanga Provinces during 2004 and 2005 with cases of mortality (NICD, 2004b). Rotaviruses have been found during 2005 to be the responsible agent in a large outbreak of watery diarrhoea in the Northern Cape (Laprap, 2005). A report compiled by the Department of Water Affairs and Forestry (DWAF) focussed on the waterborne diseases currently reported in South Africa by the Department of Health (DOH), the National Laboratory Services, DWAF and Rand Water (DWAF, 2005). In summary this report found that records in some provinces are not well kept and although information on waterborne diseases such as Hepatitis A, *Shigella* spp, cholera and typhoid fever is available, it is not reported. The report found that the number of people infected with Hepatitis A in South Africa was 231 in 2003 and 9 503 in 2004 indicating an increase in the rate of infection (DWAF, 2005). The report further showed that during 2003, 761 people and during 2004, 894 people were infected with *Shigella* spp. However, the data for *Shigella* spp are underreported because it is not on the list of notifiable diseases (DWAF, 2005). All these statistics confirm the need for the implementation of a national surveillance system to monitor waterborne disease outbreaks in South Africa.

2.3 THE MICROBIOLOGICAL QUALITY OF WATER

Water supplies in developing countries are devoid of treatment and the communities have to make use of the most convenient supply (Sobsey, 2002; Moyo *et al*., 2004). Many of these water supplies are unprotected and susceptible to external contamination from surface runoff, windblown debris, human and animal faecal pollution and unsanitary collection methods (Chidavaenzi *et al*., 1998; WHO, 2000; Moyo *et al*., 2004).

Detection of each pathogenic microorganism in water is technically difficult, time consuming and expensive and therefore not used for routine water testing procedures (Grabow, 1996). Instead, indicator organisms are routinely used to assess the

microbiological quality of water and provide an easy, rapid and reliable indication of the microbiological quality of water supplies (Grabow, 1996).

In order for a microorganism to be used as an indicator organism of pollution, the following requirements should be fulfilled (Grabow, 1986; WHO, 1993; NRC, 2004):

- The concentration of the indicator microorganism should have a quantitative relationship to risk of disease associated with exposure (ingestion/recreational contact) to the water;
- The indicator organism should be present when pathogens are present;
- The persistence and growth characteristics of the indicator organism should be similar to that of pathogens;
- Indicator organisms should not reproduce in the environment;
- The indicator organism should be present in higher numbers than pathogens in contaminated water;
- The indicator organism should be at least as resistant to adverse environmental conditions, disinfection and other water treatment processes as pathogens;
- The indicator organism should be non-pathogenic and easy to quantify;
- The tests for the indicator organism should be easy, rapid, inexpensive, precise, have adequate sensitivity, quantifiable and applicable to all types of water;
- The indicator organism should be specific to a faecal source or identifiable as to the source of origin of faecal pollution.

Although many microorganisms have desirable features to be considered as possible indicators of faecal pollution, there is no single microorganism that meets all of these requirements (Moe *et al*., 1991; Payment and Franco, 1993; Sobsey *et* al., 1993; Sobsey *et al*., 1995). Several studies have showed the limitations of some of the current indicator organisms, which include the following:

- Indicator organisms may be detected in water samples in the absence of pathogens (Echeverria *et al.*, 1987).
- Some pathogens may be detected in the absence of indicator organisms (Seligman and Reitler, 1965; Thompson, 1981). Echeverria and co-workers

(1987) have showed that *Vibrio cholera* (*V. cholera*) persists in water exposed to solar disinfection well after *E. coli* was inactivated. El-Agaby and co-workers (1988) have showed that potable water supplies in Egypt contained bacteriophages, with zero total and faecal coliform counts, which indicated the possible risk of the presence of human enteric viruses.

- Thompson (1981) has showed that *E. coli* bacteria have a short die-off curve with temperature playing an important role.
- McFeters and co-workers (1986) have showed that injured coliform bacteria can be undetected due to several chemical and physical factors and were unable to grow on commonly used media.
- LeChevallier and co-workers (1996) have showed that improper filtration, temperature, inadequate disinfection and treatment procedures, biofilms and high assimilable organic carbon (AOC) levels, could all be responsible for the regrowth of coliform bacteria in water samples.
- Regli and co-workers (1991) and Hot and co-workers (2003) have showed that the prevalence of viruses in water may differ from that of indicator organisms. Low numbers of viruses are present in water samples compared to indicator organisms, viruses are only excreted for short periods of time while coliform bacteria is excreted continuously, and the structure, size, composition and morphological differences between viruses and bacteria also had an influence on behavioural and survival patterns of these microorganisms (Regli *et al*., 1991; Hot *et al*., 2003).

In spite of the shortcomings of indicator microorganisms, it is better to use a combination of indicator microorganisms to give a more accurate picture of the microbiological quality of water (DWAF, 1996; NRC, 2004). In general, every country has its own set of guidelines for drinking water. However, most of these guidelines are similar for different countries and the same indicator microorganisms to indicate the presence of pathogenic microorganisms are used. The water quality guidelines for South Africa are shown in Table 2.2.

Table 2.2 Microbiological requirements for domestic water in South Africa (Kempster *et al*., 1997; SABS, 2001)

Indicator organism	Units	Allowable compliance
Heterotrophic plate count	Colony forming units.1 ml^{-1}	100
Total coliform bacteria	Colony forming units.100 ml^{-1}	10
Faecal coliform bacteria	Colony forming units.100 ml^{-1}	1
Escherichia coli	Colony forming units.100 ml^{-1}	0
Somatic bacteriophages	Colony forming units.10 ml^{-1}	1
Enteric viruses	Plaque forming units.100 l^{-1}	1
Protozoan parasites (*Giardia/Cryptosporidium)*	Count.100 l^{-1}	0

The most commonly used indicator microorganisms include heterotrophic plate counts, total coliform bacteria, faecal coliform bacteria, *E coli*, faecal enterococci, *C. perfringens* as well as somatic and male specific F-RNA bacteriophages (WHO, 2000). Each of these indicator microorganisms has advantages and disadvantages which will be discussed in more detail in the following sections.

2.3.1 Heterotrophic plate counts

Heterotrophic microorganisms or heterotrophs are naturally present in the environment and can be found in soil, sediment, food, water and in human and animal faeces (Collin *et al*., 1988; Olson *et al*., 1991; Standard Methods, 1995; Lillis and Bissonnette, 2001). Broadly defined, heterotrophs include bacteria, yeasts and molds that require organic carbon for growth (WHO, 2002c). Although generally considered harmless, some heterotrophic microorganisms are opportunistic pathogens, which have virulence factors that could affect the health of consumers with suppressed immune systems (Lye and Dufour, 1991; Bartram *et al*., 2003). Heterotrophic microorganisms can also survive in biofilms inside water distribution systems, water reservoirs and inside household storage containers (Momba and Kaleni, 2002; Jagals *et al*., 2003). Therefore, heterotrophic plate counts can also be used to measure the re-growth of organisms that may or may not be a health risk (WHO, 2002c).

Heterotrophic Plate Count, also known as Total or Standard Plate Count includes simple culture based tests intended to recover a wide range of heterotrophic microorganisms from water environments (Bartram *et al*., 2003). Enumeration tests for heterotrophic plate counts are simple and inexpensive giving results within 48 h to 5 days, depending on the method, type of media and the incubation temperature used (Collin *et al*., 1988; Olson *et al*., 1991; Standard Methods, 1995; Lillis and Bissonnette, 2001). The pour plate, membrane filtration or spread plate methods are used routinely in various laboratories, with either Yeast-extract agar, Plate Count Agar (PCA), Tryptone Glucose agar or R2A agar, and incubation periods either at room temperature (25ºC) for 5 to 7 days, or at 35°C to 37°C for 48 h (Collin *et al*., 1988; Olson *et al*., 1991; Standard Methods, 1995; Lillis and Bissonnette, 2001). Heterotrophic plate counts alone cannot indicate a health risk and additional studies on the presence of *E. coli* or other faecal specific indicator microorganisms need to be conducted to establish the potential health risk of the water analysed (WHO, 2002c).

2.3.2 Total coliform bacteria

Total coliform bacteria are defined as aerobic or facultative anaerobic, Gram negative, non-spore forming, rod shaped bacteria, which ferments lactose and produce gas at 35°C (Standard Methods, 1995). Total coliforms include bacteria of known faecal origin such as *E. coli* as well as bacteria that may not be of faecal origin such as *Klebsiella* spp, *Citrobacter* spp, *Serratia* spp and *Enterobacter* spp which are found in nutrient rich water, soil decaying vegetation and drinking water with relatively high levels of nutrients (Pinfold, 1990; Ramteke *et al*., 1992; WHO, 1996a). The recommended test for the enumeration of total coliforms is membrane filtration using mEndo agar and incubation at 35°C to 37°C for 24 h to produce colonies with golden-green metallic shine (Standard Methods, 1995).

In water quality studies, total coliform bacteria are used as a systems indicator, which provides information on the efficiency of water treatment (Standard Methods, 1995). The presence of total coliform in water samples are therefore, an indication that opportunistic pathogenic bacteria such as *Klebsiella* and *Enterobacter* which can multiply in water environments and pathogenic pathogens such as *Salmonella* spp,

Shigella spp, *V. cholera*, *Campylobacter jejuni*, *Campylobacter coli*, *Yersinia enterocolitica* and pathogenic *E. coli* may be present (DWAF, 1996; Grabow, 1996). These pathogens and opportunistic microorganisms could cause diseases such as gastroenteritis, dysentery, cholera, typhoid fever and salmonellosis to consumers (DWAF, 1996; Grabow, 1996). In particular, individuals who suffer from HIV/AIDS related complications are more at risk of being infected by these microorganisms (DWAF, 1996).

2.3.3 Faecal coliform bacteria

Faecal coliform bacteria are Gram negative bacteria, also known as thermotolerant coliforms or presumptive *E. coli* (Standard Methods, 1995). The faecal coliform group includes other organisms, such as *Klebsiella* spp, *Enterobacter* spp and *Citrobacter* spp, which are not exclusively of faecal origin (Standard Methods, 1995). *Escherichia coli* are specifically of faecal origin from birds, humans and other warm blooded animals (WHO, 1996a; Maier *et al*., 2000). Faecal coliform bacteria are therefore considered to be a more specific indicator of the presence of faeces (Maier *et al*., 2000).

The recommended test for the enumeration of faecal coliforms is membrane filtration using mFC agar and incubation at 44.5°C for 24 h to produce blue colored colonies (Standard Methods, 1995). Faecal coliforms are generally used to indicate unacceptable microbial water quality and could be used as an indicator in the place of *E. coli* (SABS, 2001). The presence of faecal coliforms in a water sample indicates the possible presence of other pathogenic bacteria such as *Salmonella* spp, *Shigella* spp, pathogenic *E. coli, V. cholera*, *Klebsiella* spp and *Campylobacter* spp associated with waterborne diseases (DWAF, 1996). Unfortunately faecal coliform bacteria exhibit species to species variations in their respective stability and resistance to disinfection processes; do not distinguish between faeces of human and animals origin; have low survival rates and have been detected in water sources thought to be free of faecal pollution (Goyal *et al.*, 1979; Fujioka *et al.*, 1988).

2.3.4 *Escherichia coli* bacteria

Globally *E. coli* is used as the preferred indicator of faecal pollution (Edberg *et al.*, 2000). It is a Gram negative bacterium and predominantly an inhabitant of the intestines of warm blooded animals and humans, which is used to indicate recent faecal pollution of water samples (Rice *et al.*, 1990; Rice *et al.*, 1991; WHO, 1996a; Edberg *et al.*, 2000). Confirmation tests for *E. coli* include testing for the presence of the enzyme β-glucuronidase, Gram staining, absence of urease activity, production of acid and gas from lactose and indole production (Mac Faddin, 1980; Rice *et al.*, 1991; Standard Methods, 1995).

Commercially available growth media containing the fluorogenic substrate 4-methyl-umbelliferyl-β-D-glucuronidase (MUG) is used for the isolation and identification of *E. coli* from water samples (Shadix and Rice, 1991; Covert *et al.*, 1992). The *E. coli* bacteria hydrolyse the MUG in the media, which then fluoresces under ultraviolet light (Shadix and Rice, 1991; Covert *et al.*, 1992). However, false negative results on this media have been found due to injured cells, lack of expression of the gene which codes for the enzyme β-glucuronidase by the *E. coli* bacterium isolate, and non-utilization of the MUG reagent in the media by some *E. coli* strains (Chang *et al.*, 1989; Feng *et al.*, 1991; NRC, 2004).

2.3.5 Faecal enterococci bacteria

Faecal enterococci bacteria are found in the genus *Enterococcus* and include species like *Enterococcus faecalis, Enterococcus faecium, Enterococcus durans* and *Enterococcus hirae* (Standard Methods, 1995; WHO, 1996a). The genus *Enterococcus* are differentiated from the genus *Streptococcus* by their ability to grow in 6.5% sodium chloride, pH 9.6, temperatures of 45ºC and their tolerance for adverse growth conditions (Maier *et al.*, 2000). Faecal enterococci are spherical, Gram positive bacteria, which are highly specific for human and animal faecal pollution (Standard Methods, 1995). Most of the species in the *Enterococcus* genus are of faecal origin and is regarded as specific indicators of human faecal pollution, although some species are found in the faeces of animals and plant material (WHO, 1996a).

The recommended test is membrane filtration using mEnterococcus agar and incubation at 35°C to 37°C for 48 h to produce pink colonies (Standard Methods, 1995). Faecal enterococci rarely multiply in polluted water environments and are more resistant to disinfection and treatment processes than the Gram negative faecal coliform bacteria (Standard Methods, 1995). The presence of faecal enterococci in water samples are therefore, an indication of the health risk to waterborne diseases such as meningitis, endocarditis and infections of the eyes, ears and skin (DWAF, 1996; Grabow, 1996).

2.3.6 *Clostridium perfringens* bacteria

Clostridium perfringens is a Gram positive, sulphite reducing anaerobic, rod shaped, spore forming bacteria normally present in faeces of humans and warm blooded animals (Standard Methods, 1995). However, *C. perfringens* are also found in soil and water environments (WHO, 1996a). The spores can survive much longer than coliform bacteria and are highly resistant to water disinfection and treatment processes (Standard Methods, 1995). *Clostridium perfringens* are therefore used as an indicator of faecal pollution to indicate the potential presence of enteric viruses, which may include Enteroviruses, Adenoviruses and Hepatitis viruses as well as the cysts and oocysts of protozoan parasites such as *Giardia*, *Entamoeba* and *Cryptosporidium* in treated drinking water (Payment and Franco, 1993). The enumeration test includes membrane filtration using specific medium (e.g. mCP or *Perfringens* selective OPSP medium with supplements) and incubation 35°C to 37°C for 48 h at in micro-aerophillic conditions to produce black colonies (Standard Methods, 1995).

2.3.7 Bacteriophages

Bacteriophages are viruses, which specifically infect bacteria (Grabow, 2001). Bacteriophages have been suggested as useful indicators to predict the potential occurrence of enteric viruses in water (Grabow *et al*., 1984; Leclerc *et al*., 2000). The survival of bacteriophages is affected by the densities of the host and the bacteriophages in the water sample (Grabow, 2001). In addition, the association of the bacteriophage with solids and the presence of organic matter in the water sample could influence the attachment of the bacteriophages to the host bacterium (Grabow, 2001). Several studies have shown that ultra violet light, temperature, pH of the water, and ion concentrations in the water could

affect the survival of bacteriophages in water (Brion *et al*., 2002; Schaper *et al*., 2002b; Allwood *et al*., 2003). Bacteriophages show higher resistance to environmental stress compared to bacterial indicators such as total coliforms and faecal coliforms and assays for bacteriophages can be conducted quickly, economically and quantitatively (Vaughn and Metcalf, 1975; Havelaar *et al*., 1993). There are several bacteriophages that can be used as indicator organisms which includes the somatic bacteriophages, *Bacteroides fragilis* HSP40 bacteriophages and male specific F-RNA bacteriophages (Grabow, 2001).

2.3.7.1 Somatic bacteriophages

The somatic bacteriophages are a heterogeneous group of organisms that absorbs to bacterial receptors for infection and replication on the cell wall of the laboratory host strain *E. coli* WG5 (Leclerc *et al*., 2000). Somatic bacteriophages are therefore, used as indicators of the potential presence of enteric viruses in water (Grabow, 2001). These bacteriophages can serve as models for the assessment of the behaviour of enteric viruses in water treatment and disinfection processes (Grabow, 2001). The double layer plaque assay is generally used to detect somatic bacteriophages (ISO, 2000; Mooijman *et al*., 2001). However, somatic bacteriophages are not specific to *E. coli*, and may infect and replicate in other species of the Enterobacteriaceae family, which includes the total coliform group (Leclerc *et al*., 2000). Somatic bacteriophages are therefore, not considered a specific indicator for faecal pollution (Leclerc *et al*., 2000).

2.3.7.2 *Bacteroides fragilis* HSP40 bacteriophages

Bacteroides bacteria are present in high numbers in human faeces (Leclerc *et al*., 2000). *Bacteroides* is a strict anaerobic, Gram negative, non-spore forming bacterium which is rapidly inactivated by oxygen levels in water, and needs complex growth media with antibiotics to inhibit the interference from other intestinal microorganisms (Leclerc *et al*., 2000). The *Bacteroides fragilis* HSP40 bacteriophages are a relatively homogeneous group that do not multiply in the environment (Havelaar, 1993; Jagals *et al*., 1995; Puig *et al*., 1999). In some countries, *Bacteroides fragilis* HSP40 bacteriophages is present in relatively low numbers in human faeces (Havelaar, 1993; Jagals *et al*., 1995; Bradley *et al*., 1999; Puig *et al*., 1999). Although this bacteriophage has been shown to be highly

specific for human faeces, tests are complicated and labour intensive (ISO, 2001; Sinton *et al*., 1998).

2.3.7.3 Male specific F-RNA bacteriophages

The male specific F-RNA bacteriophages have small hexagonal capsomers without tails, are approximately 30 nm long with a single RNA genome (Leclerc *et al*., 2000). Male specific F-RNA bacteriophages have been recommended as useful models for monitoring the behaviour of human enteric viruses in water treatment processes because of their size and structure, which are similar to those of the Enteroviruses (Lewis, 1995; Leclerc *et al*., 2000; Grabow, 2001). These bacteriophages are relatively resistant to disinfectants, sunlight, heat- and water treatment processes (Leclerc *et al*., 2000).

Male specific F-RNA bacteriophages specifically attach to the sex pili of the host bacterium [*E. coli* HS(pFamp)R or *Salmonella typhimirium* WG49] in temperatures higher than 30°C (Havelaar and Hogeboom, 1984; Debartolomeis and Cabelli, 1991). The F-pilli are short tube-like protrusions produced by certain bacteria for the transfer of nucleic acid to other bacteria of the same or closely related species and are only produced by the bacteria in the log growth phase which is usually above 30ºC (Havelaar *et al*., 1993; Woody and Cliver, 1995). These bacteriophages are assayed according to an International Standardization Method (ISO, 1995; Mooijman *et al*., 2002). Male specific F-RNA bacteriophages belong to the family *Leviviridae*, which contains two genera, the *Leviviridae* and the *Allolevivirida*e. Both these genera contain distinct subgroups (Watanabe *et al*., 1967; Furuse *et al*., 1979), which is useful in genotyping assays where specific probes are used to distinguish between animal (subgroups I and IV) and human (subgroups II and III) faecal pollution (Osawa *et al*., 1981; Furuse, 1987; Beekwilder *et al*., 1996).

2.4 HUMAN AND ANIMAL FAECAL POLLUTION IN WATER

Water polluted with human and animal faeces may contain potentially pathogenic microorganisms that can cause diseases in consumers (Sobsey *et al*., 1993; Gerba *et al*., 1996; Grabow, 1996; Leclerc *et al*., 2002; Theron and Cloete, 2002). The most commonly used faecal indicator microorganisms which include the total coliform

bacteria, thermotolerant coliform bacteria, *E. coli* and faecal enterococci bacteria, are found in both human and animal faeces, but do not differentiate between the origins of faecal pollution (Sinton *et al*., 1998). Human viral pathogens such as Calicivirus, Hepatitis E virus, Reoviruses, Rotaviruses, somatic bacteriophages and male specific F-RNA bacteriophages also infect other animals which can serve as reservoirs (NRC, 2004). Consequently, these animals can be important potential sources of contamination of water sources because the release of microorganisms into aquatic environments by animal hosts could lead to human exposure (NRC, 2004). Poor communities in developing countries share their water sources with cattle and other domestic animals, therefore, the risk of waterborne transmission of zoonotic pathogens to humans, increases (Pournadeali and Tayback, 1980; Meslin, 1997; Sinton *et al*., 1998; Franzen and Muller, 1999; Slifko *et al*., 2000; Enriquez *et al*., 2001; Hoar *et al*., 2001; Leclerc *et al*., 2002; Theron and Cloete, 2002; Hackett and Lappin, 2003). However, water contaminated with human faeces is regarded as a greater risk to human health since it is more likely that it would contain human specific enteric pathogens (Sinton *et al*., 1998). Although various microbial and chemical indicators have been described to identify the origin of faecal pollution in water supplies, different levels of success have been obtained (Sinton *et al*., 1998; Gilpen *et al*., 2002; Gilpen *et al*., 2003).

2.4.1 The use of microorganisms to determine the origin of faecal pollution

Several microorganisms have been suggested and tested to distinguish between human and animal faecal pollution in domestic drinking water supplies (Wheather *et* al., 1980; Mara and Oragui, 1985; Tartera and Jofre, 1987; Gavini *et al*., 1991; Arango and Long, 1998; Sinton *et al*., 1998; Gilpen *et al*., 2002). Various factors can have an effect on the specificity of microorganisms that can be used as indicators to determine the origin of faecal pollution, such as: (1) specific bacteria, viruses and protozoan parasites can have multiple hosts (not species specific) (Sinton *et al*., 1998; Gilpen *et al*., 2002); (2) different microorganisms can have similar biochemical reactions in the environment, especially within the same species or genus (Sinton *et al*., 1998; Gilpen *et al*., 2002) and (3) interspecies gene transfer may occur which include small pieces of DNA (eg. plasmids and integrons) and transposons that are carried from one bacteria to another

during sexual and asexual reproduction of bacterial cells (Sinton *et al*., 1998; Gilpen *et al*., 2002).

Microorganisms that have been used in assays to determine the origin of faecal pollution include total coliforms, faecal coliforms, faecal streptococci/enterococci, *Bacteroides* spp, *Bacteroides fragilis* HSP40 bacteriophages, *Pseudonomas aeruginosa*, *Bifidobacterium* spp, *Rhodococcus coprophilus*, male specific F-RNA bacteriophages and specific human enteric viruses (Wheather *et* al., 1980; Mara and Oragui, 1985; Tartera and Jofre, 1987; Gavini *et al*., 1991; Arango and Long, 1998; Sinton *et al*., 1998; Gilpen *et al*., 2002).

2.4.1.1 The ratio of faecal coliform bacteria to faecal streptococci bacteria

The ratio between faecal coliform (FC) and faecal streptococci/enterococci (FS) counts in water is an old method used in several earlier studies to determine the origin of faecal pollution (Wheather *et* al., 1980; Mara and Oragui, 1985; Tartera and Jofre, 1987; Gavini *et al*., 1991; Arango and Long, 1998; Sinton *et al*., 1998; Gilpen *et al*., 2002). This method is based on the fact that faecal streptococci/enterococci are more abundant in animal faeces than in human faeces while faecal coliforms are more abundant in human faeces than in animal faeces (Sinton *et al*., 1998). The test stipulates that a FC:FS ratio greater than 4 is indicative of human faeces and a FC:FS ration of less than 7 is indicative of animal faecal pollution (Sinton *et al*., 1998).

The limitation of this method is the variable survival rates of some faecal streptococci species, which make this test unreliable (Wheather *et* al., 1980; Mara and Oragui, 1985; Tartera and Jofre, 1987; Gavini *et al*., 1991; Arango and Long, 1998; Sinton *et al*., 1998; Gilpen *et al*., 2002). Sinton and Donnison (1994) have showed that *Enterococcus faecalis* survives longer than *Enterococcus faecium* which survives longer than *Enterococcus durans* which survives longer than *Streptococcus equines* and *Streptococcus bovis* in water environments.

2.4.1.2 The ratio of faecal coliform to total coliform bacteria

Faecal coliforms constitute a subset of total coliforms but grow and ferment lactose with the production of gas and acid at 44.5°C within 24 h (DWAF, 1996). The ratio of faecal coliforms to total coliforms is used to show the percentage of total coliforms that comprises of faecal coliforms which comes from the gut of warm blooded animals (Sinton *et al*., 1998). If the faecal coliforms to total coliforms ration exceeds 0.1 it may suggests the presence of human faecal contamination (Sinton *et al*., 1998). However, this method only shows the possibility of faecal pollution but do not distinguish between human and animal faecal matter (Bartman and Rees, 2000). Another disadvantage of this assay is that some faecal coliforms can multiply in soils in tropical regions and give a false positive result for water pollution (Bartman and Rees, 2000).

2.4.1.3 *Bacteroides* bacteria and *Bacteroides* HSP40 bacteriophages

Bacteroides bacterial species are among the numerous bacteria in human faeces and is also found in low numbers in animal faeces (Maier *et al*., 2000). The bacterium does not survive for long periods outside the human body making the detection of *Bacteroides* difficult (Wheather *et al*., 1980; Mara and Oragui, 1985; Tartera and Jofre, 1987; Gavini *et al*., 1991; Arango and Long, 1998; Sinton *et al*., 1998; Gilpen *et al*., 2002).

However, the *Bacteroidis fragilis* HSP40 bacteriophage strain is a highly specific indicator for human faecal pollution (Grabow, 2001) but is only present in low numbers in human sewage (Wheather *et al*., 1980; Mara and Oragui, 1985; Tartera and Jofre, 1987; Gavini *et al*., 1991; Arango and Long, 1998; Sinton *et al*., 1998; Gilpen *et al*., 2002). The assays used for the *Bacteroides* bacteria and the *Bacteroides fragilis* HSP40 bacteriophages are expensive, complicated, time consuming and require specialised equipment and skilled labour (Wheather *et al*., 1980; Mara and Oragui, 1985; Tartera and Jofre, 1987; Gavini *et al*., 1991; Arango and Long, 1998; Sinton *et al*., 1998; Gilpen *et al*., 2002).

2.4.1.4 *Pseudomona aeruginosa* bacteria

Pseudonoma aeruginosa bacteria are present in 16% of human adults but occur rarely in lower animals (Sinton *et al*., 1998; Gilpen *et al*., 2002). Unfortunately this bacterium is present in water, soil and sewage samples and can rapidly die-off in aquatic environments and is therefore not a suitable candidate to determine the source of faecal pollution (Wheather *et al*., 1980; Mara and Oragui, 1985; Tartera and Jofre, 1987; Gavini *et al*., 1991; Arango and Long, 1998; Sinton *et al*., 1998; Gilpen *et al*., 2002).

2.4.1.5 *Bifidobacterium* spp

Bifidobacteria spp are strickly anaerobic, Gram positive bacteria present in the gut of humans and animals (Nebra *et al*., 2003). Species such as *Bifidobacteria adolescentis* are specific to humans while species such as *Bifidobacteria thermophilum* are specific to animal faeces (Nebra *et al*., 2003). It is difficult to differentiate between the species based on biochemical and microbiological analysis, which complicates the interpretation of the results (Wheather *et al*., 1980; Mara and Oragui, 1985; Tartera and Jofre, 1987; Gavini *et al*., 1991; Arango and Long, 1998; Sinton *et al*., 1998; Gilpen *et al*., 2002).

2.4.1.6 *Rhodococcus coprophilus* bacteria

Rhodococcus coprophilus is a Gram positive, aerobic nocardioform actinomycete which forms a fungus-like mycelium that breaks up into bacteria-like pieces (Sinton *et al*., 1998). The bacteria contaminate grass and when eaten by herbivores these bacteria-like pieces are found in the herbivore dung (Jagals *et al*., 1995; Sinton *et al*., 1998). *Rhodococcus coprophilus* has never been found in human faeces and is therefore used as an indicator of animal faecal pollution (Jaggals *et al*., 1995). The disadvantage of this bacterium is the long growth time of 21 days (Wheather *et al*., 1980; Mara and Oragui, 1985; Tartera and Jofre, 1987; Gavini *et al*., 1991; Arango and Long, 1998; Sinton *et al*., 1998; Gilpen *et al*., 2002). Saville and co-workers (2001) have designed a PCR protocol to detect this organism in faecal specimens of animals, which showed potential to be used as a routine laboratory test, but more studies are needed to evaluate this detection technique.

2.4.1.7 Male specific F-RNA bacteriophages

Male specific F-RNA bacteriophages are a homogeneous group of microorganisms belonging to the Family *Leviviridae* (Leclerc *et al*., 2000). This family comprise of four subgroups, those predominating in humans (groups II and III), and those predominating in animals (groups I and IV) (Leclerc *et al*., 2000). Genotyping with specific probes or serotyping with specific antisera can be used to classify male specific F-RNA bacteriophages into one of the four distinct subgroups (Beekwilder *et al*., 1996). The application of these assays makes it possible to distinguish between environmental contaminations from human or animal faecal origin (Beekwilder *et al*., 1996). Grouping is based on serological and physico-chemical properties of each subgroup (Leclerc *et al*., 2000). However, antisera necessary for serotyping are expensive, not readily available and some isolates are difficult to serotype (Furuse *et al*., 1978; Havelaar *et al.*, 1986).

Genotyping of F-RNA bacteriophages are based on molecular techniques, which include specific oligonucleotide probes and nucleic acid hybridisation (Hsu *et al*., 1995; Beekwilder *et al*., 1996). Hsu and co-workers (1995) investigated genotyping with non-radioactive oligonucleotide probes as an alternative to serotyping for the grouping of male specific F-RNA bacteriophages. Beekwilder and co-workers (1996) also described a method which identifies male specific F-RNA bacteriophages quantitatively by a plaque hybridisation assay. Comparison of genotype and serotype results showed that genotyping is a more effective and technically feasible method for the grouping of male specific F-RNA bacteriophages (Hsu *et al*., 1995; Beekwilder *et al*., 1996).

Several studies have suggested that male specific F-RNA bacteriophage subgroup classification, especially subgroups II and III that predominates in human faeces, will not always distinguish between human and pig faecal contamination due to similar dietary and living conditions of pigs as well as exposure of the pigs to human faecal wastes (Osawa *et al*., 1981; Havelaar *et al.*, 1990; Hsu *et al.*, 1995). Consequently, a small percentage of overlapping between the serotypes and their expected animal sources were found with studies showing that animal samples might contain all 4 serotypes (NRC, 2004). In addition, Schaper and co-workers (2002) have showed that human samples contained serotypes I and IV that is mainly associated with animal hosts. Despite these results, various studies have used genotype and serotype analysis

successfully to distinguish between faecal pollution of either human or animal origin (Osawa *et al*., 1981; Havelaar *et al.*, 1990; Hsu *et al.*, 1995; Beekwilder *et al*., 1996; Schaper *et al*., 2002a). Rose and co-workers (1997) have used reverse transcriptase polymerase chain reaction (RT-PCR) to isolate male specific F-RNA bacteriophages from polluted marine waters. However, a study conducted by Schaper and Jofre (2000) comparing RT-PCR followed by southern blotting with plaque hybridisations on male specific F-RNA bacteriophages in sewage samples, indicated that RT-PCR was less sensitive than plaque hybridisation analysis to identify the various F-RNA bacteriophages present in the sewage water samples. Therefore, genotyping of male specific F-RNA bacteriophages using nucleic acid hybridisation seems to be the microbial method of choice to distinguish between human and animal origin of faecal pollution (Schaper and Jofre, 2000).

2.4.1.8 Human enteric viruses

Human enteric viruses associated with waterborne diseases include Adenoviruses, Caliciviruses, Enteroviruses, Hepatitis A virus and Rotaviruses (Grabow, 2001). Although excreted in high numbers in faeces by infected individuals, these viruses may be present in low numbers in environmental samples due to dilution (Grabow, 2001). The detection of specific human enteric viruses can be used to confirm the presence of human faecal pollution (Grabow, 2001). Since the detection of viruses is mostly based on molecular techniques, it is not a cost-effective method to include in routine monitoring of water (Tartera and Jofre, 1987; Gavini *et al*., 1991; Arango and Long, 1998; Sinton *et al*., 1998; Gilpen *et al*., 2002; NRC, 2004).

Viability of viruses can also not be indicated by molecular techniques and additional cell culture techniques should be included, thereby further increasing the cost and labour (Grabow, 2001; Gilpen *et al*., 2002). However, all viruses are not able to grow in cell cultures (Grabow, 2001). In addition these techniques are labour intensive and skilled personnel are required (Tartera and Jofre, 1987; Gavini *et al*., 1991; Arango and Long, 1998; Sinton *et al*., 1998; Gilpen *et al*., 2002; NRC, 2004).

2.4.1.9 Multiple antibiotic resistant analyses

Resistant bacteria have the ability to survive exposure to antibiotics or disinfectants and through rapid multiplication pass their resistant genes on to other pathogenic as well as to non-pathogenic bacteria (Sergeant, 1999). These antibiotic resistant genes are often associated with transposons (genes that can easily move from one bacterium to another bacterium or by bacteriophages) (Sergeant, 1999). Many bacteria also possess integrons and plasmids, which are small pieces of DNA that accumulate new genes (Sergeant, 1999). Over a period of time, a bacterium can build up a whole range of resistant genes, which is referred to as multiple resistances, which may be passed on within a genus or species to other strains or species (Sergeant, 1999).

The multiple antibiotic analysis (MAR) includes the use of antibiotic resistance patterns of specific microorganisms to differentiate between phenotypes within a specific genus (Krumperman, 1983; Sergeant, 1999). In *E. coli*, *Salmonella* spp and *Shigella* spp, a chromosomal locus is used to determine the intrinsic levels of these organisms for their susceptibility to structurally different antibiotics and disinfectants (Krumperman, 1983). Over expression of this chromosomal locus due to mutations or chemical induction, produces a range of new bacterial phenotypes within a bacterial species (Krumperman, 1983). Bacteria isolated from humans have different MAR profiles than isolates from domestic animals (Krumperman, 1983; Hair *et al*., 1998; Sergeant, 1999). Individual bacterial isolates can be classified into phenotypic groups when the MAR profiles are combined with discriminant statistical analyses (eg. a variation of multivariant analysis of variance) (Krumperman, 1983; Hair *et al*., 1998; Sergeant, 1999). However, MAR studies are time consuming, complicated and expensive. In addition, antibiotic resistance encoded on plasmids can be lost during isolation and there are constant population shifts in antibiotic resistance (Sergeant, 1999).

2.4.1.10 Deoxy Ribonucleic Acid based profiles of microorganisms

The microbial Deoxy Ribonucleic Acid (DNA) based profile approach provide genomic profiles of microbial communities and are used to identify the genus, species, subspecies and strains of microorganisms (Turner *et al*., 1996; Nebra *et al*., 2003). The DNA based profile techniques used to distinguish between microbial genus and species

include ribotyping, Internal Transcribed Spacer-Polymerase Chain Reaction (ITS-PCR), tRNA-PCR and 16S rRNA sequencing (Nebra *et al*., 2003). The DNA based profile techniques used to distinguish between microbial subspecies and strains include Amplified Ribosomal DNA Restriction Analysis (ARDRA), Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction (ERIC-PCR), plasmid or chromosomal restriction-fragment-length-polymorphism (RFLP), Internal Transcribed Spacer-sequencing (ITS-sequencing) and Pulsed Field Gel Electrophoresis (PFGE) methods (Nebra *et al*., 2003). These DNA profiling methods are expensive, labour intensive, require skilled personnel, need specialised equipment and are therefore not used routinely (Turner *et al*., 1996; Nebra *et al*., 2003; Wei *et al*., 2004).

Although several microbiological methods have been proposed and tested to determine the origin of faecal contamination, many of these microorganisms have proved to be difficult to use in routine laboratory procedures because of the type of equipment required, the cost and the skill necessary to perform the assay (Sinton *et al*., 1998; Gilpen *et al*., 2002; Gilpen *et al*., 2003). Genotyping of male specific F-RNA bacteriophages seems to be the most promising microbiological method presently available to distinguish between human and animal faecal pollution of water supplies in rural communities based on results obtained by various studies on animal and human faeces (Osawa *et al*., 1981; Havelaar *et al*., 1990; Hsu *et al*., 1995; Beekwilder *et al*., 1996; Schaper *et al*., 2002a).

2.4.2 The use of chemicals to determine the origin of faecal pollution

Several chemical indicators have been used to identify the source of faecal pollution in various water supplies (Sinton *et al*., 1998; Gilpen *et al*., 2002; Gilpen *et al*., 2003). However, expensive equipment and high concentrations of the chemical in the water sample is needed for accurate identification of the origin of faecal pollution (Sinton *et al*., 1998; Gilpen *et al*., 2002; Gilpen *et al*., 2003).

2.4.2.1 Direct chemical indicators

Direct chemical indicators include chemicals present in the faeces, e.g. faecal sterols, uric acid and urobilin (Sinton *et al*., 1998; Gilpen *et al*., 2002; Gilpen *et al*., 2003). The

breakdown products of sterols are stanols (Leeming *et al*., 1996). Leeming and co-workers (1996) have conducted tests on human and animal faeces and especially on sterols and stanols and found that stanols produced in animals were distinctively different than the stanols formed in humans.

Faecal sterol cholesterol is reduced in the gut of humans to coprostanol and in the gut of animals to epicoprostanol (Leeming *et al*., 1996). These compounds can be found in the environment as cholestanol (Leeming *et al*., 1996). Coprostanol is used exclusively as a marker of human faecal pollution (Leeming *et al*., 1996). Plant derived 24-ethylcholestrol is reduced to 24-ethylpicoprostanol in the intestinal tract of herbivores and found in the environment as 24-ethylcholestanol (Leeming *et al*., 1996). The 24-ethylcoprostanol is used as an exclusive marker of animal faecal pollution (Leeming *et al*., 1996).

2.4.2.2 Indirect chemical indicators

Indirect chemical indicators are specific for human faecal contamination (Sinton *et al*., 1998; Gilpen *et al*., 2002; Gilpen *et al*., 2003). These chemicals are associated with faecal discharge in wastewater and septic tank discharges (Sinton *et al*., 1998; Gilpen *et al*., 2002; Gilpen *et al*., 2003). Fluorescent whitening agents (FWA) and sodium tripolyphosphate (STP) present in washing powders, long chain alkylbenzenes (LAB) present in commercial detergents and polycyclic aromatic hydrocarbons have been used as indirect indicators of human faecal pollution (Sinton *et al*., 1998; Gilpen *et al*., 2002; Gilpen *et al*., 2003).

Although different studies have described the use of these microbiological and chemical indicators, it is apparent that no single chemical determinant could reliably distinguish human from animal faecal contamination (Jagals *et al*., 1995; Sinton *et al*., 1998). It seems that the use of a combination of these determinants may provide the best solution for identifying the origin of faecal pollution in water environments (Jagals *et al*., 1995; Sinton *et al*., 1998).

2.5 SOURCE WATER SUPPLIES

The World Health Organization (WHO) classifies source water supplies as either improved or unimproved (WHO, 2000; Gundry *et al*., 2004). Improved water sources include public standpipes, household connections, boreholes, protected dug wells, protected springs, boreholes and springs connected via a pipe system to a tap, as well as rainwater collection (WHO, 2000; Gundry *et al*., 2004). Unimproved water sources include unprotected wells, unprotected springs, vendor-provided water, rivers as well as tanker truck provision of water (WHO, 2000; Gundry *et al*., 2004).

Several studies carried out in developing countries have determined the microbiological quality of these improved and unimproved water sources and depending on the water source, different results were obtained (Pournadeali and Tayback, 1980; Obi *et al*., 2002; Sobsey *et al*., 2003; Gundry *et al*., 2004; Obi *et al*., 2004). Studies conducted in Iran (Pournadeali and Tayback, 1980) and in northern Sudan (Musa *et al*., 1999) have both showed that water at communal taps were microbiologically of a better quality than untreated irrigation canal water. Contrary to these findings, a study in Burma (Han *et al*., 1989) has showed that tube well and shallow well water supplies were microbiologically of a better quality than municipal tap water and pond water source supplies.

In South Africa, studies in the Limpopo Province (Verweij *et al*., 1991) have showed that communal standpipes were microbiologically less contaminated than borehole and unprotected spring water sources. Another study in the rural Kibi area of the Limpopo Province of South Africa (Davids and Maremane, 1998), have indicated that spring and borehole water sources were microbiologically less contaminated than river water sources.

In addition three recent studies conducted in the Vhembe region of the Limpopo Province in South Africa indicated that rivers and fountains used by rural communities for domestic water were all contaminated by enteric pathogens including *E. coli*, *Plesiomonas shigelloides*, *V. cholera*, *Enterobacter cloacae*, *Shigella* spp, *Salmonella* spp, *Aeromonas hydrophila*, *Aeromonas caviae* and *Campylobacter* spp (Obi *et al*., 2002; Obi *et al*., 2003; Obi *et al*., 2004). *Escherichia coli* isolates obtained from the

different rivers during this study were typed using molecular techniques to determine the presence of virulent genes (Orden *et al*., 1999; Kuhnert *et al*., 2000; Obi *et al*., 2004). Enterotoxigenic *E. coli* isolates (11.8%) contained heat stable and heat labile genes; Shigatoxin producing *E. coli* (4.4%) isolates contained *stx1* and *stx2* genes; Necrotoxigenic *E. coli* (35.6%) contained *cnf1* and *cnf2* genes and Enteropathogenic *E. coli* (34.1%) isolates contained *BfpA* and *EaeA* genes (Obi *et al*., 2004). Necrotoxigenic *E. coli* may play a role in possible zoonotic transmission since it has been shown that human and animal strains share similar serogroups and carry the same genes coding for fimbrial and afimbrial adhesion (Mainil *et al*., 1999). All of these studies indicated that the water sources used by communities in developing countries are microbiologically contaminated and pose a health risk to the consumers (Pournadeali and Tayback, 1980; Obi *et al*., 2002; Sobsey *et al*., 2003; Gundry *et al*., 2004; Obi *et al*., 2004).

2.5.1 Water collection from the source water supply

In most developing countries, women are responsible for the collection of water (Sobsey, 2002). The work involved in fetching the water may differ in each region, it may vary according to the specific season, it depends on the time spent queuing at the source, the distance of the household from the source and the number of household members for which the water must be collected (WHO, 1996b; WHO, 1996c). Water for domestic use is collected either by dipping the container inside the water supply Fig 2.1), collecting rainwater from a roof catchment system (Fig 2.2) or by using different types of pumps connected to the water supply system (Fig 2.3) (Sobsey, 2002). The transportation of the water from the source water supply could be either by a wheelbarrow (Fig 2.4), a donkey cart (Fig 2.5), a motor vehicle (Fig 2.6), using a rolling system (Fig 2.7) or by carrying the container by hand or on the head (Fig 2.8) (CDC, 2001). A common practice often seen in rural areas was the use of leaves or branches with leaves to stop water slopping out during transit in wide-neck storage and transport containers (Fig 2.9) (Sutton and Mubiana, 1989). Consequently, a study by Sutton and Mubiana (1989) has showed that these leaves can be an additional source of coliform bacteria to the drinking water.

Figure 2.1: **Water collection by rural people in the Vhembe region of the Limpopo Province of South Africa: Dipping containers inside theprimary water source**

Figure 2.2: **Water collection by rural people in the Vhembe region of the Limpopo Province of South Africa: Collecting rain water from the roof of the household**

Figure 2.3: **Water collection by rural people in the Vhembe region of the Limpopo Province of South Africa: Ground water pumped to a communal tap**

Figure 2.4: **Water transportation by rural people in the Vhembe region of the Limpopo Province, South Africa: Use of a wheelbarrow**

Figure 2.5: Water transportation by rural people in the Vhembe region of the Limpopo Province, South Africa: Use of a donkey cart

Figure 2.6: Water transportation by rural people in the Vhembe region of the Limpopo Province, South Africa: Use of a motor vehicle

Figure 2.7: **Water transportation by rural people in the Vhembe region of the Limpopo Province, South Africa: Use of a rolling drum**

Figure 2.8: **Water transportation by rural people in the Vhembe region of the Limpopo Province, South Africa: Use of hands and head**

Figure 2.9: Methods used by rural people in the Vhembe region of the Limpopo Province, South Africa to stop water from spilling while in transport: Use of leaves/branches

Water sources could be some distance away from the households, particularly in rural areas (WHO, 1996b; WHO, 1996c). In studies conducted in Malawi, Kenya, Uganda and Tanzania (Lindskog and Lundqvist, 1989; White *et al*., 2002), it was found that if the water taps were situated closer to the dwelling, the amount of water collected/person/day increases from 9.7 to 15.5 litres. Studies in Mosambique (Cairncross and Cliff, 1987) showed that households collect on average 11.1 litres of water/person/day if the source is less than 300 m from the dwelling, while the households who have to walk more than 4 km collected on average 4.1 litres of water/person/day. In Lesotho, Esrey and co-workers (1992) made a rough estimate of 10 litres of water/person/day based on direct observations of households in rural communities. Studies in rural communities in the Limpopo Province of South Africa (Verweij *et al*., 1991) showed that on average 11.4 litres of water/person/day was collected if the source was close to the household, compared to an average of 8.6 litre of water/person/day if the sources were more than 1 km from the household. The Department of Water Affairs and Forestry in South Africa recommends 25

litre/person/day from a source within a distance of 200 m from the dwelling (DWAF, 1994) and the WHO estimates a minimum of 20 litres of water/person/day is sufficient (WHO, 1996b), while Gleick (1998) recommends 50 litres of water/person/day is efficient. These studies indicated that more water was collected per person per day if the source was closer to the dwelling (White *et al*., 2002; Lindskog and Lundqvist, 1989; Verweij *et al*., 1991).

Very few studies have investigated the microbiological quality of water during collection and transportation. In a study in Rangoon, Burma (Han *et al*., 1989) the water at the source and during collection were analysed and indicated that the faecal coliform counts in the collection samples were higher than the counts in the source water samples (Han *et al*., 1989). The increase in faecal contamination of the water in the collection containers after collection from the source could have been due to unhygienic handling of the water and posed a potential health risk of diseases to the consumers (Sobsey, 2002). In a study in Sri Lanka (Mertens *et al*., 1990) it was found that only 5% of tube well water samples were contaminated if the pump was sterilised prior to collection of the sample compared to 50% if the pump was not sterilised. This implied that the taps were contaminated by hands or animals during collection (Mertens *et al*., 1990).

In another study in rural communities in South Africa (Verweij *et al*., 1991), water samples were taken immediately after collection from communal taps and unprotected borehole and springs. Special precautions were taken to prevent contamination during collection, which included rinsing of the container before filling, using a calabash to scoop water from the source and demarcation of a special area for water collection (Verweij *et al.*, 1991). The results from this study indicated no significant difference between faecal coliform counts at the source and immediately after collection of the water (Verweij *et al.*, 1991). The drawbacks of this study however included the sample size (only 8 households were studied), and inadequate information given regarding who collected the water samples e.g. a technician or a woman from the study households (Verweij *et al*., 1991). A study carried out in a Malawi refugee camp has found that hands are primarily responsible for contamination of collected water because the women rinses the container with small amounts of water using their hands to rub around the container opening in an effort to clean it (Roberts *et al*., 2001). A study by Dunker

(2001) has concluded that rural communities in South Africa spent little time on proper cleaning of the collection containers, especially if water has to be collected more that once a day.

These studies have shown that although the microbiological quality of the source water could be classified as safe for domestic purposes, the water collected by the households from these sources, become contaminated after collection (Sobsey, 2002). The origin of the contamination includes: transport and unhygienic collection and handling practices such as dirty utensils, dirty hands and unclean storage containers (Dunker, 2001; Sobsey, 2002).

2.5.2 Interventions to improve source water supplies

Various intervention strategies to improve the water at the source have been described in the literature (Sobsey, 2002). These improvements can include the building of reservoirs, building protective structures around boreholes and fountains, providing communities with communal taps closer to the dwelling and the treatment of the water source with a disinfectant (Sobsey, 2002). A study in Shangai (Xian-Yu and Hui-Gang, 1982) have showed that continuous chlorination rather than periodic chlorination of wells is more reliable, safes time and labour and showed a reduction in the mortality rates due to enteric diseases from 13.7 per 100 000 people to 1.1 per 100 000 people. However, Jensen and co-workers (2002) have found that in rural areas of Parkistan, where public water supply systems was chlorinated, no reduction in diarrhoea incidence in children from these villages were found compared to diarrhoea incidence in children from villages where the people used untreated ground water supplies.

Different interventions can be implemented to improve the microbiological quality of the source water supply. A study in rural Malawi (Lindskog and Lindskog, 1988) has showed that communal piped water supplies situated within a distance of 400 m from a specific household, improved the microbiological water quality used for drinking because people collected water more often and did not store water which could have become contaminated during storage. A 3 year study by Ghannoum and co-workers (1981) in Libya have showed that the installation of water treatment plants did reduce the incidence of bacillary and amoebic dysentery between 10% and 50%, but not

Giardia infections. However, studies carried out in peri-urban communities in South Africa (Genthe *et al*., 1997; Jagals *et al*., 1999) have showed that although the households were supplied with good quality water complying with South African drinking water specifications (DWAF, 1996), the water in the household storage containers had increased levels of indicator microorganisms. This implied that secondary contamination was introduced after the water collection. Consequently, many of these studies have indicated that improvements at the water source are useless as water is contaminated during collection and storage in households due to poor sanitation practices.

2.6 POINT-OF-USE WATER SUPPLIES IN THE HOUSEHOLD

Source water contamination is likely to have a wide effect on the community because it can introduce new pathogens in the home environment (Sobsey, 2002). However, several studies have reported that the microbiological quality of the water deteriorate after collection, during transport and during storage at the point-of-use due to secondary contamination factors (Rajasekaran *et al*., 1977; El Attar *et al*., 1982; Han *et al*., 1989; Lindskog and Lindskog, 1989; Sandiford *et al*., 1989; Blum *et al*., 1990; Henry and Rahim, 1990; Mertens *et al*., 1990; Pinfold, 1990; Verweij *et al*., 1991; Simango *et al*., 1992; Swerdlow *et al.*, 1992; Shears *et al*., 1995; Kaltenhaler and Drasar, 1996; Genthe *et al*., 1997; Jensen *et al*., 2002; Wright *et al*., 2004). Due to the distances and unavailability of piped water supplies on the dwelling or inside the households in many developing regions of the world, people are forced to store their drinking water (Sobsey, 2002).

Transmission of microorganisms inside the household can occur through several routes (Briscoe, 1984; Roberts *et al*., 2001). The most important transmission routes include water, food, person-to-person contact, unhygienic behaviour (eg. intra-household transmission of faeces), the storage conditions of the water storage containers at the point-of-use and the abstraction conditions of water from the storage container (Briscoe, 1984; Roberts *et al*., 2001). In addition, a number of studies (as shown in Table 2.3) suggested that inadequate storage conditions increased the risk of contamination, which can lead to infectious diseases.

Table 2.3 Summary of studies indicating increased microbiological contamination of stored water and the associated infectious disease risk due to inadequate storage conditions (Sobsey, 2002)

Study Area	Storage container	Storage time	Impact on Microbial quality	Disease Impact	Reference
Bangladesh	Water jars	1-2 days	Increased *Vibrio cholera* presence	Increased cholera rates	Spira *et al.*, 1980
Bahrain	Capped plastic vessels, jars, pitchers	Not reported	*Vibrio cholera* present in stored water and not in source water	Uncertain	Gunn *et al.*, 1981
Sudan	Clay jars (zeers)	2 days – 1 month	Increased faecal indicator bacteria over time, in summer and during dust events	Not measured	Hammad and Dirar, 1982
Egypt	Clay jars (zir)	<1- 3 days	Algae growth and accumulated sediment	Not detected	Miller, 1984
India	Wide mouth vs narrow neck	Not reported	Not measured	Cholera infections fourfold higher in wide mouth storage vessels	Deb *et al.*, 1986
Burma	Buckets	Up to 2 days	Higher levels of faecal coliform bacteria than sources	Not measured	Han *et al.*, 1989
Liberia	Large containers, open or closed	Long time	High levels of enterobacteria in stored samples compared to sources	Not measured	Molbak *et al.*, 1989
Sri Lanka	Earthen pots and others	Not reported	High levels of faecal coliforms in unboiled stored water	Not measured	Mertens *et al.*, 1990
South Africa	Plastic container	4 hours	Higher coliform levels over time	Measured; no effect	Verweij *et al.*, 1991
Africa	Traditional and metal jars	24 hours and more	High total and faecal coliform levels	Not measured	Empereur-Bisonette *et al.*, 1992
Malaysia	Various containers	Not reported	Higher levels of faecal coliforms in unboiled than boiled water	Higher diarrhoea risks from water unboiled or stored in wide neck than narrow neck containers	Knight *et al.*, 1992
Zimbabwe	Covered and uncovered containers	12 hours or more	Higher *E. coli* and *Aeromonas* levels with storage and use	Not measured	Simango *et al.*, 1992
Peru	Wide mouth containers	Not reported	Higher faecal coliform levels in stored waters than source waters	Increased cholera risks	Swerdlow *et al.*, 1992
Bangladesh	Traditional pots	Not reported	Increased faecal coliform levels and antibiotic resistance	Increased faecal coliforms and multiple antibiotic resistant flora	Shears *et al.*, 1995
Trinidad	Open drum, barrel, bucket vs tank or none	Not reported	Increased faecal bacteria levels in open storage vessels than tank	Not measured	Welch *et al.*, 2000

Some studies showed an increase in the number of *V. cholera* in stored water (Spira *et al*., 1980; Gunn *et al*., 1981), while other studies indicated an increase in faecal coliform bacteria and enterobacteriaceae (*E. coli* and *Aeromona* spp) in the stored water (Deb *et al*., 1986; Hammad and Dirar, 1982; Han *et al*., 1989; Molbak *et al*., 1989; Mertens *et al*., 1990; Verweij *et al*., 1991; Empereur-Bisonette *et al*., 1992; Knight *et al*., 1992; Simango *et al*., 1992; Swerdlow *et al*., 1992; Shears *et al*., 1995; Welch *et al*., 2000).

The geometric design of household water storage containers could play an important role in ensuring that the stored drinking water does not become contaminated during storage (Sobsey, 2002). Many different types and sizes of traditional storage containers (Fig 2.10 and 2.11) are commonly used in developing countries such as the nomadic people of Sudan which uses a container made from animal hide called a girba (Musa *et al*., 1999) and communities in Africa which use traditional African clay pots or urns (Patel and Isaacson, 1989; Sutton and Mubiana, 1989; Sobsey, 2002).

