1.1 INTRODUCTION

Intestinal parasites are more common in the developing world where untreated ground water is consumed by the majority of rural people (Dzvairo *et al.,* 2006). This is a public health problem with an estimated 3.5 billion people being infected worldwide and the majority are children (Nematian *et al.,* 2004, Quihui *et al.,* 2006). Socio-economic factors such as poor hygiene, lack of safe water and sanitation facilities and low socio-economic status are known to play a pivotal role in susceptibility to infection (Kang *et al.,* 1998).

In 2000, an estimated 1.1 billion people of the World's population did not have access to improved sources of water (Sobsey and Bartram, 2004; Nath *et al.,* 2006). There are 3 groups of pathogens excreted in faeces that may cause waterborne infections. These are bacterial, parasitic or viral. Bacteria include *Yersinia enterocolitica*, *Campylobacter jejuni, Escherichia coli, Shigella spp*., *Vibrio cholerae*, *Aeromonas spp*., enterotoxigenic *Bacteroides fragilis, Clostridium difficile, Legionella pneumophila.* Parasites include *Giardia duodenalis, Naegleria fowleri*, *Acanthamoeba spp*., *Entamoeba histolytica, Cryptosporidium parvum, Cyclospora cayetanesis, Isospora belli,* and the microsporidia. Examples of viruses are calciviruses, rotaviruses, hepatitis A or Norwalk virus (Marshall, 1997; Ted *et al.,* 1997; Leclerc *et al.,* 2002; Sharma, 2003; Ashbolt, 2004).

Studies conducted in different countries have reported contamination of different water bodies by protozoan parasite cysts. In a study done in Brazil by, 4 of the 18 (22.2 %) water samples from open and protected wells including a sample from the city water supply systems were positive

for oocyts of *Cryptosporidium* (Newman *et al*., 1993). In Mexico, they identified *Giardia intestinalis* in some of the samples from wells (Cifuentes *et al.,* 2004). In Sicily, oocysts of *Cryptosporidium* and cysts of *Giardia* were identified in surface and ground waters (Di Beneddeto *et al.,* 2005). The overall removal of these parasites at water treatment plants was 90 %, an indication that even the treated tap water could be contaminated (Di Beneddeto *et al.,* 2005).

The state of drinking water sources in Zimbabwe concerning protozoan parasitic contamination is unknown. The Zimbabwe National Water Authority (ZINWA) does not test drinking water for the presence of parasite cysts as the resources are not available such that the Zimbabwean population could be consuming treated water that may contain parasite cysts.

The main parasites dealt with in this project were *Giardia duodenalis* and *Entamoeba histolytica/dispar. Giardia duodenalis* also known as *Giardia intestinalis* or *Giardia lamblia* infects numerous mammals such as domestic, wild animals and humans (Laurent, 2005). In this thesis the term *Giardia duodenalis* will be used. The infection has a worldwide distribution, and is the most common intestinal parasite in the tropics (Desowitz, 1980; Laurent *et al.,* 2005). It has been estimated (Markell and Voge, 1981) that 10 % of the world's population was infected with *E. histolytica.* The invasive trophozoite of *E. histolytica* was reported as causing up to 100,000 deaths per year globally as it is known to destroy human tissue (McCoy *et al.,* 1994; Wang *et al.,* 2004). An estimated 40 % of the population in Africa may be infected with *E. histolytica* especially in the low socio-economic groups where it remains a major cause of morbidity and mortality (Samie *et al.,* 2003).

The WHO 'Millennium Declaration' established an ambitious goal of halving the proportion of people living without access to safe water by 2015 (Sobsey and Bartram, 2004). Studies have shown that improving the microbiological quality of household water by point-of-use treatment and safe storage in improved vessels reduces diarrhoeal and other waterborne diseases in communities and households in developing and developed countries (Nath *et al.,* 2006). Household treatment can often provide these benefits much more quickly than it will take to design, install and deliver piped community water supplies (Sobsey and Bartram, 2004). Given the current economic situation in Zimbabwe, it will definitely take years for the installation of piped water systems in rural areas. Point-of-use anti-protozoan methods involving use of sand with/or activated charcoal for the removal of parasite cysts and also use of sunlight to disinfect water were identified and tested. Solar disinfection was implemented in one of the communities in order to ascertain its efficacy in disinfecting water in a community.

1.2 OBJECTIVES

1.2.1 Objective 1

To determine the prevalence of common intestinal protozoan parasite cysts in individuals residing in urban, rural tribal trust land and commercial farming environments in Zimbabwe.

1.2.1.1 Hypothesis

Protozoan parasite cysts are prevalent in different communities.

1.2.1.2 Overview

Previous studies on human protozoan parasites carried out in Zimbabwe 1987 concentrated mainly on *Giardia duodenalis* (Mason and Patterson, 1987; Simango and Dindiwe, 1987). This study done 21 years later, will provide the current status of parasitic infections as societies change due to migration and constant change in socio-economic status. In order to understand the current status of parasitic infections in Zimbabwe, stool samples were collected from pupils from urban, rural tribal trust land and commercial farming environments.

