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

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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.

TABLE OF CONTENTS

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

LIST OF FIGURES

Page

LIST OF TABLES

LIST OF PUBLICATIONS AND CONFERENCE CONTRIBUTIONS

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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.

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