Figure 2.10: Typical 25 litre water storage containers and buckets used for point-of-use water storage by rural people in the Vhembe region of the Limpopo Province, South Africa

Figure 2.11: Typical 200 litre water storage container used for point-of-use water storage by rural people in the Vhembe region of the Limpopo Province, South Africa

The material of the container is also important because the chemical material of the storage container could be conducive to bacterial growth and survival of potentially pathogenic microorganisms if contamination of the water occurs. This was shown in a study conducted by Patel and Isaacson (1989), which showed that *Vibrio cholera* 01 survived longer in corroded iron drums than in new iron drums.

The studies in Table 2.3 have showed that water can be stored between 4 h and 1 month at the point-of-use. Faechem and co-workers (1983) indicated that the time of storage was important, with the highest increase in faecal contamination occurring if the storage time was longer than 10 h. Similar observations were reported by other studies, especially if the storage periods were longer than 12 h (Han *et al*., 1989; Mertens *et al*., 1990; Verweij *et al*., 1991; Simango *et al*., 1992 Ahmed and Mahmud, 1998; Momba

and Kaleni, 2002). These studies have showed that the microbiological quality of water deteriorates during long storage times and increased the risk of the transmission of waterborne diseases.

Other factors, which could contribute to the contamination of the water during storage at the point-of-use, included unsanitary and inadequately protected (open, uncovered, poorly covered) containers (Dunker, 2001). Many of the studies listed in Table 2.3 had either uncovered containers, containers with wide openings or buckets, which were used as storage containers. Storage containers need to be covered at all times to prevent flies, animals (Fig 2.12) and small children from touching the water (Fig 2.13) (Sobsey, 2002). It was noted by Jensen *et al*., (2002), that containers with openings of less than 10 cm were less contaminated with coliform bacteria than those with wider openings. Water was poured from these containers, while water was dipped out with hands and utensils where containers with wider openings were used. However, a study by El Attar and co-workers (1982) showed no differences in water quality between containers that were covered versus those that were uncovered.

Figure 2.12: Possible contamination route of stored drinking water in rural households in the Vhembe region of the Limpopo Province, South Africa: animals licking containers while containers are filled with water

Figure 2.13: Possible contamination route of stored drinking water in rural households in the Vhembe region of the Limpopo Province, South Africa: small children touching water storage containers which are not closed

Human faecal pollution from children and adults who do not wash their hands after being to the toilet can contribute to secondary contamination of household stored drinking water (DeWolf Miller, 1984; Dunker, 2001). Several studies have indicated that *E. coli* can survive for 10 min, *Klebsiella* spp for up to 2.5 h (Casewell and Phillips, 1977) and *Shigella sonnei* and faecal enterococci for up to 3 h (Knittle, 1975; Pinfold, 1990) on unwashed hands, which could contaminate food and water in the household.

Finally, inadequate cleaning measures of the storage containers could lead to the formation of biofilms (Fig 2.14) which could harbour potentially pathogenic and opportunistic microorganisms such as total coliforms, faecal coliforms, *E. coli*, somatic and F-RNA bacteriophages, *C. perfringens*, *Salmonella* spp and *Helicobacter pylori* (Bunn *et al*., 2002; Jensen *et al*., 2002; Momba and Kaleni, 2002; Sobsey, 2002). These

indicator and pathogenic microorganisms could survive longer than 48 h in biofilms inside household drinking water storage containers and pose a potential risk factor for humans consuming this water (Bunn *et al*., 2002; Jensen *et al*., 2002; Momba and Kaleni, 2002; Sobsey, 2002).

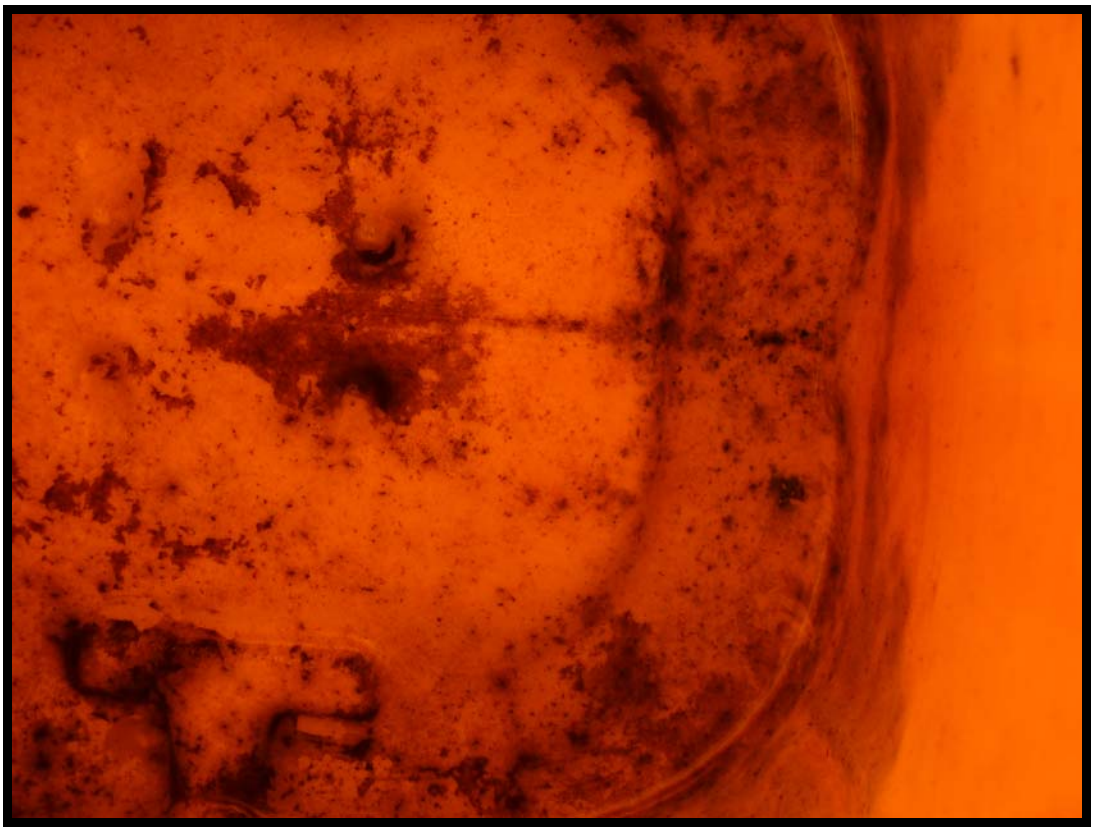


Figure 2.14: Possible contamination route of stored drinking water in rural households in the Vhembe region of the Limpopo Province, South Africa: biofilm formation inside a 25 litre water storage container

The studies mentioned in this section clearly showed that contamination of water occurred during collection and storage at the point-of-use and does contribute to the risk of disease transmission and possibly the spread of anti-microbial resistant genes (Shears *et al*., 1995; Sobsey, 2002). Therefore, the focus must be on point-of-use interventions rather than water source interventions because point-of-use interventions will be more effective in the removal and inactivation of potential disease causing microorganisms introduced during collection and storage inside a family cohort.

2.6.1 Interventions to improve point-of-use water supplies in the household

Point-of-use interventions must improve the water used for drinking at the household level (Sobsey, 2002). This can be achieved by educating household members to improve their hygienic behaviour, by improving the water storage container and by appropriate treatment of the stored water (Dunker, 2001). All of these interventions will be discussed in the following sections.

2.6.1.1 Improving the point-of-use water supply by improving hygienic practices in the household

Basic hygiene practices such as hand washing was shown to be an effective intervention in the reduction of diarrhoea in developing countries (Curtis *et al*., 2000; Trevett *et al*., 2005). A study in Burma (Han and Hlaing, 1989) showed a 30% reduction in diarrhoeal incidence if people washed their hands after defecation, prior to food preparation. Studies in Indonesia (Wilson *et* al., 1991) and Bangladesh (Shahid *et al*., 1996) have showed an 89% and 66% reduction of diarrhoea respectively after hand washing was introduced.

However, factors like the distance from the washing area and the frequency of hand washing do affect the influence of the intervention on the disease outcome (Faechem, 1984; Hoque *et al*., 1995). Faechem (1984) has showed that soap and water together removes 100% of inoculated bacteria while water alone removed less bacteria. Hoque and co-workers (1995) has showed that soap, ash and soil were equally effective hand-washing reagents, however, drying wet hands on clothing, resulted in recontamination of the hands. Proper education should therefore be given to people from rural communities to promote the correct hygiene practices and these communities should be informed on the transmission risk and the causes of waterborne diseases (Dunker, 2001).

2.6.1.2 Improving the point-of-use water supply by using an improved storage container

The United States Centres for Disease Control and Prevention (CDC) and the Pan American Health Organization (PAHO) have studied and reviewed the advantages and disadvantages of different types of water collection and storage containers from studies carried out in various regions of the world. These two organisations have written guidelines for the most desirable container to be used by households for drinking water storage. The guidelines include the following (Mintz *et al*., 1995; Reiff *et al*., 1996; CDC, 2001):

- The container must have a capacity of 15 to 25 litres, rectangular or cylindrical with one or more handles and flat bottoms for portability and ease of storage;
- Should be made of lightweight, oxidation-resistant plastic, such as high-density polyethylene or polypropylene, for durability and shock resistance;
- Should be fitted with a 6 to 9 cm screw-cap opening to facilitate cleaning, but small enough to discourage or prevent the introduction of hands or dipping utensils;
- Should have a durable, protected and preferably easily closed spigot or spout for dispensing water;
- Should have an affixed certificate of approval or authenticity;
- Should be affordable to the user.

Based on these guidelines, the CDC and PAHO designed a 20 litre container to decrease the risk of contamination during storage (Fig 2.15) (Mintz *et al*., 1995; Reiff *et al*., 1996; CDC, 2001; Sobsey, 2002). Together with the use of a sodium hypochlorite solution, this container has proved effective in several studies carried out in different developing countries in Africa, Europe and South America as indicated in Table 2.4 (CDC, 2001; Sobsey, 2002).

Figure 2.15: The CDC safe storage container designed by the CDC and PAHO in the USA for point-of-use treatment

Several of the studies mentioned in Table 2.4, have investigated the reduction of disease, especially the reduction of diarrhoea during the intervention phase (Semenza *et al*., 1998; Quick *et al*., 1999; Mong *et al*., 2001; Quick *et al*., 2002; Sobsey *et al*., 2003). The results from all of these studies showed that the diarrhoea incidences were reduced between 20% and 85%, while cholera incidence were reduced by 90% during a cholera outbreak in Madagascar (Semenza *et al*., 1998; Quick *et al*., 1999; Mong *et al*., 2001; Quick *et al*., 2002; Sobsey *et al*., 2003). Unfortunately most of these studies have only used *E. coli* and thermotolerant indicator bacteria to assess the microbiological quality of the stored household water (Semenza *et al*., 1998; Quick *et al*., 1999; Mong *et al*., 2001; Quick *et al*., 2002; Sobsey *et al*., 2003). However, none of these studies investigated the survival of pathogenic microorganisms in the CDC safe storage container nor have any study investigated the origin of the faecal contamination in the CDC safe storage container. Although, the incidence of diarrhoea decreased during the intervention studies, little information is available on the origin or the causative microorganism of the diarrhoeal diseases (Sobsey, 2002).

Table 2.4 Efficacy of chlorination and water storage in the CDC safe storage container to disinfect household water, reduce waterborne diseases and improve the microbiological quality of water (Sobsey, 2002)

Location	Water and service level	Treatment	Storage vessel	Disease reduction (%)	Significant microbe decrease?	Intervention	Reference
Uzbekistan	Household On site and off plot Mixed sources	Free chlorine	CDC safe storage container	85% diarrhoea	No But based on small number of samples	Water intervention only	Semenza *et al*., 1998
Guatamala	Street vendor water Off plot Mixed sources	Free chlorine	CDC safe storage container	No data	Yes *E. coli* positive counts decrease from >40 to <10%	Water intervention and Santation and Health intervention	Sobel *et al*., 1998
Guinea-Bisseau	Oral rehydration solution Off plot Ground water or Surface water	Free chlorine	CDC safe storage container	No data	Yes Mean *E. coli* positive counts decrease from 6200 to 0 counts.100 ml^{-1}	Water intervention and Santation and Health intervention	Daniels *et al*., 1999
Bolivia	Household On site Ground water	Electrochemical oxidant (mostly free chlorine)	CDC safe storage container	44% diarrhoea	Yes *E. coli* positive counts decrease from 94 to 22%; median E. coli counts from >20 000 to 0	Water intervention and Sanitation and Health intervention	Quick *et al.*, 1999
Parkistan	Household On site and off plot Municipal	Free chlorine	CDC safe storage container	No data	Yes Thermotolerant coliforms counts decrease by 99.8%	Water intervention and Santation and Health intervention	Luby *et al*., 2001
Madagascar	Household	Free chlorine (traditional vessel)	CDC safe storage container or traditional vessel	90% cholera (during outbreak)	Yes Median *E. coli* positive counts decrease from 13 to 0 counts.100 ml^{-1}	Water intervention and Santation and Health intervention	Mong *et al*., 2001
Zambia	Household Off plot or on site Not reported Ground water	Free chlorine	CDC safe storage container or traditional vessel	48% diarrhoea	Yes *E. coli* positive counts decrease from 95 to 31%	Water intervention and Santation and Health intervention	Quick *et al*., 2002
Bolivia and Bangladesh	Household Onsite Shallow groundwater and municipal water	Free chlorine	CDC safe storage container or traditional vessel	20.8% diarrhoea	Yes *E. coli* counts decreased in intervention households	Water intervention and Health intervention	Sobsey *et al*., 2003

The studies in Table 2.4 have also included additional interventions together with the CDC safe storage container and sodium hypochlorite solution interventions. The additional interventions included sanitation and health interventions where people were informed and educated on hygiene and handling practices (Sobel *et al*., 1998; Daniels *et al*., 1999; Quick *et al*., 1999; Luby *et al*., 2001; Mong *et al*., 2001; Quick *et al*., 2002; Sobsey *et al*., 2003). Generally all of these studies have showed that proper education will influence the compliance with point-of-use interventions (Sobsey, 2002). People should be made aware and educated on the benefit of using interventions to improve the microbiological quality of the household drinking water.

2.6.1.3 Improving the point-of-use water supply by chemical or physical treatment

Several physical and chemical treatments have been developed and tested under various field conditions in several countries as interventions to improve the water at the point-of-use (Sobsey, 2002; Nath *et al*., 2006). However, many of these treatments are not suitable for conditions in rural communities. The various advantages and disadvantages with regards to the use of some of these treatment interventions in rural regions will be discussed in the following sections.

2.6.1.3.1 Physical treatment methods

Physical treatment methods include boiling, heating, settling, filtration and exposure to ultraviolet radiation from sunlight (Gilman and Skillikorn, 1985; Mintz *et al*., 1995; Conroy *et al.,* 1996; CDC, 2001; Sobsey, 2002). Boiling is widely used since it is easy to use and effective in destroying bacteria, viruses and protozoa in all types of water (Sobsey, 2002). However, the collection of firewood is time consuming, could lead to deforestation and is an expensive method for general use (Gilman and Skillicorn, 1985; Barau and Merson, 1992). A further concern is that water is often transferred to storage containers for cooling and thus can become re-contaminated (Sobsey, 2002).

Solar disinfection such as the SOLAIR and SODIS systems, which makes use of plastic water collection bottles which is left in the sun, have been widely tested in rural African communities (Conroy *et al*., 1996; Conroy *et al*., 1999; Meyer and Reed, 2000; Conroy *et al*., 2001). Both these systems inactivates pathogens by disinfecting small quantities

of water for consumption, requires relative clear water (turbidity< 30 NTU) and the effectiveness of the inactivation is dependant on exposure times (Conroy *et al*., 1996; Conroy *et al*., 1999; McGuigan *et al*., 1999; Meyer and Reed, 2000; Conroy *et al*., 2001; Rijal and Fujioka, 2001; Sobsey, 2002; Mascher *et al*., 2003; Oates *et al*., 2003).

Sedimentation and settling is used for very turbid water (Sobsey, 2002). The turbidity is usually due to the presence of sand particles (mud) (Sobsey, 2002). After the water is collected, the container is left undisturbed for a few hours (Sobsey, 2002). The large dense particles (sands and silts) together with large microorganisms will settle out (sediment) due to the effect of gravity (Sobsey, 2002). The upper cleaner water is carefully removed without disturbing the sedimented particles (Sobsey, 2002). Unfortunately sedimentation is not very effective in reducing microbial pathogens in stored household water (Sobsey, 2002).

Filtration is a widely used method to remove particles and some microorganisms from water samples (Potgieter, 1997; Sobsey, 2002). Several types of filter media and filtration processes are available for household treatment of water (Sobsey, 2002). However, the effective removal of microorganisms, the cost and the availability of the filter media in developing countries varies from easy to moderate to difficult (Sobsey, 2002). Granular type of filters include bucket filters, barrel or drum filters and roughing filters and filter cisterns which can rapidly reduce turbidities and enteric bacteria by >90% and larger parasites by >99% efficiency, and enteric viruses by 50% to 90% (Sobsey, 2002; Clasen and Bastable, 2003). Slow sand filters, fibre, fabric and membrane filters, porous ceramic filters and diatomaceous earth filters are alternative filters that have been tested and used for household water treatment in developing countries (Sobsey, 2002; Clasen and Bastable, 2003). Many of these studies have showed to reduce turbidity by 90% and bacteria by 60%, although the cost of the filters is high (Sobsey, 2002; Clasen and Bastable, 2003). A study by Clasen and co-workers (2004) in Bolivia, indicated a reduction of diarrhoea of 70% and a 100% reduction of thermotolerant coliforms in households using ceramic filters compared to control households not using ceramic filters. Unfortunately, little information is available on the effectiveness of these filter systems in the reduction of viruses from household water (Sobsey, 2002).

2.6.1.3.2 Chemical treatment methods

Various chemical methods are available for the treatment of drinking water at the household level and include methods such as coagulation-flocculation, precipitation, adsorption, ion exchange and chemical disinfection with agents such as sodium hypochlorite (Gilman and Skillicorn, 1985; Mintz *et al*., 1995; Conroy *et al.,* 1996; CDC, 2001; Sobsey, 2002). Unfortunately most of these methods are expensive, requires technical skilled persons, regular monitoring, specific materials and the efficacy varies (Sobsey, 2002). Chemical disinfectant agents have proved to be the most successful types of treatment and include free chlorine (which will be discussed in more detail), chloramines, ozone and chlorine dioxide (Sobsey, 2002).

Several factors might play a role in the effectiveness of a chemical disinfectant. These factors include pH, turbidity, temperature, degree of microbial contamination and the contact time of the disinfectant to the water and microorganisms (LeChevallier *et al*., 1981; Reiff *et al*., 1996). According to Reiff and co-workers (1996), an ideal chemical disinfectant should have the following qualities:

- The disinfectant must be reliable and effective in the inactivation of pathogens under a range of conditions likely to be encountered;
- The disinfectant must provide an adequate residual concentration in the water as to assure safe microbial quality throughout the storage period;
- The disinfectant must not introduce nor produce substances in concentrations that may be harmful to health, nor otherwise change the characteristics of the water so as to make it unsuitable for human consumption;
- The disinfectant must be reasonable safe for household storage and use;
- The disinfectant must have an accurate, simple and rapid test for measurement of the disinfectant residual in the water, which can be performed, when required;
- The disinfectant must have an adequate shelf life without significant loss of potency;
- The disinfectant must have a cost that is affordable for the household.

A chemical disinfectant that has been used effectively since 1850, is chlorine (sodium hypochlorite) (White, 1999). During a cholera outbreak in London, chlorine was used

to disinfect water supplies (White, 1999). During the 1890's, Europe used hypochlorites against epidemics of typhoid (White, 1999). Only in the early 20th Century Great Britain and New Jersey City began treatment of potable water supplies on a continuous basis. Since then chlorine has become the most widely used water treatment disinfectant because of its potency, ease of use and cost effectiveness (White, 1999).

Chlorine reacts with water to form hypochlorous acid (HOCl) and hydrochloric acid (HCl) (Carlsson, 2003). The HOCl dissociates further into a hypochlorite ion (OCl^-) and a hydrogen atom (H^+) which are commonly referred to as the free chlorine residual (Carlsson, 2003). The main problem to overcome when chemical treatment is used is the differences in resistance of bacteria, viruses and parasites to these chemical disinfectants (Sobsey, 1989; Sobsey, 2002). The resistance of waterborne microbes to be inactivated by chemical disinfectants is influenced by several factors: (1) their physical status; (2) their physiological status; (3) the presence of microorganisms within microbial aggregates (clumps); and (4) microorganisms embedded within other matrices such as a membrane, a biofilm, another cell, or fecal matter (Sobsey, 1989; Sobsey, 2002). The microorganisms could be protected against chemical disinfectants and by the oxidant demand of the material in which they are located (Sobsey, 1989; Sobsey, 2002). Consequently it has been showed that bacteria are more susceptible to chlorine than viruses or enteric parasites (Sobsey, 1989; Sobsey, 2002).

In bacterial cells the free residual chlorine reacts with various structures on the bacterial cell (Carlsson, 2003). The free residual chlorine can also kill the microorganism by disrupting the metabolism and protein synthesis, to decrease respiration, glucose transport and adenosine triphosphate levels and to cause genetic effects by modification of the purine and pyirimidine basis (LeChevallier and Au, 2004). In viruses the free residual chlorine targets mainly the nucleic acid and do not have a noticeable effect on the protein coat (Carlsson, 2003). This means that viruses containing a protein coat are more resistant to the effect of free residual chlorine (Carlsson, 2003). Free chlorine residual is not very effective against parasites because of the tough outer coat, which makes them very resistant to the action of hypochlorous acid (Carlsson, 2003). Therefore, parasites need to be exposed for longer times to the free chlorine to be inactivated (Venczel, 1997; Carlsson, 2003). Studies have showed that *Giardia lamblia*

cysts are inactivated at 1 $mg.l^{-1}$ free chlorine in water with a pH of 6 to 7 and at temperatures of 5°C only after 1 to 2.5 h (USEPA, 1989) and *Giardia muris* cysts under the same conditions are only inactivated after exposure of 10 h (USEPA, 1989).

Studies have showed that the use of free chlorine residual together with the CDC safe storage container (Table 2.4) has improved the microbiological quality of the water and reduced the prevalence of diarrhoea (Quick *et al*., 1996; Luby *et al*., 1998; Macy and Quick, 1998; Semenza *et al*., 1998; Quick *et al*., 1999). The CDC recommends the addition of either a 0.5% or a 1.0% stabilized concentration of sodium hypochlorite solution to obtain a free chlorine residual between 0.5 and 1.5 $mg.l^{-1}$ after 60 min (WHO, 1996a; CDC, 2001; Dr R Quick, CDC, Atlanta, USA, personal communication). In South Africa, the DOH's recommendations do not specify the free chlorine residual concentration. However, the DOH do recommend the addition of 5 ml of a 3.5% stabilized concentration of sodium hypochlorite solution to a 20 or 25 litre storage container (Appendix C) (Mr H Chabalala, Department of Health, Pretoria, personal communication).

In addition, several studies have showed that the use of some chemical disinfectants resulted in the formation of chemical by-products such as trihalomethanes, haloacetonitriles, chlorinated aldehydes, chlorinated acetones, chlorinated phenols and chlorinated acetic acids (WHO, 1996a; Carlsson, 2003). Some of these by-products are potentially hazardous (carcinogenic and mutagenic) (WHO, 1996a; Carlsson, 2003). However, the health risk posed by these by-products is small in comparison to the health risk caused by waterborne pathogenic and opportunistic microorganisms (WHO, 1996a; Carlsson, 2003).

Although various point-of-use interventions have been proposed, the interventions selected for a particular community must be tailored for the needs of the community and consider the resources available to the community (Nath *et al*., 2006). The ideal solution will be to provide these communities with treated municipal tap water in the dwelling to eliminate storage of the water. However, this is not possible in many developing countries due to economical constraints. In the meantime, interventions at the point-of-use should focus on point-of-use treatments that are cost effective, easy to obtain and easy to use (Sobsey, 2002). The rural communities of the Vhembe region in

South Africa could benefit from point-of-use interventions such as the use of the CDC safe storage container together with a sodium hypochlorite solution to improve the quality of household drinking water (Sobsey, 2002).

2.6.2 Sustainability of point-of-use interventions

The microbiological effectiveness of household interventions at the point-of-use has been indicated by several studies (Sobsey, 2002; Fewtrell *et al*., 2005). However, questions on acceptability, affordability, long term utilization and sustainability of household treatments must still be answered (Nath *et al*., 2006). Only one published study on the sustainability of a point-of-use water treatment system could be obtained from the literature: Conroy and co-workers (1999) found that one year after the completion of a solar disinfection intervention in Masaai communities, almost all households were still using the intervention. The lack of adequate follow up studies on the long term utilization and sustainability of household treatments therefore, needs to be addressed in order to determine the success of point-of-use treatment systems.

2.7 SUMMARY

In South Africa almost 80% of the population are living in rural communities without adequate water and sanitation infrastructures (Statistics South Africa, 2003). Many of the communities have to share water sources with cattle and domestic animals (Dunker, 2001). Communal standpipes provide water on infrequent time schedules and the majority of communal standpipe water is untreated. The Vhembe region is situated in the Limpopo Province of South Africa. The Vhembe region was a former homeland for the Venda people in South Africa before the 1994 elections and known as the Venda homeland. In the Vhembe region, the majority of rural communities are poverty-stricken, lack access to potable water supplies and rely mainly on water sources such as rivers, streams, ponds, springs and boreholes for their daily water needs (Davids and Maremane, 1998; Obi *et al*., 2002; Obi *et al*., 2004). Water from these sources is used directly by the inhabitants and the water sources are faecally contaminated and devoid of treatment (Nevondo and Cloete, 1991; Davids and Maremane, 1998; Obi *et al*., 2002; Obi *et al*., 2004). Consequently, a significant proportion of residents are exposed to potential waterborne diseases (Central Statistics, 1995).

A pilot study, which consisted of a questionnaire survey, was conducted initially to serve as a background study before the initiation of this study. The purpose of the pilot study was to obtain information concerning the baseline microbiological quality of the source water and the storage containers as well as to observe sanitation and hygiene practices of rural people in the Vhembe region. Many of the households in rural areas of South Africa do not have individual connections to treated, piped water supplies. These households typically store water in the household. The stored water is vulnerable to contamination from handling during collection, transport and storage. Results from the pilot study indicated the need for education aimed at diseases associated with polluted water supplies and the improvement in the sanitation and hygienic behaviours of the household members during water collection and storage at the point-of-use. Based on the results obtained from the pilot study it was evident that intervention strategies at the point-of-use in the rural communities were needed as interim solutions to prevent waterborne diseases and improve the microbiological quality of domestic stored drinking water.

The literature study has showed that depending on water collection and storage practices, deterioration of the microbiological quality of the water may occur before the water is actually consumed, mostly due to secondary contamination at the point-of-use. Reviews by Sobsey (2002) and Gundry and co-workers (2004) suggested that more point-of-use intervention field studies must be conducted. The bacteriological evidence in their studies showed that improved storage containers may be effective at reducing microorganisms in stored water if the sources were of good microbiological quality or uncontaminated. However, many of the point-of-use interventions mentioned in the literature review, especially the physical and chemical treatment interventions, are impractical because of costs and sustainability and therefore not suitable for impoverished rural households in developing countries such as South Africa (Sobsey, 2002; Gundry *et al*., 2004). In addition, the literature study has also showed that improving the microbiological quality of water before consumption would reduce diarrhoeal disease together with sanitation and hygiene education (Mertens *et al*., 1990; Hoque *et al*., 1995). However, many of the studies have used indicator microorganisms to assess the effectiveness of interventions. The literature review has indicated that most of the currently used indicator microorganisms used to evaluate the microbiological quality of water have shortcomings and will only give an indication of

the potential risk associated with the transmission of waterborne diseases (Moe *et al*., 1991; Payment and Franco, 1993; Sobsey *et* al., 1993; Sobsey *et al*., 1995).

Several potentially pathogenic microorganisms in water polluted by human and animal faeces could cause diarrhoeal diseases in consumers (Sobsey *et al*., 1993; Gerba *et al*., 1996; Grabow, 1996; Leclerc *et al*., 2002; Theron and Cloete, 2002). Little information on the origin of faecal contamination in the traditional and CDC safe storage containers are presently available. Literature has showed that microbiological and chemical indicators can be used to distinguish between human and animal faecal pollution in water (Jagals *et al*., 1995; Sinton *et al*., 1998). However, no single microorganism or chemical determinant could reliably distinguish human from animal faecal contamination and therefore, the use of a combination of chemical and microbial determinants together may provide the best solution for identifying the origin of faecal pollution at the point-of-use (Jagals *et al*., 1995; Sinton *et al*., 1998).

Consequently, the literature study has indicated that the best interventions available that will be applicable to conditions in rural communities in South Africa included the use of the CDC safe storage container together with a chemical treatment such as sodium hypochlorite solution. The aim of this study was therefore to improve the microbiological quality of drinking water in rural households at the point-of-use by the implementation of intervention strategies which included the use of traditional storage containers as well as the CDC water storage container, with or without the addition of a sodium hypochlorite solution. The results obtained from this study would be used to provide information to the DOH and DWAF, which can be used in future water and health policy formulations to prevent waterborne outbreaks in these rural communities.

Chapter 3

MATERIALS AND METHODS

3.1 INFORMED AND ETHICAL CONSENT

Ethical clearance for this study was obtained from the Provincial DOH in Polokwane, the capital city of the Limpopo Province, South Africa in which the study area (Vhembe regiont) was situated. Ethical permission was also obtained from the University of Venda, Thohoyandou, South Africa from which the study was carried out and the University of Pretoria, Gauteng Province, South Africa where the study was registered. The project was also registered at the University of Venda's Research Department. In each of the two study villages, the study layout was explained to the headman or chief whom granted permission to conduct this study. In addition, the head of each study household in both rural villages gave written consent to take part in the study (Appendix A).

3.2 SCHEMATIC OUTLINE OF STUDY DESIGN

This study contained three specific objectives as indicated in Chapter 1. Objectives one and two was carried out using field based studies, while objective three was primarily a laboratory based study. Sections 3.3, 3.4 and 3.5 described the methodology used to prove each of these objectives.

3.3 OBJECTIVE ONE: TO ASSESS AN INTERVENTION STRATEGY TO IMPROVE THE DRINKING WATER QUALITY IN RURAL HOUSEHOLDS

This section described the methodology used to assess the implementation of an intervention strategy to improve the microbiological quality of drinking water in households from two typical rural communities in the Vhembe region, Limpopo Province of South Africa with little of no water and sanitation infrastructure (Fig 3.1).

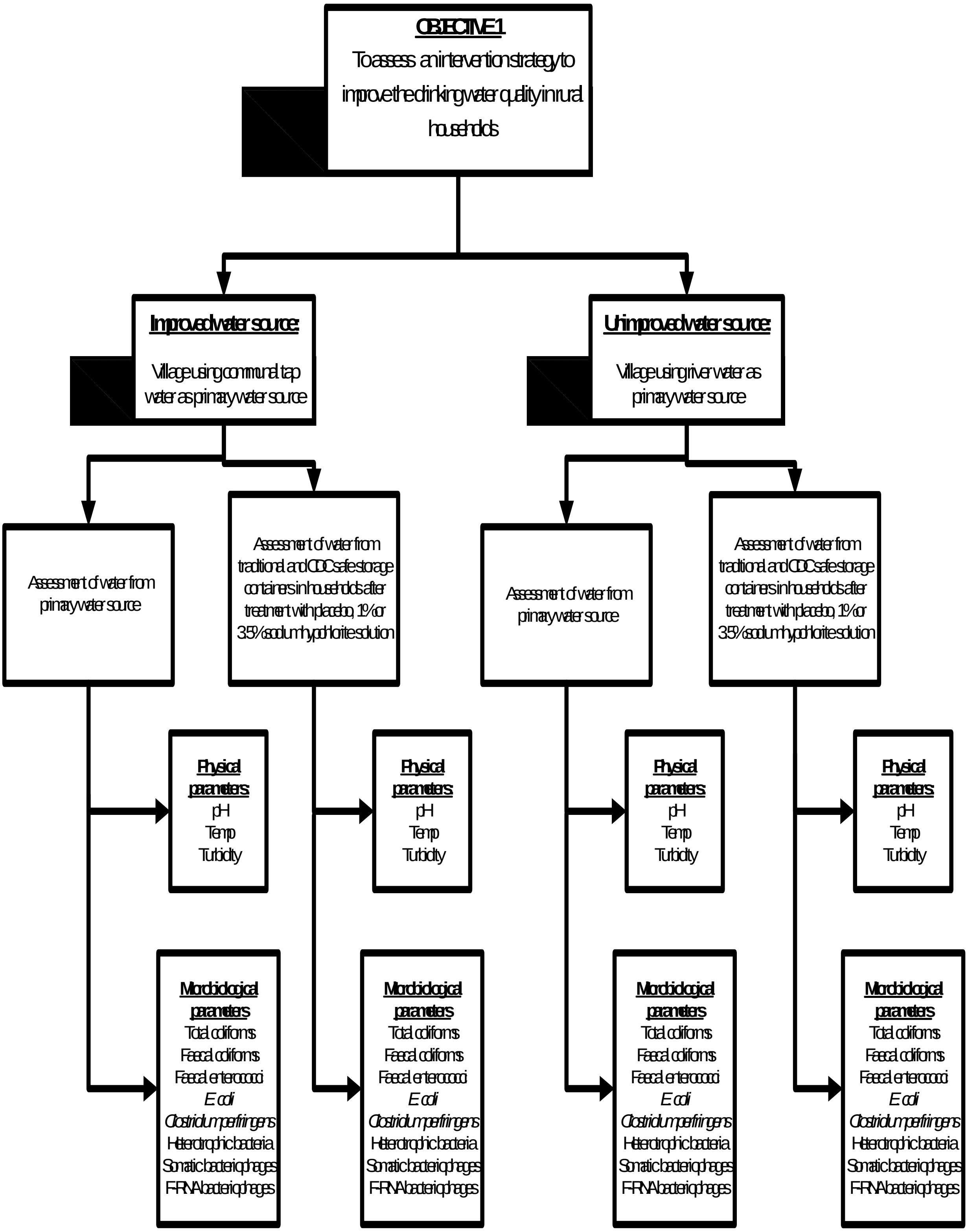


Figure 3.1: **Schematic outlay of the study design of objective one to assess an intervention strategy to improve the drinking water quality at the point-of-use in rural households of South Africa**

3.3.1 Study site and household selection

Two rural villages in the Vhembe region were selected for this study. One village used an improved water source while the other village used an unimproved water source. In village 1 the primary water source included communal taps with untreated water (Fig 3.2). The water was pump directly from a aquifer into a large open reservoir from where it was pumped to the communal taps used by the study households. In village 2 the primary water source included the Sambandou River (Fig 3.3), which was also used for livestock watering, washing of clothes and recreational activities by the community.

Figure 3.2: Typical communal taps used by rural households in village 1 in the Vhembe region of the Limpopo Province, South Africa

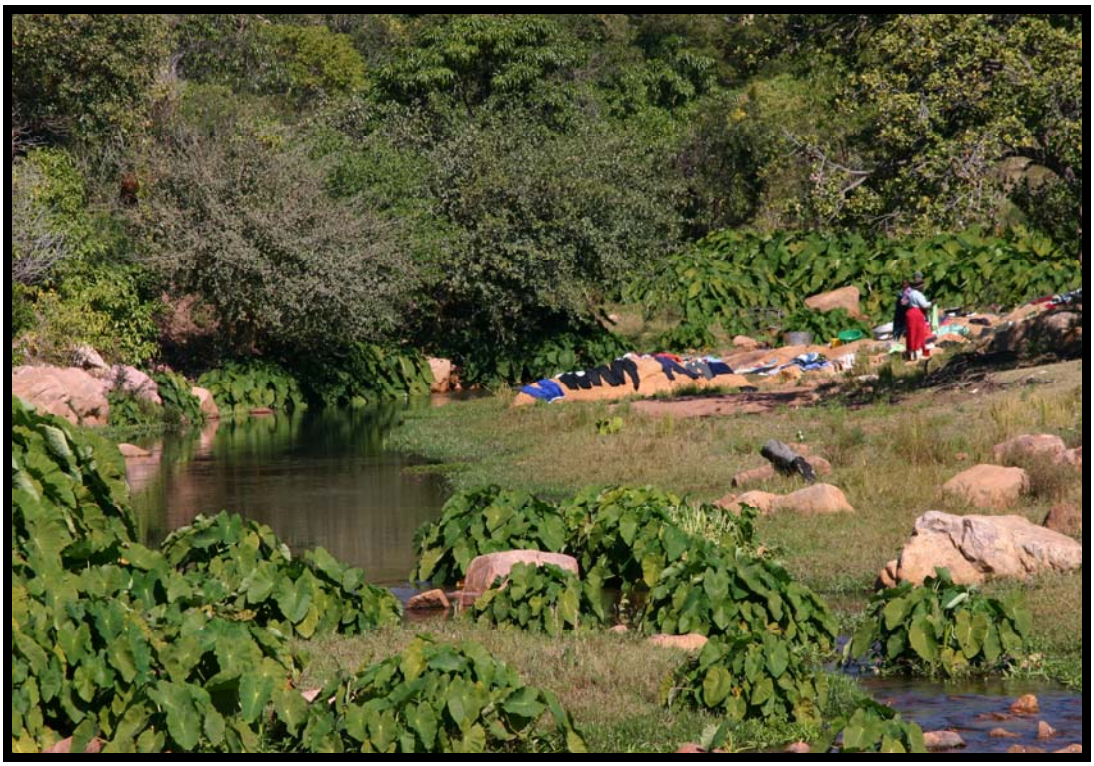


Figure 3.3: The Sambandou River used by rural households in village 2 in the Vhembe region of the Limpopo Province, South Africa

In each village 60 households were randomly recruited and assigned into one control and five intervention groups. The format of the intervention trial is presented in Table 3.1. A group meeting was held with all the selected study households in each village to explain the purpose of the study before the study commenced. Care was taken to make sure that all the study households were blinded to the concentration of the sodium hypochlorite solution. At the household group meetings, the people were only informed that different concentrations of sodium hypochlorite were going to be evaluated during the study period and that the different sodium hypochlorite concentrations will have different smells (eg. strong to weak).

The intervention study was carried out over a period of 4 months (section 3.3.2) in which the water quality of the traditional 20 litre water storage container (called a "tshigubu") was compared to water quality of the 20 litre CDC safe storage container, with the addition of either a placebo (which consisted of distilled water), 1% or 3.5% sodium hypochlorite solution. The control group of households used their traditional storage container and received the placebo solution (Table 3.1).

The households in intervention groups I and II used their traditional water storage container (Table 3.1). However, households in intervention group I received the 1% sodium hypochlorite solution, while the households in intervention group II received the 3.5% sodium hypochlorite solution (Table 3.1).

Each of the households in intervention groups III, IV and V received two CDC safe storage containers to replace their traditional household storage containers. The households in intervention group III received the placebo solution, while the households in intervention groups IV and V received the 1% and 3.5% sodium hypochlorite solutions respectively (Table 3.1). The intervention households in groups III, IV and V were visited individually and given clear instructions and education by a trained field worker (speaking the local language of the household) concerning the proper use and cleaning of the CDC safe storage container (Table 3.1).

Table 3.1: Summary of the intervention trial carried out in each of two rural villages in the Vhembe region of the Limpopo Province, South Africa

Study group	Description of each group	Number of households per group
Control	Traditional household container plus the addition of 5 ml placebo solution	10 households
Intervention I	Traditional household container plus the addition of 5 ml of a stabilized 1.0% sodium hypochlorite solution	10 households
Intervention II	Traditional household container plus the addition of a predetermined volume of a stabilized 3.5% sodium hypochlorite solution	10 households
Intervention III	CDC safe storage container plus the addition of 5 ml placebo solution	10 households
Intervention IV	CDC safe storage container plus the addition of 5 ml of a stabilized 1.0% sodium hypochlorite solution	10 households
Intervention V	CDC safe storage container plus the addition of a predetermined volume of a stabilized 3.5% sodium hypochlorite solution	10 households

In addition, all the study households in both the villages was educated on the proper storage conditions and the correct procedure of adding the placebo and sodium hypochlorite solutions to the water in the storage containers. Every third week, each

study household were given a freshly prepared bottle of placebo, 1% or 3.5% sodium hypochlorite solution. All old solution bottles were removed and replaced with fresh solutions. To be consistent, the placebo, 1% and 3.5% sodium hypochlorite solutions were distributed in similar bottles and given to the households at the same time. Using a teaspoon as measuring device, all study households in both villages were trained to add 5 ml of their given solution to the water storage container each time water from the source was collected. The volume of 5 ml was chosen as a standard for all households because:

- It is the recommended dosage stipulated by the DOH in South Africa for using the 3.5% sodium hypochlorite solution. The results of ten repeated experiments have indicated that 5 ml of the 3.5% sodium hypochlorite solution gave a free chlorine concentration of 3.8 $mg.l^{-1}$ after 60 min and 0.8 $mg.l^{-1}$ after 24 h (Appendix B).
- Laboratory studies were performed to determine the chlorine demand curve (Section 3.3.1.1) for the 1% sodium hypochlorite solution with water from both water sources. The results of ten repeated experiments have showed that 5 ml of the 1% sodium hypochlorite solution gave a free chlorine concentration of 1.5 $mg.l^{-1}$ after 60 min as stipulated by the CDC.
- To be consistent, households who received the placebo solution were advised to use a 5 ml volume of the solution provided.

3.3.1.1 Determination of the chlorine demand curve for containers receiving the 1% sodium hypochlorite solution

In South Africa, the 1% sodium hypochlorite solution as described by the CDC is not commercially available. It was therefore specially prepared for this study by a manufacturer, *TS Marketing*, situated in Polokwane, South Africa.

The volume of 1% sodium hypochlorite solution needed for each water source type had to be determined because parameters such as turbidity, pH and temperature of the water source could influence the volume of sodium hypochlorite solution needed to obtain a free chlorine residual concentration of 1.5 $mg.l^{-1}$ (Dr R Quick, CDC, Atlanta, USA, personal communication). To determine the correct dosage of the 1% sodium hypchlorite solution to the storage containers, a chlorine demand curve for each water

source was determined using the N, N-diethyl-phenylenediamine (DPD) colorimetric method according to the manufacturer's specification (Merck, Darmstadt, Germany). Briefly; different volumes (5 ml, 10 ml, 15 ml and 20 ml) of the 1% stabilized sodium hypochlorite solution was added to separate traditional and CDC safe storage containers which each contained 20 litres of the specific water source. The free chlorine residual was measured at 30 min time intervals in each container for 5 hours after thoroughly shaking the container and taking a 10 ml representative sample. The results of ten repeated experiments indicated that a volume of 5 ml of a 1% sodium hypochlorite solution corresponded to a free chlorine residual of 1.5 $mg.l^{-1}$ after 60 min for the surface and ground water sources used in this study.

3.3.1.2 Questionnaire administration at each study household

A comprehensive questionnaire was used in this study to obtain baseline characteristics of the study households in the two rural villages (Appendix C). The original questionnaire was formulated in English and the interview was conducted in either Tshivenda or Xitsonga with the female head of each household. Two postgraduate students from the University of Venda were trained as field workers to conduct the survey. Both students were fluent in English, Tshivenda and Xitsonga. Data on household demographics, water source, water collection practices, water transportation practices, water storage practices, sanitation, prevalence of diarrhoea during the past six months prior to the interview and general observations made by the interviewer during the interview, were recorded.

3.3.2 Assessment of the effectiveness, compliance and sustainability of a household intervention using an improved storage container and a sodium hypochlorite solution

The principle objective of this study was to evaluate the effectiveness, compliance and sustainability of an intervention consisting of the CDC household storage container with the addition of a 1% or 3.5% sodium hypochlorite solution in rural communities of South Africa. In order to determine the effectiveness and level of compliance of the intervention, all the visits during the intervention study were unannounced and the households were blinded with regards to the concentration of the sodium hypochlorite

solutions. This was necessary to determine if the households were using the intervention on a particular day.

Water samples were collected once a month for 4 months from the primary water source in each village and each of the study household water storage containers (traditional or CDC safe storage containers). Aseptic techniques were used to collect 2 000 ml water samples in sterile Nalgene (Merck, Darmstadt, Germany) collection bottles for microbiological analyses. During collection of communal tap water samples in village 1, the water from the tap was allowed to run for 1 min before a sample was taken. During collection of river water samples in village 2, care was taken to collect samples at the exact sites used by the study households as their water collection points in the river. All samples were transported on ice to the laboratory and processed within 8 h.

Source water samples as well as water samples collected from intervention and non-intervention households (Table 3.1) were tested for physical (section 3.3.2.1) and microbiological parameters (sections 3.3.2.2 and 3.3.2.3). In measuring and comparing the concentration of physical parameters and counts of microbiological parameters in drinking water samples in each household group (Table 3.1), the effectiveness of the intervention was assessed.

3.3.2.1 Physico-chemical analyses of water samples

Temperature and pH measurements were determined in 100 ml volumes of water samples using a Basic-20 pH meter (Crison Instruments, South Africa) and a Silberbrand laboratory thermometer (Merck, Darmstadt, Germany) respectively. The turbidity of each water sample was determined in 10 ml volumes of water samples using a portable HI93703 Microprocessor turbidity meter (HANNA Instruments, Germany). The pH and turbidity meters were calibrated according to the manufacturer's instructions. Free chlorine residuals of water samples were determined in 10 ml of each of the water samples using the N, N-diethyl-phenylenediamine (DPD) colorimetric method according to the manufacturer's specifications (Merck, Darmstadt, Germany).

3.3.2.2 Enumeration of indicator bacteria in the water samples

Indicator bacteria used to assess the microbiological quality of the water samples included heterotropic bacteria, total coliforms, faecal coliforms, faecal enterococci, *E. coli* and *C. perfringens*. These indicator microorganisms were determined in the primary water sources as well as the household water storage container samples.

Selective media were used and prepared in 90 mm Petri plates (Merck, Darmstadt, Germany) according to the manufacturer's instructions. Plate Count Agar (Difco Laboratories, Detroit, MI, USA) was used for the enumeration of heterotrophic microorganisms. Total coliform bacteria were enumerated on mEndo agar (Difco Laboratories, Detroit, MI, USA). Faecal coliform bacteria were enumerated on mFc agar (Difco Laboratories, Detroit, MI, USA). Faecal enterococci bacteria were enumerated on mEnterococcus agar (Difco Laboratories, Detroit, MI, USA). *Clostridium perfringens* OPSP agar with supplements A and B (Oxoid Ltd., Basingstoke, Hampshire, England) was used for the enumeration of *C. perfringens* vegetative cells and spores.

Water samples were assessed in duplicate for the presence of total coliforms, faecal coliforms, faecal enterococci and *C. perfringens* using the membrane filtration technique (Standard Methods, 1995). Sterile filtration membranes (0.45 µm pore size, 47 mm diameter) (Millipore, Johannesburg, South Africa) were prepared by passing 10 ml volumes of each water sample through the membranes using a vacuum pump (Model CP5PM75544; Millipore, Johannesburg, South Africa). The membranes were placed right side up on the respective agar plates. Total coliform and faecal enterococci plates were inverted and incubated (Labotec series 2000 digital incubator; Labotec, Johannesburg, South Africa) aerobically at 37°C for 24 h and 48 h respectively. Metallic green colonies were counted as positive colonies for total coliform bacteria, while pink colonies were counted as positive for faecal enterococci bacteria.

Faecal coliform plates were inverted and incubated (Labotec series 2000 digital incubator; Labotec, Johannesburg, South Africa) aerobically at 44.5ºC for 24 h and dark blue or violet colonies were considered positive colonies. *Clostridium perfringens* plates were inverted and incubated (Labotec series 2000 digital incubator; Labotec,

Johannesburg, South Africa) in micro-aerophillic conditions at 37°C for 24 h using Anaerogen sachets (Oxoid Ltd., Basingstoke, Hampshire, England). Dark brown to black colonies (both vegetative cells and spores) were counted. After incubation, all representative colonies on each plate were counted and multiplied by a factor 10 in order to report the counts as colony forming units per 100 ml (cfu.100 ml^{-1}).

Escherichia coli bacteria was enumerated as follows: membranes from the mFc agar plates containing faecal coliform bacteria were removed and placed directly onto Nutrient-MUG agar (Merck, Darmstadt, Germany) plates and incubated aerobically (Labotec series 2000 digital incubator; Labotec, Johannesburg, South Africa) at 37°C for 24 h. Plates were removed from the incubator and observed under a 366 nm ultraviolet light source (Merck, Darmstadt, Germany). Fluorescent colonies were counted as presumptive *E. coli* bacteria and the counts expressed as cfu.100 ml^{-1} (Difco Manual; Difco Laboratories, Detroit, MI, USA). Each presumptive *E. coli* colony was confirmed using Gram-staining and indole tests with Kovac's reagent (Merck, Darmstadt, Germany) according to the techniques described by Mac Faddin (1980). All Gram negative, indole positive colonies were recorded as *E. coli* (Mac Faddin, 1980).

The number of heterotrophic counts was determined as colony forming units per millilitre (cfu.ml^{-1}) using the pour plate method (Standard Methods, 1995). Briefly; ten fold serial dilutions of each water sample were prepared in sterile distilled water. One ml of each dilution was added to 9 ml Plate Count Agar that was kept in sterile 16 mm test tubes (Adcock Ingram Pty Ltd., Johannesburg, South Africa) at 55ºC in a water bath (Labotec, Johannesburg, South Africa). The test tubes were vortexed to mix the water sample and the agar and poured into sterile 90 mm Petri dishes (Merck, Darmstadt, Germany). After solidification of the agar, the plates were inverted and incubated (Labotec series 2000 incubator; Labotec, Johannesburg, South Africa) under aerobic conditions at 37°C for 48 h.

3.3.2.3 Enumeration of somatic and male specific F-RNA bacteriophages in the water samples

Standard ISO methods were used to determine the presence of somatic bacteriophages (ISO, 2000) and male specific F-RNA bacteriophages (ISO, 1995) in the water samples. The following reagents were used in the preparation of bacterial hosts and agar plates:

Calcium-Glucose solution:

The Calcium-Glucose solution contained 3 g Calsium-Chloride ($CaCl_2.2H_2O$) (Merck, Darmstadt, Germany) and 10 g Glucose (Merck, Darmstadt, Germany) dissolved in 100 ml distilled water. The solution was decontaminated by membrane filtration using 0.22 µm syringe membranes (Merck, Darmstadt, Germany) (ISO, 1995).

Nalidixic Acid solution:

Nalidixic Acid (2.5 g) (Sigma Chemicals Co., St Louis, MO, USA) was dissolved in 2 ml Sodium Hydroxide solution (1 $mol.l^{-1}$ NaOH) (Merck, Darmstadt, Germany) and 98 ml distilled water. The solution was decontaminated by using 0.22 µm syringe membrane filtration (Merck, Darmstadt, Germany) (ISO, 1995).

3.3.2.3.1 Preparation of bacterial hosts for the detection of bacteriophages

Escherichia coli strain WG5 (ISO, 2000) was used as bacterial host to isolate somatic bacteriophages. The bacterial host was grown overnight at 37ºC in a Labotec 2000 digital incubator (Labotec, Johannesburg, South Africa) in Nutrient Broth prepared according to the manufacturer's specifications (Merck, Darmstadt, Germany).

The *S. typhimurium* nalidixic acid and kanamycin resistant WG49 strain (NCTC 12484) containing an *E. coli* plasmid which codes for sex pili production was used as the bacterial host for the detection of male specific F-RNA bacteriophages (Havelaar and Hogeboom, 1984; ISO, 1995). The host was grown in Tryptone Yeast Extract which was prepared as follows: 10 g Trypticase Peptone (Difco Laboratories, Detroit, MI, USA), 1 g Yeast Extract (Difco Laboratories, Detroit, MI, USA) and 8 g Sodium

Chloride (Merck, Darmstadt, Germany) was dissolved in 1 000 ml distilled water and autoclaved at 121°C for 15 min (ISO, 1995). The agar was allowed to cool to 50ºC, the pH was aseptically adjusted to 7.2 using a Basic-20 pH meter (Crison Instruments, South Africa) and 10 ml Calcium-Glucose solution (section 3.3.2.3) was aseptically added. One vial of a host stock culture was added to 50 ml Tryptone Yeast Extract broth and incubated at 100 rpm on a Labcon Platform shaking incubator (Labotec; Johannesburg, South Africa) at 37°C until the F-pili developed onto which the bacteriophages attached to infect the bacteria cell (ISO, 1995).

The absorbance of the growth suspension was measured at 30 min intervals from time 0 min against a blank reference at 560 nm using a Spectro 22 Digital Spectrophotometer (Labomed Inc., USA) until an absorbance of 0.75 was obtained at which the sex pili were produced (Grabow, 2001). The host suspension was removed from the incubator, placed on ice and used within 2 h (ISO, 1995).

3.3.2.3.2 Preparation of bottom agar plates for the detection of somatic bacteriophages

Somatic bacteriophage bottom agar plates contained 14 g Bacto agar (Difco Laboratories, Detroit, MI, USA), 13 g Tryptone (Difco Laboratories, Detroit, MI, USA), 8 g Sodium Chloride (Merck, Darmstadt, Germany) and 1.5 g Glucose (Merck, Darmstadt, Germany) which were dissolved in 1 000 ml distilled water and autoclaved at 121°C for 15 min. The agar was allowed to cool to 50ºC and 1 ml Nalidixic Acid solution (section 3.3.2.3) were added using aseptic techniques (ISO, 2000). Twenty millilitre volumes of the prepared solution was poured into 90 mm Petri dishes (Merck, Darmstadt, Germany) and allowed to solidify (ISO, 2000).

3.3.2.3.3 Preparation of bottom agar plates for the detection of male specific F-RNA bacteriophages

Male specific F-RNA bacteriophage bottom agar plates contained 10 g Trypticase peptone (Difco Laboratories, Detroit, MI, USA), 1 g Yeast Extract (Difco Laboratories, Detroit, MI, USA), 8 g Sodium Chloride (Merck, Darmstadt, Germany), 12 g Bacto agar (Difco Laboratories, Detroit, MI, USA) which was dissolved in 1 000 ml distilled

water, autoclaved at 121°C for 15 min and adjusted to a pH of 7.2 using aseptic techniques. A volume of 10 ml of a Calcium-Glucose solution (section 3.3.2.3) was added aseptically to the medium and 20 ml volumes of the prepared agar solution was poured into 90 mm Petri dishes (Merck, Darmstadt, Germany) and allowed to solidify (ISO, 1995).