1.2.2 Objective 2

To determine the probable correlation of common intestinal protozoan parasite cysts in humans and their drinking water sources.

1.2.2.1 Hypothesis

Drinking water is contaminated with protozoan parasite cysts.

1.2.2.2 Overview

One of the problems associated with water contaminated with intestinal protozoan parasites, is that the cysts of protozoa are difficult to kill. Chlorine is the most widely used water disinfectant at water treatment plants. However, the recommended concentration of chlorine for disinfection of water does not kill enteric protozoan cysts. The amount of chlorine needed to kill these parasites would make the water almost impossible to drink (Korich *et al.,* 1990; Wallis *et al.,* 1996; Ted *et al.,* 1997). Developed countries such as the USA, Canada, UK and Sweden that consume chlorinated treated water, had outbreaks of giardiasis and amoebiasis (Korich *et al.,* 1990; Ljungstrom *et al.,* 1992; Wallis *et al.,* 1996; Barwick *et al.,* 2002; Sinclair *et al.,* 1998; Isaac-Renton *et al.,* 1999). In Africa, such waterborne outbreaks may be occurring, undetected due to lack of facilities and resources required for the identification of these parasites in drinking water sources. This study was conducted in Chiweshe rural tribal trust land. Besides collection of water samples from drinking water sources, stool samples were also collected from 113 participants, who assented or consented, age range being 2 to 89 years old. The aim was to compare parasites identified in stool with those found in drinking water sources.

1.2.3 Objective 3

The third objective was to develop and test under laboratory conditions, effectiveness of anti-protozoan methods capable of removing cysts of protozoan parasites in contaminated water. The practicality and acceptability of these methods for use in the study environments was assessed.

1.2.3.1 Hypothesis

Sand filtration and activated charcoal remove cysts of protozoan parasites in contaminated drinking water enough to make it safe for human consumption.

1.2.3.2 Overview

A sand filter was designed, that would use a combination of local sand and local partially activated wood charcoal (Morgan, 1990). Conventional activated charcoal used by the Zimbabwe National Water Authority (ZINWA), Hopkin and Williams Ltd (England) and novel activated charcoal from local firewood, amarula stones, baobab shells and macadamia nut shells, were tested for their capacity of capturing cysts of protozoan parasites namely; *Giardia duodenalis, Entamoeba histolytica/dispar, Entamoeba coli, Endolimax nana, Iodamoeba butschlii* and *Chilomastix mesnelli.*

1.2.4 Objective 4

To identify a point-of-use, anti-protozoan method capable of inactivating cysts in contaminated drinking water.

1.2.4.1 Hypothesis

Solar radiation inactivates protozoan parasite cysts in contaminated drinking water enough to render it safe for human consumption.

1.2.4.2 Overview

Solar radiation was tested for its capability to inactivate *Giardia duodenalis* and *Entamoeba histolytica/dispar* cysts. Experimentally contaminated water was exposed to sunlight for 7 hr and viability tests for parasite inactivation were conducted hourly in the laboratory. This solar disinfection (SODIS) was introduced to a rural community. This study was conducted in order to assess if there is a reduction in acquisition of protozoan parasitic infections.

1.3 LITERATURE REVIEW

1.3.1 Epidemiology of parasitic infections in humans

Parasitic infections are prevalent worldwide but more so in the tropical regions (Okyay *et al.,* 2004). A study done in 48 states of the United States of America indicated that one third of the 2 896 patients examined were infected with intestinal parasites. The highest number of individuals was from California with 859 patients and the lowest were from Mississippi with 2 patients (Amin *et al.,* 2002). Nineteen species of intestinal parasites were identified with 10 % of the patients having multiple infections of 2-4 parasitic species. Several epidemiological studies carried out in different countries have shown that the socio-economic situation of individuals is an important factor in the prevalence of intestinal parasites. It has been shown (Ahmed and Chaudhuri, 1999) that the socio-ecological risk factors of intestinal parasitic infections in an

urban squatter camp in Egypt, were lack of provision of both tap water and sewerage systems inside dwelling places. Another study conducted in Brazil, indicated that the estimated prevalence of diarrhoea decreased by 45 and 44 % respectively due to the improved water supply and sanitation, although there was no significant impact on parasitosis (Gross *et al.,* 1989). It has been shown that some Mexican children from lower income families with unemployed and less educated mothers had a higher risk of intestinal parasitism including those who defaecated in open areas (Quihui *et al.,* 2006). The results of this study agree with another done in Turkey, particularly in relation to the educational status of the mother (Okyay *et al.,* 2004). In the study there was a high prevalence of parasitic infections in children who washed their anal area after defaecation and those that sometimes or never used toilet paper, including children whose mothers did not have primary school education. There are many prevalence studies that have been carried out worldwide concerning parasitic infections (Table 1.1)

Table 1.1 : Report on studies conducted in different communities worldwide on the prevalence of parasitic infections

