

## TABLE OF CONTENTS

	Page
EXECUTIVE SUMMARY .....	i
Acknowledgements .....	viii
Abbreviations .....	xiii
List of Tables .....	xi
List of Figures .....	xii
Plate 1 .....	xii
1 INTRODUCTION .....	1
1.1 Project objectives .....	3
2 LITERATURE REVIEW .....	4
2.1 Detection and confirmation of <i>Cryptosporidium parvum</i> and <i>Giardia duodenalis</i> .....	4
2.2 PCR detection of <i>Giardia</i> .....	4
2.3 PCR detection of <i>Cryptosporidium</i> .....	5
2.4 Sources of <i>Cryptosporidium</i> and <i>Giardia</i> contamination of water.....	6
2.5 Occurrence of <i>Cryptosporidium</i> and <i>Giardia</i> in wastewater works.....	9
2.6 Significance of <i>Cryptosporidium</i> and <i>Giardia</i> in human populations.....	10
3 DEVELOPMENT OF A PCR TECHNIQUE FOR <i>CRYPTOSPORIDIUM</i> AND <i>GIARDIA</i> .....	11
3.1 Methodology .....	11
3.1.1 Protozoan cultures .....	12
3.1.2 DNA Extraction .....	12
3.1.3 Oligonucleotide Primers .....	13
3.1.4 DNA amplification .....	13
3.1.5 Product Analysis .....	14
3.1.6 Concentration Methods .....	14
3.2 Results and discussion .....	15
3.2.1 DNA extraction methods .....	15
3.2.2 DNA amplification .....	15
3.2.3 Sensitivity of PCR reactions .....	15
3.2.4 Combination of PCR and oocyst concentration methods .....	15
3.3 Conclusions .....	16
4 CATCHMENT MONITORING FOR <i>CRYPTOSPORIDIUM</i> AND <i>GIARDIA</i> .....	17
4.1 Materials and methods.....	17
4.1.1 Sampling and concentration of water, wastewater and sludge samples .....	17
4.1.2 Detection of <i>Cryptosporidium</i> and <i>Giardia</i> in water and sludge samples .....	18
4.1.3 Viability staining of oocysts and cysts in effluent and sludge samples.....	18
4.1.4 Desiccation of sludge samples .....	19
4.1.5 Rainfall and temperature data to determine seasonal variations .....	19
4.2 Results and discussion.....	19
4.2.1 Occurrence in the uMsunduze River Pietermaritzburg catchment.....	19

4.2.2	Occurrence of <i>Cryptosporidium</i> and <i>Giardia</i> in wastewater .....	24
4.2.3	Viability staining of oocysts and cysts in effluent and sludge samples .....	28
4.2.4	Desiccation of sludge .....	28
4.2.5	Collection of veterinary samples and data .....	29
4.3	Conclusions.....	30
5	OCCURRENCE OF <i>CRYPTOSPORIDIUM</i> AND <i>GIARDIA</i> IN A COMMUNITY .....	32
5.1	Methodology .....	32
5.1.1	Collection and examination of stool samples from schools .....	32
5.2	Collection of clinical information regarding positive stool samples on hospital records .....	32
5.2.1	Collection of clinical information from rural clinics in the Vulindlela area .....	33
5.2.2	Parasite analysis of household water .....	33
5.2.3	Collection of dung samples .....	33
5.3	Results and discussion .....	33
5.3.1	Presence of <i>Giardia</i> and <i>Cryptosporidium</i> in stool samples .....	33
5.3.2	Clinical information obtained from the hospital and pathology laboratory records ...	34
5.3.3	Clinical information obtained from clinics in the Vulindlela area .....	34
5.3.4	Correlating incidences of diarrhoea with the water source used .....	35
5.3.5	Parasite analysis of household water .....	36
5.3.6	Dung samples .....	36
5.4	Determination of human health risk associated with levels of (oo)cysts in river water....	37
5.4.1	Introduction to health risk assessment .....	37
5.4.2	Quantitative health risk assessment .....	38
5.4.3	Hazard identification .....	39
5.4.4	Exposure assessment .....	39
5.4.5	Dose response .....	39
5.4.6	Assumptions adopted for the risk evaluation .....	41
5.4.7	Risk characterization .....	40
5.4.8	Uncertainty analysis .....	42
5.5	Conclusions .....	46
6	GENERAL DISCUSSION .....	47
7	CONCLUSIONS .....	49
8	RECOMMENDATIONS .....	51
8.1	Health education and guidelines .....	52
8.2	General guidelines .....	52
8.3	Future research .....	52
9	TECHNOLOGY TRANSFER .....	54
9.1	Workshops .....	54
9.2	Publications .....	54
9.3	Archiving of data .....	54
10	REFERENCES .....	55