3.3.2.3.4 Preparation of top agar plates for the detection of somatic bacteriophages

The somatic bacteriophage top agar contained 8 g Bacto agar (Difco Laboratories, Detroit, MI, USA), 10 g Tryptone (Difco Laboratories, Detroit, MI, USA), 8 g Sodium Chloride (Merck, Darmstadt, Germany), 3 g Glucose (Merck, Darmstadt, Germany), 5 ml of a 1 M Sodium Carbonate (Merck, Darmstadt, Germany) solution and 1 ml of a 1 M Magnesium Chloride (Merck, Darmstadt, Germany) solution. One ml Nalidixic Acid solution (section 3.3.2.3) was added to the top agar using aseptic techniques (ISO, 2000).

3.3.2.3.5 Preparation of top agar plates for the detection of male specific F-RNA bacteriophages

The male specific F-RNA bacteriophage top agar contained 10 g Trypticase Peptone (Difco Laboratories, Detroit, MI, USA), 1 g Yeast Extract (Difco Laboratories, Detroit, MI, USA), 8 g Sodium Chloride (Merck, Darmstadt, Germany) and 6.5 g Bacto agar (Difco Laboratories, Detroit, MI, USA) which were dissolved in 1 000 ml distilled water and autoclaved at 121°C for 15 min. The pH of the agar solution was aseptically adjusted to 7.2 after autoclaving using a Basic-20 pH meter (Crison Instruments, South Africa) and 10 ml Calcium-Glucose solution (section 3.3.2.3) together with 4 ml Nalidixic Acid solution (section 3.3.2.3) added (ISO, 1995).

3.3.2.3.6 Double agar layer plate assay for the detection of somatic and male specific F-RNA bacteriophages in a water sample

Ten fold serial dilutions were made of each water sample using distilled water. The top agar was melted in a 56°C waterbath (Model GFL 1083; Labotec, Johannesburg, South Africa) and prepared as follows: three ml volumes of the top agar were aliquoted into 10

ml conical test tubes (Adcock Ingram Scientific Pty Ltd., Johannesburg, South Africa) and kept liquefied in a 56^{o}C waterbath (Model GFL 1083; Labotec, Johannesburg, South Africa). To each test tube, 1 ml of the prepared host for each bacteriophages type (section 3.3.2.3.1) and 1 ml of a tenfold dilution of the water sample was added. The test tubes were mixed by hand before pouring the solution onto a pre-marked bottom agar plate (section 3.3.2.3.2 and section 3.3.2.3.3). The plates were allowed to solidify and incubated inverted at 37ºC for 24 h in a Labotec 2000 digital incubator (Labotec, Johannesburg, South Africa).

3.3.2.3.7 Presence-Absence spot test for determination of somatic and male specific F-RNA bacteriophages in the water samples

The Presence-Absence test was used to detect somatic and male specific F-RNA bacteriophages following a procedure described by Uys (1999). The Presence-Absence test instead of the double agar layer test was used to analyse 500 ml instead of 1 ml of the water sample. The *Escherichia coli* strain WG5 (ISO, 2000) and *Salmonella typhimurium* WG49 (ISO, 1995) were used respectively as bacterial hosts to isolate somatic and male specific F-RNA bacteriophages from the water samples.

Each water sample was mixed to have a homogenous suspension and 500 ml was poured into a sterile plastic 1 000 ml water collection bottle to which 5 g Trypticase Peptone (Difco Laboratories, Detroit, MI, USA), 0.5 g Yeast Extract (Difco Laboratories, Detroit, MI, USA), 4 g Sodium Chloride (Merck, Darmstadt, Germany) and 5 ml of a Calcium-Glucose solution (section 3.3.2.3) were added. Host cultures were prepared according to ISO procedures (section 3.3.2.3.1). One millilitre of the specific host culture was added to each of the water samples and incubated (Labotec series 2000 digital incubator; Labotec, Johannesburg, South Africa) at 37°C for 24 h. The presence of either somatic or F-RNA bacteriophages were determined by spotting 5 µl from each Presence-Absence water sample onto a pre-prepared lawn of host bacteria in 90 mm Petri dishes (Merck, Darmstadt, Germany) (Uys, 1999). The plates were incubated at 37ºC for 24 h and zones of cell lysis (plaques) were considered positive and reported as Present for each water sample (Uys, 1999).

3.3.2.4 Compliance of households in two villages with the intervention using an improved storage container and a sodium hypochlorite solution

During each of the unannounced water collection visits (section 3.3.2), the free chlorine residual in household storage containers was determined as described in section 3.3.2.1. Comparisons of the free chlorine concentrations detected in the household storage containers were used to determine if the point-of-use water treatment intervention resulted in any improvement in the drinking water quality. In addition, a qualitative survey was administered at the end of the intervention study to determine the degree of satisfaction of the consumers with the point-of-use water treatment intervention. The questionnaire was used to solicit information on problems regarding the taste and smell of the water after treatment, problems in the use of the sodium hypochlorite solution or problems with the CDC safe storage containers.

3.3.2.5 Sustainability of the intervention study in two rural villages

The sustainability of the intervention introduced to the study households in each of the two rural villages was assessed twice after the 4 months intervention study was completed. No new bottles of the placebo or 1% and 3.5% sodium hypochlorite solutions were given to any of the households after the end of the formal intervention trial. The first assessment was carried out 6 months after the completion of the formal intervention trial while the second assessment was carried out 12 months after completion of the formal intervention trial. The same procedures discussed in section 3.3.2.1 to section 3.3.2.3.7 were used.

3.3.3 Statistical analyses of intervention study data

The Stata Release 8.0 (Stata Corporation, College Station, Texas, USA) statistical software package was used throughout this study for all analysis. All the raw data is kept electronically by Prof PJ Becker in the Biostatistics Unit at the Medical Research Council, in Pretoria, South Africa and could be made available on request by mutual agreement.

All parameters in the household questionnaires used on the baseline characteristics were of a categorical nature describing certain water, hygiene and sanitation practices at the household level. The Stata Release 8.0 statistical software package was used in the process of cleaning and editing the data and to do comparative analyses. Data was summarized making use of frequencies, percentages and cross-tabulations.

The results for the water samples collected from the primary water sources and water samples collected from the household storage containers were summarised for each water source and household group as outlined in Table 3.1. According to Standard Methods (1995) the best estimate of central tendency of log normal data is the geometric mean which was used in this section. In all comparison analysis differences were considered statistical significant if $P < 0.05$. In addition, the association or link between household demographic and hygiene practices and water quality, measured in terms of *E. coli* counts, was determined using Poisson regression which adequately deals with counts and zeros.

Analyses of variance (ANOVA) is a parametric test and assumes that the data analysed are normal distributed around the mean with similar variance (Helsel and Hirsch, 1995). If the assumption of equal variance was violated, the Welsch approach was used in parametric testing. In this study, the instances where data did not pass normality were considerably more than instances where data did not comply with these assumptions, non-parametric tests were employed.

Non-parametric testing has considerable power in comparing non-normal as well as normal data. The following tests were included in this study (Helsel and Hirsch, 1997):

- The *Rank Sum Test*, also referred to as the *Mann-Whitney Rank U-Test*, a non-parametric procedure was used to test for a difference in medians between two groups.
- The *Wilcoxon Matched Pairs Signed Ranks Test*, a non-parametric procedure was used on paired data sets.
- The *ANOVA on Ranks* and the *Kruskal-Wallis* tests were used to test for differences between three and more study groups. *Multiple Comparison* tests (MCT's) were used to ascertain where group differences were.

According to Helsel and Hirsch (1997), Box plots visually displayed microorganism counts in the improved and the traditional household storage container. Figure 3.4 gives a visual presentation of a Box plot.

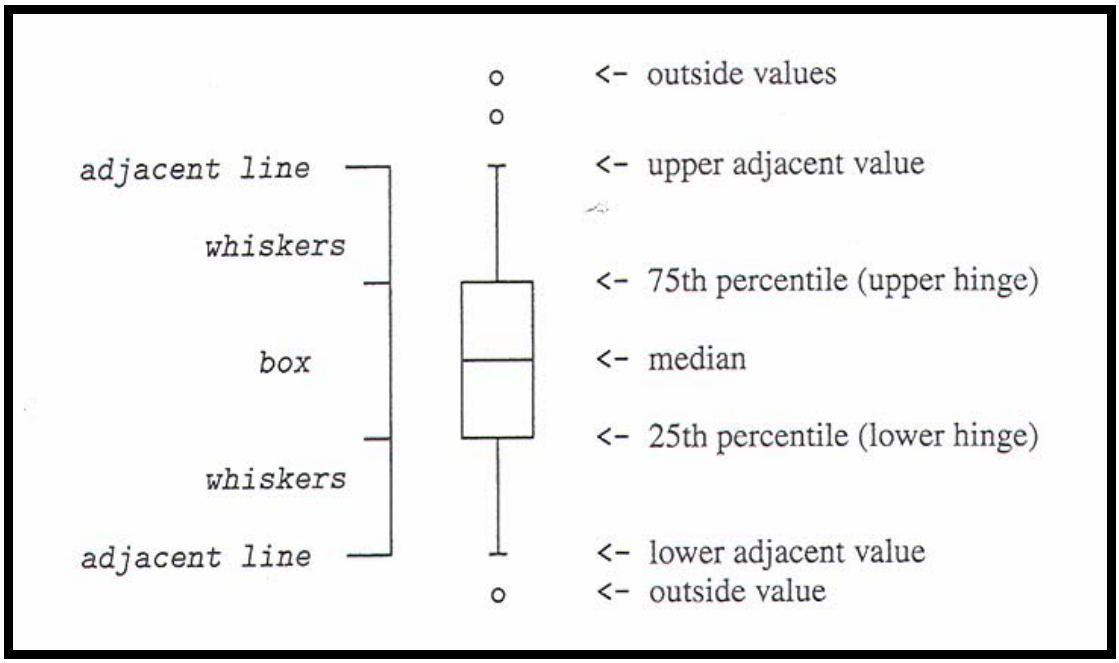


Figure 3.4: Visual presentation of a Box plot used in this study to compare the microbiological counts between the traditional and CDC safe storage containers in the study households from two rural villages in the Vhembe region of the Limpopo Province, South Africa (Helsel and Hirsch, 1995)

- The centre line in the Box plot gives the median, often the preferred measure of central tendency as it is resistant to the effects of outliers (Helsel and Hirsch, 1997).
- The inter-quartile range (variation or spread of the data) is the upper and lower boundaries forming the Box height and indicates the spread of data between the 25^{th} and the 75^{th} percentile. The closer the data are clustered to the median within the inter-quartile range, the less variation (more stable) the data set is (Helsel and Hirsch, 1997).
- The skewness (also referred to as the quartile skew) is represented by the relative size of the Box halves. The further the median line is from the middle of the box, the more skewed the data is distributed around the mean. This implies the use of non-parametric methods of analyses (Helsel and Hirsch, 1997).

- The caps whiskers on the lines protruding above and below the 75th and the 25th percentiles represent the distance 1.5 x inter-quartile range above and below the latter. The circle symbols beyond the caps and whiskers indicate outliers (Helsel and Hirsch, 1997).

3.4 OBJECTIVE TWO: TO DISTINGUISH BETWEEN FAECAL POLLUTION OF ANIMAL OR HUMAN ORIGIN USING MOLECULAR TYPING OF MALE SPECIFIC F-RNA BACTERIOPHAGE SUBGROUPS

This section described the methodology used to assess the origin of faecal contamination in the household water storage containers. Molecular genotyping of male specific F-RNA bacteriophages was used to distinguish between the four different male specific F-RNA bacteriophage subgroups.

3.4.1 Water sample collection

An additional forty households (not the same households used in section 3.3) were randomly selected in each rural village described in section 3.3.1 to participate. Two rounds of water collection from the households and the water sources were carried out over a period of 5 months. Water samples (2 000 ml) were collected aseptically in sterile Nalgene water collection bottles (Merck, Darmstadt, Germany) from 7 communal taps in village 1 (Fig 3.2) and from 4 points on the Sambandou River in village 2 (Fig 3.3).

Two water storage containers in each household were randomly selected and vigorously shaken before water samples were collected. Water samples (1 000 ml each) were collected aseptically in sterile Nalgene water collection bottles (Merck, Darmstadt, Germany) from each of the two selected storage containers in each household on both rounds of water collection. The two water samples from each household were pooled into one sample (2 000 ml) representative of the household container water, placed on ice and transported to the laboratory for further analyses within 8 h. After the first round of water sampling, 20 households in each village were randomly selected and provided with two CDC safe storage containers each. These households were requested to use the CDC safe storage containers instead of the traditional household storage containers. The water samples obtained from the households using the CDC safe storage container were compared with water samples obtained from the households in the same village using traditional storage containers in order to determine the impact of an improved storage container on the origin of faecal pollution (Fig 3.5).

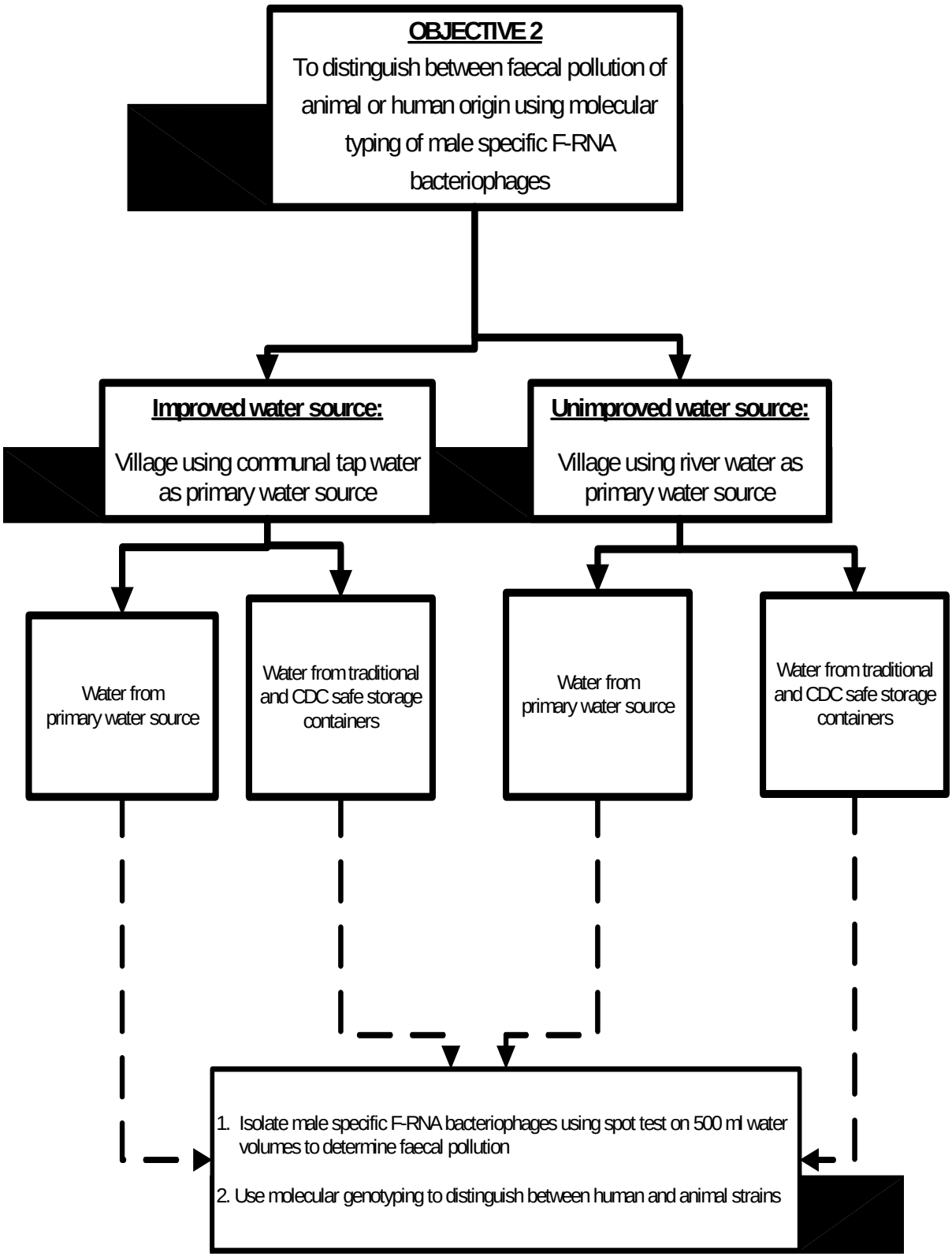


Figure 3.5: **Schematic outlay of the study design of objective two to distinguish between faecal pollution of human or animal origin in the primary water sources as well as the traditional and CDC safe storage containers**

3.4.2 Isolation and identification of male specific F-RNA bacteriophages

Preparation of the bacterial host for the detection of male specific F-RNA bacteriophages in the water samples was done as described in section 3.3.2.3. The preparation of bottom agar plates and top agar plates for direct plaque assays for the isolation of male specific F-RNA bacteriophages was carried out to the procedures described in sections 3.3.2.3.3 and 3.3.2.3.5 respectively (ISO, 1995). The Calcium-Glucose and Nalidixic Acid solutions were prepared as described in section 3.3.2.3 (ISO, 1995). The double agar plate assay procedure as described in section 3.3.2.3.6 was used for direct plaque assays for the isolation of male specific F-RNA bacteriophages.

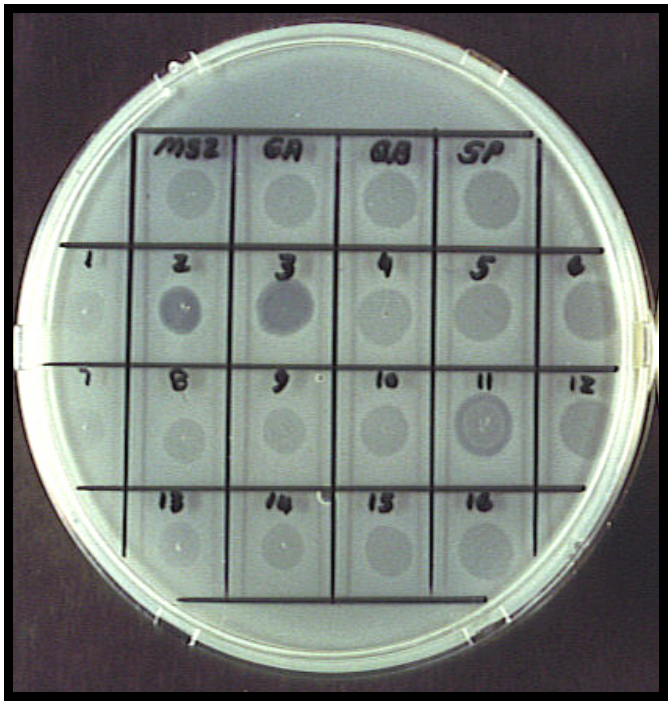


Figure 3.6: A Petri plate indicating spots of positive male specific F-RNA bacteriophage controls and water samples (Uys, 1999)

The Presence-Absence test to determine the presence of male specific F-RNA bacteriophages in the water samples was carried out as described in section 3.3.2.3.7. A total of 16 water samples were spotted on one plate (Fig 3.6). Representative strains of male specific F-RNA bacteriophage consisting of MS2 (subgroup I), GA (subgroup II), QB (subgroup III) and F1 (subgroup IV) [donated by Prof MD Sobsey, University of North Carolina, Chapel Hill, USA] were used as positive controls on each plate (Fig 3.6).

3.4.3 Preparation of phage plates for hybridisation, phage transfer and membrane fixation

The method described by Schaper and co-workers (2002a) were used. The phage spot plates (section 3.4.2) were removed from the 37ºC incubator (Labotec 2000 digital incubator; Labotec, Johannesburg, South Africa) and placed in a fridge at 4ºC for 30 min to solidify and dampen the agar to facilitate phage transfer. The plates were removed from the fridge and covered with a Nylon membrane (Roche Diagnostics, GmbH, Mannheim, Germany) for 1 min for the fixation of the bacteriophages onto the membranes. Following fixation, the bacteriophage RNA would have to be released and denatured to expose the bases to the complementary oligonucleotides. Therefore, the membranes were removed from the plates, placed in plastic containers and submerged in 40 ml of a 0.1 M Sodium Hydroxide (NaOH) (Merck, Darmstadt, Germany) solution for 5 min. This was followed by a neutralisation step where the membranes were placed into clean plastic containers and submerged in 40 ml of 0.1 M Sodium Acetate ($CH_3COOHNa$; pH 6) (Merck, Darmstadt, Germany) solution for 1 min. The nucleic acids were cross linked to the membranes by a 5 min exposure of both sides of the membranes to an ultra violet transilluminator (Model TL-302; Spectroline, Germany). The fixed membranes were used immediately for hybridisation.

3.4.4 Hybridisation of fixed male specific F-RNA bacteriophages

The hybridisation method of Beekwilder and co-workers (1996) as modified by Schaper and co-workers (2002a) was used. Each fixed membrane (section 3.4.3) was placed into a hybridisation bag (Roche Diagnostics, GmbH, Mannheim, Germany) with 5 ml of prehybridisation solution. The prehybridisation solution contained 6 X Saline Sodium Citrate (SSC) (Amfresco, Solo, Ohio, USA); 0.1% Sodium Dodecyl Sulphate (SDS) (Merck, Darmstadt, Germany); 1 x Denhardt solution (Invitrogen Ltd., Paisley, Scotland) and 0.1 $mg.ml^{-1}$ Salmon Sperm DNA (Invitrogen Ltd., Paisley, Scotland) (Sambrook *et al.*, 1989). The Salmon Sperm DNA was denatured at 99ºC for 10 min in a PCR thermocycler (Pharmacia LKB Gene ATAQ, Upsalla, Sweden) and kept at 4ºC until used.

The hybridisation bags were sealed and placed in a shaking incubator (Hub O'Matic, K Huber Engineering, South Africa) at 25ºC for 10 min. Hybridisation was carried out in the same hybridisation bags by adding 2.5 pmol.ml^{-1} of the digoxy-labelled probes (Sigma-Genosys; Sigma Chemicals Co., St Louis, MO, USA) described by Hsu and co-workers (1995) (Table 3.2).

Table 3.2: Nucleotide sequences of male specific F-RNA bacteriophage probes used in this study (Hsu *et al*., 1995)

Phage subgroup	Probe sequence	Basepairs
I	5`-CTAAGGTATGGACCATCGAGAAAGGA-3`	26
II	5`-CCATGTTATCCCCCAAGTTGCTGGCTAT-3`	27
III	5`-ATACTCAGTGAARTACTGCTGTGT-3`	24
IV	5`-GGCATAGATTCTCCTCTGTAGTGCG-3`	25

The bags were resealed and placed in a waterbath (Labotec, Johannesburg, South Africa) at 37ºC for 60 min. After hybridisation, the membranes were removed from the hybridisation bags and placed into clean plastic containers and washed twice using large volumes of a buffer containing 0.3 x SSC (Amfresco, Solo, Ohio, USA) and 0.1% SDS (Merck, Darmstadt, Germany). These washing steps were carried out in a waterbath (Labotec, Johannesburg, South Africa) at 37ºC for 10 min.

3.4.5 Chemiluminescent detection of hybridised male specific F-RNA bacteriophage plaques

A digoxigenin (DIG) Wash and Block Buffer Set (Roche Diagnostics, GmbH, Mannheim, Germany) containing washing, blocking and detection solutions was used. The membranes were washed with the washing buffer (Roche Diagnostics, GmbH, Mannheim, Germany) at 37ºC for 2 min in a waterbath (Labotec, Johannesburg, South Africa) and blocked at 37ºC for 15 min (Labotec waterbath) using 80 ml blocking solution (Roche Diagnostics, GmbH, Mannheim, Germany) per membrane.

Each membrane was incubated at 25ºC for 30 min in a shaking incubator (Hub O'Matic, K Huber Engineering, South Africa) at 100 rpm in 20 ml blocking solution. The blocking solution contained 1 µl Anti-digoxigenic-AP Fab fragments (Roche

Diagnostics, GmbH, Mannheim, Germany). The membranes were washed twice with washing solution (Roche Diagnostics, GmbH, Mannheim, Germany) at 25ºC for 15 min at 100 rpm in a shaking incubator (Hub O'Matic, K Huber Engineering, South Africa). The membranes were treated with 20 ml detection solution (Roche Diagnostics, GmbH, Mannheim, Germany) at 25ºC for 5 min at 100 rpm in a shaking incubator (Hub O'Matic, K Huber Engineering, South Africa).

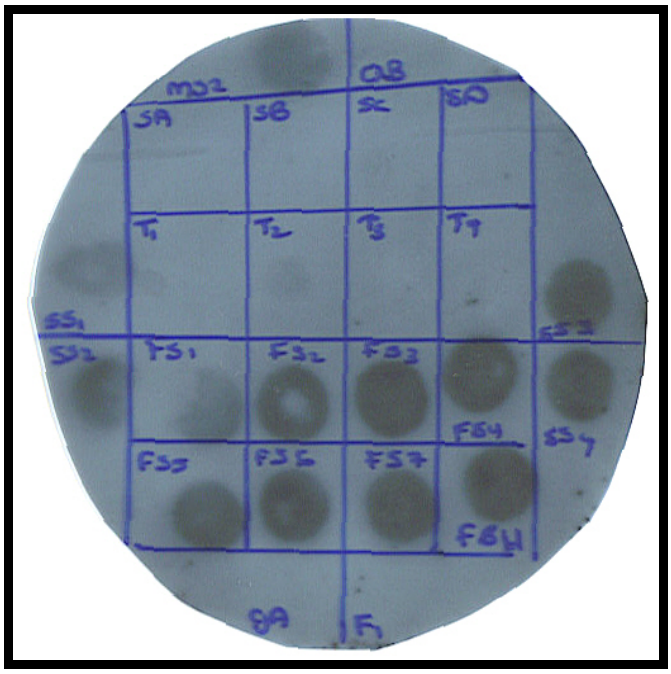


Figure 3.7: An X-Ray film showing MS2 probes hybridised to male specific F-RNA bacteriophage nucleic acid in river and tap water samples

A 1:100 dilution of a CDP (disodium 2-chloro-5-4(methoxyspiro{1,2-dioxetane-3,2'-(5'-chloro)tricyclo[3.3.1.1.3,7]decan}-4-yl)-1-phenyl phosphate) detection substrate solution (Roche Diagnostics, GmbH, Mannheim, Germany) was added to the detection solution (Roche Diagnostics, GmbH, Mannheim, Germany) and incubated with the membranes at 25ºC for 2.5 min. The membranes were sealed in new hybridisation bags (Roche Diagnostics, GmbH, Mannheim, Germany) and exposed for 5 to 8 min to X-Ray Lumi film (Roche Diagnostics, GmbH, Mannheim, Germany) in a developing cassette. The film was developed using developing, stopping and fixing solutions as described by the manufacturer (AXIM, Midrand, South Africa). Probes hybridised to F-RNA phage nucleic acid yielded black circular spots on the X-Ray film (Fig 3.7).

3.5 OBJECTIVE THREE: TO DETERMINE THE SURVIVAL OF INDICATOR AND WATERBORNE PATHOGENS IN THE IMPROVED CDC SAFE STORAGE CONTAINER

Laboratory based seeding experiments were carried out after the rural household intervention study (section 3.3) in the field. This was done to determine how long indicator and pathogenic microorganisms would survive in surface and groundwater sources used by rural communities for domestic purposes inside the 20 litre CDC safe water storage container with or without the addition of a sodium hypochlorite solution.

3.5.1 Water samples

Surface water was obtained from the Levuvhu River in the Dididi region of the Limpopo Province and groundwater was obtained from a community borehole in the Sambandou region of the Limpopo Province, South Africa. The CDC safe storage containers were filled with 20 litres of either river or borehole water directly from the respective sources and transported to the laboratory where the containers were used as outlined in section 3.5.2 and Fig 3.8.

3.5.2 Laboratory based survival study outline

The survival study was set up to contain three groups of 3 CDC safe storage containers for each water source as outlined in Figure 3.8. In each group of CDC safe storage containers, the first container was used to determine the numbers of natural occurring heterotrophic bacteria, total coliforms, faecal coliforms, *E. coli*, faecal enterococci, *C. perfringens*, somatic bacteriophages, male specific F-RNA bacteriophages, *S. typhimurium* and Enteroviruses in the respective water sources. The second container was seeded with 10^6 plaque forming units per millilitre (pfu.ml^{-1}) Coxsackie B1 virus (National Institute of Virology, Johannesburg, South Africa), 10^9 cfu.ml^{-1} male specific F-RNA subgroup II (MS2) bacteriophages [donated by Prof MD Sobsey, University of North Carolina, Chapel Hill, USA] and 10^7 cfu.ml^{-1} *E. coli* (ATCC 13706). The third container was seeded with 10^9 colony forming units per millilitre (cfu.ml^{-1}) somatic bacteriophages (ATCC 73378) and 10^6 cfu.ml^{-1} *S. typhimurium* (NCTC 12484).

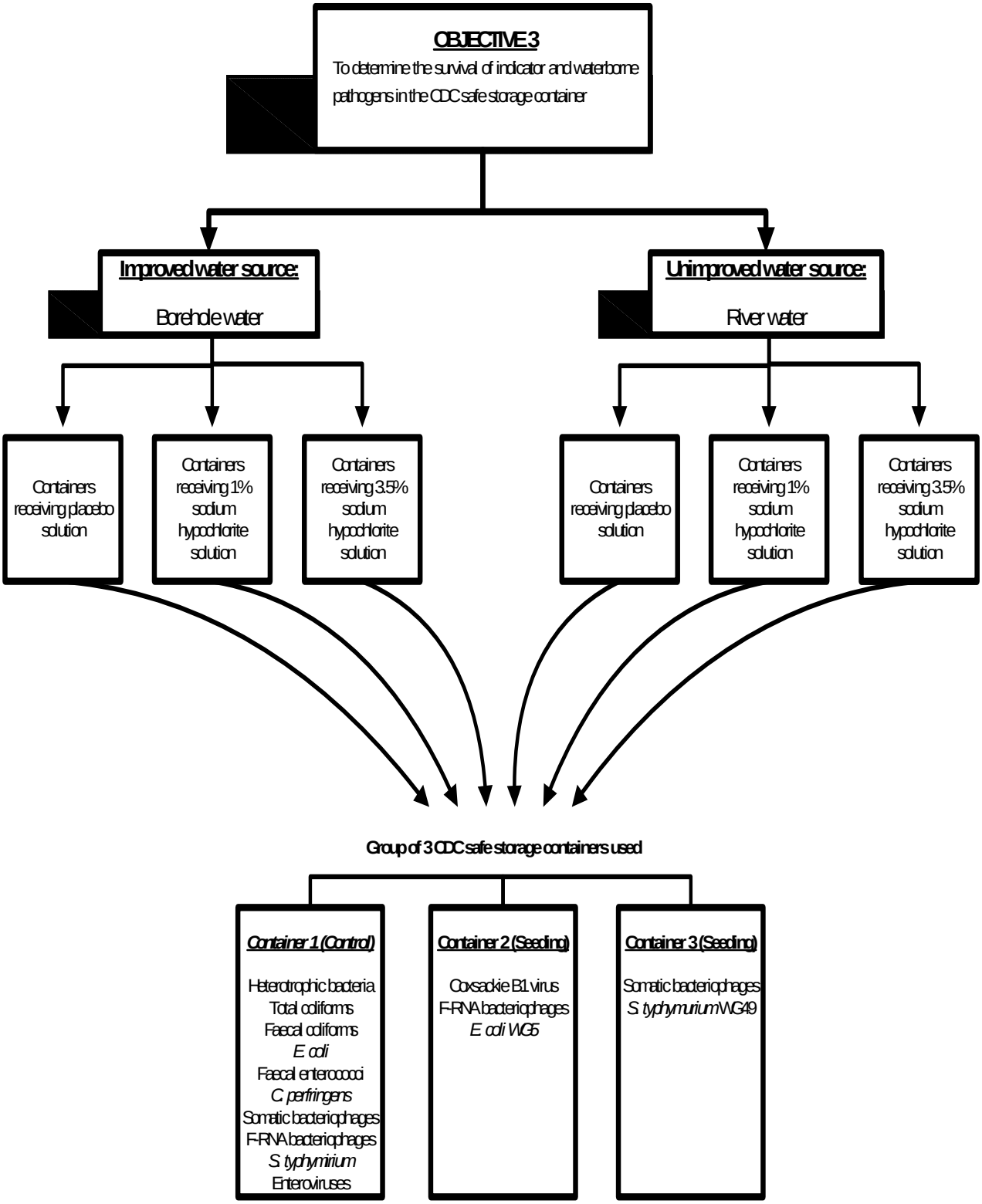

OBJECTIVE 3
To determine the survival of indicator and waterborne pathogens in the CDC safe storage container
Improved water source:
Borehole water
Unimproved water source:
River water
Containers receiving placebo solution
Containers receiving 1% sodium hypochlorite solution
Containers receiving 3.5% sodium hypochlorite solution
Containers receiving placebo solution
Containers receiving 1% sodium hypochlorite solution
Containers receiving 3.5% sodium hypochlorite solution
Group of 3 CDC safe storage containers used
Container 1 (Control)
Heterotrophic bacteria
Total coliforms
Faecal coliforms
E. coli
Faecal enterococci
C. perfringens
Somatic bacteriophages
F-RNA bacteriophages
S. typhymirium
Enteroviruses
Container 2 (Seeding)
Coxsackie B1 virus
F-RNA bacteriophages
E. coli WG5
Container 3 (Seeding)
Somatic bacteriophages
S. typhymurium WG49

Figure 3.8: Schematic outlay of the laboratory study design of objective three to determine the survival of indicator microorganisms and waterborne pathogens in the CDC safe storage container

The first group of CDC safe storage containers was referred to as the control group and 5 ml of a placebo solution was added to each of the 3 containers (section 3.3.1). The second group of CDC safe storage containers each received 5 ml of a 1% sodium hypochlorite solution (sections 3.3.1 and 3.3.1.1). The third group of CDC safe storage containers each received 5 ml of a 3.5 % sodium hypochlorite solution (section 3.3.1).

The experiment was repeated two times in triplicate using representative 1 000 ml water samples. Care was taken to collect water after thoroughly shaking each container in the same way and at the same time each day for the duration of each experiment. The numbers of different microorganisms (naturally occurring and seeded microorganisms) in the containers were determined at time zero prior to the addition of the placebo and sodium hypochlorite solutions and again 60 min after the addition of the respective placebo and sodium hypochlorite solutions. Thereafter water samples were taken at the same time from all containers after 24 h, 48 h and after five days. Chlorine was neutralized with the addition of 2 ml of a 1 M Sodium Thiosulphate (Merck, Darmstadt, Germany) solution to the collected water samples.

3.5.3 Physico-chemical analyses of water samples

Measurements of temperature, pH and turbidity of water samples were carried out as described in section 3.3.2.1. Free chlorine residuals of water samples were determined using the N, N-diethyl-phenylenediamine (DPD) colorimetric method as described in section 3.3.2.1.

3.5.4 Enumeration of naturally occurring indicator bacteria and bacteriophages in the water samples (Container 1)

Standard methods (1995) as described in sections 3.3.2.2 and 3.3.2.3 were employed in the detection of heterotrophic bacteria, total coliforms, faecal coliforms, faecal enterococci, *C. perfringens* bacteria, somatic and male specific F-RNA bacteriophages. *Escherichia coli* bacteria were enumerated on Eosin Methylene Blue (EMB) agar

(Merck, Darmstadt, Germany) prepared according to the manufacturer's specifications in 90 mm Petri plates (Merck, Darmstadt, Germany). *Salmonella typhimurium* bacteria were enumerated on MacConkey agar (Merck, Darmstadt, Germany), which were prepared according to the manufacturer's specifications in 90 mm Petri plates (Merck, Darmstadt, Germany).

Ten fold serial dilutions were made for each water sample in distilled water. The spread plate method was used and 0.1 ml of each water dilution was spread onto the individually pre-marked 90 mm Petri plates (Merck, Darmstadt, Germany) that were inverted and incubated in a Labotec series 2000 digital incubator (Labotec, Johannesburg, South Africa) at 37°C for 24 h under aerobic conditions. Typical colony growth, which included pink colonies for *S. typhimurium* and purple colonies for *E. coli* were counted and expressed as colony forming units per millilitre (cfu.ml^{-1}).

3.5.5 Enumeration of naturally occurring Enteroviruses in the water samples (Container 1)

Buffalo Green Monkey (BGM) kidney cell cultures were used to determine the prevalence of any naturally occurring Enteroviruses in the original river and borehole water samples (Grabow *et al*., 1990; Potgieter, 1997). The cells were grown in 25 cm^2 tissue culture flask (Corning, USA) to confluent monolayers, washed with 5 ml sterile Phosphate Buffered Saline (PBS; pH 7) (Sigma Chemicals Co, St Louis, MO, USA) and starved for 60 min in 1 ml serum free Eagle's Minimum Essential Media (EMEM) (Highveld Biological, Pty. Ltd, Kelvin, South Africa). After withdrawal of the starvation media, 1 ml of the water sample was inoculated onto the cells and left at 37ºC for 60 min with gentle hand rotation every 15 min.

The inoculum was removed from the cells and 5 ml EMEM (Highveld Biological, Pty. Ltd, Kelvin, South Africa) containing 1% inactivated bovine serum (Delta Bioproducts, Johannesburg, South Africa) and 1% of an antibiotic solution prepared to contain 10 000 Units.ml^{-1} Penicillin (BioWhittaker, Walkersville, MD, USA), 5 000 Units.ml^{-1} Streptomycin (BioWhittaker, Walkersville, MD, USA), and 100 Units.ml^{-1} Nystatin (Sigma Chemicals Co., St Louis, MO, USA), were added. The infected cell flasks were incubated (Galaxy CO_2 incubator, Biotech Northants, England) at 37ºC in the presence

of 5% CO_2 for 21 days and blind passages performed every three days (Grabow *et al*., 1990; Pinto *et al*., 1994; Potgieter, 1997).

A commercial viral RNA extraction kit (Qiagen, Hilden, Germany) was used to extract viral RNA from 2 ml infected BGM tissue culture fluid. Reagents used in the RT-PCR and nested PCR reactions were obtained from Promega (Promega Corp., Madison WI, USA) and Boehringer (Boehringer Mannheim GmbH, Germany) and all primers were obtained from Sigma (Sigma Genosys Ltd., Pampisford, Cambridgeshire, United Kingdom). A positive control (cell cultured coxsackie B1 virus) and a negative control (nuclease free water; Promega Corp., Madison WI, USA) were included in both the reverse transcriptase polymerase chain reaction (RT-PCR) and nested PCR reactions. A Techne Genius thermocycler (Techne, Cambridge, United Kingdom) was used for the RT-PCR and nested PCR reactions.

The published primer set (Gow *et al*., 1991, Egger *et al*., 1995) used in the RT-PCR reaction included primer EP1 (5'-$_{64}$CGGTACCTTTGTGCGCCTGT$_{83}$-3') and primer EP4 (5'-$_{459}$TTAGGATTAGCCGCATTCAG$_{478}$-3') which gave a 414 base pair (bp) product. The RT-PCR reaction was carried out in a 50 µl volume containing 50 pmol of each of the EP1 and EP4 primers, 1 µl of 5 U *Tfl* DNA polymerase; 15 µl extracted RNA, 1 µl of 5 U avian myeloblastosis virus reverse transcriptase (AMV-RT); 1x AMV/*Tfl* reaction buffer, 1.5 mM Magnesium sulphate ($MgSO_4$) and a dideoxy nucleotide triphosphates (dNTP - final concentration of 0.2 mM) mix (Gow *et al*., 1991; Kuan, 1997). A reverse transcriptase step at 48ºC for 45 min was followed by 30 cycles of DNA denaturation at 94ºC for 1 min, annealing at 56ºC for 1 min and an extension at 72ºC for 1 min. The RT-PCR reaction was ended with a final extension step at 72ºC for 10 min (Gow *et al*., 1991).

The nested PCR reaction was carried out with published primer set E1 (5'-$_{166}$AAGCACTTCTGTTTCCC$_{182}$-3') and E2 (5'-$_{447}$ATTCAGGGGCCGGAGGA$_{463}$-3') to give a final product of 297 bp (Gow *et al*., 1991; Kuan, 1997). The 50 µl nested PCR mixture contained 1 µl RT-PCR product, 50 pmol of each primer; 10 mM Tris-HCl (pH 9), 50 mM Potassium Chloride (KCl), 0.1% Triton X-100, 1.5 mM Magnesium Chloride ($MgCl_2$), dNTP mix (0.2 mM final concentration) and 1.5 U *Taq* DNA polymerase (Kuan, 1997). The nested PCR reaction started with a DNA denaturation step at 94ºC for 3 min, which was followed by 30 cycles of 94ºC for 1 min, annealing at

45ºC for 1 min and an extension at 72ºC for 1 min and ended with a final extension at 72ºC for 10 min (Kuan, 1997).

The amplified products from the RT-PCR and the nested PCR reactions were separated on a 2% agarose gel (Seakem LE agarose, Bioproducts, USA) using a Medicell Primo gel apparatus (Holbrook, NY). The size of the products was determined using a 100 bp molecular weight marker (Promega Corp., Madison WI, USA) (Gow *et al*., 1991; Kuan, 1997).

3.5.6 Enumeration of selected seeded pathogenic bacteria and bacteriophages in the water samples (Container 2 or 3)

Methods used to determine the survival of *E. coli* and *S. typhimurium* bacteria were described in section 3.5.4. Methods used to determine the survival of somatic and male specific F-RNA bacteriophages were described in section 3.3.2.3.

3.5.7 Enumeration of seeded Enteroviruses in the water samples (Container 3)

The survival of Coxsackie B1 virus in the water samples prior to and after the addition of the sodium hypochlorite solutions was determined using BGM cells (Potgieter, 1997). The BGM cells were grown in 75 cm^2 tissue culture flasks (Corning, USA) to confluent monolayers using EMEM (Highveld Biological, Pty. Ltd, Kelvin, South Africa). The EMEM contained 10% inactivated bovine serum (Delta Bioproducts, Johannesburg, South Africa) and 1% of an antibiotic solution (section 3.5.5). The flasks, containing BGM cell monolayers were trypsinised, by removing the growth medium and adding 5 ml activated Trypsine Versene solution (Highveld Biological, Pty. Ltd, Kelvin, South Africa) for 1 min. The Trypsine Versene solution was removed and the cells resuspended in fresh 10% EMEM-bovine serum-antibiotic medium (Potgieter, 1997). Cells were counted using a haemocytometer (Merck, Darmstadt, Germany) and seeded into 96 well microtitre cell culture plates (Corning, USA) in 200 µl volumes per well. The plates were incubated (Galaxy CO_2 incubator, Biotech Northants, England) overnight at 37ºC in the presence of 5% CO_2. This procedure yielded confluent cell monolayers in each well within 24 h.

End point titrations were carried out as follows: ten fold serial dilutions of each water sample were made in EMEM without serum or antibiotics. The cells in the microtitre plates were rinsed twice with sterile pH 7.2 Phosphate Buffered Saline (PBS) solution (Sigma Chemicals Co., St Louis, MO, USA). Six wells on the microtitre plate were used for each dilution, with six wells respectively for the positive and negative controls. The plates were inoculated with 20 µl of the ten fold dilutions per well. The positive control was a direct inoculation of the Coxsackie B1 virus stock. The negative control consisted of EMEM containing 2% inactivated bovine serum (Delta Bioproducts, Johannesburg, South Africa) and 1% of an antibiotic solution (section 3.5.5). The plates were incubated at 37ºC for 60 min (Galaxy CO_2 incubator, Biotech Northants, England) in the presence of 5% CO_2 with gentle hand rotation every 15 min (Potgieter, 1997).

After incubation, each well received 180 µl of a 2% EMEM-bovine serum-antibiotic solution. The plates were incubated (Galaxy CO_2 incubator, Biotech Northants, England) at 37ºC in the presence of 5% CO_2. The plates were examined daily for a period of 10 days for the presence of cytopathogenic effect (CPE). The 50% endpoints for each water sample were determined by the $TCID_{50}$ Kärber formula described by Grist and co-workers (1979).

3.5.8 Statistical analysis of the laboratory based survival study

The triplicate counts of each of the two experiments were averaged by calculating the geometric means. In cases where microbial counts were not detected, the counts were treated as 0.1 in order to calculate the geometric mean. The means were then log 10 transformed and log 10 reduction values calculated for each microorganism. A zero observation, i.e. no growth detected, was denoted by “n.d” (not detected) since log of zero is not defined.

Chapter 4

RESULTS AND DISCUSSION

4.1 AN INTERVENTION STRATEGY TO IMPROVE THE DRINKING WATER QUALITY IN RURAL HOUSEHOLDS

Home based interventions aimed at improving the quality of drinking water at the point-of-use are becoming a feasible and effective way of immediately providing potable water to people who are dependant on untreated water (Sobsey, 2002). During the pilot study (section 2.7) it was seen that the microbiological quality of household water deteriorates during storage at the point-of-use. It was therefore decided that this study will assess the efficiency of the CDC safe water system (chlorine based water treatment combined with safe storage and education) at improving the microbiological quality of stored drinking water at the point-of-use in rural households in South Africa.

4.1.1 Baseline characteristics of households in two rural villages before intervention study

The household demographics of the two villages are indicated in Table 4.1. There were few differences between the 2 study groups with regards to the total number of adult males, adult females and children under the age of 5 years. A total of 524 people lived in the 120 interviewed households and the average number of people per household varied between 4.3 and 4.5.

The majority of households in both villages had between 2 and 5 rooms (Fig 4.1 and 4.2). Approximately 3% of the female heads of households in village 1 and 5% of village 2 households had no formal education. However, 82% of the female heads of households in village 1 and 84% of village 2 households had at least secondary education (Table 4.1). In addition, 68% of the households in village 1 had children (male and female) younger than 5 years of age compared to 73% of the households in village 2 (Table 4.1).

Figure 4.1: **Traditional households in two study villages in the Vhembe region of the Limpopo Province , South Africa**

Figure 4.2: **More western type households in two study villages in the Vhembe region of the Limpopo Province , South Africa**

Table 4.1: Summary of the household demographics indicating the number of people in each household and the level of education of the female head of the household in each of two rural villages in the Vhembe region of the Limpopo Province, South Africa

Demographics	Village 1 households using tap water (n=60 households)	Village 2 households using river water (n=60 households)
People in household		
Adult females	60 (100%)	60 (100%)
Adult males	51 (85%)	51 (85%)
Female children <5 years	22 (37%)	27 (45%)
Male children <5 years	19 (32%)	17 (28%)
Educational level of female head of household		
None	2 (3%)	3 (5%)
Only pre-primary	3 (5%)	0 (0%)
Only primary	2 (3%)	3 (5%)
Only secondary	49 (82%)	50 (84%)
Diploma	3 (2%)	2 (3%)
Degree	1 (1%)	2 (3%)

The households were selected based on the water source type they were using (Fig 3.2 and 3.3). Both villages did not have a committee and none of the households paid for water. Households were asked during the survey to indicate the location of their water collection point. The distance of the water source from the household was calculated for each household by measuring the distance in steps from the household to the specific water collection point of each household. The South African government target for reasonable access to a water source is 0 m to 200 m from the place of dwelling (Republic of South Africa, 1994). In these two villages, many of the people had to walk long distances to obtain water from the source. Approximately 53% of the households in village 1 and 37% of the households in village 2 had their water source located within 100 m from the household, while 47% of the households in village 1 and 63% of the households in village 2 had a water source located within 100 m to 500 m from the household (Table 4.2).

Table 4.2: Summary of the water sources used by the study households in each of two rural villages in the Vhembe region of the Limpopo Province, South Africa

Data	Village 1 households using tap water (n=60 households)	Village 2 households using river water (n=60 households)
Source distance from household:		
< 100 m	32 (53%)	22 (37%)
> 100 m	28 (47%)	38 (63%)
Is water readily available from source?:		
Yes	28 (47%)	60 (100%)
No	32 (53%)	0 (0%)
Alternative water source:		
None	0 (0%)	59 (98%)
Rainwater	1 (2%)	1 (2%)
River	59 (98%)	0 (0%)
Busiest time at primary water source:		
Morning	49 (82%)	40 (67%)
Afternoon	2 (3%)	2 (3%)
No busy time	9 (15%)	18 (30%)
Who fetches the water for the household?		
Only children	8 (13%)	1 (2%)
Only adults	21 (35%)	19 (31%)
Both	31 (52%)	40 (67%)
Number of water collections per day:		
Once	10 (17%)	11 (18%)
Twice	16 (27%)	14 (23%)
Thrice	34 (57%)	32 (53%)
Four times	0 (0%)	3 (4%)
Source water is considered clean	23 (38%)	14 (23%)
Source water is considered clear	57 (93%)	44 (73%)
Source water don't have a smell	5 (8%)	12 (20%)
Source water don't have a taste	12 (20%)	14 (23%)
Use of the source water:		
Drinking	60 (100%)	60 (100%)
Cooking	59 (98%)	59 (98%)
Bathing	42 (70%)	43 (72%)
Treatment of water from primary water source:		
None	56 (93%)	60 (100%)
Sodium hypochlorite	0 (0%)	0 (0%)
Boiling	4 (7%)	0 (0%)

Most of the households in village 1 (82%) and village 2 (67%) reported that mornings can be busy times at the respective sources (Table 4.2). Approximately 53% of the households using tap water in village 1 complained that water was not readily available at the primary water source. Therefore, almost 98% of the households in village 1 resorted to the river in their region as an alternative water source in times when water was not readily available from the communal taps (Table 4.2).

Approximately 34 (57%) households in village 1 and 32 (53%) households in village 2 reported to collect water 3 times per day, 16 (27%) households in village 1 and 14 (23%) households in village 2 collected water twice a day and 10 (17%) households in village 1 and 11 (18%) households in village 2 collected water once a day (Table 4.2). Adults (35% in village 1; 31% in village 2) or both adults and children (52% in village 1; 67% in village 2) were responsible for collection of water for their households (Table 4.2).

All the households in both villages used the primary water source for cooking (98%) and drinking (100%) purposes (Table 4.2). In village 1, 38% of the households considered the tap water as clean; 8% of the households reported that the tap water did not smell and 20% of the households reported that the tap water did not have a taste (Table 4.2). In village 2, 23% of the households considered the water to be clean; 20% of the households reported the water did not smell and 23% of the households reported that they had no problem with the taste of the river water (Table 4.2).

Inadequate or no treatment of stored drinking water remains a problem in low socio-economic households. The majority of households in village 1 (93%) and village 2 (100%), did not use any treatment before consuming the water, while 7% of the households in village 1 indicated that they used boiling as treatment of their drinking water (Table 4.2). This indicated a lack of knowledge and education by the households on the health risks associated with waterborne diseases.

Table 4.3: Summary of the water storage practices in study households in each of two rural villages in the Vhembe region of the Limpopo Province, South Africa

Data	Village 1 households using tap water (n=60 households)	Village 2 households using river water (n=60 households)
Do you store water in your household:		
Yes	60 (100%)	60 (100%)
No	0 (0%)	0 (0%)
Container size in which water is stored inside household:		
20-50 litre	33 (55%)	25 (42%)
50-100 litre	3 (5%)	8 (13%)
100-200 litre	7 (12%)	9 (15%)
>200 litre	17 (28%)	18 (30%)
Container storage conditions:		
Closed indoors	18 (30%)	34 (57%)
Closed outdoors	1 (2%)	2 (3%)
Open indoors	34 (57%)	22 (37%)
Closed indoors	6 (10%)	1 (2%)
Open/closed indoors	1 (2%)	0 (0%)
Open/closed outdoors	0 (0%)	1 (2%)
Number of times storage container is emptied:		
Daily	8 (13%)	17 (28%)
Weekly	27 (45%)	28 (47%)
Monthly	16 (27%)	10 (17%)
Rarely	9 (15%)	5 (8%)
Cleaning of storage containers:		
Daily	7 (12%)	15 (25%)
Weekly	28 (47%)	32 (53%)
Monthly	15 (25%)	12 (20%)
Rarely	10 (17%)	1 (2%)

All the households in both study groups stored their water after collection (Table 4.3). Different size containers were used for this purpose (Fig 2.10 and Fig 2.11), ranging from 20 to 50 litres (55% village 1 households; 42% village 2 households), 50 to 100 litres (5% village 1 households; 13% village 2 households), 100 to 200 litres (12% village 1 households; 15% village 2 households), to > 200 litres (28% village 1 households; 30% village 2 households) (Table 4.3). Several studies have reported that inadequate storage conditions could result in an increase in numbers of some microorganisms such as heterotrophic bacteria and total coliform bacteria over time

(VanDerSlice and Briscoe, 1993; Reiff *et al*., 1996). According to the survey it was evident that 30% of village 1 households and 56.7% of village 2 households stored their water containers indoors with a closed lid, while 57% of village 1 households and 37% of village 2 households stored their drinking water containers indoors in open containers (Table 4.3). Further observations indicated that 15% of village 1 households and 13% of Village 2 households had loose covers on their storage containers. Approximately 58% of village 1 households and 32% of village 2 households had no cover on the storage containers. Earlier studies by Dunker (2001) and Nala and co-workers (2003) have showed that open containers were more at risk of being contaminated by human and animals than containers which were covered. Many of the households in this study kept their water storage containers on the floor which was smeared with fresh cow dung (Fig 4.3). When the cow dung becomes dry, it forms a dust layer which could contain microorganisms. The cow dung also attracted flies which could be potential vehicles of disease and can contaminate water and food supplies in these rural households (Benenson, 1995).

Figure 4.3: A female member of the study community in the Vhembe region of the Limpopo Province, South Africa busy smearing the floors of the dwelling with cattle dung using her bear hands

Most of the households (45% households in village 1; 47% households in village 2) reported to clean the storage container after 7 days (Table 4.3). Consequently, biofilm formation inside household storage containers (Fig 2.14) due to improper cleaning practices could aid in the survival and growth of potential pathogenic disease causing microorganisms (Bunn *et al*., 2002; Momba and Kaleni, 2002; Jagals *et al*., 2003). Jagals and co-workers (2003) showed that biofilm growth in storage containers can be removed or limited with effective cleaning. Bunn and co-workers (2002) and Momba and Kaleni (2002) have showed in two separate studies (South Africa and Gambia) that indicator organisms (total coliforms, faecal coliforms, *E. coli*, *C. perfringens*, somatic and male specific F-RNA bacteriophages) and pathogens (*Salmonella* spp and *Helicobacter pylori*) could survive longer than 48 h in biofilms inside household drinking water storage containers.