Name of country	Number of individuals tested	Most prevalent intestinal parasites identified	%	Type of area	Reference
Albania	277	*Trichuris trichiura* *Giardia duodenalis*	12.27 11.19	Urban	Spinelli *et al.,* 2006
Saudi Arabia	1201	*Giardia duodenalis* *Entamoeba histolytica*	18.9 9.2	Rural	Omar *et al.,* 2005
Turkey	456	*Enterobius vermicularis* *Giardia duodenalis*	18.2 10.7	Urban and rural	Okyay *et al.,* 2004
United States	916	*Blastocystis hominis* *Cryptosporidium*	72 13	Urban	Amin *et al.,* 2002
Nepal	300	*Entamoeba coli* *Giardia duodenalis*	21 13.7	Rural	Yong, 2000
India	78	*Giardia duodenalis* *Cryptosporidium*	53,8 39.7	Rural	Kang *et al*., 1998
Nicaragua	1267	*Entamoeba histolytica/dispar* *Giardia duodenalis*	18.6 15.9	Urban	Tellez *et al.,* 1997
Zimbabwe	3038	*Giardia duodenalis*	19.4	Urban and rural.	Mason *et al.,* 1986
Mexico	507	*Giardia duodenalis*	23	Rural	Quihui *et al.,* 2006
Brazil	254	*Ascaris lumbricoides*	55.4	Urban	Gross *et al.,* 1989

There is a high prevalence of intestinal parasites in different communities worldwide irrespective of the environment with *Blastocystis hominis* being as high as 72 % in an urban community of the United States and *G. duodenalis* also being prevalent (53.8 %) in a rural community in India.

1.3.2 Epidemiology of protozoan parasites in drinking water

The greatest threat associated with drinking water is the microbial risk of ingesting water contaminated with human and/or animal faecal matter harbouring potentially pathogenic microorganisms.

Table 1.2 indicates the presence of different types of microbes in water and the high resistance of protozoan parasites to chlorine. About 1.8 million people die every year from diarrhoeal diseases of microbial origin, 90 % being children under 5 years of age, mostly in developing countries (WHO, 2004).

The intestinal pathogenic protozoan parasite cysts identified in contaminated water include *Giardia duodenalis, Cryptosporidium, Entamoeba histolytica, Cyclospora cayetanensis, Isospora belli,* and *microsporidia* (Marshall *et al.,* 1997). The information on prevalence of these parasites in water sources is available from the developed countries where the capacity to do identification tests is available. Microbiological analysis of water in a developing country like Zimbabwe is achieved by identification of faecal coliforms, excluding parasitological water analysis. A study done in Zimbabwe, demonstrated no difference in rates of intestinal parasitism in children from urban areas who used municipal tap water and rural children who obtained water from surface streams (Mason *et al.,* 1986). *Giardia duodenalis* was actually more prevalent in urban children than the rural children (Mason and Patterson, 1987). The urban children may have been infected by drinking treated tap water still contaminated with these parasite cysts.

Table 1.2 : Waterborne pathogens and their significance in water supplies

Pathogen	Health Significance	Persistence in Water supplies	Resistance to chlorine	Relative Infectivity	Animal Source
Bacteria					
Burkholderia pseudomallei	Low	May multiply	Low	Low	No
Campylobacter jejuni, *C. coli*	High	Moderate	Low	Moderate	Yes
Escherichia coli – Pathogenic	High	Moderate	Low	Low	Yes
E. coli – Enterohaemorrhagic	High	Moderate	Low	High	Yes
Legionella spp	High	Multiply	Low	Moderate	No
Non-tuberculosis mycobacteria		Multiply		Low	No
Pseudomonas aeruginosa		May multiply		Low	No
Salmonella typhi	High	Moderate	Low	Low	No
Other salmonellae	High	May multiply	Low	Low	Yes
Shigella spp.	High	Short	Low	Moderate	No
Vibrio cholerae	High	Short	Low	Low	No
Yersinia enterocolitica	High	Long	Low	Low	Yes
Viruses					
Adenoviruses	High	Long	Moderate	High	No
Enteroviruses	High	Long	Moderate	High	No
Hepatitis A virus	High	Long	Moderate	High	No
Hepatitis E virus	High	Long	Moderate	Moderate	High
Noroviruses and sapoviruses	High	Long	Moderate	Moderate	High
Rotaviruses	High	Long	Moderate	High	No
Protozoa					
Acanthamoeba spp.	High	Long	High	High	No
Cryptosporidium parvum	High	Long	High	High	Yes
Cyclospora cayetanensis	High	Long	High	High	No
Entamoeba histolytica	High	Moderate	High	High	No
Giardia intestinalis	High	Moderate	High	High	Yes
Naegleria fowleri	High	May multiply	High	High	No
Toxoplasma gondii	High	Long	High	High	Yes
Helminths					
Dracunculus medinensis	High	Moderate	Moderate	High	No
Schistosoma spp.	High	Short	Moderate	High	Yes