Poor sanitation could conditions increase the risk of diseases in a household (WHO, 2002a). A study by Alam and Zurek (2004) has showed that houseflies carry virulent *E. coli* O157:H7 in areas where cattle are kept and this may play an important role in the transmission of this pathogen between cattle and to the household environment. Consequently, observations made by the interviewers included the following: 35% of the households in village 1 and 8% of the households in village 2 had a dirty yard of which 43% households in village 1 and 8% households in village 2 had flies present in the yard. In village 1, 35% households had dirty kitchens and 40% of the village 1 households had flies present in the kitchen. In village 2, 10% of the households had dirty kitchens and 5% of the households had flies present in the kitchen. Garbage containers were absent in 100% of the households in village 1 and 98% of the households in village 2. Approximately 52% of households in village 1 and 37% of households in village 2 had flies in the toilet. Approximately 63% households in village 1 and 37% households in village 2 had a pit latrine. However, 35% of the households in Village 1 and 62% of the households in village 2 had no toilet facilities and had to use the bush near their household to relieve themselves (Table 4.4).

The method used to obtain water from the storage container could contribute to contamination and the spreading of potential disease causing microorganisms between members of the same household (Jagals *et al*., 1999). Approximately 90% of village 1

households and 97% of village 2 households used a mug to collect water from the storage container (Fig 4.4).

Table 4.4: Summary of hygiene and sanitation conditions/practices in study households in each of two rural villages in the Vhembe region of the Limpopo Province, South Africa

Data	Village 1 households using tap water (n=60 households)	Village 2 households using river water (n=60 households)
Toilet facilities:		
Use bush	21 (33%)	37 (62%)
Use neighbour's toilet facilities	1 (2%)	0 (0%)
Have pit latrine	38 (63%)	23 (38%)
Hand wash facility close to toilet	1 (2%)	0 (0%)
Toilet paper available for use when going to toilet	28 (42%)	51 (85%)
Soap present in household	2 (3%)	1 (2%)
Hand washing practices:		
Before eating	58 (97%)	57 (95%)
Before food preparation	3 (5%)	7 (12%)
After being to toilet	29 (48%)	17 (28%)
After cleaning baby's butt	4 (7%)	9 (15%)
Waste storage in households:		
Daily	24 (40%)	42 (70%)
Weekly	19 (32%)	11 (19%)
Monthly	2 (3%)	3 (5%)
Rarely	15 (25%)	4 (7%)
Waste disposal by households:		
Inside or outside yard	2 (3%)	0 (0%)
Only inside yard	2 (3%)	0 (0%)
Only outside yard	56 (94%)	60 (100%)
Animals in or close to household:		
Cats	2 (3%)	8 (13%)
Dogs	10 (17%)	29 (48%)
Poultry	31 (52%)	40 (67%)
Pigs	2 (3%)	2 (3%)
Goats	21 (35%)	10 (17%)
Cattle	7 (12%)	3 (5%)
Donkeys	4 (7%)	0 (0%)

Jagals and co-workers (1999) and Sobsey (2002) indicated that faecally contaminated hands of household members who do not apply personal hygiene practices can contribute to water contamination (Fig 4.4). Observations made during the baseline survey showed that the mug was not washed every time it was used and was left next to the storage container where animals and small children had access to it.

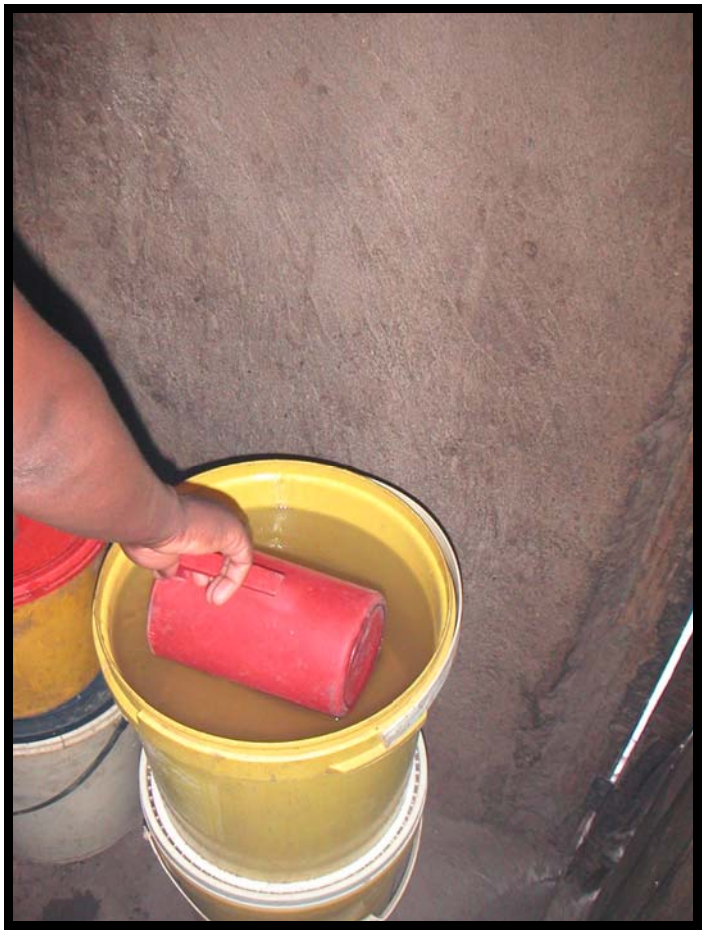


Figure 4.4: One of the study households in the two rural villages in the Vhembe region of the Limpopo Province, South Africa using a mug to collect water from a water storage container

In this study, only 2% households in village 1 and no households in village 2 had a place near the toilet to wash hands. The survey further indicated that approximately 48% households in village 1 and 28% households in village 2 washed hands after going to the toilet. Furthermore, observations during the survey indicated that only 3% of the village 1 households and 2% of the village 2 households had toilet paper available in the toilet. Generally, the toilets were not in good conditions (Fig 4.5 and Fig 4.6).

Figure 4.5: **A typical pit toilet used in both study villages in the Vhembe region of the Limpopo Province, South Africa: no toilet paper available and people used old magazines and newspapers**

Figure 4.6: **A VIP toilet used in both study villages in the Vhembe region of the Limpopo Province, South Africa**

It was also noted that between 95% and 97% of the study population in both villages, reported to wash their hands before eating, while only 5% of the households in village 1 and 12% of the households in village 2 reported to wash hands before they prepared food. This practice was considered a potential risk of faecal contamination of food and water supplies in these households. In addition, only 7% of the mothers in households from village 1 and 15% of mothers in households from village 2 reported to wash their hands after cleaning their baby's buttocks (Table 4.4). This practice was considered another risk of potential contamination of domestic drinking water supplies because studies have indicated that *E. coli* spp, *Klebsiella* spp, *Shigella sonnei* and faecal enterococci can survive between 10 min and 3.5 h on unwashed hands (Knittle, 1975; Casewell and Phillips, 1977; Pinfold, 1990).

Furthermore, 40% of the households in village 1 and 70% of the households in village 2 stored solid wastes on a daily basis (Table 4.4). Approximately 32% of village 1 households and 19% of village 2 households stored solid wastes for 7 days (Table 4.4). In general, only 25% of the study households in village 1 and 7% of study households in village 2 reported to rarely or never store solid wastes. This could be a potential breeding place for flies and pose a health risk to the communities (Table 4.4).

A close living association between the people and animals such as cattle, poultry, donkeys, pigs, goats, dogs and cats was observed in both study villages during this survey (Table 4.4). The majority of households (52% households in village 1; 67% of households in village 2) kept poultry, 35% households in village 1 and 17% households in village 2 kept goats, while 12% of the householdshouseholds in village 1 and 5% of the households in village 2 kept cattle close to the dwelling (Table 4.4). These animals generally walk free in the vicinity of the households (Fig 4.7) and the water sources which increases the risk of waterborne transmission of zoonotic pathogens (Meslin, 1997; Franzen and Muller, 1999; Slifko *et al*., 2000; Enriquez *et al*., 2001; Hoar *et al*., 2001; Leclerc *et al*., 2002; Hackett and Lappin, 2003).

Figure 4.7: Animals like goats moves freely around at one of the study households in the Vhembe region of the Limpopo Province, South Africa

Ignorance and a lack of education concerning waterborne diseases could play an important role in the general health of a household. Results from the baseline survey indicated that only 58% of the households in village 1 and 46% of the households in village 2 reported to have knowledge of waterborne diseases (Table 4.5). This is in spite of the Department of Health and Primary Health Care Clinics in the Vhembe district giving regular education sessions on waterborne diseases to the village communities. However, the clinic staff did mention they have problems reaching all the households due to transport problems and shortage of staff (personal communication with staff members at the various clinics).

During the baseline survey, it was found that 28% of the households in village 1 and 18% of the households in village 2 had a child under the age of 5 years who had suffered from diarrhoea in the last 6 months prior to the survey (Table 4.5). However, the majority of respondents (33% from village 1 households; 32% from village 2 households) had no idea what the cause of the child's diarrhoea were; 50% of the households in village 1 and 47% of the households in village 2 gave contaminated water

as reason and 12% of the households in village 1 and 10% of the households in village 2 mentioned food as a possible cause (Table 4.5).

Table 4.5: Knowledge of waterborne diseases by study households in each of two rural villages in the Vhembe region of the Limpopo Province, South Africa

Data	Village 1 households using tap water (n=60 households)	Village 2 households using river water (n=60 households)
Number of households with knowledge on waterborne diseases	30 (50%)	28 (46%)
Households with children <5 years with diarrhoea in last 6 months	17 (28%)	11 (18%)
What do head of household think caused the child's diarrhoea?		
Dirty water	30 (50%)	28 (47%)
Food	7 (12%)	6 (10%)
No idea	20 (33%)	19 (32%)
Poor hygiene	0 (0%)	2 (3%)
Seasonal change	3 (5%)	0 (0%)
Teething	0 (0%)	5 (8%)
How can diarrhoea in children be prevented?		
Clean water	25 (42%)	21 (35%)
Clean food	1 (2%)	0 (0%)
Medicine	3 (5%)	9 (15%)
No idea	31 (52%)	30 (50%)

It was alarming that 0% of the households in village 1 and 3% of the households in village 2 thought that poor hygiene could be responsible for the child's diarrhoea (Table 4.5). Similarly 52% of households in village 1 and 50% of the households in village 2 had no idea how to prevent the child from getting diarrhoea (Table 4.5). However, 42% of households in village 1 and 35% of households in village 2 did mention that clean (safe) water could prevent diarrhoea in children (Table 4.5).

4.1.2 The effectiveness of a home chlorination intervention study

The intervention households using the 1% and the 3.5% sodium hypochlorite solutions had zero counts for heterotrophic bacteria, total coliforms, faecal coliforms, *E. coli*, faecal enterococci, *C. perfringens*, somatic and male specific F-RNA bacteriophages in the water samples taken from both container types during the formal intervention trial. This indicated that both the 1% and 3.5% sodium hypochlorite solutions were effective home based treatments. Therefore, all the results discussed in this section on household water samples will be on counts obtained for the traditional and CDC safe storage containers in households receiving the placebo solution.

4.1.2.1 The physical quality of the primary water sources and the container stored water used by the two rural villages

The pH values for tap water ranged between 7.0 and 7.1 and for river water varied between 6.8 and 7.7 (Table 4.6). The pH values of both types of containers fell within the South African water quality pH guideline range for domestic use of 6.0 to 9.0 (Table 4.6) (DWAF, 1996). Several studies have indicated that pH could play a role in the survival of microorganisms during disinfection (Engelbrecht *et al.*, 1980; Schaper *et al*., 2002b). A study by Vaughn and co-workers (1986) has showed that viruses are more readily inactivated by chlorine when the water had a pH level of 6 compared to the water samples which had a pH level of 8. In this study no statistical differences (P=0.783) between the tap and river water sources with regards to the pH was found (Tables 4.7 and 4.8). In village 1 no statistical difference were found between the pH values from the communal tap water source and the traditional storage containers (P=0.354) and between the tap water sources and the CDC safe storage containers (P=0.388). In addition, no significant difference were seen in the pH measurements between the two types of water storage containers (P=0.483) (Table 4.6). Likewise, in village 2, no statistical difference were found between the pH values from the river water source and the traditional storage containers (P=0.423) and between the river water source and the CDC safe storage containers (P=0.438) (Table 4.6). In addition, no significant difference were seen in the pH measurements between the two types of water storage containers in Village 2 (P=0.350) (Table 4.6).

Table 4.6: Geometric mean values (95% confidence intervals) of the physical parameters of the water sources and the traditional and the CDC safe storage containers of two rural villages using the placebo solution in the Vhembe region of the Limpopo Province, South Africa

Physical parameters	Village 1 using tap water			Village 2 using river water		
	Communal tap water sources	Traditional water storage container	CDC safe water storage container	River water source	Traditional water storage container	Improved CDC water storage container
pH	7.0 (7.0; 7.1)	7.3 (7.0; 7.8)	7.3 (7.0; 7.8)	7.2 (6.8; 7.7)	7.0 (6.9; 7.2)	7.4 (6.7; 7.6)
Temperature (ºC)	19.4 (18.6; 20.2)	20.2 (19.2; 21.3)	19.4 (18.6; 20.2)	19.3 (15.6; 22.9)	19.3 (18.6; 19.9)	19.7 (18.7; 19.9)
Turbidity (NTU)	0.6 (0.3; 1.0)	0.6 (0.1; 1.1)	0.9 (0.2; 1.5)	5.9 (4.1; 7.7)	4.2 (3.0; 5.3)	3.5 (2.4; 4.6)

The South African recommended guideline values for temperature of domestic water ranged between 18ºC to 24ºC (DWAF, 1996). In this study the temperatures for all water source samples as well as water samples obtained from the traditional and CDC safe storage containers in both villages fell well within this range. This indicated that disinfection of the microorganisms in these water sources might be successful (Table 4.6). Several studies have shown that temperature plays an important role in the survival of microorganisms and the effectiveness of a disinfectant. Atkin and co-workers (1971) and Sattar (1981) have showed that viruses have a tendency to survive longer in groundwater sources than in surface water at similar temperatures due to the effect of temperature and ultra violet sunrays. Carlsson (2003) stated that an increase in water temperatures can result in higher rates of inactivation of microorganisms in water samples.

In this study no statistical differences (P=0.867) between communal tap and river water samples with regards to the temperature readings were seen (Tables 4.7 and 4.8). In village 1 no statistical difference were found between the temperature values from the communal tap water source, the traditional storage containers (P=0.03) and the CDC safe storage containers (P=0.281). In addition, no significant difference were seen in the temperature measurements between the traditional and CDC safe storage containers (P=0.193) (Table 4.6). In village 2, no statistical difference were found between the temperature values from the river water source and the traditional storage containers (P=0.359) and between the river water source and the CDC safe storage containers (P=0.154) (Table 4.6). In addition, no statistical difference between the traditional and CDC safe storage containers in village 2 with regards to temperature could be seen (P=0.462) (Table 4.6).

Turbidity measurements give a general indication of the concentration of suspended clay, silt, organic matter, inorganic matter, plankton and other microscopic organisms in a water source (DWAF, 1996). In this study the river water source samples had turbidity values which exceeded the recommended South African guideline value of 0.1 NTU (Table 4.6) (SABS, 2001). High turbidity values are associated with the survival of microorganisms due to association of the microorganisms with particulate matter in the water (DWAF, 1996). Tap water sources had turbidity values between 0.3 NTU and 1.0 NTU and river water sources had turbidity values ranging from 4.1 NTU to 7.7 NTU

(Table 4.6). Therefore, a significant difference ($P<0.001$) was observed in the turbidity values between the two types of water sources. This suggested that the river water had more nutrients and particulate matter, which could have assisted in the survival and transmission of waterborne diseases due to the association between microorganisms and particulate particles (DWAF, 1996). The turbidity of the water inside the traditional and improved CDC safe storage containers in households using the tap and the river water sources were higher than the South African guideline values of 0.1 NTU (SABS, 2001) (Table 4.6). This could have reduced the effectivity of the disinfectant used in this study and assisted in the survival of microorganisms due to association of the microorganisms with particulate matter in the water (DWAF, 1996).

In village 1, no statistical differences in the turbidity values between the communal tap water source and the traditional storage containers ($P=0.934$) and between the tap water and the CDC safe storage containers ($P=0.439$) were seen. In addition, in village 1, no statistical significant difference were seen between the turbidity measurements of the traditional and the CDC safe storage containers ($P=0.243$) (Table 4.6). While in village 2, a significant statistical difference between the turbidity values from the river water source and the traditional storage containers ($P=0.008$) and between the river water source and the CDC safe storage containers ($P=0.001$) were observed (Table 4.6). The lower turbidity measurements in the storage containers could be due to settlement of particular matter in the containers during storage. However, no statistical significance between the turbidity values from the two types of storage containers were observed ($P=0.814$) in village 2 (Table 4.6).

4.1.2.2 The microbiological quality of the primary water sources and the container stored water in the two rural villages

Microbiological quality of the primary water sources used in both villages was assessed using indicator microorganisms which included heterotrophic plate counts, total coliforms, *E. coli*, faecal coliforms, faecal enterococci, somatic and male specific F-RNA bacteriophages (DWAF, 1996). The presence of these indicator microorganisms in a water sample, were a general guideline to indicate the presence of potential pathogenic bacteria, viruses and parasites and to determine the health risk to consumers (DWAF, 1996).

Heterotrophic plate counts indicated the general microbiological quality of the water samples and mostly included microorganisms such as *Aeromona* spp, *Klebsiella pneumoniae, Enterococcus, Flavobacterium* spp, *Bacillus* spp and *Enterobacter* spp that required organic carbon for growth (DWAF, 1996; WHO, 2002b). Generally heterotrophic microorganisms are considered to be harmless. However, various studies have indicated that some heterotrophic microorganisms might be opportunistic pathogens (Payment *et al*., 1991; WHO, 2002b; Bartram *et al*., 2003). These opportunistic pathogens have been associated with diseases in immunocompromised individuals, infants and the elderly during exposure to or consumption of contaminated water (Payment *et al*., 1991; Bartram *et al*., 2003).

In this study, the heterotrophic bacterial counts for the communal taps and river water as well as the traditional and CDC safe storage container water samples in both villages exceeded the South African recommended guideline value of 100 cfu.ml^{-1} (Tables 4.7 and 4.8) (SABS, 2001). Heterotrophic microorganisms are found as natural inhabitants of water and soil environments and might have been present in the communal tap water and river water sources or due to biofilms inside the reservoir and pipe distribution systems or due to various animal- and human activities inside the river catchment (Bartram *et al*., 2003).

Table 4.7: Geometric mean values (95% confidence intervals) for microbiological indicators of water samples collected over a 4 month period from communal tap water sources and the stored household water in traditional and CDC safe storage containers used by households together with the placebo solution from village 1 in the Vhembe region of the Limpopo Province, South Africa

Water source and container type	Heterotrophic bacteria (cfu.1 ml^{-1})	Total coliforms (cfu.100 ml^{-1})	Faecal coliforms (cfu.100 ml^{-1})	*Escherichia coli* (cfu.100 ml^{-1})	Faecal enterococci (cfu.100 ml^{-1})	*Clostridium perfringens* (cfu.100 ml^{-1})
Communal tap source*	1.6×10^6 (6.6×10^5; 4.2×10^6)	360 (247; 525)	180 (116; 277)	84 (54; 124)	37 (18; 72)	34 (14; 83)
Traditional containers**	3.0×10^7 (7.7×10^6; 1.2×10^8)	783 (435; 1 411)	414 (221; 775)	115 (77; 170)	100 (51; 197)	98 (69; 140)
CDC safe storage containers**	1.7×10^7 (5.0×10^6; 5.4×10^7)	944 (638; 1 390)	578 (409; 816)	120 (74; 196)	105 (47; 233)	90 (40; 199)

*** n= 16 taps**

**** n = 10 households**

Table 4.8: Geometric mean values (95% confidence intervals) for microbiological indicators of water samples collected over a 4 month period from communal tap water sources and the stored household water in traditional and CDC safe storage containers used by households together with the placebo solution from village 2 in the Vhembe region of the Limpopo Province, South Africa

Water source and container type	Heterotrophic bacteria (cfu.1 ml^{-1})	Total coliforms (cfu.100 ml^{-1})	Faecal coliforms (cfu.100 ml^{-1})	*Escherichia coli* (cfu.100 ml^{-1})	Faecal enterococci (cfu.100 ml^{-1})	*Clostridium perfringens* (cfu.100 ml^{-1})
River water source *	2.1×10^6 (1.1×10^5; 3.9×10^7)	844 (691; 1 032)	538 (328; 883)	166 (90; 306)	154 (42; 582)	132 (21; 807)
Traditional containers**	5.3×10^6 (5.5×10^5; 5.1×10^7)	1 345 (1 100; 1 643)	1 025 (784; 1 341)	413 (279; 610)	139 (80; 241)	170 (106; 274)
CDC safe storage containers**	1.0×10^7 (2.2×10^6; 4.8×10^7)	1 380 (1 157; 1 646)	1 090 (855; 1 389)	343 (215; 548)	94 (62; 142)	125 (95; 165)

*** n= 4 sites on river**

**** n = 10 households**

The results further indicated that the counts detected in the household storage containers (traditional and CDC safe storage containers) were higher than the primary water source counts (Tables 4.7 and 4.8). The increase in heterotrophic plate counts in both the traditional and the CDC safe storage containers could be ascribed to: (1) secondary contamination of the stored water, (2) re-growth of some heterotrophic microorganisms, or (3) unhygienic water-handling practices (Nala *et al*., 2003). The higher heterotrophic plate counts in the storage containers indicated an increased risk to people consuming the water for infections by opportunistic pathogenic microorganisms such as *Aeromona* spp and *Pseudomonas* spp, which have been associated with diseases such as diarrhoea, skin, eye and respiratory infections (DWAF, 1996; Bartram *et al*., 2003).

The statistical analysis of the heterotrophic bacterial counts indicated the following:

- No statistical difference (P=0.272) could be seen between the heterotrophic bacterial counts of the river and tap water sources (Tables 4.7 and 4.8).
- In village 1 no statistical differences was found between the tap water source and the traditional storage containers (P=0.359) or between the tap water source and the CDC safe storage containers (P=0.968) (Table 4.7).
- In village 2 no statistical differences was found between the river water source and the traditional storage containers (P=0.196) or between the river water source and the CDC safe storage containers (P=0.303) (Table 4.8).
- In village 1 no statistical differences was found between the traditional storage containers and the CDC safe storage containers (P=0.459) (Table 4.7 and Fig 4.8).
- In village 2 no statistical differences was found between the traditional storage containers and the CDC safe storage containers (P=0.597) (Table 4.8 and Fig 4.8).
- In general no statistical difference with regards to heterotrophic bacteria could be seen between the traditional and CDC safe storage containers using the placebo solution (P=0.974). This showed that the CDC safe storage container alone did not make a difference in improving water at the point-of-use.

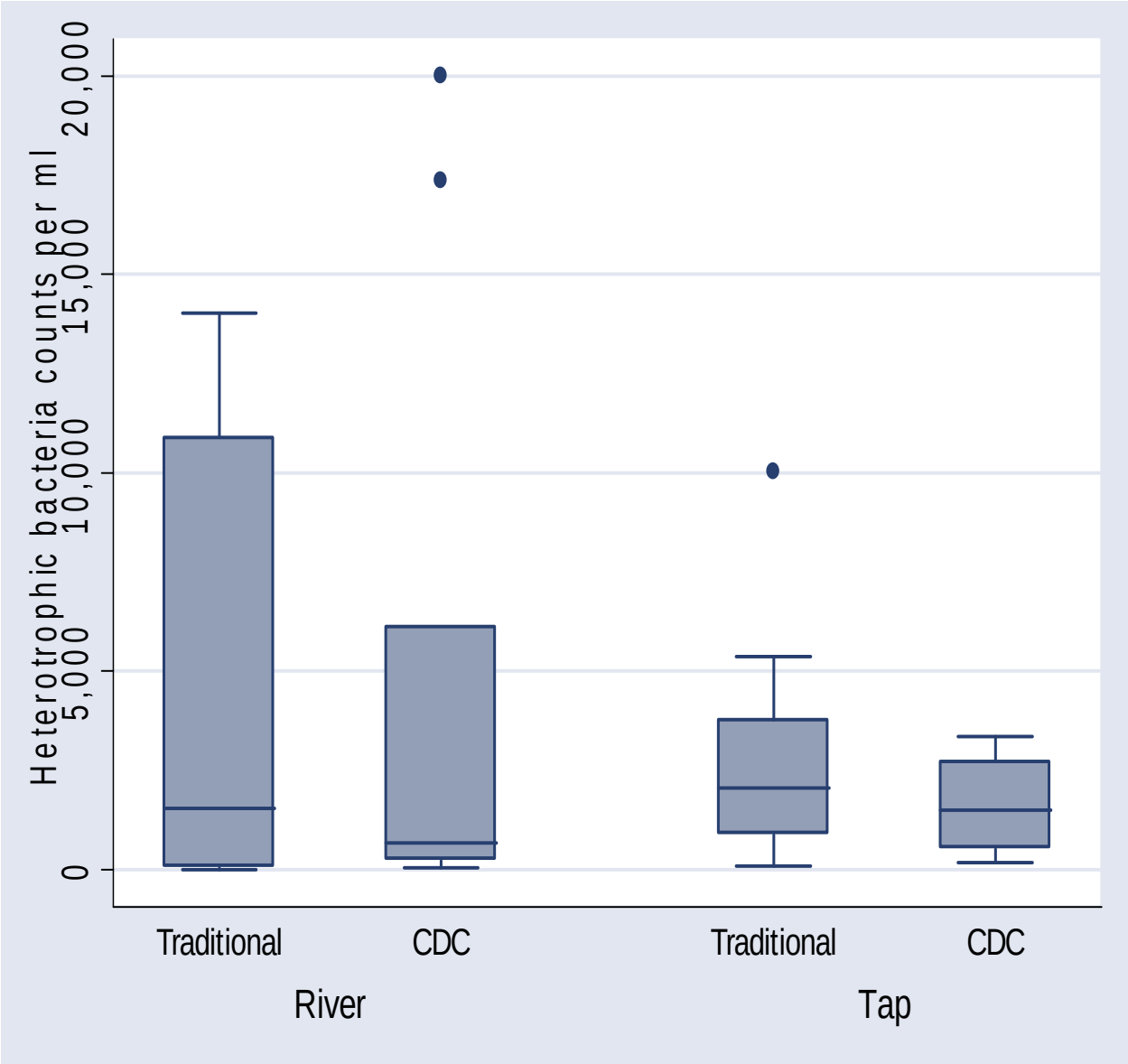


Figure 4.8: **Heterotrophic bacteria distributed by primary water sources and stored water in traditional and CDC safe storage containers from two villages in the Vhembe region of the Limpopo Province, South Africa**

Total coliforms included bacteria of known faecal origin such as *E. coli*, as well as bacteria such as *Citrobacter* spp and *Enterobacter* spp which may be found in faeces and the environment and bacteria such as *Serratia* spp which may replicate in water environments (WHO, 1996). The total coliform bacterial count for tap and river water sampling points as well as the total coliform counts for the stored water in the traditional and CDC safe storage containers in village 1 and village 2 exceeded the South African guideline value of 10 cfu.100ml^{-1} for total coliforms presence in water intended for domestic purposes (Tables 4.7 and 4.8) (SABS, 2001).

The high total coliform counts in the water sources and especially in the storage containers increased the health risk associated with waterborne diseases such as gastroenteritis, dysentery, cholera, typhoid fever and salmonellosis which are caused by pathogenic organisms such as *Salmonella* spp, *Shigella* spp, *Vibrio cholerae*, *Campylobacter jejuni*, *Campylobacter coli*, *Yersinia enterocolitica* and pathogenic *E. coli* (DWAF, 1996). In addition, the increase in the total coliform counts in the traditional and the CDC safe storage containers during storage at the point-of-use in both villages indicated secondary contamination due to unhygienic handling practices and storage conditions (Tables 4.7 and 4.8) (Jagals *et al.*, 1999; Nath *et al*., 2006).

The statistical analysis of the total coliform bacterial counts indicated the following:

- A statistical difference (P=0.004) could be seen between the total coliform bacterial counts of the river and tap water sources (Tables 4.7 and 4.8).
- In village 1 statistical differences was found between the tap water source and the traditional storage containers (P=0.02) and between the tap water source and the CDC safe storage containers (P=0.003) (Table 4.7).
- In village 2 statistical differences was found between the river water source and the traditional storage containers (P=0.0005) and between the river water source and the CDC safe storage containers (P=0.0001) (Table 4.8).
- In village 1 no statistical differences was found between the traditional storage containers and the CDC safe storage containers (P=0.557) (Table 4.7 and Fig 4.9).
- In village 2 no statistical differences was found between the traditional storage containers and the CDC safe storage containers (P=0.829) (Table 4.8 and Fig 4.9).
- In general no statistical difference with regards to total coliform bacteria could be

seen between the traditional and CDC safe storage containers using the placebo solution (P=0.557). This showed that the CDC safe storage container alone did not make a difference in improving water at the point-of-use.

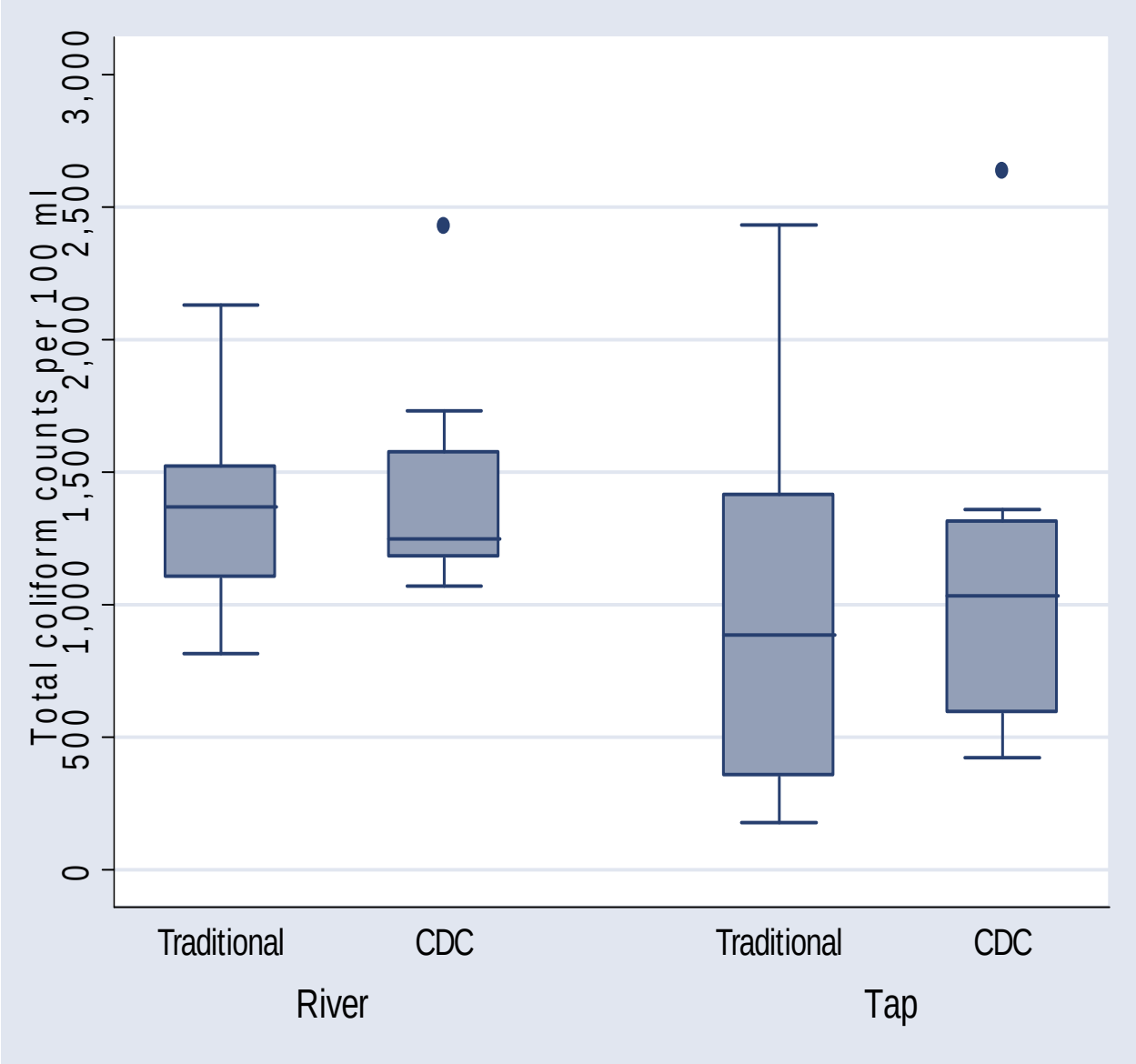


Figure 4.9: **Total coliform bacteria distributed by primary water sources and stored water in traditional and CDC safe storage containers from two villages in the Vhembe region of the Limpopo Province, South Africa**

Faecal coliform bacteria were used in this study to indicate the presence of potential pathogenic microorganisms that is transmitted through the faecal-oral route (DWAF, 1996). The faecal coliform counts in the water sources and the traditional and CDC safe storage containers in village 1 and village 2 households exceeded the South African recommended guideline value of 0 cfu.100 ml^{-1} (Tables 4.7 and 4.8) (SABS, 2001). The high faecal coliform counts in the river water samples indicated that the river has been contaminated due to direct faecal contamination from warm-blooded animals/humans or sewage run-off during rainy periods (WHO, 2002a).

In addition, the increase in faecal coliform counts in the traditional and the CDC safe storage containers in both village households during storage at the point-of-use in both villages (Tables 4.7 and 4.8) were in agreement with results from previous studies indicating the microbiological decrease in water quality after collection (Sobsey, 2002; Fewtrell *et al*., 2005). This increase in faecal coliform counts in the storage containers in both village households, indicated secondary contamination either due to human or animal faecal matter or because of unhygienic storage and handling practices at the point-of-use (DWAF, 1996).

The statistical analysis of the faecal coliform bacterial counts indicated the following:

- A statistical difference (P=0.004) could be seen between the faecal coliform bacterial counts of the river and tap water sources (Tables 4.7 and 4.8).
- In village 1 statistical differences was found between the tap water source and the traditional storage containers (P=0.012) and between the tap water source and the CDC safe storage containers (P=0.0001) (Table 4.7).
- In village 2 statistical differences was found between the river water source and the traditional storage containers (P=0.0004) and between the river water source and the CDC safe storage containers (P=0.0001) (Table 4.8).
- In village 1 no statistical differences was found between the traditional storage containers and the CDC safe storage containers (P=0.306) (Table 4.7 and Fig 4.10).
- In village 2 no statistical differences was found between the traditional storage containers and the CDC safe storage containers (P=0.708) (Table 4.8 and Fig 4.10).
- In general no statistical difference with regards to faecal coliform bacteria could be seen between the traditional and CDC safe storage containers using the placebo

solution (P=0.364). This showed that the CDC safe storage container alone did not make a difference in improving water at the point-of-use.

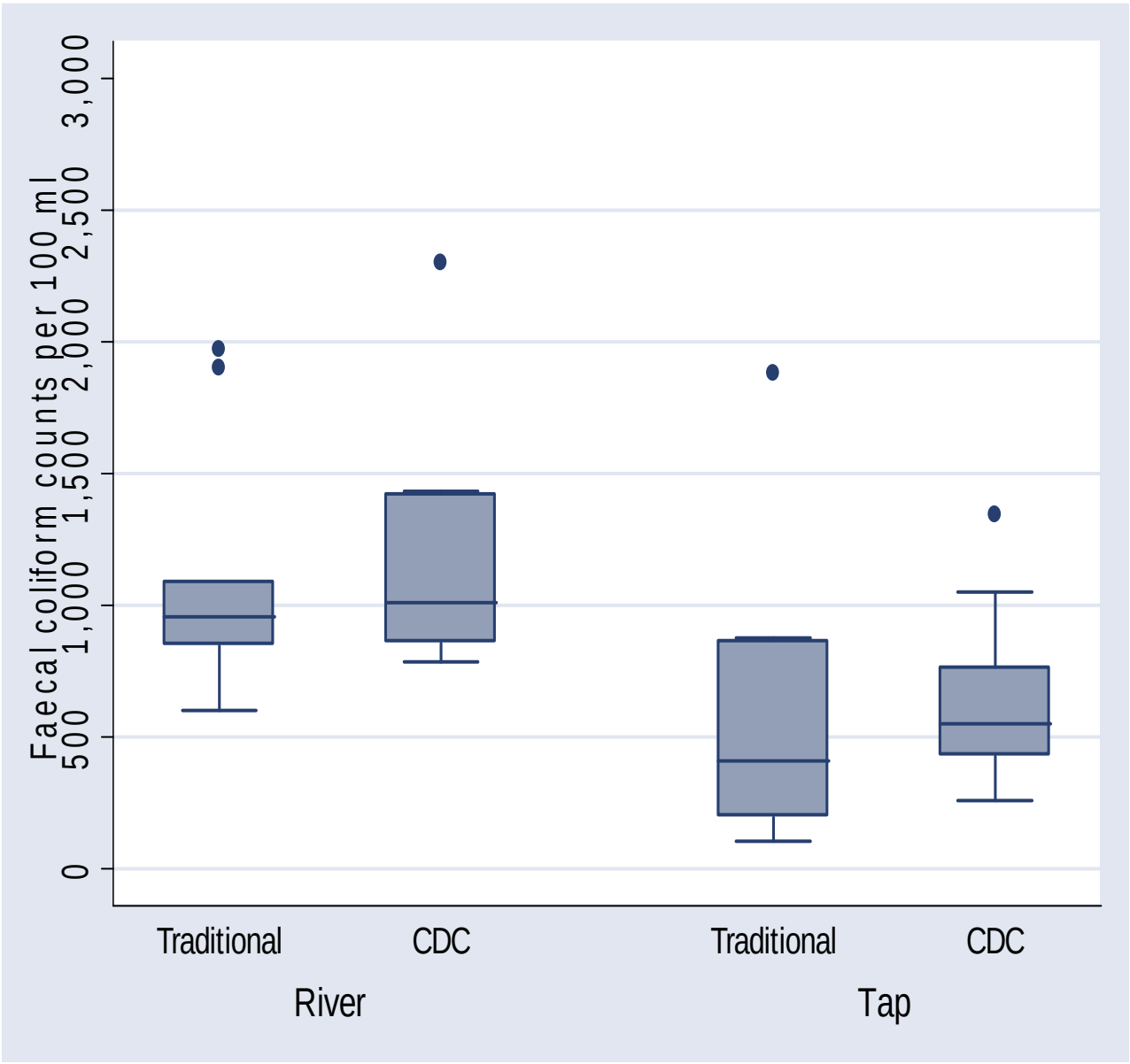


Figure 4.10: Faecal coliform bacteria distributed by primary water sources and stored water in traditional and CDC safe storage containers from two villages in the Vhembe region of the Limpopo Province, South Africa

Although *E. coli* bacteria are found in the faeces of humans and animals, pathogenic *E. coli* strains have virulence factors, which could be responsible for the cause of diseases and therefore implicate a potential risk to the consumers (Kuhnert *et al*., 2000). The detection of *E. coli* in the water samples indicated the presence of faecal pollution from warm blooded animals and humans (Kuhnert *et al*., 2000). During this study the *E. coli* counts exceeded the recommended guideline value of 0 cfu.100 ml^{-1} for both the water sources and the two types of storage containers in both villages (Tables 4.7 and 4.8) (Edberg *et al*., 2000; SABS, 2001). The results of this study showed *E. coli* counts increased after collection and indicated secondary contamination of the stored household water at the point-of-use (Tables 4.7 to 4.8).

The statistical analysis of the *E. coli* bacterial counts indicated the following:

- A statistical difference (P=0.010) could be seen between the *E. coli* bacterial counts of the river and tap water sources (Tables 4.7 and 4.8). This indicated that unimproved sources (River water) were more prone to faecal contamination than improved sources (communal taps) due to human and animal activities in the vicinity of the source.
- In village 1 no statistical differences was found between the tap water source and the traditional storage containers (P=0.109) and between the tap water source and the CDC safe storage containers (P=0.131) (Table 4.7).
- In village 2 statistical differences was found between the river water source and the traditional storage containers (P=0.0005) and between the river water source and the CDC safe storage containers (P=0.007) (Table 4.8).
- In village 1 no statistical differences was found between the traditional storage containers and the CDC safe storage containers (P=0.861) (Table 4.7and Fig 4.11).
- In village 2 no statistical differences was found between the traditional storage containers and the CDC safe storage containers (P=0.501) (Table 4.8 and Fig 4.11).
- In general no statistical difference with regards to *E. coli* bacteria could be seen between the traditional and CDC safe storage containers using the placebo solution (P=0.802). This showed that the CDC safe storage container alone did not make a difference in improving water at the point-of-use.

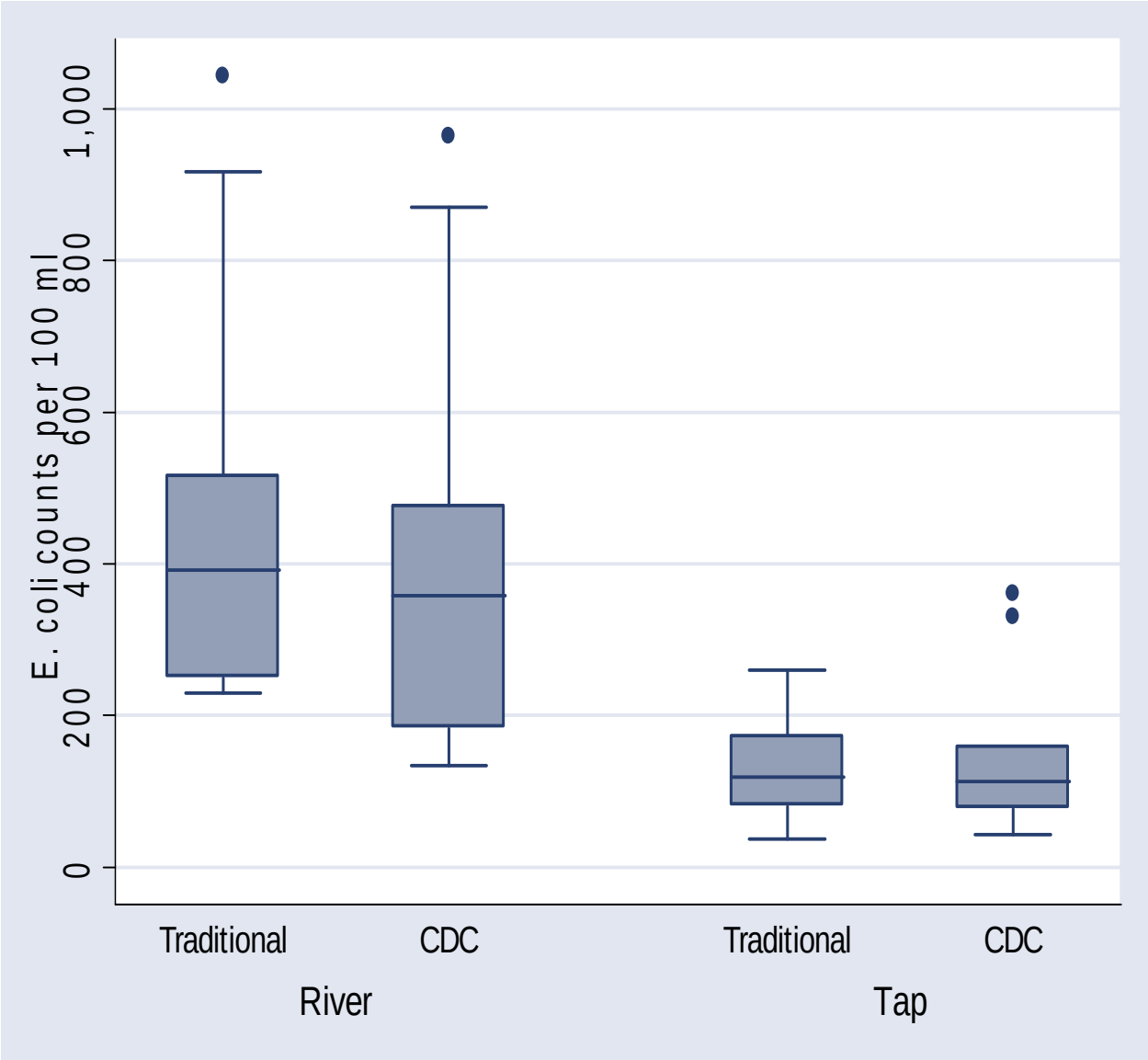


Figure 4.11: ***Escherichia coli*** **bacteria distributed by primary water sources and stored water in traditional and CDC safe storage containers from two villages in the Vhembe region of the Limpopo Province, South Africa**

Faecal enterococci counts in this study were used to indicate the presence of human faecal contamination in the water samples (SABS, 2001). The South African water quality guideline value for faecal enterococci in water intended for domestic use is 0 cfu.100 ml^{-1} (SABS, 2001). However, the faecal enterococci counts for both the water sources exceeded the South African guideline value for safe drinking water (Tables 4.7 and 4.8). In addition it was seen that faecal enterococci counts increased in the traditional and CDC safe storage containers in village 1 in households using communal tap water indicating secondary contamination through unhygienic practices during collection and storage at the point-of-use (Table 4.7). In village 2 households, the faecal coliform counts were similar to that of the water source and even decreased in the CDC safe storage containers which indicated that the collected water was already contaminated or the reduced counts were due to the natural die-off of the bacterial cells in the containers (Table 4.8) (Moyo *et al*., 2004).

The statistical analysis of the faecal enterococci bacterial counts indicated the following:

- A statistical difference (P=0.001) could be seen between the faecal enterococci bacterial counts of the river and tap water sources (Tables 4.7 and 4.8).
- In village 1 statistical differences was found between the tap water source and the traditional storage containers (P<0.001) and between the tap water source and the CDC safe storage containers (P<0.001) (Table 4.7).
- In village 2 no statistical differences was found between the river water source and the traditional storage containers (P=0.597) while for the CDC safe storage containers there was a significant reduction in the faecal enterococci counts (P=0.0001) (Table 4.8).
- In village 1 no statistical differences was found between the traditional storage containers and the CDC safe storage containers (P=0.917) (Table 4.7 and Fig 4.12).
- In village 2 no statistical differences was found between the traditional storage containers and the CDC safe storage containers (P=0.216) (Table 4.8 and Fig 4.12).
- In general no statistical difference with regards to faecal enterococci bacteria could be seen between the traditional and CDC safe storage containers using the placebo solution (P=0.532). This showed that the CDC safe storage container alone did not make a difference in improving water at the point-of-use.

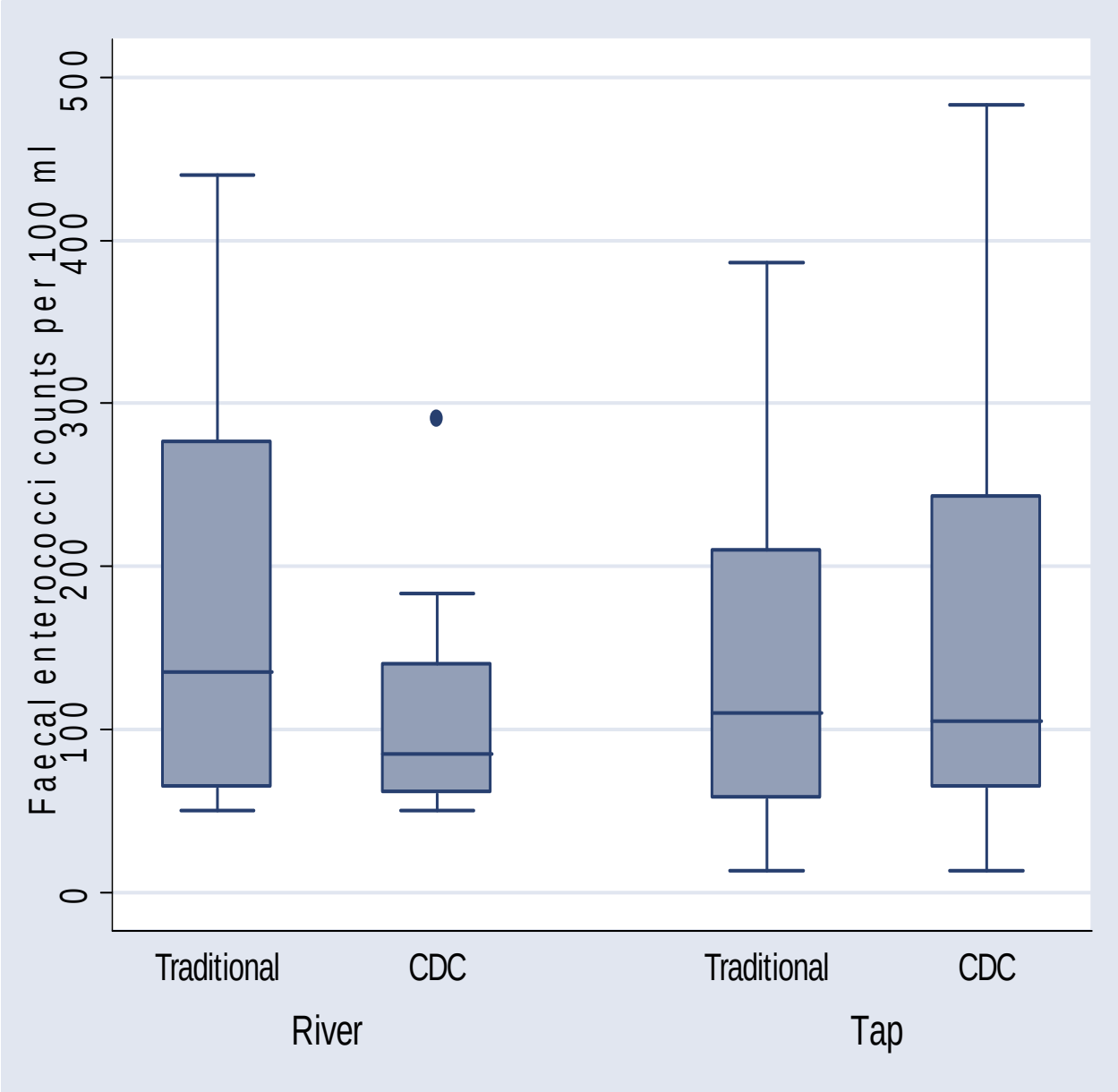


Figure 4.12: **Faecal enterococci bacteria distributed by primary water sources and stored water in traditional and CDC safe storage containers from two villages in the Vhembe region of the Limpopo Province, South Africa**

The direct detection of viruses in water samples would be preferred. However, viral isolation and detection methods are expensive, labour intensive and require skilled personnel. Therefore, indicator organisms such as *C. perfringens*, somatic and male specific F-RNA bacteriophages were used in this study to indicate the potential presence of pathogenic enteric viruses (Grabow *et al*., 1993; Leclerc *et al*., 2000). *Clostridium perfringens* is associated with soil as well as with animal and human faeces and the spores could survive for long periods in the environment such as sediments (Listle *et al*., 2004). Therefore, the presence of *C. perfringens* in the water sources and the traditional and CDC safe storage containers indicated that potential pathogenic viruses (eg. Enteroviruses and Hepatitis A virus) and parasites (eg. *Giardia* and *Cryptosporidium*) could have been present in the water. These pathogens could cause diseases such as hepatitis, meningitis and gastroenteritis (Payment and Franco, 1993).

The statistical analysis of the *C. perfringens* bacterial counts indicated the following:

- A significant statistical difference ($P<0.001$) could be seen between the *C. perfringens* bacterial counts of the river and tap water sources (Tables 4.7 and 4.8).
- In village 1 statistical differences was found between the tap water source and the traditional storage containers ($P=0.0001$) and between the tap water source and the CDC safe storage containers ($P=0.022$) (Table 4.7).
- In village 2 no statistical differences was found between the river water source and the traditional storage containers ($P=0.247$) and between the river water source and the CDC safe storage containers ($P=0.684$) (Table 4.8).
- In village 1 no statistical differences was found between the traditional storage containers and the CDC safe storage containers ($P=0.829$) (Table 4.7 and Fig 4.13).
- In village 2 no statistical differences was found between the traditional storage containers and the CDC safe storage containers ($P=0.216$) (Table 4.8 and Fig 4.13).
- In general no statistical difference with regards to *C. perfringens* bacteria could be seen between the traditional and CDC safe storage containers using the placebo solution ($P=0.401$). This showed that the CDC safe storage container alone did not make a difference in improving water at the point-of-use.