Waterborne transmission of pathogens confirmed by epidemiological studies and case histories. The relative infectivity and resistance to chlorine is highest for parasites compared to bacteria and viruses. (Adapted from WHO Guidelines for Drinking-Water Quality 2002)

In a study done in Canada in 72 municipalities, of which 58 treated their water with chlorine, *Giardia* cysts and oocysts of *Cryptosporidium* were found in 18.2 % and 3.5 % respectively, of the treated water samples (Wallis *et al.,* 1996). Positive samples were found during all seasons. In a study done in Russia in Cherepovets city and three other cities on the European side in Russia, 9.4 % of the patients who had diarrhoea were infected with *G. duodenalis* while 6.9 % were positive for *C. parvum.* It was also demonstrated that some of the treated drinking water supplies from these cities and 4 other cities were contaminated with both *Giardia* and *Cryptosporidium* although no associations were made between the water and people infected (Egorov *et al.,* 2002). Thirty-one water samples were collected from 9 portable water treatment plants in Taiwan. Thirteen out of the 31 were from treated water and 10 had cysts of *G. duodenalis* and 5 had oocysts of *Cryptosporidium* (Hsu *et al.,* 1999). All these studies clearly indicated that drinking water that had met the safety standards for that country was still contaminated with protozoan parasite cysts. *Entamoeba histolytica* has not received much attention and yet it caused the devastating effects of 98 deaths after the Chicago World Fair in 1933 where 1400 were infected after drinking contaminated water with the invasive amoebic species (Ted *et al.,* 1997).

1.3.3 Protozoa of the gastrointestinal tract of man

Protozoa are unicellular organisms found in different environments such as the soil and water, and also within living organisms such as mammals. Some are non-pathogenic, whilst others are responsible for human morbidity and mortality, particularly in developing countries (Mandell *et al.,* 2000). The burden of these protozoan parasitic infections is high in tropical countries.

Several studies have clearly documented that the provision of safe water alone will reduce diarrhoeal and other enteric diseases, even in the absence of improved sanitation or other hygiene measures (Semenza *et al.,* 1998; Mintz *et al.,* 2001; Quick *et al.,* 2002).

Waterborne protozoan parasite cysts that cause diarrhoeal diseases in both immunocompetent and immunocompromised individuals include *G. duodenalis,* and *E. histolytica* whilst *C. parvum* and *C. cayetanensis* cause diarrhoea in mostly immunocompromised individuals (Marshall *et al.,* 1997). *Giardia duodenalis* and *E. histolytica* are among the most important intestinal parasites worldwide (WHO, 2002). Weekly reports from provincial, district and mission hospitals around Zimbabwe that come to the National Microbiology Reference Laboratory (NMRL) concerning organisms that cause diarrhoea, indicate that *G. duodenalis* and *E. histolytica* are the most common causes of diarrhoea out of all intestinal parasites. In some parts of the country such as Beitbridge, Karanda, Gokwe and especially Mberengwa and Zvishavane were more than 20 % of people that had diarrhoea were infected with these two parasites (NMRL, 2004).

1.3.3.1 History of *G. duodenalis* and *E. histolytica*

The flagellate, *G. duodenalis* flagellate were first discovered by Leeuwenhoeck who recognised it in his own stool specimens in 1681 (Beaver *et al.,* 1984). It was then described by Lambl in 1859 who named it *Giardia intestinalis.* Then in 1915, Stiles, named it *Giardia lamblia* in honour of Professor A. Giard of Paris and Dr. F Lambl of Prague. The name *Giardia duodenalis* is currently agreed upon worldwide according to Thompson and Monis, (2004). This parasite is cosmopolitan, more common in children than in adults and is the most common diagnosed intestinal flagellate in humans.

Entamoeba histolytica was first described by Losch in 1875 from a young peasant in Leningrad, Russia (Markell and Voge, 1981; Beaver *et al.,* 1984). Losch found the protozoa at autopsy and infected a dog *per rectum* with the patient's bloody-mucoid stools. He was unable to correlate the relationship between the acute colitis and the organism. Kartulis (1886) in Cairo, Hlava (1887) in Prague, and Councilman and Lafleur (1891) in Baltimore provided clinical and pathological evidence that this organism was responsible for a certain type of dysentery and liver abscess (Beaver *et al.,* 1984). They also cited that Quinke and Roos (1893) discovered the cysts and Schaudinn (1903) named it *Entamoeba histolytica* differentiating it from *E. coli.* Some ten years later Walker and Sellards in the Phillipines (1913) obtained experimental evidence that *E. histolytica* is the cause of amoebic colitis and *Entamoeba coli* is a harmless commensal of the large intestine (Beaver *et al.,* 1984). Infection with *G. duodenalis* and *E. histolytica* could be due to ingestion of contaminated food, soil or water but only water sources were investigated in the current study (Fig 1.1).