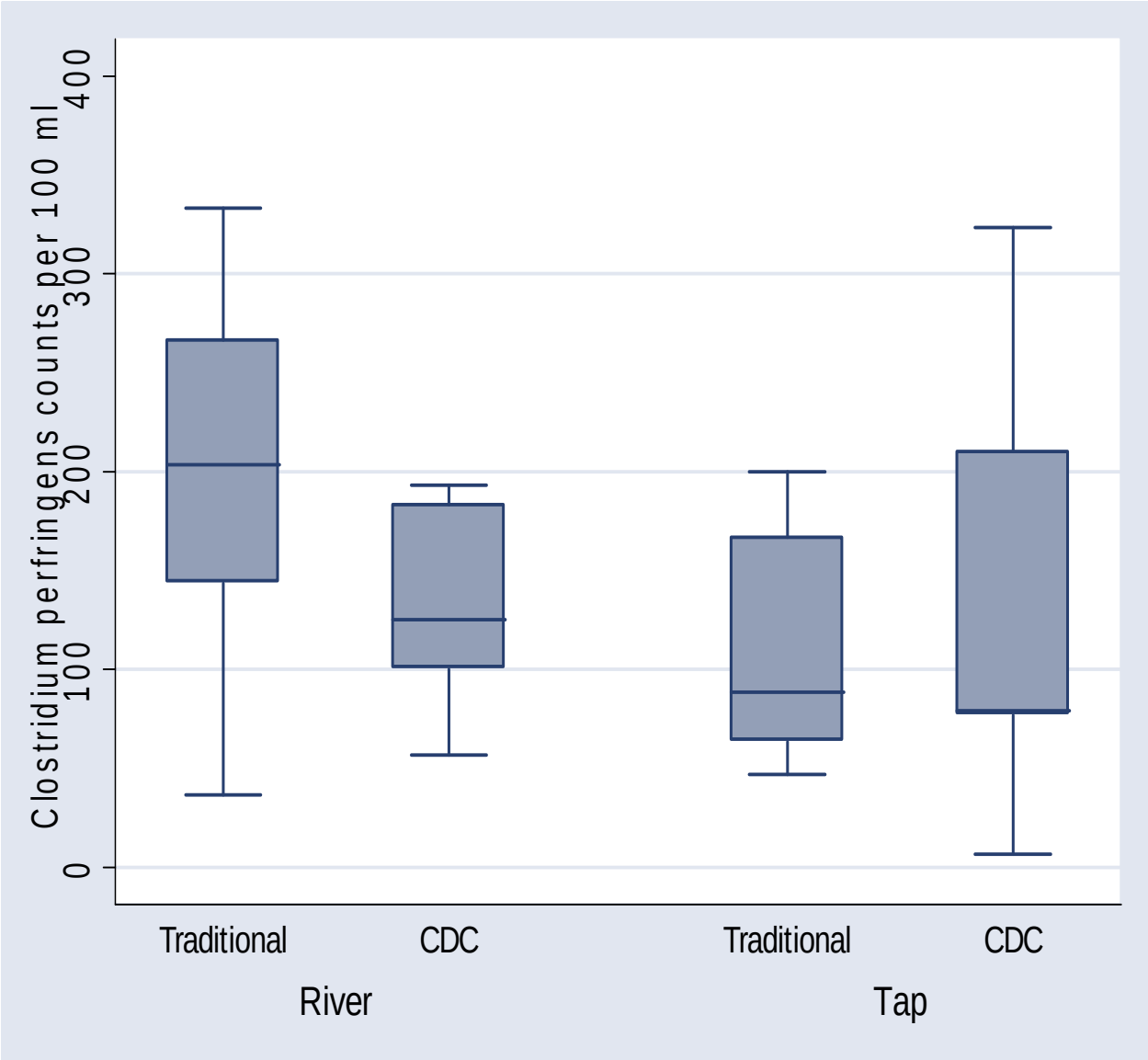


Figure 4.13: ***Clostridium perfringens*** **bacteria distributed by primary water sources and stored water in traditional and CDC safe storage containers from two villages in the Vhembe region of the Limpopo Province, South Africa**

According to the South African guidelines, no somatic bacteriophage counts should be detected in water intended for drinking purposes (SABS, 2001). Table 4.9 showed the presence of somatic and male specific F-RNA bacteriophages in the primary water sources which indicated the potential risk of the presence of human viruses such as Adenoviruses, Astroviruses, Caliciviruses, Enteroviruses, Hepatitis A virus and Rotaviruses which could have caused diseases such as hepatitis, myocarditis and gastroenteritis to consumers (Grabow *et al*., 1993b). The increase in somatic and male specific F-RNA bacteriophage prevalence in household storage containers of the households using communal tap water indicated secondary contamination after collection and during storage at the point-of-use due to unhygienic practices (DWAF, 1996).

Table 4.9: Presence-Absence analyses of source water (communal tap and river water) and stored water (traditional and CDC safe storage containers), from households using the placebo solution in two rural villages in the Vhembe region of the Limpopo Province, South Africa.

Bacteriophages	Village 1			Village 2		
	Communal tap sources (n = 16 taps)	Traditional storage containers (n = 10 HH)	CDC safe storage containers (n = 10 HH)	River water source (n = 4 sites)	Traditional storage container (n = 10 HH)	CDC safe storage container (n = 10 HH)
Somatic	1/16 (6.3%)	9/10 (90%)	10/10 (100%)	4/4 (100%)	10/10 (100%)	10/10 (100%)
Male specific F-RNA	1/16 (6.3%)	8/10 (80%)	9/10 (90%)	4/4 (100%)	10/10 (100%)	10/10 (100%)

In general, the results discussed in this section indicated that the 1% and 3.5% sodium hypochlorite solutions were effective water treatment interventions. Both the traditional and CDC safe water storage containers showed similar results with regards to treatment effectivity in households using either the 1% or 3.5% sodium hypochlorite solutions. Furthermore, the microbial counts obtained from the traditional and CDC safe storage containers in households using the placebo solution, indicated that the container without a sodium hypochlorite solution treatment, do not improve the stored drinking water. Therefore, more intensive marketing of sodium hypochlorite as a water treatment

intervention should be pursued especially in communities where point-of-use water treatments could make a difference in the microbiological quality of drinking water.

4.1.2.3 Association between household demographics and hygiene practices and water quality in the study population

The association (link) between household demographic and hygiene practices and water quality, measured in terms of *E. coli* counts, were assessed using Poisson regression which adequately deals with counts and zeros. All factors included in the baseline household questionnaire (Appendix C) were considered. The factors that were included into the final regression were the following: (1) practice of hand washing before food preparation, (2) container type in use and (3) a compounded variable of source and the distance the household is away from the source. The results are shown in Table 4.10.

Table 4.10 Poisson regression analysis with *E. coli* average counts in households using the placebo solution as measure for water quality

***E. coli* average counts**	**IRR (Incidence Rate Ratio)**	**P-value**	**95% confidence interval for IRR**
Hand washing vs no hand washing	0.58	0.031	(0.349 ; 0.950)
CDC storage containers vs traditional storage container	0.98	0.941	(0.646 ; 1.499)
Living far (>100 m) vs living close (<100 m) to the river source	0.85	0.623	(0.453 ; 1.607)
Living close (<100 m) to a tap source vs living close (<100 m) to the river source	0.26	0.000	(0.132 ; 0.493)
Living far (>100 m) from a tap source vs living close (<100 m) to the river source	0.29	0.005	(0.121 ; 0.681)

Based on the incidence rate ratios obtained in the analysis in Table 4.10, the following could be concluded:

- If hands were washed before food preparation *E. coli* counts were reduced to 58% (P=0.031) of the *E. coli* counts when hands were not washed. Hand washing after defecation and before food preparation is fundamental to food hygiene and several studies have showed that hands could play an important role in the transmission of *E. coli* species (Boyer *et al*., 1975; Harris *et al*., 1985). In addition, Lin and co-workers (2003) have showed that *E. coli* bacteria are harboured under the fingernails and proper washing with soap could decrease the incidence. This was confirmed by studies showing that hand washing decrease diarrhoeal prevalence by 89% (Han *et al*., 1989).
- When living further (> 100 m) away from the river, the *E. coli* counts were 85% (P=0.623) of that when living close (within 100 m) to the river, i.e. relative to living close, however, a 15% reduction in *E. coli* counts were observed in households further than 100 m of the river source. This was contrary to the expectation that it should have been higher. One of the reasons could be that households living far from the primary water source tend to collect more water and store water for longer periods. The storage containers these households are using are larger than 25 litres. The results from this analysis could be explained due to the possible settling of the microorganisms at the bottom of these larger containers. A second explanation could be due to natural die-off of *E. coli* bacteria during the long periods of storage inside these larger containers (Moyo *et al*., 2004).
- When living close (within 100 m) to a tap source, *E. coli* counts was only 26% (P<0.000) of that of *E. coli* counts when living close (within 100 m) to the river. This implied that people using an improved source such as the communal taps, will have less *E. coli* bacteria compared to people using an unimproved water source such as a river.
- When living further (> 100 m) away from a tap source, *E. coli* counts was only 29% (P=0.005) of that of *E. coli* counts when living close (within 100 m) to the river. This implied that people using an improved source such as the communal taps, will have less *E. coli* bacteria compared to people using an unimproved water source such as a river.

- In the CDC container, *E. coli* counts were 98% that of traditional container (P=0.941). The latter is evident from Fig 4.11 and Tables 4.7 and 4.8.

4.1.3 Compliance of study households in the two villages with the intervention

During the intervention study, the presence of a free chlorine residual in both the traditional and the CDC safe containers in the households which used the 1% and 3.5% sodium hypochlorite solutions were measured to determine if these households complied with a point-of-use treatment such as the use of the sodium hypochlorite solutions. In general, the levels of compliance in households for both villages were in agreement with other studies (Table 4.11) (Quick *et al*., 1999; Quick *et al*., 2002; Reller *et al*., 2003; Crump *et al*., 2005). Generally the households in village 1 complied between 60% and 100% (Table 4.11). Households in village 1 not always using the sodium hypochlorite solutions gave two reasons for the low levels of compliance. The first reason was because the people believed tap water was microbiologically cleaner than river water (which they have been using before the introduction of communal taps) and therefore it was not necessary to treat the water (indicated in Table 4.12). The second reason was that households using the 3.5% sodium hypochlorite solution did not like the taste of the sodium hypochlorite in the water, which could be due to the high free chlorine residual of the 3.5% sodium hypochlorite water samples that ranged between 3.8 and 4.5 $mg.l^{-1}$ after 60 min (indicated in Table 4.12). This free chlorine residual is higher than the recommended free chlorine residual level of 0.8 $mg.l^{-1}$ as suggested by the WHO (2004). Unfortunately it was found during this study that the stipulated free chlorine residual level was only achieved after 24 h and not 2 h as implicated by the DOH and DWAF. These high concentrations of chlorine in drinking water can lead to the formation of trihalomethanes (THMs) which have been associated with various types of cancers (Freese and Nozaic, 2004). However, the intervention study indicted that the households in village 2 complied between 90% and 100% and that these households had no complaints about the taste of the sodium hypochlorite in the treated stored water during the intervention study. In households where free chlorine residuals were not detected on the unannounced visits of the research teams to the households, it was due to the households having collected water the previous day and in which the free chlorine residual levels have already dropped to undetected levels.

Table 4.11: Compliance by intervention households who used either a 1% or a 3.5% sodium hypochlorite solution as an intervention strategy together with their traditional or CDC safe water storage containers

Study Population	Container Type	Round 1 water collection		Round 2 water collection		Round 3 water collection	
		1% sodium hypochlorite solution	3.5% sodium hypochlorite solution	1% sodium hypochlorite solution	3.5% sodium hypochlorite solution	1% sodium hypochlorite solution	3.5% sodium hypochlorite solution
Village 1 households using communal taps as primary water source	Traditional	80% (n = 10 households)	70% (n = 10 households)	70% (n = 10 households)	70% (n = 10 households)	70% (n = 10 households)	90% (n = 10 households)
	CDC	70% (n = 10 households)	70% (n = 10 households)	60% (n = 10 households)	100% (n = 10 households)	80% (n = 10 households)	100% (n = 10 households)
Village 2 households using the Sambandou River as primary water source	Traditional	100% (n = 10 households)	100% (n = 10 households)	90% (n = 10 households)	90% (n = 10 households)	100% (n = 10 households)	100% (n = 10 households)
	CDC	90% (n = 10 households)	90% (n = 10 households)	90% (n = 10 households)	100% (n = 10 households)	100% (n = 10 households)	100% (n = 10 households)

A total of 103 (86%) households from village 1 (n = 54 households) and village 2 (n = 49 households) completed the qualitative survey at the end of the intervention study. The survey consisted of observations made by the interviewers and a short questionnaire regarding the use of the intervention and degree of satisfaction or dissatisfaction with the intervention. The results are shown in Table 4.12.

Table 4.12: Summary of the qualitative survey at the end of the formal intervention study by households in each of two rural villages in the Vhembe region of the Limpopo Province, South Africa

Data	Village 1 households using tap water (n=54 households)	Village 2 households using river water (n=49 households)
Use the same container to collect and store water	49 (91%)	43 (88%)
Number of water collections per day:		
Once	16 (30%)	14 (29%)
Twice	4 (7%)	8 (16%)
Thrice	25 (46%)	25 (51%)
Four times	5 (9%)	2 (4%)
Water have a taste after treatment	5 (9%)	33 (67%)
Water have a smell after treatment	1 (2%)	16 (33%)
Like the taste of the water after treatment	48 (88%)	36 (73%)
Will buy sodium hypochlorite for treatment of water in containers	4 (7%)	3 (6%)
Reasons for not buying sodium hypochlorite solution:		
Government must provide	0 (0%)	5 (10%)
Insufficient funds	31 (57%)	7 (14%)
No reason	6 (11%)	35 (71%)
Believe water is already clean	17 (31%)	0 (0%)
Don't want to use it/ don't need it	0 (0%)	2 (4%)
If CDC safe storage container is available at shops – I will replace my traditional containers:	51 (94%)	26 (53%)
Overall satisfaction with CDC safe storage container	54 (100%)	45 (92%)
Problems encountered with CDC safe storage container:		
Broken tap/spigot	0 (0%)	4 (6%)

In general, no problems were reported by the study population concerning the use of the CDC safe water protocol (chlorine based water treatment combined with safe storage). The overall consensus of households in village 1 (100%) and households in village 2 (92%) was that they were satisfied with the CDC safe storage container (Table 4.12). However, 6% of households in village 2 complained about broken taps (spigot) (Table 4.12).

At baseline characteristics of the households, it was seen that the households from both villages were not used to treat their domestic water (Table 4.2). Although this intervention showed a high level of compliance with the sodium hypochlorite solution during the intervention trial (Table 4.11), the survey showed that only 7% of the households in village 1 and 6% households in village 2 are willing to buy the sodium hypochlorite solution to continue treating their drinking water. This indicated that more intensive education interventions are needed to help people understand why they need to change their behaviour (Wilson and Chandler, 1993). It will be necessary to incorporate cultural believes around hygiene behaviours and diarrhoea which is caused by improper hygiene and sanitation practices and faecal contaminated water (Kaltenhaler and Drasar, 1996). It was found that people in rural Vhembe region of South Africa do not consider diarrhoea as a health problem. These communities see diarrhoea as something that is natural and even induce it to “clean” their gastrointestinal systems (both adults and children). Another reason for not continuing in the use of the sodium hypochlorite solution was that 31% of the households from village 1 believed that the water from the communal taps are clean/safe and not in need of treatment (Table 4.12). This could be seen in the addition of the sodium hypochlorite solutions during the intervention trial (Table 4.11). Although 67% of the households in village 2 reported that the water had a different taste after treatment with the 1% and 3.5% sodium hypochlorite solutions, 73% of the households reported to like the taste of the treated water irrespective of the concentration of the sodium hypochlorite solution (Table 4.12). In comparison, only 9% of the households from village 1 reported that water had a taste after treatment with the 1% and 3.5% sodium hypochlorite solutions, while 88% of the households reported to like the taste of the treated water irrespective of the concentration of the sodium hypochlorite solution (Table 4.12).

4.1.4 Sustainability of intervention strategy in two rural villages

The sustainability of the intervention introduced to the study households in each of the two rural villages was assessed twice after the formal intervention trial of 16 weeks. The first visit to the households was unannounced and was carried out 6 months and the second visit was carried out unannounced 12 months after the intervention trial. During both visits to all the households, water samples were collected from the traditional and CDC safe storage containers (depending which containers were given to the specific household) and free chlorine residuals were tested as described in section 3.3.2.1.

The results from the water samples collected from all study households in village 1 for the first and second visits are shown in Tables 4.13 and Table 4.14. The households in village 1 complied with the use of the sodium hypochlorite and this was reflected in the free chlorine residual results and microbiological counts as shown in Tables 4.13 and 4.14. The results from the households using the placebo solution were similar to results seen during the formal intervention trial. Counts for heterotrophic bacteria, total coliforms, faecal coliforms, *E. coli*, faecal enterococci and *C. perfringens* bacteria still exceeded the recommended guideline values for water used for domestic purposes (DWAF, 1996; SABS, 2001) as specified in Table 2.2. The counts for total coliform, faecal coliform and *E. coli* bacteria did increase in the CDC safe storage containers compared to the traditional containers in the households from village 1 using the placebo solution after 6 months (Table 4.13).

However, the results from the 12 month follow up visit (Table 4.14) indicated that the counts for these microorganisms were higher in the traditional containers compared to the CDC safe storage containers. This increase could have been due to biofilm formation inside the containers or natural die-off of the various microorganisms (Momba and Notshe, 2003; Moyo *et al*., 2004). No microbial counts for any of the indicator microorganisms could be detected in households using the 1% and 3.5% sodium hypochlorite solutions in both villages indicating compliance and susceptibility of the intervention protocol (Tables 4.13 and 4.14).

Table 4.13: Geometric mean values (95% confidence intervals) for microbiological indicators of tap water samples collected 6 month after the formal intervention study in traditional and CDC safe water storage containers used by households from village 1 in the Vhembe region of the Limpopo Province, South Africa

Sodium hypochlorite solution	Container type	Heterotrophic bacteria (cfu.1 ml^{-1})	Total coliforms (cfu.100 ml^{-1})	Faecal coliforms (cfu.100 ml^{-1})	*Escherichia coli* (cfu.100 ml^{-1})	Faecal enterococci (cfu.100 ml^{-1})	*Clostridium perfringens* (cfu.100 ml^{-1})
Placebo	**Traditional containers (n = 10 households)**	2.3×10^6 (6.1×10^5; 8.8×10^6)	844 (691; 1 032)	538 (328; 883)	166 (90; 306)	154 (42; 582)	132 (21; 807)
	CDC containers (n = 10 households)	2.2×10^5 (5.2×10^4; 9.5×10^5)	1 345 (1 100; 1 643)	1 025 (784; 1 341)	413 (279; 610)	139 (80; 241)	170 (106; 274)
1%	**Traditional containers (n = 10 households)**	Computation of geometric means and 95% confidence intervals was not feasible due to large number of households with 0 counts					
	CDC containers (n = 10 households)						
3.5%	**Traditional containers (n = 10 households)**						
	CDC containers (n = 10 households)						

Table 4.14: Geometric mean values (95% confidence intervals) for microbiological indicators of tap water samples collected 12 months after the formal intervention study in traditional and CDC safe storage containers used by households from village 1 in the Vhembe region of the Limpopo Province, South Africa

Sodium hypochlorite solution	Container type	Heterotrophic bacteria (cfu.1 ml^{-1})	Total coliforms (cfu.100 ml^{-1})	Faecal coliforms (cfu.100 ml^{-1})	*Escherichia coli* (cfu.100 ml^{-1})	Faecal enterococci (cfu.100 ml^{-1})	*Clostridium perfringens* (cfu.100 ml^{-1})
Placebo	**Traditional containers (n = 10 households)**	1.2×10^6 (4.0×10^5; 4.0×10^6)	606 (304; 1 206)	354 (152; 830)	62 (25; 153)	148 (45; 489)	74 (41; 135)
	CDC containers (n = 8 households)	4.6×10^4 (3.8×10^3; 5.6×10^5)	376 (160; 888)	133 (46; 382)	65 (17; 122)	82 (11; 608)	50 (15; 169)
1%	**Traditional containers (n = 6 households)**	Computation of geometric means and 95% confidence intervals was not feasible due to large number of households with 0 counts					
	CDC containers (n = 8 households)						
3.5%	**Traditional containers (n = 10 households)**						
	CDC containers (n = 6 households)						

The results from the water samples collected from all study households in village 2 for the first and second visits are shown in Tables 4.15 and Table 4.16. Although the formal intervention trial clearly showed effectivity of the intervention strategy and compliance by the households in these villages in the use of the sodium hypochlorite solutions, the results from the two follow up visits indicated a different scenario (Tables 4.15 and 4.16). The results from both visits showed that no household was using the sodium hypochlorite solution after the intervention trial. No free chlorine residual levels were detected in any of the water samples tested during both visits. The microbiological counts for all indicator bacteria in all 60 households exceeded the recommended guideline values for water used for domestic purposes (DWAF, 1996; SABS, 2001) as specified in Table 2.2. The microbiological counts of the water stored at these households indicated a potential risk for waterborne diseases (WHO, 2004).

Several studies have reported on the success of point-of-use devices in communities all over the world (Chapter 2). However, there is still a large gap in the literature on studies which have tested the sustainability of point-of-use interventions. These type of studies are important in order to determine if communities have change their behaviour and adopted the point-of-use intervention as a way of life. Consequently, this is the first study in South Africa to test the sustainability of a point-of-use intervention in a rural setting.

Although it was assumed before the study commenced, that the use of sodium hypochlorite by the rural communities will not be a problem because during diarrhoeal outbreaks the DOH and DWAF provided 3.5% sodium hypochlorite solution to all households in affected communities. Several awareness campaigns and pamphlets are available in all 11 official languages in the Primary Health Care clinics in the rural regions (Appendix B). However, the results of this study have clearly indicated that more should be done to have people change their usual habits which could be harmful for the members inside a close relationship, such as a household.

Table 4.15: Geometric mean values (95% confidence intervals) for microbiological indicators of river water samples collected 6 month after the formal intervention study from traditional and CDC safe storage containers used by households from village 2 in the Vhembe region of the Limpopo Province, South Africa

Sodium hypochlorite solution	Container type	Heterotrophic bacteria (cfu.1 ml^{-1})	Total coliforms (cfu.100 ml^{-1})	Faecal coliforms (cfu.100 ml^{-1})	*Escherichia coli* (cfu.100 ml^{-1})	Faecal enterococci (cfu.100 ml^{-1})	*Clostridium perfringens* (cfu.100 ml^{-1})
Placebo	**Traditional containers (n = 10 households)**	3.4×10^6 (2.9×10^5; 3.7×10^7)	1 196 (973; 1 467)	697 (510; 951)	151 (106; 217)	137 (59; 313)	133 (77; 225)
	CDC containers (n = 10 households)	8.8×10^6 (1.0×10^6; 7.4×10^7)	534 (433; 661)	233 (153; 354)	113 (65; 198)	176 (113; 275)	108 (66; 178)
1%	**Traditional containers (n = 10 households)**	1.6×10^6 (2.7×10^5; 9.1×10^6)	1 392 (630; 3 075)	587 (319; 1 081)	183 (109; 306)	149 (63; 349)	106 (63; 178)
	CDC containers (n = 10 households)	4.9×10^6 (6.1×10^5; 3.7×10^7)	485 (371; 636)	246 (169; 359)	92 (62; 136)	74 (39; 139)	109 (50; 238)
3.5%	**Traditional containers (n = 10 households)**	1.2×10^6 (1.3×10^5; 1.1×10^7)	903 (548; 1 489)	518 (278; 965)	96 (56; 165)	113 (66; 196)	102 (50; 210)
	CDC containers (n = 10 households)	4.5×10^7 (4.3×10^5; 4.6×10^7)	551 (415; 733)	252 (201; 317)	132 (86; 202)	123 (60; 250)	161 (86; 304)

Table 4.16: Geometric mean values (95% confidence intervals) for microbiological indicators of river water samples collected 12 months after the formal intervention study in traditional and CDC safe storage containers used by households from village 2 in the Vhembe region of the Limpopo Province, South Africa

Sodium hypochlorite solution	Container type	Heterotrophic bacteria (cfu.1 ml^{-1})	Total coliforms (cfu.100 ml^{-1})	Faecal coliforms (cfu.100 ml^{-1})	*Escherichia coli* (cfu.100 ml^{-1})	Faecal enterococci (cfu.100 ml^{-1})	*Clostridium perfringens* (cfu.100 ml^{-1})
Placebo	**Traditional containers (n = 9 households)**	2.5×10^6 (1.4×10^5; 4.4×10^7)	1 303 (892; 1 904)	1 086 (733; 1 608)	473 (275; 812)	386 (30; 250)	106 (48; 230)
	CDC containers (n = 7 households)	2.6×10^6 (9.1×10^4; 7.0×10^7)	1 410 (957; 2 077)	1 082 (588; 1 994)	396 (147; 1 066)	99 (49; 202)	113 (64; 199)
1%	**Traditional containers (n = 3 households)**	8.1×10^4 (1.2×10^3; 6.0×10^6)	669 (25; 17 777)	391 (10; 14 693)	86 (8; 892)	193 (69; 538)	113 (40; 320)
	CDC containers (n = 4 households)	1.3×10^5 (1.5×10^3; 1.2×10^7)	638 (138; 2 952)	330 (36; 3 098)	143 (19; 1 068)	155 (23; 1 075)	154 (32; 750)
3.5%	**Traditional containers (n = 6 households)**	2.6×10^5 (8.0×10^3; 8.5×10^6)	1 092 (506; 2 358)	564 (239; 1 334)	203 (104; 400)	188 (111; 312)	93 (64; 136)
	CDC containers (n = 6 households)	1.7×10^5 (2.0×10^4; 1.4×10^6)	617 (74; 2 190)	392 (108; 1 430)	92 (39; 218)	173 (79; 377)	91 (54; 153)

Several reasons could be listed why the intervention was not sustainable in village 2 and was not continued in village 1. Firstly, it will be the cost of the sodium hypochlorite solution. Poor households would rather buy bread and maize meal before spending money on something such as sodium hypochlorite. In addition, these communities are conditioned to the effect that "if" or "when" their water source is found to be contaminated like in the case of a cholera outbreak, the government will provide sodium hypochlorite for them and they don't have to buy it themselves. Secondly, the people in these rural communities are used to the water they consume and don't get ill possibly due to a higher immunity. However, they are not considering the health implications it has on immunocompromised individuals, young children and the elderly. South Africa has a high prevalence of HIV/AIDS infected individuals which could seriously be affected by poor water quality, poor and inadequate sanitation infrastructures and unhygienic practices at the point-of-use. It was found that the study households could not understand why the water should be treated if it does not affect their health. This implied that more vigorous educational programmes should be launched in these rural communities in South Africa. Lastly, in village 2 where the intervention was not sustainable after the initial intervention trial, it did not seem that the community leaders (who were all men) had any interest in water quality issues. In comparison, in village 1, the chief was involved in all community research activities and the results indicated the intervention was sustainable as long as households had a supply of sodium hypochlorite. This clearly showed that the environment must be supportive to make an intervention sustainable in the long run. The results from this study have clearly showed that people need to be educated and behaviour change interventions must be incorporated into point-of-use intervention trials.

4.1.5 Summary of the efficiency of the CDC protocol (CDC safe storage container with a sodium hypochlorite solution) at improving the microbiological quality of stored drinking water in rural households in South Africa

The microbiological quality of the water sources used for domestic purposes by the two study populations were unacceptable and posed a potential health risk to the consumers. The counts for all indicator microorganisms exceeded the SABS (2001) stipulated water quality guideline values and indicated that the water might be harbouring potential opportunistic and pathogenic microorganisms.

This was the first study carried out in South Africa to evaluate the impact of the CDC safe storage container with or without the addition of a 1% or a 3.5% sodium hypochlorite solution on water supplies stored in rural households in the Vhembe region of the Limpopo Province. The results indicated that both the 1% and 3.5% sodium hypochlorite solution interventions was effective and reduced the potential risk of waterborne diseases by improving the microbiological quality (based on indicator microbial counts) of stored household drinking water in the CDC safe storage containers to undetectable counts. These results are in agreement with other studies conducted in developing countries where the CDC safe storage container together with a sodium hypochlorite solution was assessed as a combined intervention strategy (Macy and Quick, 1998; Semenza *et al*., 1998; CDC, 2001; Sobsey, 2002; Sobsey *et al*., 2003).

It was seen that even in the traditional household water storage containers, the numbers of indicator organisms of stored drinking water were reduced to undetectable counts with the use of the 1% and the 3.5% sodium hypochlorite solutions. This is in agreement with earlier studies suggesting that when the traditional household storage container is handled correctly and covered properly, the microbiological quality of the stored drinking water can be protected and the traditional storage container can be used effectively by households which cannot afford the CDC safe storage container (Hammad and Dirar, 1982; Deb *et al*., 1986; Pinfold, 1990).

The increase in the indicator microorganism counts in the traditional and CDC safe storage containers in the households using the placebo solution indicated secondary faecal contamination at the point-of-use due to unhygienic water-handling practices and unsanitary use of utensils and contaminated hands touching the water. In addition, no statistical differences were seen in the prevalence of indicator microorganisms between the traditional and the CDC safe storage containers using the placebo solution in both the study populations. This indicated that the CDC safe container as a single intervention without a sodium hypochlorite solution was not effective in the prevention of secondary contamination and did not significantly improved the microbiological quality of the stored drinking water. This is in agreement with an earlier study conducted by Quick and co-workers (1996) who indicated that the CDC safe storage container without the sodium hypochlorite intervention is not effective in reducing the risk associated with waterborne diseases.

Although this study included an education intervention on the use and cleaning of the CDC safe storage container and the correct addition of sodium hypochlorite solutions to the stored water, the survey indicated an urgent need for behavioural changes in these communities. It seemed that appropriate hygiene practices were not practiced due to cultural believes and financial burdens on the family and the lack of proper sanitation and water infrastructures. In addition, several studies have shown that the addition of sodium hypochlorite to stored drinking water reduced diarrhoea between 44% and 48% (Quick *et al*., 1999; Quick *et al*., 2002). It is however, essential that interventions at the household level should be implemented and promoted by goverment on a larger scale in rural communities to prevent the outbreak of waterborne diseases.

It was evident from this study that the intervention was effective and households complied with the use of sodium hypochlorite as long as they knew that their water will be tested by the research team. However, the results showed that the intervention was not sustainable after 12 months, especially in village 2 where households used the river as a primary water source. The households in village 1 using the tap water continued using the sodium hypochlorite solutions until the bottles were finished but did not purchase new stock to treat the water. The sustainability of the intervention in village 1 could also be biased because of various research activities carried out in the Vhembe region during the past few years which could have alarmed the households that the research team might pitched up at their homes to take a water sample. Consequently, the results suggested that without behaviour change and people taking ownership of the intervention, point-of-use intervention might not be sustainable (Nath *et al*., 2006).

4.2 DETERMINATION OF FAECAL SOURCE ORIGIN IN STORED DRINKING WATER FROM RURAL HOUSEHOLDS IN SOUTH AFRICA USING MALE SPECIFIC F-RNA BACTERIOPHAGE SUBGROUP TYPING

The use of male specific F-RNA bacteriophages genotyping assisted in differentiating between faecal contamination of human and animals, which was used in the determination of intervention strategies, aimed at improving household stored drinking water supplies. This study assessed the prevalence (using the Presence-Absence spot test) and the origin (using oligonucleotide subgroup typing) of male specific F-RNA bacteriophages in water sources and household storage containers in rural communities of the Vhembe region of the Limpopo Province, RSA.

4.2.1 Prevalence of male specific F-RNA bacteriophages in the primary water sources and the household water storage containers in rural households

The prevalence of male specific F-RNA bacteriophages in the primary water sources and in the stored water collected from the traditional household storage containers in the two study villages were assessed using methods describe in section 3.4. All 4 (100%) of the river water and all 7 (100%) of the tap water samples collected during the first and second trips tested positive for the presence of male specific F-RNA bacteriophages (Fig 4.14). During the first water collection trip, only 26 (65%) of the traditional storage containers in the 40 households that used tap water as a primary water source were positive for the presence of male specific F-RNA bacteriophages. In comparison, 36 (90%) of the traditional storage containers in the 40 households that used river water as a primary water source were positive for the presence of male specific F-RNA bacteriophages (Fig 4.14). During the second water collection trip, 12 (30%) of the traditional storage containers in the 40 households using tap water contained male specific F-RNA bacteriophages (Fig 4.14). In comparison 34 (85%) of the traditional storage containers in the 40 households using river water contained male specific F-RNA bacteriophages during the second water collection trip (Fig 4.14).

Generally more of the traditional household water storage containers filled with river water tested positive for the prevalence of male specific F-RNA bacteriophages compared to the traditional household water storage containers filled with tap water (Fig 4.14). This could be due to animals frequently using the river catchment for drinking and then defecating near or in the river water. In village 2 many of the women also use the river for bathing and washing clothes. Consequently the animal and human activities in or near the river in village 2 could have contributed to the presence of male specific F-RNA bacteriophages in the river water samples.

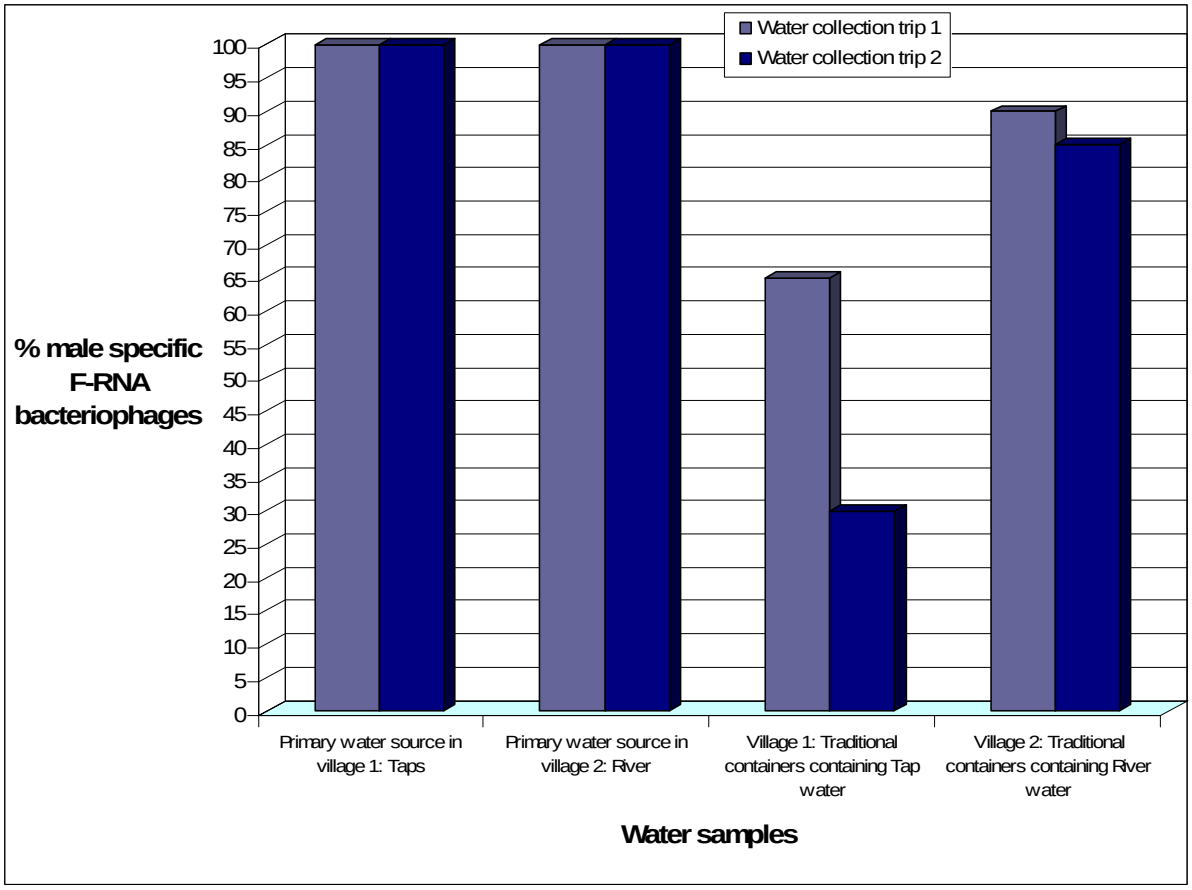


Figure 4.14: Prevalence of male specific F-RNA bacteriophages in primary water sources and stored water in traditional household water storage containers from two villages using different primary water sources

In order to determine the impact of an improved storage container on the origin of faecal pollution the presence of male specific F-RNA bacteriophages in the traditional and CDC safe storage containers were determined (Fig 4.15) during the second water collection trip (section 3.4). In the households which used the tap water as their primary

water source, 6 (30%) of the 20 households contained male specific F-RNA bacteriophages in their traditional storage containers, compared to only 4 (20%) of the 20 households which were provided with the CDC safe storage containers (Fig 4.15).

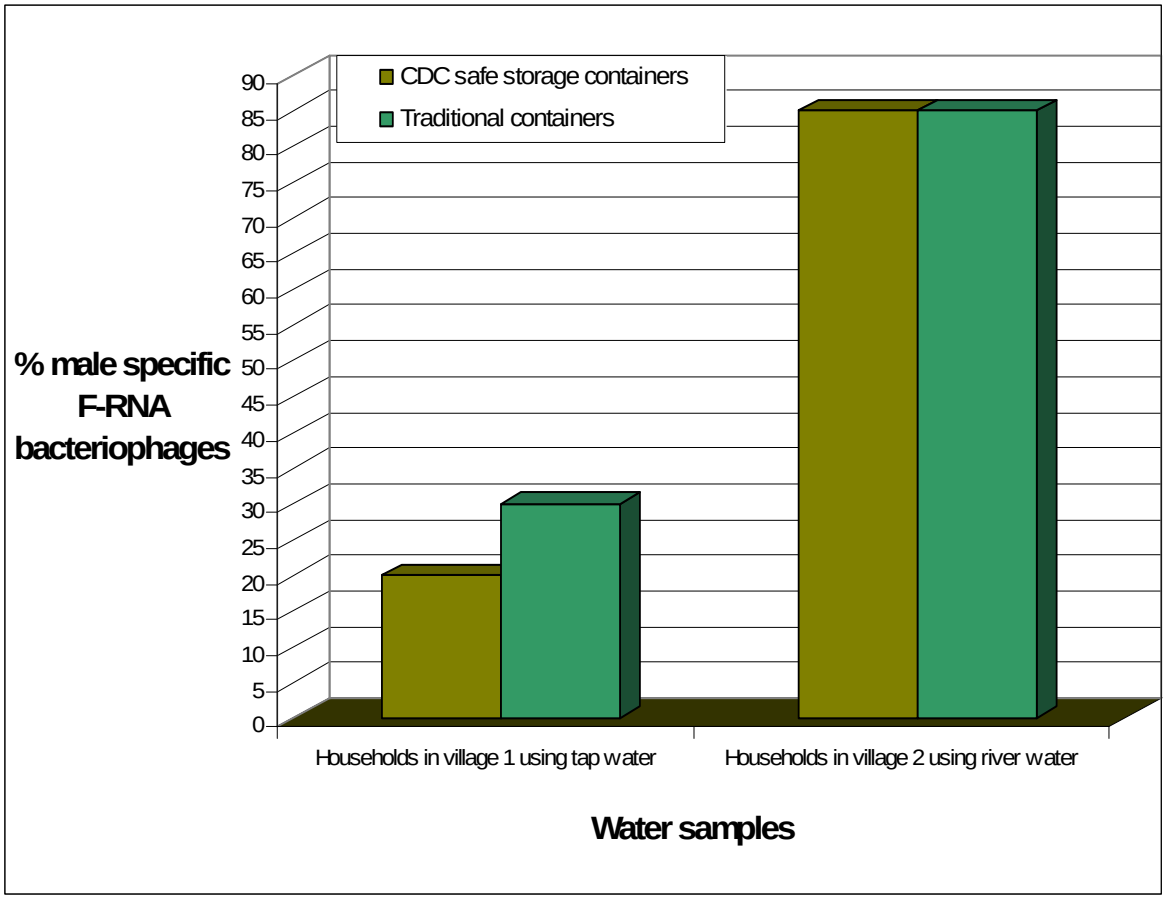


Figure 4.15: Presence of male specific F-RNA bacteriophages in the traditional and CDC safe storage containers in rural households from two villages using different water sources

In the households which used the river water as their primary water source, male specific F-RNA bacteriophages were prevalent in 17 (85%) of the 20 households respectively using the traditional storage containers and the households which were provided with the CDC safe storage containers (Fig 4.15). This indicated that the containers with water from an unimproved source (eg. River water) used in village 2 was more contaminated with male specific F-RNA bacteriophages compared to containers with water from an improved source (eg. tap water) used in village 1 (Fig 4.15).

4.2.2 Origin of male specific F-RNA bacteriophage subgroups in the primary water sources

Genotyping of F-RNA isolates from the communal tap and river water sources for both the villages identified subgroup I male specific F-RNA bacteriophages as the predominant bacteriophage subgroup present (Table 4.17). Subgroup I male specific F-RNA bacteriophages are indicative of animal faecal pollutions, specifically cattle, sheep and pig faeces which are in agreement with earlier studies conducted by Hsu and co-workers (1995), Beekwilder and co-workers (1996) and Uys, (1999). In village 1 using communal tap water sources it was observed that faeces of animals such as pigs, goats and cattle were lying next to the taps. The water reservoir in village 1 was also exposed to small animals, bird droppings and dust particles which might have contained faeces from animals grazing in the vicinity of the reservoir (Fig 4.16).

Figure 4.16: Animals near groundwater reservoir pumping water to communal taps used by study households in village 1 in the Vhembe region of the Limpopo Province, South Africa

The Sambandou River used by households in village 2 was frequently used by domestic animals and cattle for drinking purposes and it was common to find animal faeces (Fig 4.17) in the vicinity of the drinking water sources or close to the areas where people collect their drinking water or even in the water source (Fig 4.18) (Table 4.17). All these animal activities in the vicinity of the water sources contributed to the presence of subgroup I male specific F-RNA bacteriophage contamination that was identified in the water sources. The National Research Council (NRC, 2004) has reported that subgroup I male specific F-RNA bacteriophages are found in both human and animals faeces and sewage. Therefore, it could be possible that the predominance of subgroup I male specific F-RNA bacteriophages in both the water sources and especially in high concentrations in the river source could be due to both animal and human activities in and near the river source (Table 4.17).

Figure 4.17: Animal dung seen in the river water source used by study households in village 2 in the Vhembe region of the Limpopo Province, South Africa

Figure 4.18: Animals drinking and defecating in the river water source used by study households in village 2 in the Vhembe region of the Limpopo Province, South Africa

No male specific F-RNA bacteriophages belonging to subgroups II and III (associated mainly with human faecal pollution) and subgroup IV (associated mainly with animal faecal pollution) have been isolated from the communal tap water source samples. These bacteriophage groups may have had a fast die-off curve or were just not present at all. A study carried out by Schaper and co-workers (2002b) have shown that subgroup I male specific F-RNA isolates were more resistant than subgroup II F-RNA isolates followed by subgroup III male specific F-RNA isolates and lastly subgroup IV male specific F-RNA isolates to chlorine, temperature, pH and salt concentrations in water samples (Schaper *et al*., 2002b). The absence of subgroups II, III and IV in the tap water sources could therefore give a false indication that the subgroup I isolates were primarily of animal faecal origin and not from human origin (Hsu *et al*., 1995; Beekwilder *et al*., 1996; Uys, 1999).

Table 4.17: **Prevalence of male specific F-RNA bacteriophages in river and communal tap water sources in two rural villages in the Vhembe region of the Limpopo Province, South Africa**

Village 1 using communal tap water					**Village 2 using Sambandou River water**				
Male specific F-RNA bacteriophages genotype isolated (percentage %)					**Male specific F-RNA bacteriophages genotype isolated (percentage %)**				
Number of water samples tested	Subgroup I (MS2)	Subgroup II (GA)	Subgroup III (QB)	Subgroup IV (F1)	**Number of water samples tested**	Subgroup I (MS2)	Subgroup II (GA)	Subgroup III (QB)	Subgroup IV (F1)
14*	14 (100%)	0 (0%)	0 (0%)	0 (0%)	8*	8 (100%)	4 (50%)	0 (0%)	0 (0%)

* Water samples collected for round and round 2

However, male specific F-RNA bacteriophages belonging to subgroup II were found in the river water samples (50%) which could indicate possible human pollution of the source water (Fig 4.19) (Hsu *et al*., 1995; Beekwilder *et al*., 1996; Uys, 1999; Brion *et al*., 2002). Subgroup IV bacteriophages have been shown to be associated with bird faeces (Brion *et al*., 2002; Schaper *et al*., 2002a) and even though no subgroup IV male specific F-RNA bacteriophages were identified during this study, both the river and communal tap reservoirs were exposed to faecal contamination from small animals and birds (Table 4.17).

Figure 4.19: People washing clothes in the river water source used by study households in village 2 in the Vhembe region of the Limpopo Province, South Africa

4.2.3 Origin of male specific F-RNA bacteriophage subgroups in the stored household water at the point-of-use in the traditional and CDC safe water storage containers in rural households

A total of 4 (7%) male specific F-RNA bacteriophages belonging to subgroup I male specific F-RNA bacteriophages (associated with animal faecal pollution), was identified in the traditional storage containers in the study households using the tap water source (Table 4.18). Similarly only 1 (5%) of the CDC safe storage containers in the households using tap water sources tested positive for the presence of subgroup I male specific F-RNA bacteriophages (associated with animal faecal pollution) (Table 4.18).

In the study households using the river water source, a total of 37 (62%) of the traditional storage containers contained subgroup I male specific F-RNA bacteriophages (associated with animal faecal pollution) (Table 4.18). Similarly, 9 (45%) of the CDC safe storage containers tested positive for the presence of subgroup I male specific F-RNA bacteriophages (associated with animal faecal pollution) (Table 4.18). Since animals were observed during this study to lick the communal taps in village 1 (Fig 4.16) and defecate in the vicinity of the taps and river water area where people collect their domestic water from, the presence of subgroup I male specific F-RNA bacteriophages (associated with animal faecal pollution) was similar to the results obtained for the two water sources analysed (Table 4.17). This is in agreement with similar studies, which found that the presence of subgroup I male specific F-RNA bacteriophages in water samples primarily indicated animal faecal pollution (Hsu *et al*., 1995; Beekwilder *et al*., 1996; Uys, 1999).

Since it was observed that the storage containers were left out in the yard or stored inside a traditional hut (Table 4.3), in many instances without a cover, the exposure to dust and faecal contamination from domestic animals, insects and poultry could have introduced subgroup I male specific F-RNA bacteriophages to the containers (Rosas *et al*., 2006). Many of these households also used fresh cow dung to smear the floors of their huts (Fig 4.3). The dust that originates from the dried cow dung could have contributed to the contamination of the open water storage containers (Benenson, 1995; Rosas *et al*., 2006).

No subgroup II male specific F-RNA bacteriophages (associated with human faecal pollution) were isolated from the tap water sources (Table 4.17), or in any of the storage containers in village 1 households (Table 4.18). However, subgroup II male specific F-RNA bacteriophages (associated with human faecal pollution) were isolated in both the traditional and the CDC safe storage containers in households from village 2 (Table 4.18). Nine (15%) of the sixty households (40 households from round 1 water collection and 20 households from round 2 water collections) using the traditional water storage containers and nine (45%) of the twenty households (from second water collection trip) using the CDC safe storage containers contained subgroup II male specific F-RNA bacteriophages associated with human pollution (Table 4.18).

Brion and co-workers (2002) have stated that the presence of subgroup II male specific F-RNA bacteriophages was an indication of distant or sporadic faecal pollution of human origin. Studies conducted by Hsu and co-workers (1995), Beekwilder and co-workers (1996) and Uys (1999), have confirmed that subgroup II male specific F-RNA bacteriophages are predominantly found in human faeces and sewage. Consequently, contamination of the stored water in this study by humans might have occurred when members of the households used dirty utensils to transfer the stored water from these large open storage containers to a smaller storage container or directly through faecally contaminated hands – especially by small children touching the storage containers (Fig 2.13) and utensils (Jagals *et al*., 1999). In addition, a study conducted in South Africa and Spain (Schaper *et al*., 2002a), analysed various sewage and faecal samples and showed that faeces from poultry, cattle and pigs could also contribute to the presence of subgroup II male specific F-RNA bacteriophages.

Table 4.18: **Prevalence of male specific F-RNA bacteriophages in stored drinking water containers from rural households in two villages in the Vhembe region of the Limpopo Province, South Africa**

Household storage container	Village 1 using communal tap water Male specific F-RNA genotypes isolated (percentage %)				Village 2 using Sambandou River water Male specific F-RNA genotypes isolated (percentage %)			
	Subgroup I (MS2)	Subgroup II (GA)	Subgroup III (QB)	Subgroup IV (F1)	Subgroup I (MS2)	Subgroup II (GA)	Subgroup III (QB)	Subgroup IV (F1)
Traditional storage containers (n = 60)*	4 (7%)	0 (0%)	0 (0%)	0 (0%)	37 (62%)	9 (15%)	0 (0%)	0 (0%)
CDC safe storage containers (n = 20)**	1 (5%)	0 (0%)	0 (0%)	0 (0%)	9 (45%)	9 (45%)	0 (0%)	0 (0%)

*40 households selected in each village (first water collection round) using traditional storage containers + 20 households (second water collection round) used as control group in each village using traditional storage containers (Household as described in section 3.4.1)

**20 households selected in each village (second water collection round) using CDC safe storage containers (Household as described in section 3.4.1)

A close human to animal association were observed in these rural communities and domestic animals and poultry were frequently seen walking into the household area where the water containers were stored (Fig 4.7). Consequently, the presence of the subgroup II male specific F-RNA bacteriophages in the traditional and especially in the CDC safe storage containers suggested that faecal contamination could also have originated from these domestic animals and cattle at the households as well as from the primary water sources (Jagals *et al*., 1999; Schaper *et al*., 2002a). No subgroup III (associated mainly with human faecal pollution) or subgroup IV (associated mainly with animal faecal pollution such as poultry and pig faeces) (Schaper *et al*., 2002a) were detected in any of the traditional or CDC safe storage containers during the study period (Table 4.18). These results were similar to the results obtained for the primary water sources (Table 4.18). However, according to a 2004 review on *Indicators for Waterborne Pathogens* by the National Research Council of the National Academies of Science (NRC, 2004), subgroup I was found in both human and animals faeces and sewage. Therefore, the absence of subgroup III and IV from the water samples tested during this study, might mean that isolates belonging to these two subgroups might not persist in water as long as subgroups I and II (Schaper *et al*., 2002b). Consequently, subgroups I and II isolates present in these water samples might have been introduced into the water due to both human and animal contamination (NRC, 2004).

4.2.4 Summary of the use of male specific F-RNA bacteriophages subgroup typing to determine the faecal source origin in primary water sources and drinking water stored in traditional and CDC safe storage containers in rural households

This is the first study to use male specific F-RNA bacteriophages to determine the origin of faecal pollution in household storage containers in rural households without adequate water and sanitation infrastructures. The results demonstrated that water from the water sources and the household storage containers were primarily contaminated by animal faecal matter because mainly subgroup I F-RNA bacteriophages (associated with animal faecal pollution) were isolated. In addition, households using an unprotected water source also had subgroup II male specific F-RNA bacteriophages present in the household stored water which could have been either due to poor sanitation and hygienic conditions during storage and handling or due to contamination by animal

faeces (Rosas *et al*., 2006). It was difficult to determine the reason for the human faecal contamination because this study did not focussed on household hygiene practices. It was however, observed that people removed the taps and the caps of the containers because they were afraid the children would break them. This happened in spite of the educational intervention on the proper use of the CDC safe storage container. In addition a recent study suggests that subgroup II male specific F-RNA bacteriophages could have been from faecal samples of poultry and cattle (Schaper *et al*., 2002a). Consequently, it could be speculated that both subgroups I and II isolates could have been introduced to the stored drinking water from both human and animal origin (NRC, 2004). However, it is important to note that Schaper and co-workers (2002a) concluded that the association between the specific subgroups can not be used for absolute distinction between human and animal faecal pollution. Genotyping, therefore, seems not to be such an accurate tool to determine the origin of faecal pollution due to the potential for cross-reactions between some human and animal subgroups (NRC, 2004). This indicated the need for more intensive studies to confirm the specificity of the four subgroups of male specific F-RNA bacteriophages.

The absence of subgroups III and IV male specific F-RNA bacteriophage isolates in both the sources and storage containers indicated (1) no human contamination of the household stored water, (2) isolates from these subgroups does not survive for long periods in the environment and (3) temperature, pH and turbidity of the water could affect the survival of this specific subgroup isolates (Schaper *et al*., 2002b). More studies are therefore needed to investigate the prevalence of male specific F-RNA subgroups in human and animal faeces especially in rural communities where a close living relationship exists between humans and animals.

Although the CDC safe storage container was specifically designed to reduce external microbial pollution of stored drinking water, it was observed that the households did not at all times put the caps and/or the taps/spigot on the CDC safe storage containers exposing the water in these containers to potential faecal pollution. One of the reasons was that the parents were scared that the children would break the tap or through away the cap because children loved to play with the tap which could have increased the risk of faecal contamination of the water. Although this study reported on a small study group, the results clearly illustrated the need to provide these households with proper

water and sanitation infrastructures to reduce the storage period of household drinking water and in the process try to prevent the possible faecal contamination of the stored water. In general this study has found that the use of male specific F-RNA bacteriophage genotyping could be used to some extend to distinguish between human and animal faecal pollution. However, this is an expensive technique which requires skilled personnel and more studies in rural settings are needed. This was however the first study according to the literature to describe the origin of faecal pollution in household stored drinking water in a rural setting.

4.3 SURVIVAL OF INDICATOR AND PATHOGENIC MICROORGANISMS IN DRINKING WATER STORED IN AN IMPROVED HOUSEHOLD STORAGE CONTAINER WITH OR WITHOUT THE ADDITION OF A SODIUM HYPOCHLORITE SOLUTION

Very little information on the survival of pathogenic microorganisms in the CDC safe storage container is currently available. Therefore this study investigated the survival of naturally occurring indicator and selected seeded pathogenic microorganisms in the CDC safe storage container before and after the use of specific concentrations of a sodium hypochlorite solution.

4.3.1 Physical quality of improved and unimproved water sources inside the CDC safe storage container over a period of 5 days

Turbidity, pH and temperature of a water source play an important role in the complete removal of microorganisms during the chemical treatment of the water with sodium hypoclorite (Allwood *et al.*, 2003; Skraber *et al.*, 2004). Additionally, factors such as virus aggregation, viral attachment to surfaces or suspended matter, the initial free chlorine dose and free chlorine residual after disinfection also influence the survival of microorganisms during disinfection (Floyd and Sharp, 1977; Carlsson, 2003).

Studies have showed that viruses tend to survive longer in groundwater than in surface water at similar temperatures (Atkin *et al.*, 1971; Sattar, 1981). A study by Carlsson (2003) indicated that increased temperatures produced higher rates of bacterial and viral inactivation in water. Lund and Ormerod (1995), LeChevallier and co-workers (1996) and Power and Nagy (1999) have showed that temperatures above 5ºC could attribute to the formation of biofilms in drinking water systems which could aid in the survival of microorganisms. In addition, several studies have reported that attachment of organisms to surfaces makes them more resistant to starvation and disinfection due to biofilm formation (Kjellberg *et al.*, 1983; Baker, 1984; LeChevallier *et al.*, 1984; Herson *et al.*, 1987; John and Rose, 2005). In this study, the temperature for both borehole and river water samples ranged between 19 ºC and 24°C and fell within the South African recommended guideline values of 18ºC to 24ºC (DWAF, 1996).

In two separate studies, Engelbrecht and co-workers (1980) and Schaper and co-workers (2002b) have showed that bacteriophages and viruses were affected differently in their susceptibility to chlorine disinfection due to changes in the temperatures and pH parameters of water sources. Grabow and co-workers (1993b) have showed that the higher the pH of the solution, the more resistant microorganisms become to chlorine disinfection. This was confirmed by Vaughn and co-workers (1986) whom have showed that viruses are more readily inactivated by chlorine in pH levels of 6 compared to pH levels of 8. In this study the pH values for borehole water samples ranged between 7.0 and 7.1 and for river water samples varied between 6.8 and 7.7 which fell within the South African water quality pH guideline range for domestic use of 6.0 to 9.0 (DWAF, 1996).