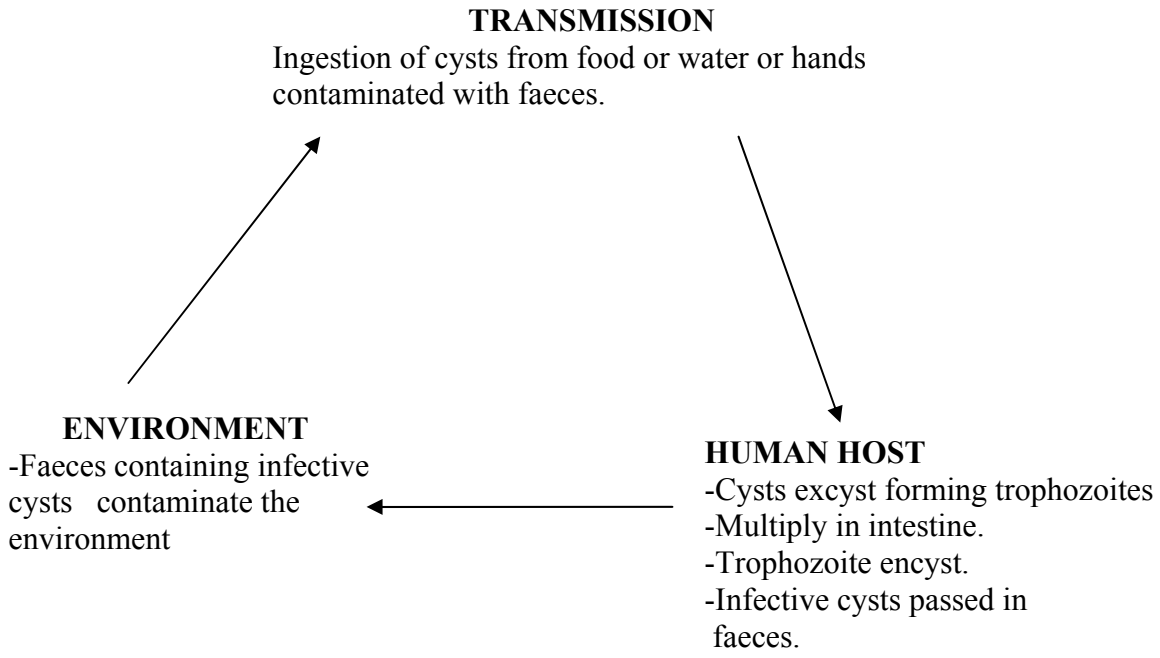

Figure 1.1 : Life cycle and transmission of *G. duodenalis* and *E. histolytica*

(Adapted from Cheeseborough 2005)

Both parasites have a direct life cycle with no intermediate host, but have a resistant cyst.

1.3.3.2 Pathology of *G. duodenalis*

Giardia infections affect the activity of gut enzymes such as lactose disaccharidase, damage the mucosal surface causing shortening of crypts and villi and give rise to overgrowth of bacteria and yeasts in the small intestine (WHO, 2002). Clinical symptoms of giardiasis include diarrhoea, epigastric pain, flatulence, malabsorption with lactose intolerance, weight loss, weakness, cramps, vomiting, mucus in stool and bloody foul smelling stool (Desowitz, 1980; Garcia, 1999; Laurent *et al.,* 2005). *Giardia* infections cause impairment of growth in children and a wide range of symptoms depending on the host's immunity (Cheesebrough, 2005). These include asymptomatic cyst passage, a chronic syndrome or acute self-limiting diarrhoea, or illness that requires hospitalisation (Marshall *et al.,* 1997; Laurent *et al.,* 2005). Symptoms in AIDS patients are known to be severe, whilst infection in children causes failure to thrive by impairing the uptake of nutrients such as fats, vitamins A and B_{12} (Faubert, 1996; WHO, 2002).

Worldwide outbreaks of giardiasis have occurred, with as many as 2.5 million cases in the USA of which 25 % were waterborne (Wang *et al.,* 2004; McGuigan *et al.,* 2006). Both healthy and the vulnerable population such as those that are HIV positive, the young and the old are being exposed to these parasites causing an increase in mortality and morbidity. It is prevalent especially in children and is known to be common in day care centres (Mandell *et al.,* 2000; Laurent *et al.,* 2005). Studies in a shanty town in Peru, suggested that *G. duodenalis* was hyperendemic in children < 10 yr old, and despite treatment 98 % of the children became re-infected within 6 months (Ashbolt, 2004). Studies done in Zimbabwe (1985) on the nutritional status of children showed that there was a strong association between giardia infection and under-nutrition and stunted growth (Loewenson *et al.,* 1985). The most common intestinal

protozoan pathogen in Zimbabwean children, especially in urban as compared to rural community was *G. duodenalis* as indicated by Mason and Patterson, (1987).

1.3.3.3 Laboratory diagnosis of *G. duodenalis*

The stool specimen is usually mucoid and foul smelling. Trophozoites of *G. duodenalis* can be identified in fresh diarrhoeic specimens or duodenal aspirates. The protozoan is a pear-shaped flagellate with a rapid tumbling and spinning motility likened to a falling leaf. It measures 10-25 µm in length and 5-15 µm in width. The parasite possesses a concave sucking disc on the ventral surface, 4 pairs of flagella, 2 axonemes and 2 nuclei (Fig 1.2) (Cheesebrough, 2005).