Turbidity in water could be caused by the presence of suspended matter such as clay, silt, organic matter, inorganic matter, plankton and other microscopic organisms (LeChevallier *et al*., 1981; DWAF, 1996). The recommended South African guideline value for turbidity in water to be used for domestic purposes is 0.1 NTU (DWAF, 1996). During this study, the turbidity values for borehole water varied between 0.74 and 1.75 NTU and for river water between 7.04 and 8.30 NTU, which exceeded the South African guideline values. These high turbidity values suggested that microorganisms present in the water source could possibly be associated with particulate matter in the water, which can protect and assist in their survival and reduce the effect of the sodium hypochlorite disinfectant (DWAF, 1996).

4.3.2 Free chlorine residuals in the improved CDC safe storage containers after addition of 1% or 3.5% sodium hypochlorite solutions

Throughout this study, the free chlorine residual of the containers receiving the 1% and 3.5% sodium hypochlorite solutions after sixty minutes were in the order of 0.8 $mg.l^{-1}$ for containers which received the 1% sodium hypochlorite solution and 3.8 $mg.l^{-1}$ for containers which received the 3.5% sodium hypochlorite solution. After 24 h the free chlorine residual levels had dropped to 0 $mg.l^{-1}$ and 0.8 $mg.l^{-1}$ respectively for the 1% and 3.5% sodium hypochlorite solutions. On day 2 no more free chlorine residual were detected in any of the containers. The 3.5% sodium hypochlorite solution had a higher free chlorine residual compared to the 1% sodium hypochlorite solution. Consequently,

the 3.5% sodium hypochlorite solution was more effective for longer periods as would be expected compared to the 1% sodium hypochlorite solution in both the borehole and the river water containers. The free chlorine residuals in the containers receiving the 1% sodium hypochlorite solution indicated that the water was no longer protected after 24 h against secondary contamination, which could be introduced by unhygienic handling and storage practices, dust and animals at the point-of-use (Sobsey, 2002).

4.3.3 Survival of naturally occurring indicator and pathogenic microorganisms in the CDC safe storage container before and after the addition of a sodium hypochlorite solution

The microbiological analyses of the borehole water and the river water samples indicated that ground water was microbiologically of a better quality and less contaminated than surface water when looking at the prevalence of naturally occurring indicator microorganisms in both the water sources (Tables 4.19 to 4.23). Borehole water contained initial counts of heterotrophic bacteria and total coliforms, while river water only contained initial counts of several indicator bacteria which included heterotrophic bacteria, total coliforms, faecal coliforms, faecal enterococci and *C. perfringens* (Tables 4.19 to 4.23). This was in agreement with similar studies conducted by Lehloesa and Muyima (2000) on ground water and communal tap water sources used by rural communities in the Eastern Cape, South Africa. No Enteroviruses were detected in any of the water samples after amplification in BGM cell cultures and molecular detection methods (section 3.5.5), although Enteroviruses have been shown to be sporadically present in untreated water sources (WHO, 1996). In addition, no *Salmonella* spp were detected in any of the original water samples after selective enrichment and enumeration steps (section 3.5.4).

The presence of heterotrophic microorganisms in both water sources indicated the general microbiological quality of water samples (DWAF, 1996; WHO, 2002b). Although heterotrophic bacteria is generally not considered harmful, various studies have indicated that some heterotrophic bacteria such as *Aeromona* spp, *Klebsiella pneumoniae*, *Enterococcus*, *Bacillus* spp and *Enterobacter* spp might be opportunistic pathogens and have been associated with diseases of the respiratory tract and wound

infections (Payment *et al*., 1991; WHO, 1996; WHO, 2002b; Bartram *et al*., 2003; Ehlers *et al*., 2003).

The recommended South African guideline value for heterotrophic bacteria in domestic water is less than 100 cfu.1 ml^{-1} or less than 2 log_{10} (SABS, 2001). The initial heterotrophic plate counts of 9 log_{10} present in both the water sources indicated that the water was unacceptable for human consumption because of the possible presence of opportunistic and pathogenic microorganisms which could cause diseases (Table 4.19) (DWAF, 1996; SABS, 2001; Ehlers *et al*., 2003). Over the 5 day period, the heterotrophic microorganisms declined respectively to 8 log_{10} in borehole water and 7 log_{10} in river water in the containers receiving the placebo solution (Table 4.19).

In the containers filled with borehole water, the 1% sodium hypochlorite solution reduced the numbers of heterotrophic organisms within 60 min to undetectable levels (Table 4.19). However, in the containers filled with river water, the heterotrophic microorganisms were not inactivated within 60 min and were even detected for 5 days during which the heterotrophic microorganism counts decreased from 9 log_{10} to 5 log_{10} (Table 4.19).

The results from this laboratory study for borehole water were in agreement with results from section 4.1 on the effectiveness of the 1% and 3.5% sodium hypochlorite solutions in the CDC safe storage containers for heterotrophic bacteria. However, the turbidity of river water used in the laboratory studies were higher (7.14 NTU to 8.3 NTU) than the turbidity of the river water samples during the field studies (2.4 NTU to 4.4 NTU). This indicated that the higher turbidity of the water used in the laboratory studies could have reduced the effectivity of the 1% sodium hypochlorite solution in killing the heterotrophic bacteria (Table 4.19) (WHO, 1996; Tree *et al*., 2003). It was possible that some of the heterotrophic microorganisms used the nutrients in the turbid water to survive (WHO, 1996). However, in the containers receiving the 3.5% sodium hypochlorite solution, no heterotrophic bacteria survived in the river or borehole water samples after 60 min (Table 4.19). This was in agreement with the results obtained during the field intervention trial studies for heterotrophic bacterial counts in water samples assessed in the CDC safe storage containers (section 4.1).

Table 4.19: The survival of naturally occurring heterotrophic bacteria over a 5 day period detected in the borehole and river water samples before and after the addition of a placebo, 1% or 3.5% sodium hypochlorite solution

Sodium hypochlorite solution	Water source									
	Borehole water					River water				
	Day 0*	Day 0**	Day 1	Day 2	Day 5	Day 0*	Day 0**	Day 1	Day 2	Day 5
Placebo***	9.5	-	9.4	9.0	8.5	9.3	-	8.6	8.5	7.5
1%	9.3	n.d	n.d	n.d	n.d	9.3	6.9	6.2	5.5	5.4
3.5%	9.3	n.d	n.d	n.d	n.d	9.3	n.d	n.d	n.d	n.d

* time = 0 minutes before the addition of the sodium hypochlorite solution
** time = 60 minutes after the addition of the sodium hypochlorite solution
*** placebo = distilled water
n.d = not detected
- = not tested

The presence of total coliforms in both water sources indicated the presence of bacteria which can originate from faecal contamination or from environmental sources such as sewage run offs (Pinfold, 1990). The South African recommended guideline value for total coliforms in drinking water is less than 10 cfu.100 ml^{-1} or 1 $\log_{10}$ (SABS, 2001). In this study the levels of naturally occurring total coliform bacteria determined in borehole (1 $\log_{10}$) and river (4 $\log_{10}$) water samples indicated the likelihood that the water was faecally contaminated by human and animal faeces (Table 4.20) (DWAF, 1996).

Total coliform bacteria decreased in both water sources in the containers receiving the placebo solution over a 5 day period with a higher decline rate in the river water containers (decrease from 4 $\log_{10}$ to 2 $\log_{10}$) (Table 4.20). The higher decline rate could have been due to the decrease in nutrient levels because of competition between microorganisms (LeChevallier and McFeters, 1985; Momba and Notshe, 2003). In the containers receiving the 1% and the 3.5% sodium hypochlorite solutions, no total coliform bacteria in the water samples survived after 60 min (Table 4.20). These results were in agreement with the results obtained during the field intervention trial studies for total coliform counts in water samples assessed in the CDC safe storage containers receiving the 1% and 3.5% sodium hypochlorite solutions (section 4.1).

Table 4.20: The survival of naturally occurring total coliform bacteria over a 5 day period detected in the borehole and river water samples before and after the addition of a placebo, 1% or 3.5% sodium hypochlorite solution

Sodium hypochlorite solution	Water source									
	Borehole water					River water				
	Day 0*	Day 0**	Day 1	Day 2	Day 5	Day 0*	Day 0**	Day 1	Day 2	Day 5
Placebo***	1.9	-	1.7	1.6	1.4	4.1	-	3.9	3.6	2.2
1%	1.9	n.d	n.d	n.d	n.d	4.1	n.d	n.d	n.d	n.d
3.5%	1.9	n.d	n.d	n.d	n.d	4.1	n.d	n.d	n.d	n.d

* time = 0 minutes before the addition of the sodium hypochlorite solution
** time = 60 minutes after the addition of the sodium hypochlorite solution
*** placebo = distilled water
n.d = not detected
- = not tested

The South African guideline value for the prevalence of faecal coliform bacteria in water used for domestic purposes is 0 cfu.100 ml^{-1} or not detected (SABS, 2001). Faecal coliform bacteria were detected only in the river water (3 $\log_{10}$) and not in any of the borehole water samples (Table 4.21). The presence of faecal coliform bacteria in the river water samples in the containers receiving the placebo solution indicated the presence of potential pathogenic microorganisms such as *Salmonella* spp, *Shigella* spp, pathogenic *E. coli* and *V. cholerae* which are associated with waterborne diseases such as salmonellosis, dysentery, gastroenteritis and cholera (DWAF, 1996; SABS, 2001).

The faecal coliform bacteria were still detected after 5 days in the containers receiving the placebo solution with a 1 $\log_{10}$ decrease from day 1 (Table 4.21). In the containers receiving the 1% and the 3.5% sodium hypochlorite solutions, no faecal coliform bacteria in the river water samples survived after 60 min (Table 4.21). These results were in agreement with the results obtained during the field intervention trial studies for faecal coliform counts in water samples assessed in the CDC safe storage containers receiving the 1% and 3.5% sodium hypochlorite solutions (section 4.1). In addition, several previous studies indicating that coliform bacteria are more sensitive to chlorine disinfection than male specific F-RNA bacteriophages and Enteroviruses (Sobsey, 1989; Morris, 1993; Tree *et al.*, 1997).

Table 4.21: The survival of naturally occurring faecal coliform bacteria over a 5 day period detected in the borehole and river water samples before and after the addition of a placebo, 1% or 3.5% sodium hypochlorite solution

Sodium hypochlorite solution	Water source									
	Borehole water					River water				
	Day 0*	Day 0**	Day 1	Day 2	Day 5	Day 0*	Day 0**	Day 1	Day 2	Day 5
Placebo***	n.d	-	n.d	n.d	n.d	3.3	-	3.2	2.9	2.4
1%	n.d	n.d	n.d	n.d	n.d	3.4	n.d	n.d	n.d	n.d
3.5%	n.d	n.d	n.d	n.d	n.d	3.5	n.d	n.d	n.d	n.d

* time = 0 minutes before the addition of the sodium hypochlorite solution
** time = 60 minutes after the addition of the sodium hypochlorite solution
*** placebo = distilled water
n.d = not detected
- = not tested

Naturally occurring faecal enterococci were only detected in river water samples (2 $\log_{10}$) during this study (Table 4.22). The presence of faecal enterococci in water indicates the presence of human faecal contamination in the water samples as well as the potential risk of waterborne diseases (DWAF, 1996). The South African guideline value for faecal enterococci in water to be used for domestic purposes is 0 cfu.100 ml^{-1} or not detected (SABS, 2001). The counts (2 $\log_{10}$) of faecal enterococci in the river water containers receiving the placebo solution exceeded the recommended South African guideline values (0 cfu.100 ml^{-1}) for faecal enterococci counts in water to be used for domestic purposes and indicated the potential risk of transmission of waterborne pathogens which may include viruses and parasites that can survive for longer periods of time in water (DWAF, 1996).

Faecal enterococci bacteria could still be detected in the river water samples receiving the placebo solution after 5 days, although a 1 $\log_{10}$ decrease in the survival could be detected between day 1 and day 5 (Table 4.22). In the containers receiving the 1% and the 3.5% sodium hypochlorite solutions, no faecal enterococci bacteria in the river water samples survived after 60 min (Table 4.22). These results were in agreement with the results obtained during the field intervention trial studies for faecal enterococci counts in water samples assessed in the CDC safe storage containers receiving the 1% and 3.5% sodium hypochlorite solutions (section 4.1). A study by Tree and co-workers (2003) indicated that enterococci bacteria are more resistant than *E. coli* to chlorine

disinfection. However, this was not seen in this study (Table 4.26), which could have been due to the initial differences in the naturally occurring bacterial counts of enterococci and the higher seeded counts for *E. coli* in the CDC safe storage containers.

Table 4.22: The survival of naturally occurring faecal enterococci bacteria over a 5 day period detected in the borehole and river water samples before and after the addition of a placebo, 1% or 3.5% sodium hypochlorite solution

Sodium hypochlorite solution	Water source									
	Borehole water					River water				
	Day 0*	Day 0**	Day 1	Day 2	Day 5	Day 0*	Day 0**	Day 1	Day 2	Day 5
Placebo***	n.d	-	n.d	n.d	n.d	2.8	-	2.5	2.2	1.4
1%	n.d	n.d	n.d	n.d	n.d	2.8	n.d	n.d	n.d	n.d
3.5%	n.d	n.d	n.d	n.d	n.d	2.7	n.d	n.d	n.d	n.d

* time = 0 minutes before the addition of the sodium hypochlorite solution
** time = 60 minutes after the addition of the sodium hypochlorite solution
*** placebo = distilled water
n.d = not detected
- = not tested

Clostridium perfringens is normally present in human and animal faeces, survives longer than indicator microorganisms and serves as an indicator for the presence of resistant microorganisms such as viruses, protozoan cysts and oocysts (Payment and Franco, 1993; WHO, 1996). No *C. perfringens* spores or vegetative cells were detected in the borehole water receiving the placebo solution (Table 4.23). However, containers with river water receiving the placebo solution did have *C. perfringens* vegetative cells and spores present (2 $\log_{10}$) over the 5 day period (Table 4.23).

In the containers receiving the 1% sodium hypochlorite solution *C. perfringens* were not inactivated in 60 min (Tables 4.23). The resistance of the *C. perfringens* bacteria spores and vegetative cells in the storage containers might have been due to survival ability of the spores or the high turbidity values of the river water which influenced the effectiveness of the 1% sodium hypochlorite solution (WHO, 1996; Tree *et al*., 2003). The extended survival of *C. perfringens* in the river water samples indicated the possible presence of more resistant microorganisms such as enteric Adenoviruses, Caliciviruses, Enteroviruses, Hepatitis A virus and Rotaviruses, as well as protozoan

parasites such as *Cryptosporidium*, *Entamoeba* and *Giardia* (WHO, 1996; Carlsson, 2003).

The results from this laboratory study for borehole water indicates that higher turbidity affects the efficiency of a disinfectant such as the 1% sodium hypochlorite solution. The higher turbidity (7.14 NTU to 8.3 NTU) of the water used in the laboratory studies could have assisted the *C. perfringens* bacterial spores to survive (Table 4.23).

Table 4.23: The survival of naturally occurring *Clostridium perfringens* bacteria over a 5 day period detected in the borehole and river water samples before and after the addition of a placebo, 1% or 3.5% sodium hypochlorite solution

Sodium hypochlorite solution	Water source									
	Borehole water					River water				
	Day 0*	Day 0**	Day 1	Day 2	Day 5	Day 0*	Day 0**	Day 1	Day 2	Day 5
Placebo***	n.d	-	n.d	n.d	n.d	2.7	-	2.5	2.3	1.5
1%	n.d	n.d	n.d	n.d	n.d	2.6	1.6	n.d	n.d	n.d
3.5%	n.d	n.d	n.d	n.d	n.d	2.6	n.d	n.d	n.d	n.d

* time = 0 minutes before the addition of the sodium hypochlorite solution
** time = 60 minutes after the addition of the sodium hypochlorite solution
*** placebo = distilled water
n.d = not detected
- = not tested

In the containers receiving the 3.5% sodium hypochlorite solution, no *C. perfringens* vegetative cells or spores in the water samples survived after 60 min (Table 4.23). This indicated that the 3.5% sodium hypochlorite solution was more effective than 1% sodium hypochlorite solution against spore forming microorganisms and could be used successfully for the disinfection of resistant microorganisms in water with high turbidity (Payment and Franco, 1993; WHO, 1996). This was in agreement with the results obtained during the field intervention trial studies for *C. perfringens* bacteria in water samples assessed in the CDC safe storage containers using the 3.5% sodium hypochlorite solution (section 4.1).

4.3.4 Survival of seeded indicator and pathogenic microorganisms in the CDC safe storage container before and after the addition of a sodium hypochlorite solution

To date the only information available on the effect of disinfection procedures on microorganisms in the CDC safe storage container is based on *E. coli* and faecal coliforms (Sobsey, 2002; Sobsey *et al*., 2003). Ashbolt (2004) has shown that the survival of many enteric pathogens is different to the survival of indicator microorganisms. Therefore, the survival of seeded indicator microorganisms (somatic and male specific F-RNA bacteriophages) and pathogenic microorganisms (*S. typhimurium*, *E. coli* and Coxsackie B1 virus) before and after the addition of a placebo, 1% or 3.5% sodium hypochlorite solution in the CDC safe storage container was assessed (Tables 4.24 to 4.28).

Somatic and male specific F-RNA bacteriophages were used in this study as indicators of enteric viruses (Grabow, 2001). These bacteriophages closely resembled human enteroviruses with regard to size, morphology, nucleic acid structure and failure to replicate in water environments (Grabow, 2001). The survival of both somatic and male specific F-RNA bacteriophages during the 5 day period implicated that when pathogenic enteric viruses were present, they could survive in these storage containers for periods longer than 24 h in temperatures of 25ºC (Tables 4.24 and 4.25) (Duran *et al*., 2003).

The somatic bacteriophages decreased from 9 $\log_{10}$ to 5 $\log_{10}$ in the borehole water and from 9 $\log_{10}$ to 6 $\log_{10}$ in the river water samples in the containers receiving the placebo solutions (Table 4.24). The South African water quality guidelines state that somatic bacteriophages must be present in the water sample in concentrations not exceeding 1 cfu.10 ml^{-1} (SABS, 2001). The results in this study have showed that somatic bacteriophages were sensitive to both the 1% and the 3.5% sodium hypochlorite solutions and did not survive longer than 60 min after addition of the solutions (Table 4.24). These results were in agreement with the results obtained during the field intervention trial studies for somatic bacteriophages in water samples assessed in the CDC safe storage containers receiving the 1% and 3.5% sodium hypochlorite solutions (section 4.1).

Table 4.24: The survival of seeded somatic bacteriophages over a 5 day period detected in the borehole and river water samples before and after the addition of a placebo, 1% or 3.5% sodium hypochlorite solution

Sodium hypochlorite solution	Water source									
	Borehole water					River water				
	Day 0*	Day 0**	Day 1	Day 2	Day 5	Day 0*	Day 0**	Day 1	Day 2	Day 5
Placebo***	9.1	-	8.1	6.0	5.3	9.2	-	9.2	8.0	6.2
1%	9.1	n.d	n.d	n.d	n.d	9.2	n.d	n.d	n.d	n.d
3.5%	9.1	n.d	n.d	n.d	n.d	9.1	n.d	n.d	n.d	n.d

* time = 0 minutes before the addition of the sodium hypochlorite solution
** time = 60 minutes after the addition of the sodium hypochlorite solution
*** placebo = distilled water
n.d = not detected
- = not tested

Schaper and co-workers (2002b) have showed that temperature and pH play an important role in the survival of the different genotype groups of male specific F-RNA bacteriophages. In the containers receiving the placebo solution, the male specific F-RNA bacteriophages decreased from 9 $\log_{10}$ to 7 $\log_{10}$ in the borehole water and from 9 $\log_{10}$ to 8 $\log_{10}$ in the river water containers respectively (Table 4.25). In general male specific F-RNA bacteriophages counts were higher over the 5 days in the storage containers receiving the placebo solution in both borehole and the river water samples, compared to somatic bacteriophages (Tables 4.24 and 4.25).

The results indicated that male specific F-RNA bacteriophages were more resistant to environmental conditions than somatic bacteriophages. This is in agreement with earlier laboratory studies carried out during 2003 by two different groups: (1) Allwood and co-workers (2003) have shown that F-RNA bacteriophages are a good indictor for the survival of Noroviruses in water free from disinfectants because it survived longer than Noroviruses during laboratory studies; and (2) Duran and co-workers (2003) have shown that somatic bacteriophages were inactivated significantly easier than male specific F-RNA bacteriophages and *Bacteroides fragilis* bacteriophages in ground water samples.

In the borehole water containers, the 1% sodium hypochlorite solution effectively reduced the male specific F-RNA bacteriophages after 60 min to undetectable levels

(Table 4.25). These results were in agreement with the results obtained during the field intervention trial studies for male specific F-RNA bacteriophages in water samples assessed in the CDC safe storage containers using the 1% sodium hypochlorite solution (section 4.1). However, in river water containers, F-RNA bacteriophages were not inactivated by the 1% sodium hypochlorite solution within 60 min of exposure and could be detected for all 5 days with a decrease in the survival from 9 $\log_{10}$ to 1 $\log_{10}$ (Table 4.25). However, the higher turbidity (7.14 NTU to 8.3 NTU) of the water used in the laboratory studies could have reduced the effectivity of the 1% sodium hypochlorite solution in killing the male specific F-RNA bacteriophages (Table 4.25).

This study showed that male specific F-RNA bacteriophages survived longer compared to Coxsackie B1 viruses (Table 4.28) with the addition of the 1% sodium hypochlorite solution. This was in agreement with a study by Tree and co-workers (2003) which indicated that Poliovirus was more susceptible to chlorine than male specific F-RNA bacteriophages and more resistant to chlorine than bacterial indicators. Consequently the survival of both the male specific F-RNA bacteriophages and Coxsackie B1 viruses during chlorination with 1% sodium hypochlorite solution indicated the suitability of the male specific F-RNA bacteriophages as indicators for the presence of potentially pathogenic enteric viruses in drinking water sources (Grabow, 2001; Allwood *et al*., 2003; Duran *et al*., 2003; Tree *et al*., 2003).

The results further indicated that the 3.5% sodium hypochlorite solution were the most effective sodium hypochlorite solution because no male specific F-RNA bacteriophages survived longer than 60 min after addition of the solution in both water sources (Table 4.25). This was in agreement with the results obtained during the field intervention trial studies for male specific F-RNA bacteriophages in water samples assessed in the CDC safe storage containers after the addition of the 3.5% sodium hypochlorite solution (section 4.1).

Table 4.25: The survival of seeded male specific F-RNA bacteriophages over a 5 day period detected in the borehole and river water samples before and after the addition of a placebo, 1% or 3.5% sodium hypochlorite solution

Sodium hypochlorite solution	Water source									
	Borehole water					River water				
	Day 0*	Day 0**	Day 1	Day 2	Day 5	Day 0*	Day 0**	Day 1	Day 2	Day 5
Placebo***	9.2	-	8.3	8.2	7.3	9.9	-	9.0	9.1	8.0
1%	9.1	n.d	n.d	n.d	n.d	9.1	6.8	5.4	4.5	1.5
3.5%	9.2	n.d	n.d	n.d	n.d	9.2	n.d	n.d	n.d	n.d

* time = 0 minutes before the addition of the sodium hypochlorite solution
** time = 60 minutes after the addition of the sodium hypochlorite solution
*** placebo = distilled water
n.d = not detected
- = not tested

Escherichia coli (ATCC 13706) bacteria were used to indicate the survival of pathogenic microorganisms that can multiply in the gastrointestinal tracts of warm blooded humans and animals (DWAF, 1996). *Salmonella typhimurium* (NCTC 12484) bacteria were used in the study as a typical waterborne pathogen to give information on the possible survival of waterborne pathogens in household water storage containers (Theron and Cloete, 2002). The seeded studies on *E. coli* (Table 4.26) and *S. typhimurium* (Table 4.27) bacteria indicated that these bacteria could survive in the environment because counts for both bacteria were detected during the 5 days in the river and borehole water containers without any sodium hypochlorite solutions. Generally, these two bacteria had a faster die-off than male specific F-RNA bacteriophages (Table 4.25) and Coxsackie B1 viruses (Table 4.28). This natural die-off curve is in agreement with studies carried out by Nasser and Oman (1999), Allwood and co-workers (2003) and Skraber and co-workers (2004) which have showed with laboratory studies that *E. coli* cells decreased faster than male specific F-RNA bacteriophages, Hepatitis A virus or Polio virus type 1.

The survival of *E. coli* bacterial cells in containers containing borehole and river water samples is indicated in Table 4.26. In the river and borehole water containers receiving the placebo solution, *E. coli* bacterial cells were able to survive for 5 days with a decrease in survival from 7 $\log_{10}$ to 3 $\log_{10}$ (Table 4.26). In the borehole water

containers, the 1% sodium hypochlorite solution effectively reduced the *E. coli* bacteria after 60 min to undetectable levels (Table 4.26). However, in river water containers, *E. coli* bacteria were not inactivated by the 1% sodium hypochlorite solution within 60 min of exposure and the bacterial cells survived for 24 h in the river water containers (Table 4.26). The laboratory studies indicated that the higher turbidity of the river water samples could have reduced the effectivity of the 1% sodium hypochlorite solution. However, the containers receiving the 3.5% sodium hypochlorite solution showed complete inactivation of all *E. coli* bacterial cells within 60 min (Table 4.26). The results seen in this study for *E. coli* bacteria is in agreement with results reported by Duran and co-workers (2003) whom have showed that chlorination inactivated bacteria more efficiently than bacteriophages and Enteroviruses. In addition, the temperature could also have played a major role in the survival of the bacteria. During this study the temperatures in the containers ranged between 19°C and 24°C. Flint (1987) has showed that *E. coli* cells survived better at 4ºC compared to 15ºC, 25ºC or 37ºC. Lim and Flint (1989) have shown that *E. coli* can survive up to 12 days without loss of viability dependant on the water temperatures which ranged between 15°C to 37ºC. Both these two studies have showed that *E. coli* bacteria survive better at lower temperatures (Flint, 1987; Lim and Flint, 1989). In addition, this was in agreement with the results obtained during the field intervention trial studies for *E. coli* bacteria in water samples assessed in the CDC safe storage containers after the addition of the 1% and 3.5% sodium hypochlorite solution (section 4.1).

Table 4.26: The survival of seeded *Escherichia coli* bacteria over a 5 day period detected in the borehole and river water samples before and after the addition of a placebo, 1% or 3.5% sodium hypochlorite solution

Sodium hypochlorite solution	Water source									
	Borehole water					River water				
	Day 0*	Day 0**	Day 1	Day 2	Day 5	Day 0*	Day 0**	Day 1	Day 2	Day 5
Placebo***	7.0	-	5.0	4.8	3.2	7.0	-	6.9	4.6	3.9
1%	7.1	n.d	n.d	n.d	n.d	7.0	3.8	n.d	n.d	n.d
3.5%	6.9	n.d	n.d	n.d	n.d	7.1	n.d	n.d	n.d	n.d

* time = 0 minutes before the addition of the sodium hypochlorite solution
** time = 60 minutes after the addition of the sodium hypochlorite solution
*** placebo = distilled water
n.d = not detected
- = not tested

The survival of *S. typhimurium* bacterial cells in containers containing borehole and river water samples is indicated in Table 4.27. In both the river and borehole water containers receiving the placebo solution, *S. typhimurium* bacterial cells were able to survive for 5 days with a decrease in survival from 6 $\log_{10}$ to 3 $\log_{10}$ (Table 4.27). The high turbidity of the river water in this study could have assisted in the survival of the bacteria and protected them from the effect of the sodium hypochlorite solution. The survival of *S. typhimurium* as a typical waterborne microorganism indicated that other waterborne microorganisms such as *Shigella* spp, *V. cholera*, *Yersinia enterocolitica* and *Campylobacter jejuni* could also survive in household storage containers without treatment (WHO, 1996). In the borehole water containers, the 1% sodium hypochlorite solution effectively reduced the *S. typhimurium* bacteria after 60 min (Table 4.27). However, in river water containers, *S. typhimurium* bacteria were not inactivated by the 1% sodium hypochlorite solution within 60 min of exposure and the bacterial cells survived for 24 h in the river water containers (Table 4.27). The containers receiving the 3.5% sodium hypochlorite solution showed complete inactivation of all *S. typhimurium* bacterial cells within 60 min in both types of water samples (Table 4.27). Generally, results from this study is in agreement with a study by Mitchell and Starzyk (1975) which have showed that *S. typhimurium* and *E. coli* cells in river water samples have similar survival patterns.

Table 4.27: The survival of seeded *Salmonella typhimurium* bacteria over a 5 day period detected in the borehole and river water samples before and after the addition of a placebo, 1% or 3.5% sodium hypochlorite solution

Sodium hypochlorite solution	Water source									
	Borehole water					River water				
	Day 0*	Day 0**	Day 1	Day 2	Day 5	Day 0*	Day 0**	Day 1	Day 2	Day 5
Placebo***	6.9	-	4.8	3.5	3.3	6.9	-	5.8	4.5	3.3
1%	6.9	n.d	n.d	n.d	n.d	6.9	2.3	n.d	n.d	n.d
3.5%	6.8	n.d	n.d	n.d	n.d	6.9	n.d	n.d	n.d	n.d

* time = 0 minutes before the addition of the sodium hypochlorite solution
** time = 60 minutes after the addition of the sodium hypochlorite solution
*** placebo = distilled water
n.d = not detected
- = not tested

Although a vaccine strain of Poliovirus type 1 was included in the original protocol, studies on Poliovirus type 1 during this research study were excluded due to the global Poliovirus-containment. Therefore, Coxsackie B1 virus was the only virus used in this study as representative of human Enteroviruses. The Enteroviruses are Picornaviruses containing a single stranded RNA and particles containing 60 molecules each of 4 distinct proteins designated VP1 through VP4 (Rueckert, 1985). The Picornaviruses group contains the Polioviruses, Coxsackie viruses (A and B), Echoviruses and several Enteroviruses (WHO, 1996). Coxsackie B1 virus was used as a representative indicator virus to indicate the survival of human Enteroviruses in stored water containers. Several studies have indicated that human enteric viruses not only survived longer than bacterial indicators, but can also be present when indicator microorganisms are absent (Bosch *et al.*, 1991; Bosch, 1998). Therefore, it was deemed necessary to include a representative viral indicator in this study to assess the survival of viruses in the CDC safe storage container with or without the treatment of a sodium hypochlorite solution.

The results of this study have indicated that Coxsackie B1 virus particles were more persistent and have been detected through-out the 5 day period in the containers receiving the placebo solution (Table 4.28). This is in agreement with a study by Skraber and co-workers (2004) whom have showed that Enteroviruses such as Poliovirus type 1 were more persistent and survived longer than thermotolerant coliforms at various temperatures and pH values. It is however important to highlight that Shuval and co-workers (1971) have shown that Enteroviruses have different stabilities in water. Shuval and co-workers (1971) have found that Polio type 3 and Coxsackie A13 viruses were more readily inactivated than Polio type 1 or Coxsackie B1 virus at different water temperatures. The study of Shuval and co-workers (1971) have showed that Coxsackie B1 virus survived longer than Poliovirus type 1 at temperatures ranging between 23°C to 27 ºC.

The results from this study indicated that in the containers containing the borehole water with much lower turbidity values (between 0.74 and 1.75 NTU) than river water containers (between 7.04 and 8.30 NTU), the 1% and the 3.5% sodium hypochlorite solutions effectively reduced the Coxsackie B1 virus particles after 60 min to undetectable levels (Table 4.28). However, in river water containers, Coxsackie B1 virus particles were not inactivated by the 1% sodium hypochlorite solution within 60

min of exposure, but survived for 2 days in the containers (Tables 4.28). These results were in agreement with several earlier laboratory-seeding studies (Duran *et al*., 2003; Tree *et al*., 2003). Duran and co-workers (2003) have showed that Enteroviruses and bacteriophages were more resistant to chlorination inactivation compared to bacterial cells. Additionally, studies carried out by Kelly and Sanderson (1958) and Shaffer and co-workers (1980) have showed that different strains of Poliovirus type 1 have different rates of chlorine inactivation which enables them to survive chlorine treatment. This indicated the need to conduct more intensive studies on a range of viruses that could potentially affect these rural communities in order to assess the survival of viruses in point-of-use intervention systems.

Table 4.28: The survival of seeded Coxsackie B1 viruses over a 5 day period detected in the borehole and river water samples before and after the addition of a placebo, 1% or 3.5% sodium hypochlorite solution

Sodium hypochlorite solution	Water source									
	Borehole water					River water				
	Day 0*	Day 0**	Day 1	Day 2	Day 5	Day 0*	Day 0**	Day 1	Day 2	Day 5
Placebo***	6.9	-	6.7	6.5	6.0	6.9	-	6.8	6.5	6.2
1%	5.8	n.d	n.d	n.d	n.d	5.8	5.3	2.7	n.d	n.d
3.5%	5.8	n.d	n.d	n.d	n.d	5.8	n.d	n.d	n.d	n.d

* time = 0 minutes before the addition of the sodium hypochlorite solution
** time = 60 minutes after the addition of the sodium hypochlorite solution
*** placebo = distilled water
n.d = not detected
- = not tested

Several other factors could also have influenced the survival of Coxsackie B1 viruses in the river water. One of these factors could be the adhesion of virus particles to the walls of storage containers which was showed to happen when the pH of the water is at 7 or lower (Taylor *et al*., 1981). Ward and Winston (1985) have showed that Poliovirus type 1 adheres to the walls of containers filled with ground water. Bixby and O'Brien (1979) as well as Chattopadhyay and co-workers (2002) have found that virus particles are in competition with organic matter for adsorption sites on the walls of polypropylene storage containers. A study by John and Rose (2005) has showed that the effect of attachment of viruses to solid surfaces is virus dependant. They have however showed

that the survival of Poliovirus and Hepatitis A virus increased when attached to solid surfaces (John and Rose, 2005).

Another factor which could have aided in the survival of Coxsackie B1 virus particles in the river water was the high turbidity values (7.03 NTU to 8.3 NTU). Suspended matter in the water could act as adsorption sites for virus particles and protect them from the effect of disinfectants. Floyd and Sharp (1977) and Young and Sharp (1977) have showed that Enteroviruses in their normal state in fresh water clump together to form aggregates which are capable of protecting viable particles from disinfection and increase their survival. The high turbidity values of the river water used in this study did indicate the presence of particulate matter, which might have influenced the effectiveness of the 1% sodium hypochlorite solution. The survival of Coxsackie B1 virus particles in the river water containers during the 1% sodium hypochlorite solution treatment could therefore be ascribed to either aggregation, high turbidity of the water or due to chlorine resistance (Jensen *et al*., 1980; Hejkal *et al*., 1981). The 3.5% sodium hypochlorite was more effective in killing all viable viruses in both the river and borehole water containers after the addition of the solution (Table 4.28). It is however important to mention the study of Tree and co-workers (2003) whom have showed that indigenous Enteroviruses are more resistant to chlorination than laboratory adapted strains. The Coxsackie B1 virus strain used in this study was a laboratory adapted strain. Therefore, laboratory studies may overestimate the level of human enteric virus inactivation in the field and should only be used as a guideline to assess the efficiency of a disinfection process.

4.3.5 Summary of the survival of selected indicator and pathogenic microorganisms in drinking water stored in an improved household storage container with or without the addition of a sodium hypochlorite solution

In general, the CDC safe storage container proved to be convenient to handle, store the water and protect it from external contamination during storage. The reduction in the numbers of total coliforms, faecal coliforms, *C. perfringens*, somatic bacteriophages, *E. coli* and *S. typhimurium* in the control CDC safe storage containers not treated with a sodium hypochlorite solution (containers receiving the placebo solution) reflected the natural die-off curve of microorganisms under the prevailing storage conditions.

Consequently, this study indicated that even without the addition of a disinfectant, the counts of indicator and pathogenic microorganisms in water stored in the CDC safe storage containers decreased with time if the containers were not exposed to secondary contamination factors such as flies, insects, dust and faecally polluted hands and utensils (Jagals *et al*., 1999; Rose *et al*., 2006). However, microorganisms have been shown to survive in biofilms, which forms inside household storage containers (Fig 2.14) (Momba and Kaleni, 2002). These biofilms might harbour potentially pathogenic microorganisms, which can pose a health risk to consumers (Bunn *et al*., 2002; Jensen *et al*., 2002; Momba and Kaleni, 2002).

The 1% sodium hypochlorite solution was effective in reducing the counts of indicator and the seeded pathogenic microorganisms in the borehole water containers within 60 min to undetectable levels. However, in the river water samples, the 1% sodium hypochlorite dosage did not reduce the numbers of heterotrophic bacteria, *C. perfringens*, *E. coli*, *S. typhimurium*, male specific F-RNA bacteriophages and Coxsackie B1 viruses within 60 min. More resistant microorganisms such as heterotrophic bacteria, male specific F-RNA bacteriophages and Coxsackie B1 viruses were still present after 1 day and male specific F-RNA bacteriophages were detected up to 5 days after treatment with 1% sodium hypochlorite solution. It was evident that the high turbidity levels (7.04 to 8.30 NTU) in the river water did influence the effectivity of the 1% sodium hypochlorite solution. The river water could have contained particulate matter to which microorganisms could have attached for protection (WHO, 1996; Carlsson, 2003). Turbid water could also contain nutrients, which support microbial growth (LeChevallier *et al*., 1981). LeChevallier and co-workers (1981) have showed that water with turbidity between 1 and 10 NTU can result in an eight-fold decrease in efficiency of disinfection and were eight times more likely to carry pathogenic microorganisms.

The results obtained in this study confirmed that the 3.5% sodium hypochlorite dosage successfully reduced the number of a spectrum of microorganisms to undetectable levels within 60 min in the CDC safe storage container. This is the first evidence of successful disinfection by the sodium hypochlorite solution and dosage recommended by the South African Department of Health in the improved CDC safe storage containers.

Although seeding experiments provided valuable information on the inactivation of organisms, the seeded microorganisms used during this study may not always be representative of naturally occurring microorganisms in ground and surface water samples (Tree *et al*., 2003; Schaper *et al*., 2002b). The chlorine resistant parasitic protozoa such as the oocysts of *Cryptosporidium parvum* and various enteric viruses (Hambidge, 2001; Li *et al*., 2002) are of particular concern. Future studies should therefore, investigate the survival of parasites such as *Giardia* and *Cryptosporidium*.

The findings of this study confirmed that the CDC protocol (chlorine based water treatment combined with safe storage and education) offers a user-friendly and relatively inexpensive intervention strategy to control the transmission of enteric waterborne pathogens. The results from this study clearly indicated that the 3.5% sodium hypochlorite concentration was more effective against resistant pathogenic microorganisms compared to the 1% sodium hypochlorite solution used by the CDC. The 3.5% sodium hypochlorite solution, which is prescribed by the South African DOH and DWAF, provided a relatively high free chlorine residual of 3.8 $mg.l^{-1}$ after 60 min which is effective in reducing the health risk associated with waterborne pathogens in households with limited or no existing water and sanitation infrastructures. However, the water is not considered safe to drink before a free chlorine residual level of 0.8 $mg.l^{-1}$ is detected which in this study was the case only after 24 h for the water sources used in this study. Therefore, during this study all households were told to add their sodium hypochlorite solution, shake the container, closed it and let it stand for 24 h before the water was used. The main concern was that water with high concentrations of chlorine can lead to the formation of trihalomethanes (THMs) which have been closely linked with increased incidences of bladder, rectal and colon cancers in older individuals of the world population (Mills *et al*., 1999; Edstrom Industries, 2003; Freese and Nozaic, 2004). However, in these rural communities, the risk of death due to waterborne diseases is far greater than the relatively small risk of people dying from a small risk of getting cancer in their old days (WHO, 2004).

With proper education and follow-up studies the use of the 3.5% sodium hypochlorite solution together with the CDC safe storage containers could benefit the rural communities in South Africa. The CDC safe storage container is currently produced by a company in South Africa for the CDC and their intervention projects in other African

countries. Subsequently with governmental and non-govermental organisation (NGO) sponsorships, it could be available to rural communities in South Africa for less than R10 a container which is an affordable price for the low socio-econnomic communities in desperate need for point-of-use treatment. In addition, the 3.5% sodium hypochlorite solution is already available in all supermarkets in South Africa and most of the rural population have the knowledge on how to use it because of informative pamphlets distributed by the DOH and DWAF during environmental disasters and waterborne disease outbreaks (Appendix B). The combination of an affordable container and sodium hypochlorite solution could improve point-of-use water quality in rural communities in South Africa where problems such as inadequate water and sanitation infrastructures are present.

Chapter 5

GENERAL CONCLUSIONS AND RECOMMENDATIONS

5.1 INTRODUCTION

Almost 1.1 billion people in the world do not have access to improved water supplies and many of these people are without access to "safe drinking water" supplies (WHO, 2005). In addition, burden of disease data from the World Health Organisation, suggests that 1.8 million deaths and 61.9 million disability-adjusted life years worldwide are due to unsafe water, sanitation and hygiene (WHO, 2004). In developing countries, 98% of deaths are due to unsafe water, sanitation and hygiene of which 90% of these deaths are children (WHO, 2004).

The Millennium Development Goal of the United Nations aimed to halve by 2015 the proportion of people without sustainable access to safe drinking water and basic sanitation (UN, 2000). Unfortunately the definition of "safe drinking water" is not clearly understood and is interpreted differently in various countries. Even if a household is supplied by a standpipe within 200 m from the dwelling, the water the tap provides may still be contaminated because of the poor microbiological quality of the source (Chapter 2). In addition, the potential for water contamination during transport from the source to the dwelling and subsequent storage makes the challenge of providing "safe drinking water" even greater. Therefore point-of-use treatment systems is seen as providing "safe drinking water" to communities, households and individuals who are in desperate need for clean water (Sobsey, 2002).

This study was the first of its kind to be conducted in the rural communities of the Vhembe region of the Limpopo Province of South Africa. The results obtained from this study may be used to investigate the water quality of other rural communities on the African continent with similar environmental conditions. The microbiological quality of water sources in rural communities were assessed to determine the microbiological deterioration of household stored water at the point-of-use and evaluated the use of a

simple user friendly, affordable intervention system consisting of the CDC safe storage container together with a sodium hypochlorite solution was evaluated. The CDC safe storage container with or without a sodium hypochlorite solution was further assessed in a laboratory based study to determine the survival of indicator microorganisms and pathogenic waterborne microorganisms over a period of 5 days. In addition, genotyping of male specific F-RNA bacteriophage subgroups were used to determine the origin of animal or human faecal contamination inside the household stored water supplies. All three objectives as outlined in Chapter 1 have been achieved and several important findings from the results will be highlighted in this chapter.

5.2 AN INTERVENTION STRATEGY TO IMPROVE THE DRINKING WATER QUALITY IN RURAL HOUSEHOLDS

Point-of-use water treatment systems should be safe, affordable, free of bacteria and effective (Sobsey, 2002). The results obtained in this study have showed that the CDC protocol (chlorine based water treatment combined with safe storage and education) did fulfill all these criteria for rural households in South Africa. Therefore, this study contributes to the existing literature on the use of the Safe Water System developed by the CDC.

Microbiological assessment of the water from the primary water sources (river and communal tap sources) used by the two study populations, indicated that these sources were already faecally contaminated and had unacceptable high counts for heterotrophic bacteria, total coliform bacteria, faecal coliform bacteria, faecal enterococci and *Clostridium perfringens* according to the recommended South African guidelines for potable water (SABS, 2001). No statistical differences in the Heterotrophic bacterial counts (P=0.272) was seen between the river and tap water sources. However, statistical differences were seen in the total coliform bacterial counts (P=0.004), faecal coliform bacterial counts (P= 0.004), *E. coli* counts (P=0.010), faecal enterococci bacterial counts (P=0.001) and *C. perfringens* bacterial counts (P=0.001) between the river and tap water sources. Implications are that contamination of these water sources could mostly be due to human faecal pollution. A clear difference between improved (communal tap) and unimproved (river) sources (Gundry *et al*., 2004) could be seen in

the microbial counts of these two sources during this study. The unimproved water source (river) had higher counts of total coliform bacteria, faecal coliform bacteria and faecal enterococci bacteria. However, the results indicated that the definition of what constitutes an improved water source should be revised. In this study the communal tap water sources had indicator bacterial counts all exceeding the South African water quality guideline limits for safe drinking water. The results of this study are indicating that although communities are provided with communal taps, the water is not necessarily microbiologically acceptable or safe to drink as the general perception is.

Water samples from the traditional and CDC safe storage containers in the households using the placebo solution, indicated that water further deteriorated after collection and during storage at the point-of-use. Various reasons have been proposed for the deterioration of water quality between the source and point-of-use of which the two leading factors include the hygiene condition of the storage container and the environment in which these storage containers are stored (Jagals *et al*., 1999; Gundry *et al.*, 2004; Jagals *et al*., 2003; Trevett *et al*., 2005; Maraj *et al.*, 2006). These studies have showed that uncovered containers are exposed to environmental conditions such as dust and dirt, children and animals which could be potential sources of faecal contamination (Jensen *et al*., 2002; Rosas *et al*., 2006). The baseline characteristics of the households in the two villages implied that various factors could have played a role in the increase of the water at the point-of use in both the traditional and CDC safe storage containers without the addition of the 1% or 3.5% sodium hypochlorite solutions. These factors included dust and dirt (Rosas *et al*., 2006), biofilm growth and/or bacterial regrowth (Vanderslice and Briscoe, 1993; Momba and Notshe, 2003), storage and handling conditions of the water storage containers as well as hygiene and sanitation practices Jagals *et al*., 1999).

The results of the efficiency of the intervention in the households from the two villages clearly indicated that no statistical significant difference in the counts of heterotrophic bacteria could be seen between the water source and the household storage containers in both study villages. However, the results clearly showed that in households using tap water, a statistical significant difference ($P<0.05$) could be seen between the water source and the household storage containers in the counts for total coliform bacteria,

faecal coliform bacteria, faecal enterococci and *C. perfringens*. However, no statistical differences (P>0.05) were seen in the *E. coli* counts in households using tap water and their household stored water.

Finding *E. coli* in water primarily means such water is faecally polluted. From a water-suitability perspective, one would then manage this by discouraging ingestion of such waters not only because of faecal pollution, but also because of the potential presence of other bacterial pathogens *E. coli* are reported to indicate. Finding *E. coli* in water is practically the same as to finding other pathogens in there as well. This is the fundamental reason why most water quality guidelines use *E. coli* bacteria as the common indicator of microbiological quality of water that people use. The use of *E. coli* as an indicator is firmly entrenched in many water quality guidelines as well as in institutional approaches towards managing water quality. However, current *E. coli* tests for water are designed to test for the indicator value based on the fact that most strains of *E. coli* are actually harmless commensals from the gut of warm-blooded animals and humans. It is reported that pathogenic *E. coli* are not cultured in the faecal flora of health individuals. Certain strains of *E. coli* do in themselves actually become pathogenic depending on circumstances between excretion (into faecally polluted water) and infection of a naïve host. This has lead to a growing realisation that these strains of *E. coli* may even be the dominant bacterial pathogen species in faecally polluted water. This implies that technologies that were originally intended for simply indicating the potential presence of bacterial pathogens in water can, to a large extent, also confirm the presence of at least a substantial portion of bacterial pathogens that could commonly occur in water contaminated with faecal material. However, we do not know which *E. coli* strains are reflected in our indicator tests and whether they are pathogenic. If we develop an index of which *E. coli* strains dominate in a given water environment, we could anticipate the strains people would ingest should they use the water untreated. However, these households did not have any diarrhoea incidence during the intervention study. These are signs that the *E. coli* in that environment were indeed of the harmless strains and that the pathogens these were supposed to indicate were absent. Or it shows human immune systems that can deal with infection. To complicate matters further, we found that in other households, stored water contains lower *E. coli* numbers but diarrhoea is prevalent and even persistent. This could well be from the other carrier

media such as food, but it still shows that there are sub-populations in areas that do not cope immunologically. Or it might imply that the *E. coli* strains in that area are pathogenic. We have no way of telling. This implies a weakness in the classical *E. coli* indicator approach. The presumptive pathogens (indicated by detection *E. coli*) may potentially cause diarrhoea when ingested, but the actual effect (predicted by guideline values) may then not turn out as predicted in the consumer population. Using guidelines based on microbiological water quality alone is following a no-adverse-effect-level approach. In other words we should begin to observe certain health effects in populations if the water that they access (and ingest) for their daily needs, contain numbers of the indicator organism (*E. coli*) above a certain level.

Likewise the results from the intervention trial showed that in households using river water, a statistical significant difference ($P<0.05$) could be seen between the water source and the household storage containers in the counts for total coliform bacteria, faecal coliform bacteria and *E. coli* bacteria. However, no statistical differences ($P>0.05$) were seen in the faecal enterococci and *C. perfringens* counts in households using river water and their household stored water, which suggested that resistant spores and vegetative cells were present in the river water.

Furthermore, the results from this study have indicated no statistical differences ($P>0.05$) between the traditional and the CDC safe storage containers using the placebo solution in both study populations with regards to the prevalence of indicator microorganisms. This indicated that the CDC safe container as a single intervention without a sodium hypochlorite solution was not effective in the prevention of secondary contamination and therefore did not improve the microbiological quality of the stored drinking water. This was in agreement with an earlier study conducted by Quick and co-workers (1996) who indicated that the CDC safe storage container without the sodium hypochlorite intervention is not effective in reducing the risk associated with waterborne diseases. Therefore, other pathways of faecal contamination of the domestic water at the point-of-use must be research in the households. The role of zoonoses and biofilms inside the storage containers needs to be investigated further.

Consequently, this study has showed that treatment of water at the point-of-use with a sodium hypochlorite solution (1% or 3.5%) was 100% effective and people complied with the use of the sodium hypochlorite solutions when provided. The effectivity of the 1% and 3.5% sodium hypochlorite solutions are in agreement with the laboratory studies concerning the survival of total and faecal coliform bacteria, faecal enterococci bacteria, *E. coli* bacteria, *C. perfringens* bacteria and somatic bacteriophages in both the unimproved (river) and improved (groundwater/communal tap) water samples.

It was also found during this study that people did not generally wanted to wait 2 h or longer after the addition of a chlorine treatment before drinking or using the water. The households usually collected enough water for their daily household needs and then used the water immediately for the intended purpose. Therefore, educational interventions are needed to give the communities knowledge on behavior changes and health benefits.

The DOH in South Africa is promoting the use of the 3.5% sodium hypochlorite solution during disease outbreaks. Results of this study indicated that free chlorine residual of 0.8 $mg.l^{-1}$ as specified by the WHO (2004) was only obtained after 24 h for the 3.5% sodium hypochlorite solution which is recommended by the DOH in South Africa. The questions that needs to be asked concerning this aspect: *Is it rather a case of overkill and not effective assessment of the health risks of the high sodium hypochlorite solution?* This study has, however, showed that home treatment of drinking water with a sodium hypochlorite solution is a viable option to provide "safe drinking water" in rural communities and households in South Africa without adequate water and sanitation infrastructures.

In this study diarrhoea was not used as a health outcome because the VhaVenda and Shangaan communities in the Vhembe region of the Limpopo Province, South Africa, do not consider or perceive diarrhoea as a health threat, except for serious diseases such as cholera. In fact, diarrhoea was seen as necessary to clean the body and was even induced by taking traditional medicine. The main concern with regards to these rural communities was the lack of knowledge on the effect of diarrhoea on the most vulnerable group namely young children (Ashbolt, 2004). Inquiries into the prevalence

of diarrhoea in the two communities by looking at the Primary Health Care (PHC) clinic's data on diarrhoea incidences, indicated that diarrhoea was not a serious problem in these communities. Basically there could be two conclusions drawn from this: (1) Diarrhoea is underreported because mothers only take the child to the PHC clinic when the child is dehydrated. In general the mother treat the child at home with indigenous medicines (personal communication with several community members and PHC clinic staff) and (2) Adults and children has a natural immunity towards the microorganisms in their drinking water due to exposure at an early age. These findings could have serious implications for future intervention studies where the risk of diarrhoeal diseases will be used as an outcome to determine the effectiveness of the water treatment system. Cultural believes and living conditions must be taken into consideration before implementing intervention systems within a community.

Although many studies have reported on the effectiveness of household interventions, data on the sustainability of these interventions are scarce and warrants further investigation (Wilson and Chandler, 1993; Conroy *et al*., 1999). This study has investigated the sustainability of the intervention at 6 and 12 months intervals respectively after the initial intervention trial. It was found that households in village 1 using the improved water source (communal tap water), complied with the intervention protocol even 12 months after the original trial. These households used the free supply of 1% and 3.5% sodium hypochlorite solutions provided. However, households in village 2 using the unimproved water source (river water), did not comply with the intervention protocol even though they were also supplied with free bottles of 1% and 3.5% sodium hypochlorite solutions. The microbiological quality of the stored household water of households in this study indicated an increases health risk. Generally, the only difference between the two study villages, apart from the primary water source, was the fact that the chief in village 1 took a keen interest in the study and supported the idea of providing "safe drinking water" to his people. The chief was an educated person and the head of the secondary school in the community. The results from this study showed that households from village 1 were motivated and their behavior around water issues has changed. However, the chief from village 2, was not interested in the study because according to him it was a woman's issue to look at the household drinking water.

Of particular concern is the rising population in South Africa that is vulnerable to infection such as people infected with HIV/AIDS, young children and old people with declining immune systems. Safe drinking water also depends on hygiene practices which keep faecal matter from reaching stored domestic water supplies. It is important that facilities for the safe disposal of feces and hand washing close to the toilet are available (Trevett *et al*., 2005). Waterborne pathogens could also be transmitted within a household by ingestion of contaminated food and beverages, person-to-person contact and direct/indirect contact with faeces (Trevett *et al*., 2005). A study by Trevett and co-workers (2005) has indicated that the type of storage container and hand contact with drinking water was associated with increased risk of disease in the household. In this study the overall risk estimate of disease with regards to *E. coli* counts was 0.58 (95% CI 0.349 – 0.950) for people who washed their hands before food preparation. This highlights the need of proper education of rural communities on the benefits of hand washing. The study by Trevett and co-workers (2005) has also indicated that cultural believes, sanitary conditions and poverty affects the pathogen load in the household. It is understandable that people who have to walk far to collect water for household purposes, would be careful not to waste water unnecessary. In such cases, regular washing of hands are not a high priority in the household.