Cysts are found in formed, semi-formed and sometimes diarrhoeic stools (Desowitz, 1980). The formol-ether concentration method and zinc sulphate technique can be carried out to identify the cysts (Desowitz, 1980; Cheeseborough, 2005). Cysts are oval measuring 11-14 µm. They may occur in enormous numbers or can be extremely scanty as they are shed periodically. The later makes it necessary to examine at least 3 stool samples over a period of 2 weeks as a routine diagnostic procedure (Garcia, 1999). Internal structures of cysts include 4 nuclei grouped at one end, axonemes, median bodies and remains of flagella (Figure 1.3).

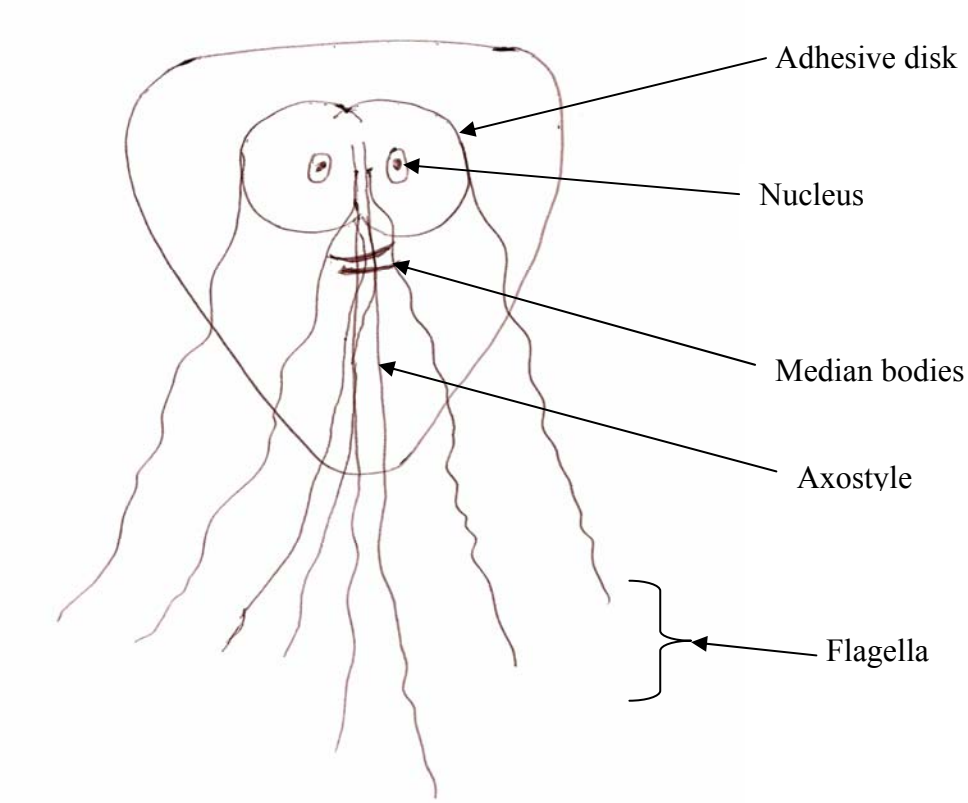

Figure 1.2 : Trophozoite of *Giardia duodenalis*

The 'tear drop shape' of the trophozoite, has adhesive disks. Below this is a single or double median body which is unique to this genus.

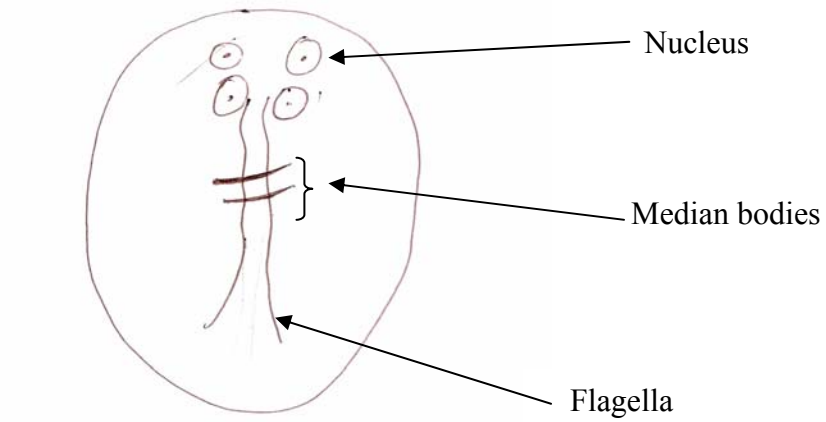

Figure 1.3 : Cyst of *Giardia duodenalis*

The oval cyst has disintegrated flagella in the middle with a single or double median body as above.

Other more specific and sensitive tests that can be carried out include the direct fluorescent antibody assay (DFA). The test kit and a fluorescent microscope are needed for this test. The enzyme immunoassay (EIA) does not require a microscope and is useful in analyzing large numbers of specimens. It detects antigens of *Giardia* in the stool specimen, using a micro-plate reader and the test kit. There are also rapid immunochromatographic cartridge assay where no special equipment is required and antigens of *Giardia* can be identified even on preserved specimens.

1.3.3.4 Treatment of *G. duodenalis*

Giardia can be treated with metronidazole, tinidazole, paromomycin, nitromidazoles, quinacrine and furazolidone (Mandell *et al.,* 2000; WHO, 2002).

1.3.3.5 Pathology of *E. histolytica*

The pathogenic strain of *E. histolytica* may cause ulcerative and inflammatory lesions of the colon (Mandell *et al.,* 2000; Thompson *et al.,* 2005). This occasionally leads to invasion of extraintestinal organs such as the liver and lungs where marked tissue destruction may occur. Approximately 34 to 50 million symptomatic cases of amoebiasis and 100 000 deaths occur worldwide each year, making *E. histolytica* second to malaria as a cause of mortality due to protozoan parasites (Ali *et al.,* 2003).

One of the well documented outbreaks concerning invasive amoebiasis that occurred during the Chicago World's Fair in 1933, caused 1400 infections including 98 deaths (Ted *et al.,* 1997). An outbreak of gastroenteritis occurred at a school in Taiwan in 2001 whereby 730 students were

infected with *E. histolytica* and the bacterium, *Shigella sonnei.* This was due to drinking well water that had been contaminated with sewage from a nearby toilet (Chen *et al.,* 2001).

1.3.3.6 Laboratory diagnosis of *E. histolytica*

Trophozoites are found in a fresh (still warm) dysenteric faecal specimen whereby a small amount of blood and mucus are placed on a slide without adding saline (Cheesebrough, 2005). The average size of a trophozoite is 20 × 25 µm. There is active directional amoeboid movement using pseudopodia and the amoebae may contain ingested red blood cells (Figure 1.4). The single nucleus has a central karyosome. Cysts are round measuring 10-15 µm in diameter, possessing 1-4 nuclei (Fig 1.5). Chromatoid bars which are aggregations of ribosomes can be seen particularly in immature cysts. *Entamoeba histolytica,* the cause of invasive amoebiasis, is morphologically identical to *E. dispar* which is noninvasive and *Entamoeba moshkovskii* a free living amoeba, ubiquitous in anoxic sediments and has so far been shown to rarely infect humans. These three identical organisms can be distinguished from each other by polymerase chain reaction (PCR) (Ali *et al.,* 2003). Another test that can be done for identification of *E. histolytica* is an EIA. The kit requires fresh or frozen unpreserved stool. There is also a rapid immunochromatographic cartridge assay that detects antigens of *E. histolytica/dispar.* This kit requires fresh or frozen samples and no special equipment is needed.

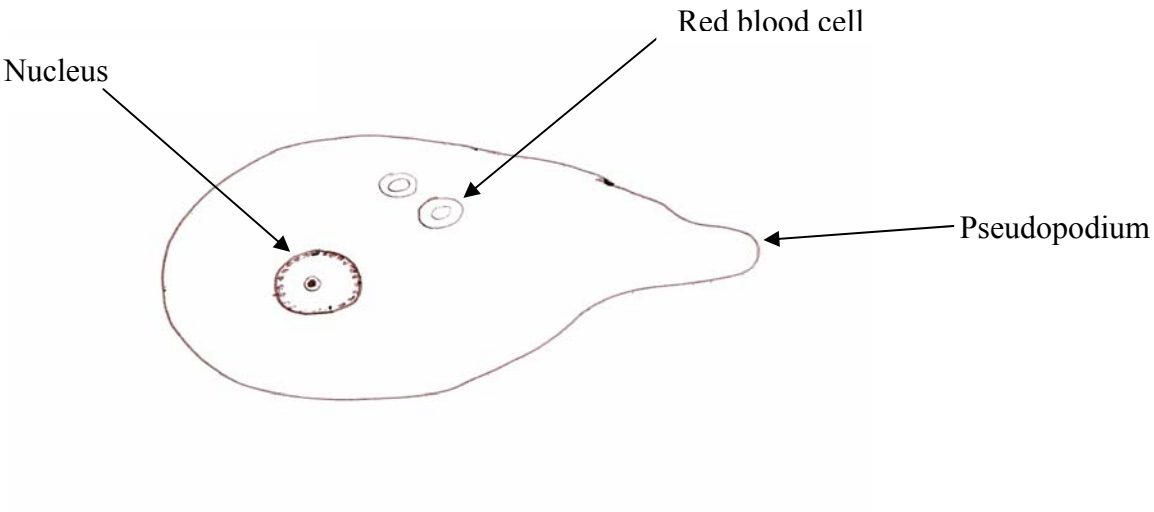

Figure 1.4 : Trophozoite of *E. histolytica* with an ingested red blood cell

The pseudopodium formed by the trophozoite is used for movement.