The long term plan of the South African government is to improve accessibility of all households to municipal treated standpipe water in the household or at least inside the dwelling. In the interim, household point-of-use interventions are needed to improve the microbiological quality of drinking water. Fewtrell and co-workers (2005) have reviewed 46 published publications on household interventions to determine the effectiveness of each type on intervention. According to this review, multiple interventions (combined water, sanitation and hygiene measures) were not more effective than interventions with single focus such as point-of use water quality interventions (Fewtrell *et al*., 2005). This could have been due to the fact that studies showing negative outcomes on water, sanitation and hygiene aspects were not published (not accepted) or not even submitted for publication by the researchers (Fewtrell *et al*., 2005).

In order for any intervention to be sustainable in a community, the environment must be supportive and the community must take ownership. Therefore, the chiefs and elders of the community must take the initiative to be part of the support system because the community respects their viewpoints. This aspect needs to be investigated further because several factors could play a role in the continued use of the system. These factors include: (1) knowledge of health, (2) knowledge of waterborne diseases, (3) hygiene, (4) proper storage of water containers and (5) proper handling of water containers (Sobsey, 2002). From the baseline survey it was evident that these communities have a lack of knowledge on all these factors. In order for any household water treatment and safe storage interventions to be successful, it must involve community education, participation and motivation (Nath *et al*., 2006). Consequently, the communities must take responsibility for the treatment and safe storage of water in their own homes (Nath *et al*., 2006).

5.3 TO DISTINGUISH BETWEEN FAECAL POLLUTION OF ANIMAL OR HUMAN ORIGIN USING MOLECULAR TYPING OF MALE SPECIFIC F-RNA BACTERIOPHAGE SUBGROUPS

It is important to determine the pathways of faecal contamination within the domestic household to decide on an effective point-of-use treatment system. Male specific F-RNA bacteriophage subgroups were used in this study to determine the origin of faecal pollution. The study was carried out specifically in rural households with a close living association with domestic animals and cattle. Differences in male specific F-RNA bacteriophages prevalence in the storage containers of households using different water sources were seen in this study. The prevalence of male specific F-RNA bacteriophages ranged between 30% and 65% for households using tap water and between 85% and 90% for households using river water. The higher prevalence of phages in the river water could have been due to animal and human activities in or near the river source. In addition, no difference between the traditional and CDC safe storage container water samples were seen with regards to the prevalence of male specific F-RNA bacteriophages. This is in agreement with the results from the formal intervention study indicating that the container without a sodium hypochlorite treatment is not improving the microbiological quality of the stored drinking water.

The results further demonstrated that water from the communal tap water and the household storage containers in village 1 were primarily contaminated by animal faecal matter because the majority of samples contained subgroup I male specific F-RNA bacteriophages (associated with animal faecal pollution). However, water from the river water sources and the household storage containers in village 2 were primarily contaminated by animal and human faecal matter because the samples contained subgroup I male specific F-RNA bacteriophages (associated with animal faecal pollution) and subgroup II male specific F-RNA bacteriophages (associated with human faecal pollution).

Consequently the results did give some indication of the origin of faecal pollution, but it was not conclusive due to the small sample size. In addition the results implied that the storage container does not prevent faecal contamination of stored drinking water in the absence of improved hygiene and sanitation behavior practices by the household members.

However, this is the first study to use male specific F-RNA bacteriophages to determine the origin of faecal pollution in household storage containers in rural households and the following aspects were identified for further research:

(1) Survival of male specific F-RNA subgroups in households water storage containers. It would be important to investigate factors such as container type, storage conditions, role of temperature, pH and turbidity, water type, prevalence and role of biofilm in container and the survival period of different male specific F-RNA subgroups
(2) Compare male specific F-RNA subgroups genotyping with new molecular PCR technique (Ogorzaly and Zantzer, 2006) to compare effectivity and costs
(3) Determine the male specific F-RNA subgroups present in different animals from these rural communities to assess the specificity of male specific F-RNA subgroups typing as a source tracking technique.

5.4 TO DETERMINE THE SURVIVAL OF INDICATOR AND WATERBORNE PATHOGENS IN THE IMPROVED CDC SAFE STORAGE CONTAINER

This study demonstrated that home treatment of drinking water using the 3.5% sodium hypochlorite solution as stipulated by the DOH is a viable option for households without access to safe water supplies. Laboratory studies on the survival of indicator and seeded pathogens in the CDC safe storage container with or without the addition of a sodium hypochlorite solution indicated that the 3.5% sodium hypochlorite was more effective than the 1% sodium hypochlorite solution as expected. The 3.5% solution effectively reduced all the indicator and pathogenic microorganisms in the ground and river water samples within 60 min. However, the 1% solution was not as effective. In the ground water samples, the 1% sodium hypochlorite solution was effective in reducing heterotrophic bacteria, total coliforms, faecal coliforms, faecal enterococci, *E. coli*, *S. typhimurium*, somatic and male specific F-RNA bacteriophages within 60 min. While, in river water samples with a higher turbidity level (7.04 and 8.30 NTU), the 1% sodium hypochlorite solution was not effective and heterotrophic bacterial counts, *E. coli*, *S. typhimurium*, *C. perfringens*, male specific F-RNA bacteriophages and Coxsackie B1 virus were still detected from one to five days.

To date the only information available on the effect of disinfection procedures on microorganisms in the CDC safe storage container is based on *E. coli* and faecal coliforms (Sobsey, 2002; Sobsey *et al*., 2003). Although seeding experiments from this study provided valuable information on the inactivation of organisms, the seeded microorganisms used may not be representative of naturally occurring microorganisms in ground and surface water samples (Tree *et al*., 2003; Schaper *et al*., 2002b). Additional studies on the survival of chlorine resistant parasitic protozoa (C*ryptosporidium* and *Giardia*) and various other enteric viruses (Hepatitis A, Rotavirus, Adenoviruses, Astroviruses and Noroviruses) are needed (Hambidge, 2001; Li *et al*., 2002). However, it is difficult to detect and to determine viability of viruses from environmental samples since it requires cell culture methods and molecular based assays which are expensive. In addition skilled personnel are required to perform the viral and parasite analysis (WHO, 2005)

A comprehensive review by Sobsey (2002) has concluded that chlorination with storage in an improved vessel was one of five point-of-use technologies considered promising to be explored for communities without safe drinking water supplies. This study has showed that the CDC safe storage container together with a sodium hypochlorite solution can be promising for South African communities. In this study the CDC container and sodium hypochlorite solution as a point-of-use treatment system was accepted by the study communities and showed to be affordable for South African standards. However, more studies are needed on the long term utilization and sustainable use of this treatment system in rural communities of South Africa. It could therefore, be concluded that point-of-use treatments of water at the household level could provide effective health benefits to rural communities in the Vhembe region of the Limpopo Province of South Africa.

5.5 FUTURE RESEARCH NEEDS

In addition to the research needs mentioned in the previous section, important areas for further research have been identified. The prevalence of pathogenic microorganisms (eg. *E. coli* 0157:H7, *Salmonella* spp, *Shigella* spp, *Vibrio cholerae,* Adenoviruses, Astroviruses, Noroviruses, Enteroviruses, Hepatitis A, Hepatitis E, Rotaviruses, *Giardia* and *Cryptosporidium*) in various water sources and stored water in household storage containers used by rural households should be determined. There is a lack of information regarding the prevalence of viruses, parasites and virulent bacterial strains in water sources and container stored water in rural communities. Pathogenic microorganisms have evolved mechanisms to rapidly adjust to changes in the environment (WHO, 2005). This may have implications regarding the infectivity, antibiotic sensitivity and pathogenicity of the microorganism. Research on microbial ecology and the investigation of virulence factors of the various heterotrophic microorganisms and other pathogenic bacteria such as *E. coli* and especially *E. coli* O157:H7, *Salmonella* spp and *Shigella* spp might assist in determining the health risk. This may have major health implications for high risk individuals such as the young, the elderly and immunocompromised people consuming this water.

Advanced analytical methods should be used to help discriminate between introduced pathogenic and naturally occurring non-pathogenic strains of waterborne microorganisms and to characterise the emergence of new strains of pathogens as a result of genetic changes. This analysis could be conducted using molecular and genotyping techniques. Molecular typing and sequencing of the isolates will provide valuable information on the origin of the specific microbial species and their relatedness (Lebuhn *et al*., 2004; Rousselon *et al*., 2004; Wang *et al*., 2004; Wei *et al*., 2004). Consequently, if a link between environmental and clinical isolates could be established in rural communities, appropriate action can be taken by the DOH and the Department of Water Affairs to prevent the risk of waterborne diseases. Burden of disease and risk-associated studies can also be conducted if more information on bacterial pathogens, viruses and parasites are available.

Additional studies are needed based on the antibiograms of the isolated opportunistic and pathogenic bacterial isolates from unprotected and protected water sources as well as for bacterial isolates obtained from water and biofilms inside the storage containers. Although antibiograms are known to vary from place to place and with time, necessitating the need for periodic updates in order to uncover resistance patterns, there are no baseline data on antibiograms of potential bacterial pathogens of diarrhoea isolated from diarrhoeic stool specimens in rural communities in the Vhembe region of South Africa. An urgent need, therefore, exists to ascertain the incidence of enteric pathogens in diarrhoic stools, as well as antibiograms of these bacterial isolate .These studies would assist in assessing the presence of resistant microorganisms circulating in a community. Additional information will be provided regarding the health risk these resistant bacteria hold for high risk individuals that are exposed to these microorganisms (Obi *et al*., 2002; Obi *et al*., 2004).

The possible zoonotic risk prevalent in these communities has not received much attention. Several studies have reported a link between animal pathogens and isolates obtained from humans (Meslin, 1997; Sinton *et al*., 1998; Franzen and Muller, 1999; Slifko *et al*., 2000; Enriquez *et al*., 2001; Hoar *et al*., 2001; Leclerc *et al*., 2002; Theron and Cloete, 2002; Hackett and Lappin, 2003). Genetic and phenotypic characteristics of pathogenic microorganisms are needed to explain zoonotic relationships of

microorganisms with their animal hosts to determine factors that may influence their transmission to humans. Most of these communities are at risk of contracting diseases from animals due to the close living association between domestic animals, cattle and people in rural areas of South Africa.

In addition the effect of human and animal activities on water sources should also be investigated in more detail: (1) human sewage and animal excreta in surface water in communities with inadequate sanitation infrastructure could increase the nitrogen and phosphate levels of water used for drinking , (2) phosphates levels in water where rural woman wash their clothes or people bath and (3) irrigation of crops with pesticides and fungicides increases the levels of organophosphates, copper and mercury. These same water sources are used for drinking water collection and little is known on the health effect of these activities on people in rural areas. Data on these factors will assist in effective water treatment and intervention policies.

Another aspect is the lack of information on the role of toxins produced by bacteria such as the *Cyanobacteria* as well as their role in waterborne diseases (WHO, 2005). *Cyanobacteria* have been identified at causing hay fever, eye irritations, skin rashes, vomiting and diarrhoea (WHO, 2005). In addition, research on different chemical compounds, heavy metals, endocrine disrupting compounds (EDC) to determine the health risk to consumers in regions without adequate water infrastructures are important. Mining activities increase mineral and salts in water, affects the pH of the water and increase the presence of metals such as nickel, zinc, cadmium and lead which can build up in fish and animals which are eaten by the communities (DNR, 2006). Insecticides such as DDT which are used in South Africa for control of the malaria mosquito could also be washed into surface and groundwater sources during rains. Accumulation in fish and animals drinking the water can occur, ultimately reach humans who consumes these animals as part of their daily food intake.

Finally, an important aspect that is not addressed adequately in intervention studies is the promotion of sustainable behavior changes to improve basic hygiene and sanitation practices in these rural communities (Fewtrell *et al*., 2005). The only way the behavior of a household or community will be sustained is when (1) the environment is

supportive (media involvement, policy makers involvement and resources provided); (2) delivery systems is sufficient (services must be available, products must be available and the Department of Health must promote behavior changes); (3) communities must take ownership and have support groups and (4) individual household members must be motivated, have positive attitudes about behavior changes and proper resources must be available to the household (knowledge and skills impartation). It is necessary to involve the female head of the household in all intervention strategies and involve community women groups and faith based organisations with which people can associate to effect behavior changes. Studies are needed which will investigate the integration of education on health aspects and training on basic hygiene and sanitation practices of the existing health infrastructure. These educational studies need to address and monitor behavioral patterns in the households. Although people know that water can be contaminated, they are ignorant of the effect of how some of their actions could contribute to the faecal pollution of the drinking water at the point-of-use (Dunker, 2001). Very little information on how households allocate water to different purposes within the household is available. It is important to establish the sequence of the type of water supply, sanitation and hygiene interventions produce the greatest health benefits for these communities. These studies need to provide information on the prevalence and survival of a broad spectrum of selected pathogenic microorganisms in stored household water particularly in households where high-risk individuals are living. This information will assist in formulating policies on health and sanitation for developing communities to assess rural water supply needs and to determine whether the water is used efficiently.

Research on all these aspects will be of extreme importance in water quality studies and will provide valuable data to improve the microbiological quality of water stored at the rural households, prevent the transmission of waterborne diseases and provide people living with immunocompromised diseases with safe drinking water. The results of these studies will assist various role players in the South African government in the formulation of policies regarding water, sanitation and hygiene aspects and changes in South Africa to improve the general well being of the people of South Africa.

Chapter 6

REFERENCES

Ahmed S and Mahmud A (1998) Water management practices in rural and urban homes: A case study from Bangladesh on ingestion of polluted water. *Public Health* **112,** 317-321

Alam MJ and Zurek L (2004) Association of *Escherichia coli* O157:H7 with houseflies on a cattle farm. *Applied and Environmental Microbiology* **70,** 7578-7580.

Alarly M and Nadeau D (1990) An outbreak of *Campylobacter enteritis* associated with a community water supply. *Canadian Journal of Public Health* **81,** 268-271.

Allwood PB, Yashpal SM, Hedberg CW and Goyal SM (2003) Survival of F-specific RNA coliphage, feline calici virus and *Escherichia coli* in water: A comparative study. *Applied and Environmental Microbiology* **69,** 5705-5710.

Arango CP and Long SC (1998) Alternative bacterial indicators of sources of microbial inputs to watersheds of drinking water sources. *Annual Conference Proceedings: American Water Works Association* **C,** 670-687.

Ashbolt NJ (2004) Microbial contamination of drinking water and disease outcomes in developing regions. *Toxicology* **198,** 229-238.

Atkin E, Benton WH and Hill WF (1971) Enteric viruses in ground and surface waters: A review of their occurrence and survival. Proceedings of the 13th Water Quality Conference, University of Illinois, Bullitin **69,** 59-74.

Baker KH (1984) Protective effect of turbidity on *Escherichia coli* during chlorine disinfection. In Crusberg TC, Cheetham RD and Hoffman AH (eds). Water quality and

the public health. Worcester Consortium for Higher Education. Worcester, Massachuchets, USA.

Barau D and Merson M (1992) Prevention and control of cholera. In: Cholera. Barau D and Greenbough W (eds). Plenum Medical Book Co., New York. Pp 329-346.

Bartman J and Rees G (2000) Monitoring bathing water. In: Guidelines for safe recreational water environments. Chapter 9. World Health Organization, Geneva. Pp 159-167.

Bartram J, Cotruvo J, Exner M, Fricker C, and Glasmacher A (2003) Heterotrophic plate counts and drinking-water safety. *World Health Organization* **X,** 1-256.

Beekwilder J, Nieuwenhuizen R, Havelaar AH and Van Duin J (1996) An oligonucleotide hybridization assay for the identification and enumeration of F-specific RNA bacteriophages in surface water. *Journal of Applied Bacteriology* **80,** 179-86.

Benenson AS (1995) Control of communicable diseases in man. (16th Ed). Washington. American Public Health Association. Pp 530.

Bifulco JM, Shirey JJ and Bissonnette GK (1989) Detection of Actinetobacter spp. In rural drinking water supplies. *Applied and Environmental Microbiology* **55,** 2214-2219.

Bixby RC and O'Brien DJ (1979) Influence of fulvic acid on bacteriophage adsorption and complexation in soil. *Applied and Environmental Microbiology* **38,** 840-845.

Blum D, Emeh R, Huttly S, Dosunmu-Ogunbi O, Okeke N, Ajala M, Okoro J, Akujobi C, Kirkwood B and Faechem R (1990) The Imo State (Nigeria) drinking water supply and sanitation project, 1. Description of the project, evaluation methods, and impact on intervention variables. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **84,** 309-315.

Bosch A, Lucena F, Diez JM, Gajardo R, Blasi M and Jofre J (1991) Waterborne viruses associated with hepatitis outbreak. *Journal of American Water Works Association* **83,** 80-83.

Bosch A (1998) Human enteric viruses in the water environment: A minireview. *International Microbiology* **1,** 191-196.

Boyer Km, Petersen NJ, Farzaneth I, Pattison CP, Hart MC and Maynard JE (1975) An outbreak of gastroenteritis due to *E. coli* 0142 in a neonatal nursery. *Journal of Pediatrics* **86,** 919-927.

Bradley G, Carter J, Gaudie D and King C (1999) Distribution of the human faecal bacterium *Bacteroides fragilis*, its bacteriophages and their relationship to current sewage pollution indicators in bathing water. *Journal of Applied Microbiological Symposium Supplement* **85,** 90S-100S.

Brion GM, Meschke JS and Sobsey MD (2002) F-specific RNA coliphages: Occurrence, types, and survival in natural waters. *Water Research* **36,** 2419-2425.

Briscoe J (1984) Intervention studies and the definition of dominant transmission routes. *American Journal of Epidemiology* **120,** 449-455.

Bunn JEG, Mackay WG, Thomas JE, Reid DC and Weaver LT (2002) Detection of *Helicobacter pylori* DNA in drinking water biofilms: Implications for transmission in early life. *Letters in Applied Microbiology* **34,** 450-454.

Cairncross S and Cliff JL (1987) Water use and health in Mueda, Mozambique. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **81,** 429-437.

Carlsson FHH (2003) Elementary handbook of water disinfection. WRC Report TT205/03. Water Research Commission, Pretoria, South Africa.

Casewell M and Phillips I (1977) Hands as a route of transmission of *Klebsiella* species. *British Medical Journal* **2,** 1315-1317.

CDC (2001) Safe water systems for the developing world: A handbook for implementing household based water treatment and safe storage projects. Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, USA. Pp 187.

Central Statistics (1995) Living in the Northern Province. Pretoria, South Africa.

Chang GW, Brill J and Lum R (1989) Proportion of ß-D-glucunoridase negative *Escherichia coli* in human fecal samples. *Applied and Environmental Microbiology* **55,** 335-339.

Chattopadhyay D, Chattopadhyay S, Lyon WG and Wilson JJ (2002) Effect of surfactants on the survival of viruses. *Environmental Science and Technology* **36,** 4017-4024.

Chidavaenzi MT, Jere M, Chandara C, Chingundury D and Bradley M (1998) An evaluation of water urns to maintain domestic water quality. 24th WEDC Conference. Sanitaion and Water for all, Islamabad, Pakistan. Pp 249-253.

Clasen F and Bastable A (2003) Faecal contamination of drinking water during collection and household storage: The need to extend protection to the point of use. *Journal of Water and Health* **3,** 109-115.

Clasen T, Brown J, Suntura O and Collins S (2004) Reducing disrrhoea through household-based ceramic filtration of drinking water: A randomised controlled trial in Bolivia. *American Journal for Tropical Medicine and Hygiene* **70,** 651-657.

Collin IF, Zmirou D, Ferley JP and Charrel M (1988) Comparison of bacterial indicators and sampling program for drinking water systems. *Applied and Environmental Microbiology* **54,** 2073-2077.

Conroy RM, Elmore-Meegan M, Joyce T, McGuigan KG and Barnes J (1996) Solar disinfection of drinking water and diarroea in Masai children: A control field trial. *Lancet* **348,** 1695-1697.

Conroy RM, Meegan ME, Joyce T, McGuigan K and Barnes J (1999) Solar disinfection of water reduces diarrhoeal disease: An update. *Archives of Diseases in Children* **81,** 337-338.

Conroy RM, Meegan ME, Joyce T, McGuigan K and Banes J (2001) Solar disinfection of drinking water projects against cholera in children under 6 years of age. *Archives of Diseases in Children* **85,** 293-295.

Covert TC, Rice EW, Johnson SA, Berman D, Johnson CH and Mason PJ (1992) Comparing defined-substrate coliform tests for the detection of *Escherichia coli* in water. *Journal of the American Water Works Association* **84,** 98-104.

Crump JA, Otieno PO, Slutsker L, Keswick BH, Rosen DH, Hoekstra RM, Valule JM and Luby SP (2005) Household based treatment of drinking water with flocculant disinfectant for preventing diarrhoea in areas with turbid source water in rural western Kenya: cluster randomised controlled trial. Internet URL: BMJ, doi:10.1136/bmj.38512.618681.EO (published 26 July 2005).

Curtis V, Cairncross S and Yonli R (2000) Review: Domestic hygiene and diarrhoea – pinpointing the problem. *Tropical Medicine and International Health* **5,** 22-32.

Daniels DL, Cousens SN, Makoae LN and Faechem RG (1990) A case study control of the impact of improved sanitation on diarrhoeal morbidity in Lesotho. *Bulletin of the World Health Organization* **68,** 455-463.

Daniels NA, Simons SL, Rodriques A, Gunnlaugsson G, Forester T, Wells J, Hutwagner (1999) First do no harm: Making oral rehydration solutions safer in a cholera epidemic. *American Journal of Tropical Medicine and Health* **60,** 1051-1055.

Davids AS and Maremane KMA (1998) Physical environmental factors and infantile diarrhoea in rural Northern Province. *Infectious Control Journal of South Africa* **3,** 21-23.

Deb BC, Sircar BK, Sengupta PG, De SP, Saha MR and Pal SC (1986) Intra-familial transmission of *Vibrio cholera* biotype Eltor in Calcutta Slums. *Indian Journal of Medical Research* **76,** 814-819.

Debartolomeis J and Cabelli VJ (1991) Evaluation of an *Escherichia coli* host strain for enumeration of F male-specific bacteriophages. *Applied and Environmental Microbiology* **57,** 1301-1305.

Deetz TR, Smith EM, Goyal SM, Gerba CP, Vollet JJ, Tsai C, Du Pont H and Keswick BH (1984) Occurrence of rota and enteroviruses in drinking and environmental water in a developing country. *Water Research* **18,** 567-571.

DeWolf Miller F (1984) Problems of water storage in the rural village home: The Egyptian zir. *Journal of Tropical Medicine and Hygiene* **87,** 53-59.

DNR – Department of Natural Resources (2006) Volatile organics in drinking water. Internet URL: www.dnr.state.wi.us/org/water/dwg

DOH – Department of Health (2000) Cholera in KwaZulu Natal. Internet URL: http://www.doh.gov.za.

DOH – Department of Health (2002) Cholera in the Limpopo Province. Internet URL: http://www.doh.gov.za/docs/reports/2002/cholera/limpopo.html.

DOH – Department of Health (2003) Cholera outbreak in the Mpumalanga. Internet URL: http://www.doh.gov.za/docs/reports/2003/cholera/mpumalanga.html.

Dunker L (2001) The KAP tool for Hygiene. A manual on: knowledge, attitudes and practices in the rural areas of South Africa. Water Research Commission Report TT144/00. Water Research Commission, Pretoria, South Africa.

DWAF - Department of Water Affairs and Forestry (1994) White paper on Water and Sanitation. Internet URL: www-dwaf.pwv.gov.

DWAF - Department of Water Affairs and Forestry (1996) South African Water Quality Guidelines. Volumes 1 and 2. Domestic use. The Goverment Printer, Pretoria, South Africa.

DWAF – Department of Water Affairs and Forestry (2005) Waterborne diseases: Incidence and Reporting Process Assessment for Waterborne Diseases. August 2005.

Duran AE, Muniesa M, Moce-Llivina L, Campos C, Jofre J and Lucena F (2003) Usefulness of different groups of bacteriophages as model micro-organisms for evaluating chlorination. *Journal of Applied Microbiology* **95,** 29-37.

Echeverria P, Taylor D, Seriwatnana J, Leksomboon U, Chaicumpa W, Tirapat C and Rowe B (1987) Potential sources of enterotoxigenic *Eschericia coli* in homes of children with diarrhoea in Thailand. *Bulletin of the World Health Organization* **65,** 207-215.

Edberg SC, Rice EW, Karlin RJ and Allen MJ (2000) *Escherichia coli*: The best biological drinking water indicator for public health concern. *Journal of Applied and Environmental Microbiology* **88,** 106S-116S.

Edstrom Industries (2003) Chlorination of drinking water. Edstrom Industries, Wisconsin, USA. 4230-MI4174.

Egger D, Passamontes L, Ostermeyer M and Bienz K (1995) Reverse transcription and multiplex PCR for differentiating between polio and Enteroviruses from clinical and environmental samples. *Journal of Clinical Mirobiology* **33,** 1442-1447.

Ehlers MM, Pavlov DN, Grabow WOK and De Wet CME (2003) Cytotoxicity and invasiveness of Heterotrophic Bacteria. Water Research Commission Report 1069/1/03. ISBN 1 77005 007 8.

El-Agaby MM, Dutka BJ and Kamel M (1988) Incidence of coliphage in potable water supplies. *Applied and Environmental Microbiology* **54,** 1632-1633.

El Attar L, Gawad AA, Khairy AEM and El Sebaie O (1982) The sanitary condition of rural drinking water in a Nile Delta village II. Bacterial contamination of drinking water in a Nile Delta village. *Journal of Hygiene in Cambodia* **88,** 63-67.

Empereur-Bisonette P, Salzman V and Monjour L (1992) Application of a new transport and storage material for improving tha quality of drinking water in rural African areas. *Bulletin de la Society Pathologie Exotique* **85,** 390-394.

Engelbrecht RS, Weber MJ, Salter BL and Schmidt CA (1980) Comparative inactivation of viruses by chlorine. *Applied and Environmental Microbiology* **40,** 249-256.

Enriquez C, Nwachuku N and Gerba CP (2001) Direct exposure to animal enteric pathogens. *Reviews in Environmental Health* **16,** 117-131.

Esrey SA and Habicht JP (1986) Epidemiological evidence for health benefits from improved water and sanitation in developing countries. *Epidemiological Reviews* **8,** 117-128.

Esrey SA, Habicht JP and Casella G (1992) The complimentary effect of latrines and increased water usage on the growth of infants in rural Lesotho. *American Journal of Epidemiology* **135,** 659-666.

Faechem RG (1984) Interventions for the control of diarrhoeal diseases among young children: Promotion of personal and domestic hygiene. *Bulletin of the World Health Organization* **62,** 467-476.

Faechem RG, Bradley DJ, Garelick H and Mara DD (1983) Sanitation and disease: Health aspects of excreta and wastewater management. *Bulletin of the World Health Organization* **6,** 67-76.

Feng P, Lum R and Chang GW (1991) Identification of *uidA* gene sequences in ß-D-glucuronidase negative *Escherichia coli*. *Applied and Environmental Microbiology* **57,** 320-323.

Fewtrell L, Kaufmann RB, Enanaoria W, Haller L and Colford JM (2005) Water, sanitation and hygiene interventions to reduce diarrhoea in less developed countries: a systematic review and meta-analysis. *Lancet* **5,** 42-52.

Flint KP (1987) The long-term survival of *Escherichia coli* in river water. *Journal of Applied Bacteriology* **63,** 261-270.

Floyd R and Sharp DG (1977) Aggregation of poliovirus and reovirus by dilution in water. *Applied and Environmental Microbiology* **33,** 159-167.

Franzen C and Muller A (1999) Cryptosporidia and microsporidia-waterborne diseases in the immunocompromised host. *Diagnostic Microbiological Infectious Diseases* **34,** 245-262.

Freese SD and Nozaic DJ (2004) Chlorine: Is it really so bad and what are the alternatives? *Water SA* **30,** 18-24.

Fujioka RS, Tenno K and Kansako S (1988) Naturally occurring fecal coliforms and fecal enterococci in Hawaii's freshwater streams. *Toxicity Assessment* **3,** 613-630.

Furuse K, Sakurai T, Hirashima A, Katsuki M, Ando A and Watanabe I (1978) Distribution of RNA coliphages in South and East Asia. *Applied and Environmental Microbiology* **35,** 995-1002.

Furuse K, Hirashima A, Harigai H, Ando A, Watanabe K, Kurosawa K, Inokuchi Y and Watanabe I (1979) Grouping of RNA coliphages based on analyses of the sizes of their RNAs and proteins. *Virology* **97,** 328-341.

Furuse K (1987) Distribution of coliphages in the environment: General considerations. In: Goyal SM, Gerba CP, and Bitton G (eds). Phage ecology. New York, Wiley. Pp 87-124.

Gavini F, Pourcher A, Neut C, Monget D, Romond C, Oger C and Izard D (1991) Phenotypic differentiation of bifidobacteria of human and animal origins. *International Journal of Systematic Bacteriology* **41,** 548-557.

Genthe B, Strauss N, Seager J, Vundule C, Maforah F and Kfir R (1997) The effect of water supply on water quality in a developing community in South Africa. *Water, Science and Technology* **35,** 35-40.

Gerba CP, Rose JB, Haas CN and Crabtree KD (1996) Waterborne rotavirus: A risk assessment. *Water, Science and Technology* **30,** 2929-2940.

Ghannoum MA, Moore KE, Al-Dulaimi M and Nasr M (1981) The incidence of water related diseases in the Brak area, Libya from 1977 to 1979, before and after yhe installation of water treatment plants. *Zentralbl Babteriol Microbiol Hyg B* **173,** 501-508.

Gilman RH and Skillicorn P (1985) Boiling of drinking water. Can a fuel-scarce community afford it? *Bulletin of the World Health Organization* **63,** 157-163.

Gilpen BJ, Gregor JE and Savill MG (2002) Identification of the source of faecal pollution in contaminated rivers. *Water, Sciene and Technology* **46,** 9-15.

Gilpen B, James T, Nourozi F, Saunders D, Scholes P and Savill M (2003) The use of chemical and molecular microbial indicators for source identification. *Water, Science and Technology* **47,** 39-43.

Gleick PH (1998) The World's Water 1998-1999. Island Press, Washington, DC, USA.

Gow JW, Behan WMH, Clements GB, Woodall C, Riding M, and Behan PO (1991) Enteroviral RNA sequences detected by polymerase chain reaction in muscle of patients with post viral fatigue syndrome. *British Medical Journal* **302,** 692-696.

Goyal SM, Gerba CP and Melnick JL (1979) Human enteroviruses in oysters and their overlaying waters. *Applied and Environmental Microbiology* **37,** 572-581.

Grabow WOK (1996) Waterborne diseases: Update on water quality assessment and control. *Water SA* **22,** 193-202.

Grabow WOK (2001) Bacteriophages: Update on applications as models for viruses in water. *Water SA* **27,** 251-257.

Grabow WOK, Botma KL, De Villiers JC, Clat CG and Erasmus B (1990) An assessment of cell culture and PCR procedures for the detection of polio viruses in water. *Bulletin of the World Health Organization* **77,** 973-980.

Grabow WOK, Coubrough P, Nupen EM and Bateman BW (1984) Evaluation of coliphages as indicators of the virological quality of sewage-polluted waters. *Water SA* **10,** 7-14.

Grabow WOK, Holtzhausen CS and De Villiers JC (1993) Research on bacteriophages as indicators of water quality. WRC Report 321/1/93. Water Research Commission, Pretoria, South Africa.

Grabow WOK, Vrey A, Uys M and De Villiers C (1993) Evaluation of the application of bacteriophages as indicators of water quality, 1 January 1990 - 31 December 1992. Final report No 321/1/93. Water Research Commission, Pretoria, South Africa.

Grist NR, Bell EJ, Follet EAC and Urquhart GED (1979) Diagnostic methods in clinical virology. Blackwell Scientific Publications, London. Pp 61-80.

Guerrant RL (1997) Cryptosporidiosis: An emerging, highly infectious threat. *Emerging Infectious Diseases* **3,** 51-57.

Gundry S, Wright J and Conroy R (2004) A systematic review of the health outcomes related to household water quality in developing countries. *Journal of Water and Health* **2,** 1-13.

Gunn R, Kimball A, Mathew P, Dutta S and Rifatt AHM (1981) Cholera in Bahrain: Epidemiological characteristics of an outbreak. *Bulletin of the World Health Organization* **59,** 61-66.

Hackett T and Lappin MR (2003) Prevalence of enteric pathogens in dogs in north-central Colorado. *Journal of Animal Hospital Association* **39,** 52-56.

Hair JF, Anderson RE, Tatham RL and Black WC (1998) Multivariate data analysis. (5th Ed). Prentice Hall, Upper Saddle River, New York.

Hambidge A (2001) Reviewing efficacy of alternative water treatment techniques. *Health Estate* **55,** 23-25.

Hammad ZH and Dirar HA (1982) Microbiological examination of Sebeel water. *Applied and Environmental Microbiology* **43,** 1238-1243.

Han AM and Hlaing T (1989) Prevention of diarrhoea and dysentery by handwashing. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **83,** 128-131.

Han A, Oo K, Midorikawa Y and Shwe S (1989) Contamination of drinking water during collection and storage. *Tropical and Geographical Medicine* **41,** 138-140.

Handzel T (1998) The effect of improved drinking water quality on the risk of diarrhoeal disease in an urban slum of Dhaka, Bangladesh. A home chlorination intervention trial. PhD dissertation, Department of Environmental Sciences and Engineering, University of North Carolina at Chapel Hill, North Carolina, USA.

Hänninen ML, Haajanen H, Pummi T, Wermundsen K, Katila MC, Sarkinnen H, MiettinenI and Rautelin (2003) Detection and typing of *Campylobacter jejuni* and *Campylobacter coli* and analysis of indicator microorganisms in three waterborne outbreaks in Finland. *Applied and Environmental Microbiology* **69,** 1391-1396.

Harris JR, Mariano J, Wells JG, Payne BJ, Donnell HD and Cohen ML (1985) Person-to-person transmission in an outbreak of enteroinvasive *Escherichia coli*. *American Journnal of Epidemiology* **122,** 245-252.

Havelaar AH (1993) Bacteriophages as models of human enteric viruses in the environment. *American Society of Microbiology News* **59,** 614-619.

Havelaar AH and Hogeboom WM (1984) A method for enumeration of male-specific bacteriophages in sewage. *Journal of Applied Bacteriology* **56,** 439-447.

Havelaar AH, Furuse K and Hogeboom WM (1986) Bacteriophages and indicator bacteria in human and animal faeces. *Journal of Applied Bacteriology* **60,** 255-262.

Havelaar AH, Pot-Hogeboom WM, Furuse K, Pot R and Hormann MP (1990) F-specific RNA bacteriophages and sensitive host strains in feces and wastewater of human and animal origin. *Journal of Applied Bacteriology* **69,** 30-37.

Havelaar AH, Van Olphen M and Drost YC (1993) F-specific RNA bacteriophages are adequate model organisms for enteric viruses in fresh water. *Applied and Environmental Microbiology* **59,** 2956-2962.

Hejkal TW, Wellings FM, Lewis AL and LaRock PA (1981) Distribution of viruses associated with particles in waste water. *Applied and Environmental Microbiology* **41,** 628-634.

Helsel DR and Hirsch RM (1997) Statistical Methods in water resources. Elsevier Science, BV Amsterdam, Netherlands.

Henry F and Rahim Z (1990) Transmission of diarrhoea in two crowded areas with different sanitary facilities in Dhaka, Bangladesh. *Journal of Tropical Medicine and Hygiene* **93,** 121-126.

Herson DS, McGonigle B, Payer MA and Baker KH (1987) Attachment as a factor in the protection of *Enterobacter cloacae* from chlorination. *Applied and Environmental Microbiology* **53,** 1178-1180.

Hoar BR, Atwill ER, Elmi C and Farver TB (2001) An examination of risk factors associated with beef cattle shedding pathogens of potential zoonotic concern. *Epidemiological Infections* **127,** 147-155.

Hopkins RS, Gaspard GB, Williams FP, Karlin RJ, Cukor G and Blacklow NR (1984) A community waterborne gastroenteritis outbreak: evidence of rotavirus as the agent. *American Journal of Public Health* **74,** 263-265.

Hoque B, Mahalanabis D, Alam M and Islam M (1995) Post-defecation handwashing in Bangladesh: Practice and efficiency perspectives. *Public Health* **109,** 15-24.

Hot D, Legeay O, Jaques J, Gantzer C, Caudrelier Y, Guyard K, Lange M and Andreoletti (2003) Detection of somatic bacteriophages, infectious enteroviruses and enterovirus genomes as indicators of human enteric viral pollution in surface water. *Water Research* **37,** 4703-4710.

Hoxie NJ, Davies JP, Vegeront JM, Nashold RD and Blair KA (1997) Cryptosporidiosis-associated mortality following a massive waterborne outbreak in Milwaukee, Wisconsin. *American Journal of Public Health* **87,** 2032-2035.

Hrudey SE, Payment P, Huck PM, Gillman RW and Hrudey EJ (2003) A fatal waterborne disease epidemic in Walkerton, Ontorio: Comparison with other waterborne outbreaks in the developed world. *Water, Science and Technology* **47,** 7-14.

Hsu F, Shieh YC, van Duin J, Beekwilder MJ and Sobsey MD (1995) Genotyping male-specific RNA coliphages by hybridization with oligonucleotide probes. *Applied and Environmental Microbiology* **61,** 3960-3966.

International Water Association (2004) News headlines: Thousands suffer effects of waterborne disease in wake of Bangladesh flood. Internet URL: http://217.77.141.80/portal/publish/article_147.shtml.

ISO (1995) Water quality-Detection and enumeration of bacteriophages-Part 1: Enumeration of F-specific RNA bacteriophages. Doc ISO 10705-1. International Organization for Standardization, Geneva.

ISO (2000) Water quality-Detection and enumeration of bacteriophages-Part 2: Enumeration of somatic bacteriophages. Doc ISO 10705-2. International Organization for Standardization, Geneva.

ISO (2001) Water quality-Detection and enumeration of bacteriophages-Part 4: Enumeration of *Bacteroides fragilis* bacteriophages. Doc ISO 10705-4. International Organization for Standardization, Geneva.

Jagals P, Bokato TC and Grabow WOK (1999) Changing consumer water-use patterns and their effect on microbiological water quality as a result of an engineering intervention. *Water SA* **25,** 297-300.

Jagals P, Grabow WOK and De Villiers JC (1995) Evaluation of indicators for assessment of human and animal faecal pollution of surface run-off. *Water, Science and Technology* **31,** 235-241.

Jagals P, Jagals C and Bokaka TC (2003) The effect of container-biofilm on the microbiological quality of water used from plastic household containers. *Journal of Water and Health* **1,** 101-108.

Jensen PK, Ensink JHJ, Jayasinghe G, Van der Hoek W, Cairncross S and Dalsgaard A (2002) Domestic transmission routes of pathogens: The problem of in-house contamination of drinking water during storage in developing countries. *Tropical Medicine and International Health* **7,** 604-609.

Jensen H, Thomas K and Sharp DG (1980) Inactivation of coxsackieviruses B3 and B5 in water by chlorine. *Applied and Environmental Microbiology* **40,** 633-640.

John DE and Rose JB (2005) Review of factors affecting microbial survival in groundwater. *Environmental Science and Technology* **39,** 7245-7356.

Kaltenhaler E and Drasar B (1996) The study of hygiene behaviour in Botswana: a combination of qualitative and quantitative methods. *Tropical Medicine and International Health* **1,** 690-698.

Kelly S and Sanderson WW (1958) The effect of chlorine in water on enteric viruses. *American Journal of Public Health* **48,** 1323-1334.

Kempster PL, Van Vliet HR and Kuhn A (1997) The need dor guidelines to bridge the gap beteeb ideal drinking water quality and that quality which is practical achievable and acceptable. *Water SA* **23,** 163-167.

Kirkwood A (1998) Safe water for Africa. DFID Conference Features. *Africa Health* 9-11.

Kjellberg S, Humphrey BA and Marshall KC (1983) Initial phases of starvation and activity of bacteria at surfaces. *Applied Environmental Microbiology* **46,** 978-984.

Knight S, Toodayan W, Caique J, Kyi W, Barnes A and Desmarchelier P (1992) Risk factors for the transmission of diarrhoea in children: A case-control study in rural Malaysia. *International Journal of Epidemiology* **21,** 812-818.

Knittle M (1975) Role of hand contamination of personnel in the epidemiology of Gram negative nosocomial infections. *Journal of Pediatrics* **86,** 433-437.

Kosek M, Bern C and Guerrant RL (2003) The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. *Bulletin of the World Health Organization* **81,** 197-204.

Krumperman PH (1983) Multiple antibiotic resistance indexing of *Escherichia coli* to identify high risk sources of fecal contamination of foods. *Applied and Environmental Microbiology* **46,** 165-170.

Kuan MM (1997) Detection and rapid identification of human Enteroviruses following genomic amplification. *Journal of Clinical Microbiology* **35,** 285-291.

Kuhnert P, Boerlin P and Frey J (2000) Target genes of virulence assessment of *Escherichia coli* isolates from water, food and environment. *Federation of the European Microbiological Society Reviews* **24,** 107-117.

Kukula M, Arstila P, Klossner ML, Maunula L, Von Bonsdorf CH and Jaathinen P (1997) Waterborne outbreak of viral gastroenteritis. *Scandanavian Journal of Infectious Diseases* **29,** 415-418.

Kukula M, Maunula L, Silvennoinen E and Von Bonsdorff CH (1999) Outbreak of viral gastroenteritis due to drinking waterncontaminated by Norwalk-like viruses. *Journal of Infectious Diseases* **180,** 1771-1776.

Labrap (2005) Newsletter of the NHLS: Outbreak news. July/Augustus 2005. ISSN: 1812-545X.

Lebuhn M, Effenberger M, Garces A and Wilderer PA (2004) Evaluating real-time PCR for the quantification of distinct pathogens and indicator microorganisms in environmental samples. *Water, Science and Technology* **50,** 263-270.

LeChevallier MW and Au KK (2004) Water treatment and pathogen control. Process efficiency in achieving safe drinking water. World Health Organization, Geneva. ISBN 924-156255-2.

LeChevallier MW and McFeters GA (1985) Interactions between heterotrophic plate count bacteria and coliform organisms. *Applied and Environmental Microbiology* **49,** 1338-1341.

LeChevallier MW, Evans TM and Seidler RJ (1981) Effect of turbidity on chlorination efficiency and bacterial persistence in drinking water. *Applied and Environmental Microbiology* **42,** 159-167.

LeChevallier MW, Hassenauer TS, Camper A and McFeters GA (1984) Disinfection of bacteria attached to granular activated carbon. *Applied and Environmental Microbiology* **48,** 918-923.

LeChavellier MW, Welch NJ and Smith DB (1996) Full scale studies of factors related to coliform regrowth in drinking water. *Applied and Environmental Microbiology* **62,** 2201-2211.

Leclerc H, Edberg S, Pierzo V and Delattre JM (2000) A review: Bacteriophages as indicators of enteric viruses and public health risk in groundwaters. *Journal of Applied Microbiology* **88,** 5-21.

Leclerc H, Schwartzbrod L and Dei-Cas E (2002) Microbial agents associated with waterborne diseases. *Critical Reviews in Microbiology* **28,** 371-409.

Leeming R, Ball A, Ashbolt N and Nichols P (1996) Using fecal sterols from humans and animals to distinguish fecal pollution in receiving waters. *Water Research* **30,** 2893-2900.

Lehloesa LJ and Muyima NYO (2000) Evaluation of the impact of household treatment procedures on the quality of groundwater supplies in the rural community of the Victoria district, Eastern Cape. *Water SA* **26,** 285-290.

Lewis GD (1995) F-specific bacteriophages as an indicator of human viruses or natural viruses in natural waters and sewage effluents in Northern New Zealand. *Water, Science and Technology* **31,** 231-234.

Li JW, Xin ZT, Wang XW, Zheng JL and Chao FH (2002) Mechanisms of inactivation of Hepatitis A virus by chlorine. *Applied and Environmental Microbiology* **68,** 4951-4855.

Lillis TO and Bissonnette GK (2001) Detection and characterisation of filterable heterotrophic bacteria from rural groundwater supplies. *Letters in Applied Microbiology* **32,** 268-272.

Lim CH and Flint KP (1989) The effects of nutrients on the survival of *Escherichia coli* in lake water. *Journal of Applied Microbiology* **66,** 559-569.

Lin J, Biyda PT and Puckree (2004) Antibiotic resistance profiles and environmental isolates from Mhlathuze River, KwaZulu-Natal (RSA). *Water SA* **30,** 23-28.

Lin CM, Wu FM, Kim HK, Doyle MP, Michael BS and Williams LK (2003) A comparison of hand washing techniques to remove *Eswcherichia coli* and Caliciviruses under natural or artificial fingernails. *Journal of Food Protection* **66,** 2296-2301.

Lindskog RUM and Lindskog PA (1988) Bacteriological contamination of water in rural areas: An intervention study from Malawi. *Journal of Tropical Medicine and Hygiene* **91,** 1-7.

Lindskog P and Lundqvist J (1989) Why poor children stay sick: The human ecology of child health and welfare in rural Malawi. Scandanavian Institute of African Studies, Uppsala, Sweden.

Listle JT, Smith JJ, Edwards DD and McFeters GA (2004) Occurrence of microbial indicators and *Clostridium perfringens* in wastewater, water column samples, sediments, drinking water and Weddell seal feces collected at McMurdo Station, Antarctica. *Applied and Environmental Microbiology* **70,** 7269-7276.

Luby SP, Agboatwalla M, Raza A, Mintz E, Sobel J, Hussain S, Husan R, Ghouri F, Baier K and Gangarosa G (1998) Microbiologicl evaluation and community acceptance of a plastic water storage vessel, point-of-use water treatment and hand washing in Karachi, Parkistan. Presented at International Night, 47th Annual EIS Conference, Atlanta, USA. April 1998.

Luby SP, Agboatwalla M, Raza A, Sobel J, Mint ED, Baier K, Hoekstra RM, Rahbar MH, Hassan R, Qureshi SM and Gangarosa EJ (2001) Microbiological effeciveness of hand washing with soap in an urban squatter settlement, Karachi, Pakistan. *Epidemiology and Infection* **127,** 237-244.

Luby SP, Agboatwalla M, Raza A, Sobel J, Mintz E, Baier K, Rahbar M, Qureshi SM, Hassan R, Ghouri F, Hoekstra RM and Gangarosa E (2001) A low-cost intervention for cleaner drinking water in Karachi, Pakistan. *International Journal of Infectious Diseases* **5,** 144-150.

Lund V and Ormerod K (1995) The influence of disinfection processes on b iofilm formation in water distribution systems. *Water Research* **29,** 1013-1021.

Lye DJ and Dufour AP (1991) A membrane filter procedure for assaying toxic activity in heterotrophic bacteria isolated from drinking water. *Journal of Applied Microbiology* **70,** 89-94.

Macy JT and Quick RE (1998) Letter to the Editors: Evaluation of a novel drinking water treatment and storage intervention in Nicaragua. *Pan American Journal of Public Health* **3,** 135-136.

Mac Faddin JF (1980) Biochemical tests for identification of medical bacteria (2nd Ed) Williams and Wilkons, Baltimore, USA. Pp 527.

Maier RM, Pepper IL and Gerba CP (2000) Environmental Microbiology. Academic Press, New York. Pp 585.

Mainil JG, Jaquemin E, Pohl P, Fairbrother JM, Ansuini A, Le Bouguenec C, Ball HJ, De Rycke J, and Oswald LD E (1999) Comparison of necrotoxigigenic *Escherichia coli* isolates from farm animals and from humans. *Veterinary Microbiology* **70,** 123-135.

Mara DD and Oragui JI (1985) Occurance of *Rhodococcus coprophilus* and associated actinomycetes in feces, sewage and freshwater. *Applied and Environmental Microbiology* **51,** 85-93.

Maraj S, Rodda N, Jackson S, Buckley C and Macleod N (2006) Microbial deterioration of stored water for users supplied by stand pipes and ground tanks in a peri-uban community. *Water SA* **32,**693-699.

Masago Y, Katayama H, Hashimoto A, Hirata T and Ohgaki S (2002) Assessment of risk of infection due to *Cryptosporidium parvum* in drinking water. *Water, Science and Technology* **46,** 319-324.

Mascher F, Dellar S, Picher-Semmelrock FP, Roehen S and Math E (2003) The significance of sunlight for the elimination of indicator bacteria in small scale bathing ponds in Central Europe. *Water, Science and Technology* **47,** 211-213.

McFeters GA, Kippen JS and LeChevallier MW (1986) Injured coliforms in drinking water. *Applied and Enviironmental Microbiology* **51,** 1-5.

McGuigan KG, Joyce TM and Conroy RM (1999) Solar disinfection: Use of sunlight to decontaminate drinking water in developing countries. *Journal of Medical Microbiology* **48,** 785-787.

Meslin FX (1997) Global perspective of emerging and potential zoonoses: A WHO perspective. *Emerging Infectious Diseases* **3,** 223-228.

Mertens TE, Fernando MA, Marshal TF, Kirkwood BR, Cairncross S and Radalowich A (1990) Determinants of water quality, availability and use in Kurenegala, Sri Lanka. *Tropical Medical Parasitology* **41,** 89-97.

Meyer V and Reed RH (2000) SOLAIR disinfection of hand drawn drinking water. Oral presentation at WISA 2000 Biennial Conference, Sun City, South Africa. 28 May to 01 June.

Miller FD (1984) Problem of water storage in the rural village home: The Egyptian Zir. *Journal of Tropical Medicine and Hygiene* **87,** 53-59.

Mills CJ, Bull RJ, Cantor KP, Reif J, Hrudey SE and Huston P (1999) Health risks of drinking water chlorination by-products: Report of an expert working group. *Chronicles of Diseases in Canada* **19,** 91-102.

Mintz ED, Reiff FM, Robert V and Tauxe MD (1995) Safe water treatment and storage in the home: A practical new strategy to prevent waterborne disease. *Journal of the American Medical Association* **273,** 948-953.

Mitchell DO and Starzyk MJ (1975) Survival of *Salmonella* and other indicator microorganisms. *Canadian Journal of Microbiology* **21,** 1420-1421.

Moe CL, Sobsey MD, Samsa GP and Mesolo V (1991) Bacterial indicators of risk of diarrhoeal disease from drinking water in the Phillipines. *Bulletin of the World Health Organization* **69,** 305-317.

Molbak K, Hojlyng N, Jepsen S and Gaarsler K (1989) Bacterial contamination of stored water and stored food. A potential same source of diarrhoea in West Africa. *Epidemiology and Infection* **102,** 309-316.

Momba MNB and Kaleni P (2002) Regrowth and survival of indicator microorganisms on the surfaces of household containers used for the storage of drinking water in rural communities of South Africa. *Water Research* **36,** 3023-3028.

Momba MNB and Notshe TL (2003) The microbiological quality of groundwater-derived drinking water after long storage in household containers in a rural community of South Africa. *Journal of Water Supply: Research and Technology-AQUA* **52**, 67-77.

Momba MNB and Mnqumevu BV (2000) Detection of fecal coliform bacteria and heterotrophic plate count bacteria attached to household containers during the storage of drinking groundwater in a rural community. Paper presented at the WISA 2000 Biennial Conference, Sun City, South Africa, 28 May – 01 June 2000.

Mong Y, Kaiser R, Rasoatiana DI, Razafimbololona I and Quick RE (2001) Impact of the safe water system on water quality in cyclone-affected communities in Madagascar. *American Journal of Public Health* **91,** 1577-1579.

Mooijman KA, Bahar M, Conteras N and Havelaar AH (2001) Optimisation of the ISO ruite on enumeration of somatic bacteriophages. *Water, Science and Technology* **43,** 205-208.

Mooijman KA, Bahar M, Munesa M and Havelaar AH (2002) Optimisation of ISO 10705-1 on enumerationof F-specific phages. *Journal of Virological Methods* **103,** 129-136.

Morris R (1993) Reduction of microbial levels in sewage effluents using chlorine and paracetic acid disinfectants. *Water, Science and Technology* **27,** 387-393.

Moyo S, Wright J, Ndamba J and Gundr S (2004) Realising the maximum health benefit from water quality improvements in the home: A case study from Zaka District, Zimbabwe. *Physics and Chemistry of the Earth* **29,** 1295-1299.

Musa HA, Shears P, Kafi S and Elsabong SK (1999) Water quality and Public health in northern Sudan: A study of rural and peri urban communities. *Journal of Applied Microbiology* **87,** 676-682.

Nala NP, Jagals P and Joubert G (2003) The effect of a water-hygiene educational programme on the microbiological quality of container-stored water in households. *Water SA* **29,** 171-176.

Nasser AM and Oman SD (1999) Quantitative assessment of the inactivation of pathogenic and indicator viruses in natural water sources. *Water Research* **33,** 1748-1752.

Nath KJ, Bloomfield S and Jones M (2006) Household water storage, handling and point-of-use treatment. International Scientific Forum on Home Hygiene. Internet URL: http://www.ifh-homehygiene.org

Nebra Y, Bonjoch X and Blanch AR (2003) Use of *Bifidobacterium dentium* as an indicator of the origin of fecal water pollution. *Applied and Environmental Microbiology* **69,** 2651-2656.

NICD - National Institute for Communicable Diseases (2004a) Communicable Diseases Surveillance Bulletin. Cholera. November 2004. Internet URL: http://www.nicd.ac.za.

NICD - National Institute for Communicable Diseases (2004b) *Communicable Diseases Communique* **3**, November 2004.

NRC (2004) Indicators for waterborne pathogens. Committee on Indicators for Waterborne Pathogens, National Research Council. Internet URL: http://www.nap.edu/catalog/11010.html.

Oates PM, Shanahan P and Polz MF (2003) Solar disinfection (SODIS): Simulation of solar radiation for global assessment and application for point-of-use water treatment in Haiti. *Water Research* **37,** 47-54.

Obi CL, Potgieter N, Bessong PO and Matsaung G (2002) Assessment of the microbial quality of river water sources in rural Venda communities in South Africa. *Water SA* **28,** 287-292.

Obi CL, Potgieter N, Bessong PO and Matsuang G (2003) Scope of potential bacterial agents of diarrhoea and microbial assessment of quality of river water sources in rural Venda communities in South Africa. *Water, Science and Technology* **47,** 59-64.

Obi CL, Green E, Bessong PO, De Villiers B, Hoosen AA, Igumbor EO and Potgieter N (2004) Gene encoding virulence markers among *Escherichia coli* isolates from diarrhoic stool samples and river sources in rural Venda communities of South Africa. *Water SA* **30,** 37-42.

Ogorzaly L and Zanther C (2006) The development of real time RT-PCR for specific detection of F-specific RNA bacteriophage genotypes. *Journal of Virological Methods* **138,** 131-139.

Olson BH, Clark DL, Milner BB, Stewart MH and Wolfe RL (1991) Total coliform detection in drinking water: Comparison of membrane filtration with *Colilert* and *Coliquick. Applied and Environmental Microbiology* **57,** 1535-1539.

Orden JA, Ruiz-Santa Quiteria JA, Cid D, Garcia S and Lafuerter DA (1999) Prevalence and characteristics of necrotoxigenic *Escherichia coli* (NTEC) isolated from diarrhoeic calves. *Veterinary Microbiology* **66,** 265-273.

Osawa S, Furuse K and Watanabe I (1981) Distribution of ribonucleic acid coliphages in animals. *Applied and Environmental Microbiology* **41,** 164-168.

Patel M and Isaacson M (1989) Survival of *Vibrio cholera* in African domestic water storage containers. *South African Medical Journal* **76,** 365-367.

Payment P and Franco E (1993) *Clostridium perfringens* and somatic coliphages as indicators of the efficiency of drinking water treatment for viruses and protozoan cysts. *Applied and Environmental Microbiology* **59,** 2418-2424.

Payment P, Richardson L, Siemiatychi J, Dewar R, Edwardes M and Franco E (1991) A randomised trial to evaluate the risk of gastrointestinal disease due to consumption of drinking water meeting current microbiological standards. *American Journal of Public Health* **81,** 703-708.

Pinfold JV (1990) Faecal contamination of water and fingertip-rinses as a method for evaluating the effect of low-cost water supply and sanitation activities on faeco-oral disease transmission (II). A hygiene study in rural north-east Thailand. *Epidemiological Infections* **105,** 377-389.

Pinto RM, Diez JM and Bosch A (1994). Use of the colonic carcinoma cell line CaCo-2 for in vivo amplification and detection of enteric viruses. *Journal of Medical Virology* **44,** 310-315.

Potgieter N (1997) Recovery of small number of viruses from large volumes. Masters of Science degree in Medical Virology, Department of Medical Virology, University of Pretoria, Pretoria, South Africa.

Pournadeali E and Tayback M (1980) Potable water and village health: Is primary prevention affordable? *Public Health Report* **95,** 291-294.

Power KG and Nagy LA (1999) Relationships between bacterial regrowth and some physical and chemical parameters within Sydney's drinking water distribution system. *Water Research* **33,** 741-750.

Puig A, Queralt N, Jofre J and Araujo R (1999) Diversity of Bacteroides fragilis strains in their capacity to recover phages from human and animal wastes and from fecally polluted wastewater. *Applied and Environmental Microbiology* **65,** 1772-1776.

Quick RE, Gerber ML and Palacois AM (1996) Using a knowledge, attitude and practices survey to supplement findings of an outbreak investigation: Cholera prevention measures during the 1991 epidemic in Peru. *International Journal of Epidemiology* **25,** 872-878.

Quick RE, Kimura A, Thevus A, Tembo M, Shamputa I, Hutwagner L and Mintz E (2002) Diarrhea prevention through household-level water disinfection and safe storage in Zambia. *American Journal of Tropical Medicine and Hygiene* **66,** 584-589.

Quick RE, Venczel LV, Mintz ED, Soleto L, Aparicio J, Gironaz M, Hutwagner L, Greene K, Bopp C, Maloney K, Chavez D, Sobsey M and Tauxe RV (1999) Diarrhoea prevention in Bolivia through point-of-use water treatment and safe storage: A promising new strategy. *Epidemiological Infections* **122,** 83-90.

Rajasekaran P, Dutt P and Pisharoti K (1977) Impact of water supply on the incidence of diarrhoea and shigellosis among children in rural communities in Madurai. *Indian Journal of Medical Research* **66,** 189-199.

Ramteke PW, Bhattacharjee JW, Pathak SP and Kalra N (1992) Evaluation of coliforms as indicators of water quality in India. *Journal of Applied Bacteriology* **72,** 352-356.

Raphael RA, Sattar SA and Springthorpe VS (1985) Longterm survival of human rotavirus in raw and treated river water. *Canadian Journal of Microbiology* **31,** 124-128.

Regli S, Rose JB, Haas CN and Gerba CP (1991) Modeling the risk from *Giardia* and viruses in drinking water. *Journal of the American Water Works Association* **83,** 76.

Reiff FM, Roses M, Venczel L, Quick R and Witt VM (1996) Low cost safe water for the world: A practical interim solution. *Journal of Public Health Policy* **17,** 389-408.

Reller ME, Mendoza CE, Lopez MB, Alvarez M, Hoekstra RM, Olson CA, Baier KG, Keswick BH and Luby SP (2003) A randomised trial of household based flocculant disinfectant drinking water treatment for diarrhoea prevention in rural Guatemala. *American Journal of Tropical Medicine and Hygiene* **69,** 411-419.

Republic of South Africa (RSA) (1994). Water supply and sanitations policy for South Africa. Department of Water Affairs, Pretoria, South Africa.

Rice EW, Allen MJ, Brenner DJ and Edberg SC (1991) Assay for ß-glucuronidase in species of the genus *Escherichia coli* and its applications for drinking water analysis. *Applied and Environmental Microbiology* **57,** 592-593.

Rice EW, Allen MJ and Edberg SC (1990) Efficacy of ß-glucuronidase assay for the identification of *Escherichia coli* by the defined substrate technology. *Applied and Environmental Microbiology* **56,** 1203.

Rijal GK and Fujioka RS (2001) Synergistic effect of solar radiation and solar heating to disinfect drinking water sources. *Water, Science and Technology* **43,** 155-162.

Roberts L, Chartier Y, Chartier O, Malenga G, Toole M and Rodka H (2001) Keeping clean water clean in a Malawi refugee camp: A randomised intervention trial. *Bulletin of the World Health Organization* **79,** 280-287.

Rosas I, Salinas E, Martinez L, Calva E, Cravioto A, Eslava C and Amabile-Cuevas CF (2006) Urban dust fecal pollution in Mexico City: Antibiotic resitance and virulence factors of *Escherichia coli*. *International Journal of Hygiene and Environmental Health* **209,** 461-470.

Rose JB, Zhou X, Griffen DW and Paul JH (1997) Comparison of PCR and plaque assay for detection and enumeration of coliphage in polluted marine waters. *Applied and Environmental Microbiology* **63,** 4564-4566.

Rousselon N, Delgenes JP and Godon JJ (2004) A real time PCR (TaqMan PCR) system for detection of 16S rDNA gene associated with fecal bacteria. *Journal of Microbiological Methods* **59,** 15-22.

Rueckert RR (1985) Picornaviruses and their replication. In Fields BN, Knipe DM, Chanock RM, Melnick JL, Roizman B and Shope RM (Eds). Fields Virology. Raven Press, New York. P705-738.

SABS (2001) Specifications for drinking water (Ed 5). SANS 241-2001. South African Bureau of Standards, Pretoria. Pp 9.

Sacks JL, Lieb S, Baldy LM, Berta S, Patton CM, White MC, Bigler WJ and Witte JJ (1986) Epidemic Campylobacteriosis associated with a community water supply. *American Journal of Public Health* **76,** 424-429.

Sandiford P, Gorter A, Davey Smith G and Pauw J (1989) Determinants of drinking water quality in rural Nicaragua. *Journal of Epidemiology and Infection* **102,** 429-438.

Sattar SA (1981) Virus survival in receiving waters. In Goddard M and Butler M (eds). Viruses and wastewater treatment. Pergamon Press Inc., Elmsford, New York. P91-108.

Saville MG, Murray SR, Scholes P, Maas EW, McCormick RE, Moore EB and Gilpin BJ (2001) Application of polymerase chain reaction (PCR) and TaqMan PCR techniques to the detection and identification of *Rhodococcus coprophilus* in faecal samples. *Journal of Microbiological Methods* **47,** 355-368.

Schaper M and Jofre J (2000) Comparison of methods for detecting genotypes of F-specific RNA bacteriophages and fingerprinting the origin of fecal pollution in water samples. *Journal of Virological Methods* **89,** 1-10.

Schaper M, Jofre J, Uys M and Grabow WOK (2002a) Distribution of genotypes of F-specific RNA bacteriophages in human and non-human sources of faecal pollution in South Africa and Spain. *Journal of Applied Microbiology* **92,** 657-667.

Schaper M, Duran AE and Jofre J (2002b) Comparative resistance of phage isolates of four genotypes of F-specific RNA bacteriophages to various inactivation processes. *Applied and Environmental Microbiology* **68,** 3702-3707.

Seligman R and Reitler R (1965) Enteropathogens in water with low *Escherichia coli* counts. *Journal of American Water Works Association* **57,** 1572-1574.

Semenza JC, Roberts L, Henderson A, Bogan J and Rubin CH (1998) Water distribution system and diarrheal disease transmission: A case study in Uzbekistan. *American Journal of Tropical and Medical Hygiene* **59,** 941-946.

Sergeant D (1999) Fecal contamination source identification methods in surface water. Washington State Department of Ecology, Washington, USA.

Shadix CC and Rice EW (1991) Evaluation of ß-glucuronidase assay for the detection of *Escherichia coli* from environmental waters. *Canadian Journal of Microbiology* **37,** 908-911.

Shaffer PTB, Metcalf TG and Sproul OJ (1980) Chlorine resistance of poliovirus isolants recovered from drinking water. *Applied and Environmental Microbiology* **40,** 1115-1121.

Shahid N, Greenough E, Samadi A, Huq M and Rahaman N (1996) Hand washing with soap reduces diarrhoea and spread of bacterial pathogens in a Bangladesh village. *Journal of Diarrhoeal Research* **14,** 85-89.

Shears P, Hussein A, Chowdhurryu A and Mamun K (1995) Water sources and environmental transmission of multiply resistant enteric bacteria in rural Bangladesh. *Annual Tropical Medical Parasitology* **89,** 297-303.

Shuval HI, Thompson A, Fattal B, Cymbalista S and Wiener Y (1971) Natural virus inactivation processes in sea water. *Proceedings of the American Society of Civil Engineering* **5,** 587-600.

Simango C, Dindiwe J and Rukere G (1992) Bacterial contamination of food and household stored drinking water in a farmworker community in Zimbabwe. *Central African Journal of Medicine* **34,** 143-149.

Sinton LW and Donnison AM (1994) Characterization of faecal streptococci from some New Zealand effluents and receiving waters. *New Zealand Journal of Marine and Freshwater Research* **28,** 145-156.

Sinton LW, Finley RK and Hannah DJ (1998) Distinguishing human from animal faecal contamination in water: A review. *New Zealand Journal of Marine and Fresh Water Research* **32,** 323-348.

Skraber S, Gassilloud B, Schwartzbrod L and Gantzer C (2004) Survival of infectious poliovirus-1 in river water compared to the persistence of somatic coliphages, thermotolerant coliforms and poliovirus-1 genome. *Water Research* **38,** 2927-2933.

Slifko TR, Smith HV and Rose JB (2000) Emerging parasite zoonoses associated with water and food. *International Journal of Parasitology* **30,** 1379-1393.

Sobel J, Mahon B, Mendoza CE, Passaro D, Cano F, Baier K, Racioppi F, Hutwagner L and Mintz E (1998) Reduction of fecal contamination of street-vended beverages in Guatemala by a simple system for water purification and storage, handwashing and beverage storage. *American Journal of Tropical Medicine and Hygiene* **59,** 380-387.

Sobsey MD (1989) Inactivation of Health related microorganisms in water by disinfection processes. *Water Science and Technology* **21,** 179-196.

Sobsey MD (2002) Managing water in the home: Accelerated health gains from improved water supply. World Health Organization Sustainable Development and Healthy Environments. World Health Organization, Geneva. WHO/SDE/WSH/02.07.

Sobsey MD, Dufour AP, Gerba CP, LeChavallier MW and Payment P (1993) Using a conceptual framework for assessing risks to health from microbes in drinking water. *Journal of the American Water Works Association* **85,** 44-48.

Sobsey MD, Battigelli D, Handzel T and Schwab K (1995) Male-specific coliphages as indicators of viral contamination of drinking water. AWWA Research Foundation, Denver, CO, USA.

Sobsey MD, Handzel T and Venczel L (2003) Chlorination and safe storage of household drinking water in developing countries to reduce waterborne disease. *Water, Science and Technology* **47,** 221-228.

Spira W, Khan M, Saeed Y and Sattar M (1980) Microbiological surveillance of intra-neighbourhood El Tor cholera transmission in rural Bangladesh. *Bulletin of the World Health Organization* **58,** 731-740.

Standard Methods (1995) Standard methods for the examination of water and wastewater (19th Ed). Eaton AD, Clesceri LS and Greenberg AE (eds). American Public Health Association, Washington DC, USA.

Statistics South Africa (2003) Census in brief. Report 03-02-03 (2001). Pretoria, South Africa.

Sutton S and Mubiana D (1989) Household water quality in rural Zambia. *Waterlines* **8,** 88-89.

Swerdlow DL, Mintz ED, Rodriquez M, Tejada E, Oxcampo C, Espejo L, Greene KD, Saldana W, Seminario L and Tauxe RV (1992) Waterborne transmission of epidemic cholera in Trujillo, Peru: Lessons for a continent at risk. *Lancet* **340,** 28-33.

Tartera C and Jofre J (1987) Bacteriophages active against *bacteroides fragilis* in sewage-polluted waters. *Applied and Environmental Microbiology* **53,** 1632-1637.

Taylor DH, Moore RS and Sturman LS (1981) Influence of pH and electrolyte composition on adsorption of poliovirus by soils and minerals. *Applied and Environmental Microbiolgy* **42,** 976-984.

Theron J and Cloete TE (2002) Emerging waterborne infections: contributing factors, agents and detection tools. *Critical Reviews in Microbiology* **28,** 1-26.

Thompson JA (1981) Inadequacy of *Escherichia coli* as an indicator of water pollution in a tropical climate: A preliminary study in Botswana. *South African Journal of Science* **77,** 44-45.

Tree JA, Adams MR and Lees (DN (1997) Virus inactivation during disinfection of wastewater by chlorination and UV irradiation and the efficacy of F+ bacteriophage as a viral indictor. *Water, Science and Technology* **35,** 227-232.

Tree JA, Adams MR and Lees DN (2003) Chlorination of indicator bacteria and viruses in primary sewage effluent. *Applied and Environmental Microbiology* **69,** 2083-2043.

Trevett AF, Carter RC and Tyrell SF (2005) The importance of domestic water quality management in the context of faecal-oral disease transmission. *Journal of Water and Health* **3,** 259-270.

Turner SJ, Lewis GD and Bellamy AR (1996) Use of multiplex PCR and automated DNA detection for tracing sewage derive *Escherichia coli* in a rural stream. Abstract, IAWQ, Health Related Water Microbiology Conference, Mallorca, Spain, 6th to 10th October. Pp 55.

UN (2000) United Nations Millennium Declaration. General Assembly Res. 55/2 (18 September 2000)

USEPA (1989) Health effects of drinking water treatment technologies. Drinking water task team (Eds). Lewis Publishers Inc., New York.

Uys M (1999) Molecular characterization of F-specific RNA phages in South Africa. Masters of Science degree in Medical Virology, Department of Medical Virology, University of South Africa, Pretoria, South Africa.

Vanderslice J and Briscoe J (1993) All coliforms are not created equal – a comparison of the effects of water source and in-house water contamination on infantile diarrhoeal disease. *Water Resources Research* **29,** 1983-1995

Vaughn JM and Metcalf TG (1975) Coliphages as indicators of enteric viruses in shellfish and shellfish-raising estuarine waters. *Water research* **9,** 613-616.

Vaughn JM, Chen YW and Thomas MZ (1986) Inactivation of human and simian rotaviruses by chlorine. *Applied and Environmental Microbiology* **51,** 391-394.

Venczel L (1997) Evaluation and application of a mixed oxidant disinfection system for waterborne disease prevention. Doctoral Dissertation, Department of Environmental

Sciences and Engineering, University of North Carolina, Chapel Hill, North Carolina, USA.

Verweij P, Van Egnod M, Bac DJ, Van der Schroef JG and Mouton RP (1991) Hygiene, skin infections and types of water supply in Venda, South Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **85,** 681-684.

Wang M, Ahrne S, Antonsson M and Molin G (2004) T-RFLP combined with principal component analysis and 16S rRNA gene sequencing: An effective strategy for comparison of fecal microbiota in infants of different ages. *Journal of Microbiological Methods* **59,** 53-69.

Ward RL and Winston PE (1985) Development of methods to measure virus inactivation in fresh waters. *Applied and Environmental Microbiology* **50,** 1144-1148.

Watanabe I, Miyake T, Sakurai T, Shiba T and Ohno T (1967) Isolation and grouping of RNA bacteriophages. *Proceedings of the Japanese Academy* **43,** 204-209.

Wei G, Pan L, Du H, Chen J and Zhao L (2004) ERIC-PCR fingerprinting-based community DNA hybridization to pinpoint genome-specific fragments as molecular markers to identify and tract populations common to healthy human guts. *Journal of Microbiological Methods* **59,** 91-108.

Welch P, David J, Clarke W, Trinidade A, Penner D, Bernstein S, McDougall L and Adesiyn AA (2000) Microbial quality of water in rural communities in Trinidad. *Pan American Journal of Public Health* **8,** 172-180.

Wheather DWF, Mara DD, Jawad L and Oragui J (1980) *Pseudomonas aeruginosa* and *Escherichia coli* in sewage and fresh water. *Water Research* **14,** 713-721.

White GC (1999) Handbook on chlorination and alternative disinfectants (3rd ED) Van Nostrand Reinhold Com., New York. Pp 1308.

White GF, Bradley DJ and White AU (2002) Drawers of water: Domestic use in East Africa (1972). *Bulletin of the World Health Organization* **80,** 63-73.

WHO (1993) Improving water and sanitation hygiene behaviours for the reduction of diarrhoeal disease: The report of an informal consultation. World Health Organization, Geneva. WHO/CDD/CWS WHO.

WHO (1996a) Guidelines for drinking-water quality. (2nd Ed). Volume 2. Health criteria and other supporting information. World Health Organization, Geneva. Pp 973.

WHO (1996b) Global Health-for-All indicators. Internet URL: http://www.who.ch/hst/hsp/a/globindi.htm.

WHO (1996c) Water and Sanitation. Fact sheet no. 112. Internet URL: http://www.who.org/inf/fs/fact112.html.

WHO (2000) Global Water Supply and Sanitation Assessment Report. World Health Organization, Geneva. ISBN 92-4156202-1.

WHO (2002a) The world health report: Reducing risks, promoting healthy life. World Health Organization, Geneva. Internet URL: http://www.who.int/whr/en.

WHO (2002b) Water for Development: A practical advocacy guide for World Water Day 2002. Internet URL: http://www.worldwaterday.org/advocacy/adv.html.

WHO (2002c) Heterotrophic plate count measurement in drinking water safety management, Protection of the Human environment. Water, Sanitation and Health. World Health Organization, Geneva. WHO/SDE/WSH/02.10.

WHO (2004) Guidelines for drinking water quality, 3rd Ed, Volume 1. Recommendations. World Health Organisation, Geneva.

WHO (2005) World Health Statistics. World Health Organisation Press, Geneva, Switserland. ISBN 92 4 159326 1.

Wilson JM and Chandler GN (1993) Sustained improvements in hygiene behaviour amongst village woman in Lombok, Indonesia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **87,** 615-616.

Wilson JM, Chandler GN, Muslihatun and Jamiluddin (1991) Hand-washing reduces diarrhoea episodes: A study in Lombok, Indonesia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **85,** 819-821.

Woody MA and Cliver DO (1995) Effects of temperature and host cell growth phase on replication of F-specific RNA coliphage Qß. *Applied and Environmental Microbiology* **61,** 1520-1526.

Wright JA, Gundry SW and Conroy R (2004) Household drinking water in developing countries: A systematic review. *Tropical Medicine and International Health* **9,** 106-117.

Xian-Yu F and Hui-Gang Z (1982) Rural drinking water supply. *American Journal of Public Health* **75,** 52-53.

Yatsuyanagi J, Saito S, Miyajima Y, Amano KI and Enomoto K (2003) Characterization of atypical enteropathogenic *Escherichia coli* strains harboring the *astA* gene that were associated with a waterborne outbreak of diarrhoea in Japan. *Journal of Clinical Microbiology* **41,** 2033-2039.

Young DC and Sharp DG (1977) Poliovirus aggregates and their survival in water. *Applied and Environmental Microbiology* **33,** 168-177.

APPENDIX A

HOUSEHOLD CONSENT FORM

WATER STORAGE IN RURAL HOUSEHOLDS: INTERVENTION STRATEGIES TO PREVENT WATERBORNE DISEASES

HOUSEHOLD NUMBER:……..
NAME:……...
VILLAGE:…….

This study will investigate the quality of stored drinking water from various containers in 60 randomely chosen households in the village. Every household will be given a sodium hypochlorite solution (household bleach) which will either be 3.5%, 1.0% or 0% in order to determine the effectiveness of a chlorine based intervention. The other intervention that will be running together with the sodium hypochlorite solution intervention will be the addition of the CDC safe storage container to 30 of the households. This part of the study will determine the effectiveness of the storage container in improving the microbiological quality of the stored drinking water. The household members agree to participate in this study and will at the end of the study each receive 2 CDC safe storage containers for their participation. A group meeting will also be held at the end of the study to inform all the households taking part in the study, the chief of the village, the clinic staff and other relevant stakeholders like the Department of Water Affairs and the Department of Health, of the outcome of the interventions and to find a common goal to improve water quality in rural households. Each household is free to withdraw from the study at any time. Any personal information on the households and the household members will also be kept anonymous. The results of the study will strictly be used for scientific purposes only.

I…… agree to be part of the study.

Sign:…… Witness:…….

MADI O VHEWAHOMIDINI: DZI TSHANDUKO DZI NO THUSA U THIVHELA U PHADALALA HA MALWADZE A NO PHADALADZWA NGA MADI O TSHIKAFHADZEAHO

MUDI:……..
DZINA:……...
KUSI:…….

Dzingudo dzi khou ita thoduluso kha madi a unwa a ne a vha a zwigubuni mahayani. Ri do nanga midi ya 60 nga mamvate. Mudi munwe na munwe u do wana sodium hypochloride (bleach) (ine yavha 3.5%, 1.0% kana 0%zwi vha zwo sedzana na u sedza kushumele kwayo kha u kunakasi madi). Dzinwe ngudo dzine ra khou ita ndi dza zwigubu zwa CDC zwine ra khou fha madi a 30. Heyi ivha I tshi khou sedza vhudi ha zwigubu zwa CDC ri tshi zwi vhambedza na zwe vha vha vha tshi khou zwi shumisa u vhea khazwo madi. Na zwauri vhudi ha madi a hone (u vha na zwitshili) vhu a fana naa.

Midi yo tenda u dzhenelela kha idzi ngudo ido fhiwa zwigubu zwvhili zwa CDC magumoni a iyi ngudo. Hudo farwa mutangano magumoni a ngudo u ita muvhigo kha vhathu vho dzhenelelaho kha idzi ngudu, Vha-Musanda, manese, vha muhasho wa mutakalo na vhulonda vhathu. Uri vhathu vha hadzimane mihumbulo kha u kunakisa madi a unwa. Munwe na munwe o tendelwa udi bvisa kha dzingudo tshifhinga tshinwe na tshinwe.

Nne:……, ndi khou tenda uvha tshipida tsha idzi ngudo.

Tsiano:…….. Thanzi:……..

APPENDIX B

PAMPHLETS DISTRIBUTED BY THE DEPARTMENT OF HEALTH AND THE DEPARTMENT OF WATER AFFAIRS ON THE USE OF JIK IN SOUTH AFRICA

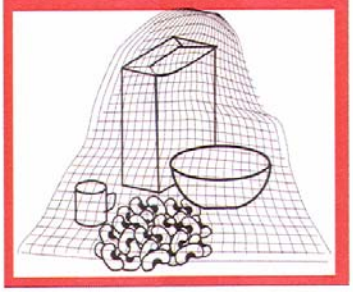
PREVENT AND TREAT CHOLERA

DRINK COOL BOILED WATER OR TREATED WATER

Boil for 1 Minute

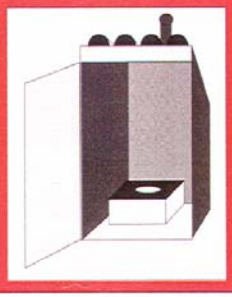
Add 1 teaspoon of Bleach (Jik) to 25 litres of water
Leave standing for at least 2 hours, but it is best to leave it over night, before drinking.

COVER FOOD

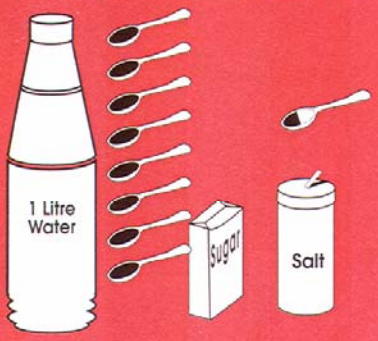


WASH HANDS WITH SOAP AND COOL BOILED OR TREATED WATER

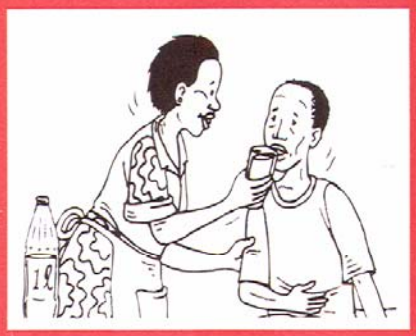
BUILD SAFE TOILETS



TREAT CHOLERA



mix 1 l clean water + 8 teaspoons sugar + half teaspoon salt



drink mixture as often as possible

FOR MORE INFORMATION CONTACT YOUR LOCAL CLINIC

DEPARTMENTS:

WATER AFFAIRS AND FORESTRY • HEALTH • LOCAL AND PROVINCIAL GOVERNMENT
• GOVERNMENT COMMUNICATION AND INFORMATION SYSTEM

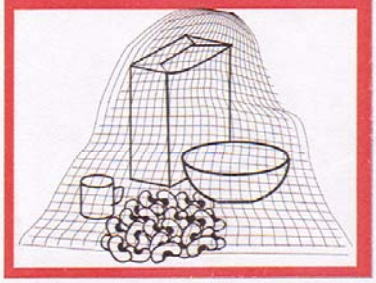
KHA VHA THIVHELE KHOLERA VHA DOVHE VHA I ALAFHE

KHA VHA NWE MADI O VHILISWAHO KANA O SHELWAHO MUSHONGA

Kha vha vhilise minithi muthihi

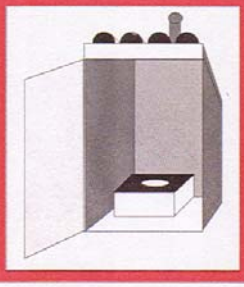
Kha vha shele thisipuni nthihi ya Bulitshi (dzhiki) kha lithara dza 25 dza madi. Vha a litshe awara mbili, thedzi ndi khwine u a litsha vhusiku hothe, phanda ha musi vha tshi a nwa.

VHA TIBE ZWILIWA

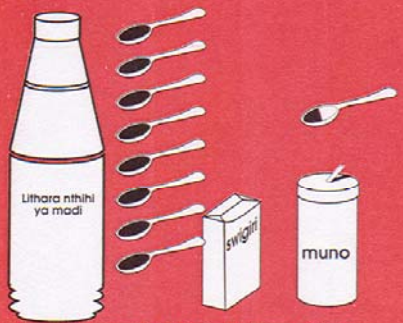


VHA TAMBE ZWANDA NGA TSHISIBE NA MADI O VHILISWAHO O FHOLAHO KANA O SHELWAHO MUSHONGA

KHA VHA FHATE MABUNGA O TSIRELEDZEAHO



KHA VHA ALAFHE KHOLERA

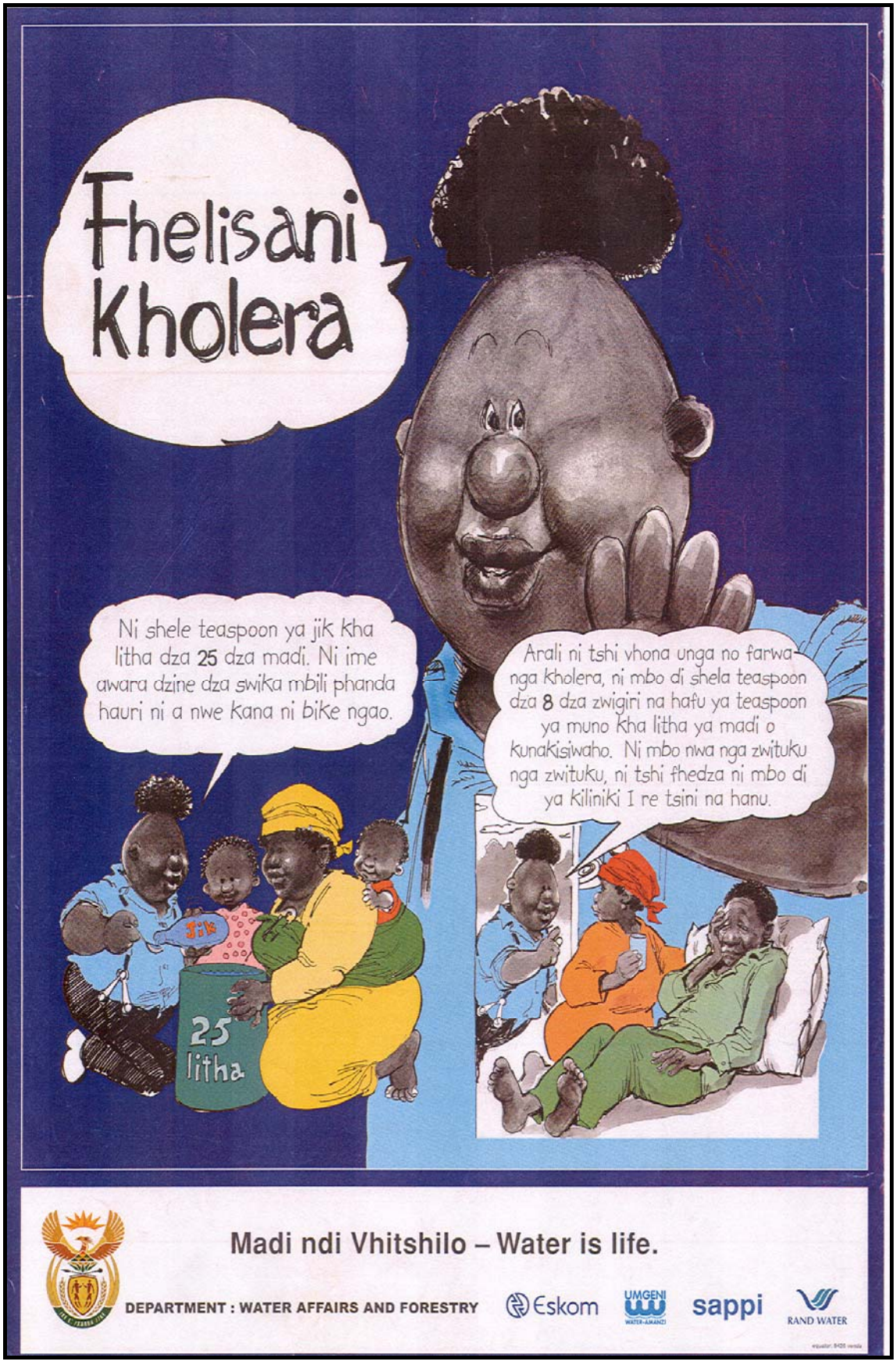


Lithara nthihi ya madi + thisipuni dza malo (8) dza swigiri + hafu ya thisipuni ya muno

kha vha nwe muvango mu nzhi nga hune vha nga kona

ARALI VHA TSHI TODA MAFHUNGO NGA VHUDALO, KHA VHA KWAME KILINIKI YA HAVHO

MIHASHO
ZWA MADI NA ZWA MADAKA. ZWA MUTAKALO. ZWA MUVHUSO WAPO NA WA PHUROVINTSI.
SISITEME YA ZWA MAFHUNGO NA VHUDAVHIDZANI YA MUVHUSO


Fhelisani Kholera
Ni shele teaspoon ya jik kha litha dza 25 dza madi. Ni ime awara dzine dza swika mbili phanda hauri ni a nwe kana ni bike ngao.
Arali ni tshi vhona unga no farwa nga kholera, ni mbo di shela teaspoon dza 8 dza zwigiri na hafu ya teaspoon ya muno kha litha ya madi o kunakisiwaho. Ni mbo nwa nga zwituku nga zwituku, ni tshi fhedza ni mbo di ya kiliniki I re tsini na hanu.
Jik
25 litha
Madi ndi Vhitshilo – Water is life.
DEPARTMENT : WATER AFFAIRS AND FORESTRY
Eskom
UMGENI WATER
sappi
RAND WATER


CHOLERA
WHAT IS CHOLERA?
Cholera is an acute bowel infection caused by a bacterium called Vibrio cholerae. The bacteria cause a severe watery diarrhoea that can be fatal. You can be infected by drinking contaminated water.
SYMPTOMS
Vomiting, severe watery diarrhoea (rice water stool), exhaustion and dehydration.
PREVENTION
Children and elderly people are at greatest risk.
Water must be disinfected by boiling it for at least 2 minutes or bleaching it. Add a teaspoon of bleach (such as Jik) for every 20-25 litres of water and leave it to stand for two hours, preferably overnight.
Always cover food from flies.
HOW DOES CHOLERA AFFECT THE BODY?
• People sometimes drink contaminated water containing cholera bacteria.
• These bacteria travel to the small intestine (gut) where they multiply.
• The bacteria destroy the salt balance of the body and dehydration occurs.
• Water is no longer transported across the intestine and is flushed straight out of the body and as a result the person becomes dehydrated.
• If rehydration does not occur immediately it can result in the failure of organs, coma and death.
Toilets should be far away from the river or stream which is used for cooking and washing.
Do not swim in cholera contaminated rivers.
Fruit, vegetables, utensils, clothing and bedding must be washed in treated water (boil water or use bleach) before use.
Wash hands before handling food, eating and after using the toilet.
If you are in a cholera risk area, do not eat uncooked oysters and shellfish.
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NORTHERN PROVINCE
DEPARTMENT OF HEALTH AND WELFARE

HELPING SOMEONE WHO IS SICK WITH CHOLERA:

- A person can die quickly from loss of body water and salt.
- Quickly give them lots more to drink.
- Make the special drink with sugar and salt (below).
- OR you can use a watery rice or mealie porridge. Mix 1/2 cup rice or mealie meal with 1 litre water and boil for 6 - 7 minutes.
- Children and adults should drink as much as they want but at least one to two cups after each watery stool.
- Continue to feed babies with breast milk and porridge or rice. Also give half a cup of the special drink, slowly by spoon, after each watery stool.

TAKE PEOPLE SICK WITH DIARRHOEA TO YOUR CLINIC OR DOCTOR.

If a person loses lots of water and feels or looks weak, take her to the clinic or doctor immediately. Give more of the special drink or water on the way. This will help to keep the person alive.

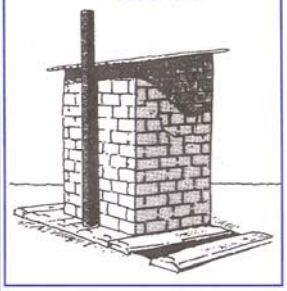
A SPECIAL DRINK FOR TREATING DIARRHOEA AT HOME

Take:	AND	AND
One litre clean water	8 level teaspoons sugar	Half level teaspoon of salt

MIX ALL THREE TOGETHER AND GIVE THE PERSON TO DRINK

4 Adapted by Department of Community Health, 2001: English

Use a toilet



Stop

CHOLERA

Northern Province Department of Health and Welfare

Wash your hands

Sudden watery diarrhoea

WHAT IS CHOLERA?

Cholera germs in the stomach cause diarrhoea. This can lead to rapid loss of body fluids.

HOW DO WE KNOW SOMEONE HAS THE DISEASE?

- The first sign is watery diarrhoea, sometimes with vomiting.
- The person loses a lot of water very quickly.
- They feel weak and can have cramps in the arms and legs.
- The skin can become cold and wrinkled.
- The person may collapse and die.

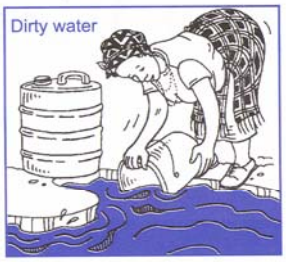
WHERE DO THE GERMS COME FROM?

- Cholera germs live in the intestines of some people.
- Sometimes the germs make people sick and sometimes not.
- Those that do not get sick can carry the germs for a few weeks before the body's natural defences gets rid of them.

HOW DO THE GERMS SPREAD FROM ONE PERSON TO ANOTHER?

- When infected people empty their bowels, the germs come out in the stool (faeces).
- Some stool gets onto a person's hands or into drinking water or food.
- Water or food can look OK but contain the germs. Cholera germs spread to other people who consume this dirty water or food.
- Once inside the body, it can make that person sick.

Dirty water



HOW CAN WE STOP CHOLERA?

- Make sure that germs do not spread from one person to another.

2

Wash fruit and vegetables

- Wash your hands each time after you have been to the toilet.
- Wash your hands before eating or working with food.
- Wash fruit and vegetables before eating.
- Build and use a toilet.
- The toilet should be kept clean.
- It should be far from a borehole or stream used for drinking.
- Do not use water unless you know it is clean.

Boil dirty water

WHAT WATER IS CLEAN AND SAFE?

Tap or borehole water is usually safe. Water from rivers or dams is NOT safe.

To make water safe to drink:
Boil the water, OR
Add bleach (Jik or Javel) to the water as shown below. Use clear water or filter it through a cloth first.
DANGER: Do not drink pure bleach!

If you need a lot of water, add 1 teaspoon or capful of bleach to 25 litres and wait for at least 2 hours or overnight.

Add 1 teaspoon bleach

If a little water is needed, add 5 drops of bleach to one litre and leave for half an hour before using it.

Add 5 drops bleach

3

KU PFUNA MUNHU LOYI A NGA KHOMIWA HI VUVABWI BYA KHOLERA:

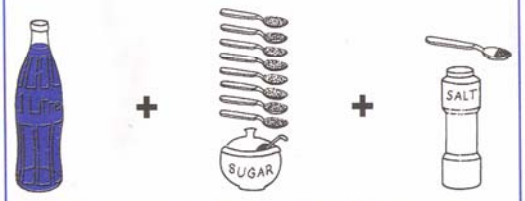
- Munhu a nga fa hi ku hatlisa hi ku heleriwa hi mati ni munyu e mirini wa yena.
- Nyika munhu ya loye swo nwa swo hlawuleka le swi nga cheriwa tshukela ni munyu (xikombiso).
- KUMBE u tirhisa rhayisi ya mati kumbe mukapu wa mapa. Hlanganisa hafu ya khapu ya rhayisa kumbe mapa na litara yinwe ya mati, uswi virisa ku ringana 6-7 wa timinete.
- Vana na lavakulu va nga nwa hi ku rhandza xinwo lexi, kambe va fanele ku nwa tikhapu timbiri endzaku ka ku chuluka.
- Mi nga yimi ku mamisa vana, yanani mahlweni no nyika vana mukhapu kumbe rhayisi leyo vevuka. Tlhelani mi va nyika hafu ya khapu ya xinwo lexo hlawuleka hi lepula endzaku ka nchuluko unwana na unwana.

VANHU LAVA CHULUKAKA VA FANELE KU YISIWA E KLINIKI KUMBE EKA DOKODELA.

Munhu loyi a titwaka a hela matimba hikokwalaho ka ku lahlekeriwa hi mati yo tala emirini u fanele a yisiwa ekliniki kumbe eka dokodela hi ku hatlisa. Munhu loyi u fanele ku nyikiwa mati ku hlayisa vutomi bya yena.

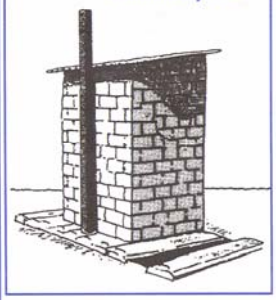
XINWO XO HLAWULEKA XA KU SIVELA KU CHULUKA EKAYA.

Teka:	NA	NA
Litara yinwe ya mati yo basa	swilepulana swa nunghu swa chukele	hafu ya xilepulana xa munyu



USWI HLANGANYISA U NYIKA MUNHU LOYI A CHULUKAKU LESWAKU A NWA

Tirhisani toyilete



Yimisa KHOLERA

Northern Province Department of Health and Welfare

Hlambani mavoko

Nchuluko wa mati wa xihatla

XANA KHOLERA I YINI?

Xitsongwatsongwani xa Kholera e khwirini xi vanga ku chuluka ku fika laha munhu a heleriwaka hi mati e mirini.

XANA HI NGA SWI TIVA NJHANI LESWAKU MUNHU U NA KHOLERA?

- Xikombiso xo sungula i ku chuluka swo vevuka kumbe (mati); kumbe ku hlanta.
- Munhu yaloye u lahlekeriwa hi mati yo tala hi ku hatlisa.
- Va hela matimba va tlhela va khomiwa hi tikirempe e mavokweni ni le milengeni.
- Nhlonge yi nga fika la ha yi titimelaka yi tlhela yi khonyana.
- Munhu a nga titivala kumbe a fa.

XANA XITSONGWATSONGWANA LEXI XI HUMA KWIHI?

- Xitsongwatsongwana xa Kholera xi tshama e marhumbwini ya vanhu vanwana.
- Nkarhi wunwana kholera yi endla kuri vanhu va vabya, kumbe vanga vabyi.
- Vanhu lava va nga vabyiki va nga tshama na xitsongwatsongwana lexi nkarhinyana ku fikela loko miri wu lwa na xitsongwatsongwana lexi.

XANA XITSONGWATSONGWANI LEXI XI TLULELA NJHANI?

- Xitsongwatsongwani xi kumeka eka mahuma ya vanhu lava va nga na xona.
- Xi nga nghena hi le mavokweni kumbe e matini yo nwa kumbe e swakudyeni.
- Mati kumbe swakudya swi nga tikomba swi ri le swinene. Xitsongwatsongwana lexi xi tlulela hi ku nwa mati yo thyaka kumbe swakudya swo thyaka.
- Loko xi nghenile e mirini xi nga endla leswaku munhu a vabya.

Mati ya thyaka

Hlantswa mihandzu ni matsavu

XANA KHOLERA HI NGA YIMISA KU YINI?

Langutisisa leswaku xitsongwatsongwana lexi a xi tluleli vanwana:

- Hlamba mavoko e ndzaku ka loko u huma e xiyindlwanini.
- Hlamba mavoko u nga si dya kumbe ku khoma khoma swakudya.
- Hlantswa mihandzu ni miroho u nga si dya.
- Akani xiyindlwana xo hambukela ka xona mitlhelamixitirhisa.
- Xiyindlwana xi fanele ku tshama xi basile

Virisa mati ya thyaka

- Xiyindlwana xi fanele ku va kule ni laha ku nga ni minkhele ya mati yo nwiwa.
- U nga tirhisi mati loko u nga ri na ntiyiso wa leswaku mati ya basile.

XANA MATI YO TENGA HI WANI?

Mati ya le phayiphini ni mati ya le borho ya sirhelekile. Mati ya le milambwini A YA SIRHELEKANGA.
Ku sirhelela mati yo nwa:
Virisa mati, KUMBE
U chela Jik kumbe Javela e ka mati tani hi laha swi nga kombisiwa laha hansi. Tirhisa mati yo basa kumbe u hluta mati hi lapi ro basa loko u nga si ya tirhisa.

Chela xilepulana xinwe xa Jik

NGHOZI: u nga nwi Jik yi ri yoxe!

Loko u lava mati yo tala, chela xilepulana xinwe kumbe xipfalo xinwe xa Jik e ka mati yo ringana 25 litres, nkarhi wo ringana ti awara ti mbirhi kumbe vusiku byinwe.

Loko ku laveka mati mantsanana, engeta mathonsi ya ntlhanu ya Jik e ka litre yinwe ya mati, yima nkarhi wo ringana xiphemu xa awara.

Chela 5 wa mathonsi ya Jik

APPENDIX C

QUESTIONNAIRRE

THE IMPACT OF WATERBORNE DISEASES IN RURAL COMMUNITIES OF THE VHEMBE REGION IN THE LIMPOPO PROVINCE

PARTICIPANT IN THIS STUDY:

I am aware that the information obtained through this questionnaire will be treated as anonymous and will be used strictly for scientific purposes. I am free to withdraw from the study at any time.

I ..... agree to be part of this study.

Sign:..... Wittness:.....

INSTRUCTIONS TO THE INTERVIEWER:

1. Ask questions and match the answer to the choices. Do not give the choices.
2. Write an X in the appropriate box.
3. If there is no match, choose other and ask the respondent to describe.

A. DEMOGRAPHIC DATA:

1. General household information:

1.1. Name of village

_____

1.2. House number for future visit (any type of identification)

_____

_____

2. How many people live in your household?

2.1. adult females	
2.2. adult males	
2.3. female children <5	
2.4. female children 6 - 10	
2.5. female children 11 - 18	
2.6. male children < 5	
2.7. male children 6 - 10	
2.8. male children 11 - 18	

3. What is the highest educational standard of the female adult head of the family?

3.1. degree	
3.2. diploma	
3.3. std. 8 - 10	
3.4. std 4 - 7	
3.5. std 1 - 3	
3.6. grade 1 - 2	
3.7. not educated	

4. How many rooms does your house have?

B. WATER SOURCE

5. Does the village have a water committee?	yes	no

6. What is your main source of domestic water in your village?

6.1. rain	
6.2. dam	
6.3. river	
6.4. private borehole	
6.5. outdoor tap at home	
6.6. indoor tap	
6.7. communal tap for < 100 people	
6.8. communal tap for > 100 people	
6.9. communal borehole (windmill)	
6.10. other (please specify)	

7. Is water readily available from the source?	yes	no

8. If your answer to the above question is NO, state alternative source

8.1. buy water	
8.2. private source	
8.3. pond	
8.4. river/ stream	

9. Do you pay for water?	yes	no

10. How much do you pay for water per month?

10.1. R5.00	
10.2. R10.00	
10.3. R20-00 or more	

11. If the water source is a private communal, how many households use the source?

11.1. 1 - 10	
11.2. 11 - 20	
11.3. 20 - 50	

12. How far is the water source from your house (in meters)?

12.1. 0 (at home)	
12.2. 50 - 100	
12.3. 100 - 500	
12.4. 500 - 1000	
12.5. > 1000	

13. What time is the water source the busiest?

13.1. morning	
13.2. afternoon	
13.3. no busy time	

C. WATER COLLECTION AND STORAGE

14. What type of container do you use to fetch or store water?

14.1. plastic	
14.2. unpainted metal	
14.3. painted metal	
14.4. fibreglass	

14.5. stainless steel ☐

14.6. other (please specify) ☐

15. How big is the container (litres) you use to collect the water with?

15.1. 5 - 10 litre ☐

15.2. 10 - 25 litre ☐

15.3. 25 - 50 litre ☐

16. Indicate the shape of the container

16.1. drum ☐

16.2. bucket ☐

16.3. bottle ☐

16.4. other (please specify) _____

_____

17. How do you remove the water from the water source?

17.1. dipping into it with a container (cup/jar) ☐

17.2. hand pump ☐

17.3. tap ☐

17.4. diesel pump ☐

17.5. electric pump ☐

17.6. use piece of hosepipe ☐

17.7. other (please specify) _____

_____

18. How do you take the water home? (Transportation)

18.1. hand carried container	
18.2. vehicle	
18.3. rolling the container	
18.4. wheelbarrow	
18.5. use donkey cart	

19. How many times do you fetch water each day?

19.1. once	
19.2. twice	
19.3. thrice	

20. Who fetches water?

20.1. adults	
20.2. children	
20.3. both	

21. Do you store water at home?	yes	no

22. What is the size of your storage tank?

22.1. 20 - 50 litres	
22.2. 50 - 100 litres	
22.3. 100 - 200 litres	
22.4. 200 litres and more	

23. What type of storage container do you use?

23.1. plastic	
23.2. unpainted metal	
23.3. painted metal	
23.4. fibre glass	
23.5. stainless steel	
23.6. glass	

24. Is the storage container kept.....?

24.1. open	
24.2. closed	
24.3. outdoors	
24.4. indoors	

25. How is the water obtained from the storage container?

25.1. tap	
25.2. mug	
25.3. other (please specify)	

26. How often is the storage container emptied or nearly emptied?

26.1. daily	
26.2. weekly	
26.3. monthly	
26.4. rarely or not at all	

27. How often is the storage container cleaned?

27.1. daily	
27.2. weekly	
27.3. monthly	
27.4. rarely or not at all	

28. What do you use to clean the storage container?

28.1. water only	
28.2. soap and water	
28.3. bleach	
28.4. sand and water	

29. Do you treat water used for drinking by .....

29.1. boiling	
29.2. straining	
29.3. adding chemicals e.g. chlorine tablets	
29.4. other (please specify)	_____

D. WATER QUALITY OF STORED WATER

30. Is the water clear	yes	no
31. Does the water have a smell?	yes	no
32. Does the water have any taste?	yes	no

33. Does your household use water for each of the following?

33.1. drinking	
33.2. cooking	
33.3. bathing	
33.4. laundry	
33.5. watering the garden	
33.6. watering animals	
33.7. home industry/business	

E. ATTITUDES/KNOWLEDGE TOWARDS WATERBORNE DISEASES

34. Do you know any diseases caused by contaminated water?	yes	no

35. Which of the following waterborne diseases have you suffered from?

35.1. Cholera

35.2. Dysentery

35.3. Typhoid fever

35.4. Diarrhoea

36. Have any of your children had diarrhoea (loose tummy) at any time in the past six months? (loose tummy = more than 3 stools/day for at least 2 days).	yes	no

List their ages:.....

37. What do you think caused the diarrhoea?

38. For the most severe cases of stomach problems, which symptoms applied in your case?

38.1. Stomach ache	
38.2. Passing blood	
38.3. Vomiting	
38.4. Fever	
38.5. More than 4 looses stools in 24 hours	
38.6. Other (please specify)	

39. Did you report your health problems to the clinic nurse?	yes	no

40. Were you given medication for your health problems?	yes	no

41. For how many days did this bout of diarrhoea last?

41.1. 1 - 3 days	
41.2. 4 - 6 days	
41.3. More than 7 days	

42. How do you think diarrhoea may be prevented?

_____

_____

_____

_____

43. Have your family suffered from stomach ache in the last six months?

yes	no

F. SANITATION

44. What type of toilet does the household have?

44.1. In-house flush	
44.2. Outdoor flush	
44.3. Bucket system	
44.4. Pit latrine	
44.5. Other (please specify)	

45. How many people use your toilet?

45.1. 1 - 5	
45.2. 6 - 10	
45.3. More than 10	

46. If your household does not have a toilet, where does your family normally defecate?

46.1. Neighbours	
46.2. Hole dug in the yard	
46.3. Other (please specify)	

47. Are there times when the toilet is unavailable and household members relieve themselves in the vicinity of the house?

yes	no

48. Did your household have any problems with the toilet in the last four weeks which made it necessary to use other toilet facilities?

yes	no

49. How is water including waste from flush toilets disposed of?

49.1. Pipeline to sewage works	
49.2. Septic tank	
49.3. Poured into yard in the vicinity of house	
49.4. Poured outside yard	
49.5. Other (please specify)	

50. How do you dispose of your domestic rubbish?

50.1. Rubbish is collected	
50.2. Dump in the yard	
50.3. Bury in the yard	
50.4. Dump outside the yard	
50.5. Bury outside the yard	
50.6. Burn	
50.7. Other (please specify)	

51. For how long do you store solid waste in the house before taking it outside?

51.1. Daily	
51.2. Weekly	
51.3. Monthly	
51.4. Rarely or not at all	

52. How often is solid waste removed from the outside of your house?

52.1. Daily	
52.2. Weekly	
52.3. Monthly	

53. Is there a problem in your area of people dumping solid waste?	yes	no

54. Do you keep the following animals at home?

54.1. Cat	
54.2. Dog	
54.3. Poultry	
54.4. Pigs	
54.5. Goats	
54.6. Cattle	
54.7. Other (please specify)	_____

55. What do you use to clean your baby's anus/buttocks?

55.1. Water and hand wash	
55.2. Cotton wool	
55.3. Toilet paper	
55.4. Washing rag	
55.5. Newspaper	

56. List occasions when you usually wash your hands each day

56.1. Before eating food	
56.2. Before preparing food	
56.3. After toilet use	
56.4. After waking up in the morning	
56.5. After cleaning baby's buttocks	

57. Do you have soap in your household?	yes	No

58. Where do you keep soap for washing your hands after using the toilet?

58.1. In the toilet	
58.2. In the yard	
58.3. In the bathroom	
58.4. In the kitchen	
58.5. In the bedroom	

G. ECONOMIC IMPACT

59. How often have children in your household been ill with diarrhoea the past 6 months?

A. How often did you take these children for medical care?	
B. How far are medical services from your home?	
C. How do you get to the medical services?	
D. How much does it cost you to get to the medical services	
E. How much does the medical treatment cost you each time?	
F. How many days did you have to stay away from work to take children for medical care?	

60. How often have adults in your household been ill with diarrhoea the past 6 months?

A. How often did you take these ill adults have to go for medical care?	
B. How much does the medical treatment cost you each time?	
C. How many days did ill adults stay away from work because of this illness?	

61. What is the total monthly income of your household?

H. OBSERVATION

62. How are the water containers covered?

62.1. No cover	
62.2. Tightly	
62.3. Loose	
62.4. No containers	

63. What is the hygienic condition of the yard?

63.1. Clean	
63.2. Dirty	
63.3. Very dirty	

64. Fly count in yard

64.1. Numerous	
64.2. Many	
64.3. Few	
64.4. None	

65. What is the hygienic status of the kitchen?

65.1. Clean	
65.2. Dirty	
65.3. Very dirty	

66. Fly count in kitchen

66.1. Numerous	
66.2. Many	
66.3. Few	
66.4. None	

67. What is the general condition of the latrine?

67.1. Faecal matter in the toilet	
67.2. Toilet paper available	
67.3. Toilet is ventilated	
67.4. Smell of urine	
67.5. Presence of flies	

68. Is there a place for washing hands next to the toilet?	yes	no

69. Cleanliness of children?

69.1. Hands	
69.2. Face	
69.3. Clothes	

70. Garbage container in house?

70.1. None	
70.2. Closed	
70.3. Open	

71. Are there a lot of flies in your kitchen during the day?

71.1. No	
71.2. Usually/ almost always	
71.3. Occasionally	

72. Does your toilet attract flies during the day?

72.1. Almost never	
72.2. Occasionally	
72.3. Usually	