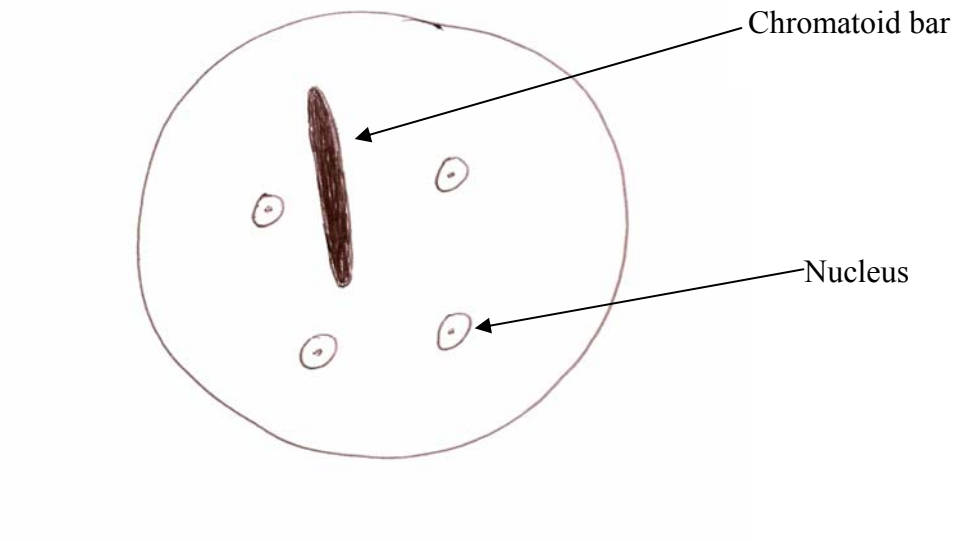

Figure 1.5 : *Entamoeba histolytica/dispar* cyst

This cyst contains characteristic 1 – 4 nuclei ± distinguishing chromatoid bars.

1.3.3.7 Treatment of *E. histolytica*

Entamoeba histolytica is treated with metronidazole, tinidazole, emetine hydrochloride or dehydroemetine (Mandell *et al.,* 2000).

1.3.4 Water disinfection methods

The physical methods that can be employed in order to make water safe for drinking include boiling, exposure to sunlight, use of ultraviolet lamps, sedimentation, filtration and aeration (Sobsey and Bartram, 2004; McGuigan *et al.,* 2006). Boiling water for 1 to 5 minutes is effective in destroying all classes of waterborne pathogens (Feachem *et al.,* 1983; Ted *et al.,* 1997). The method is simple and accessible. However it is taxing to the world's poorest people due to scarcity of firewood, time consumed and has been discouraged in many countries for environmental reasons (Joyce *et al.,* 1996; WHO/AFRO, 2001).

1.3.4.1 Filtration

There are a variety of filters and filtration processes available for household point-of-use treatment of water. These are listed in the Table 1.3.

Table 1.3 : Filters and filtration media for treatment of household water at point-of-use

Type of filters	Media
Granular media, rapid rate depth	Sand, gravel, diatomaceous earth, coal and other minerals.
Slow sand	Sand
Vegetable and animal derived depth.	Coal, sponge, charcoal and cotton.
Fabric, paper, membrane, canvas.	Cloth, other woven fabric, synthetic polymers and wick siphons.
Ceramic and other porous cast.	Clay and other minerals
Septum and body feed.	Diatomaceous earth and other fine media.

Adapted from WHO report by Sobsey and Bartram 2004

Different media can be used ranging from sand, diatomaceous earth, clay, coal or woven fabric to treat household water.

1.3.4.1.1 Sand filtration

Sand has been used to purify water for over 1000 years. It still remains one of the dependable methods for water purification and the majority of water treatment plants utilize it in combination with other processes (Chaudhuri and Satter, 1986; Logsdon, 1990; Morgan, 1990). The first slow sand filter for a small water treatment plant was built by John Gibb in 1804, for his bleachery in Paisley, Scotland as reported by Huisman and Wood, (1974). He sold the excess treated water to the public for half a penny per gallon. In 1852 his filtration process was adapted by James Simpson for the Chelsea Water Company that supplied public water in London from the River Thames. At that time they regarded this process as a mechanical means of removing turbidity and also suspended solids. In 1892, when outbreaks of cholera occurred, those that drank filtered water did not acquire the disease whilst amongst those that drank unfiltered water some deaths occurred. These events clearly indicated the importance of water filtration as it improved not only the physical, chemical quality of water but also the microbiological quality (WHO, 1997).

Filtration is a physical process that involves the removal of suspended solids from water by passing it through a porous medium (Morgan, 1990; Sangware, 2005). There are 3 main processes that come into play in sand filtration. These comprise the mechanical trapping of the suspended solids which is related to the pore size of the spaces created by the sand granules, adsorption of suspended materials on to the sand granules then finally the biological activity that takes place at the interface of the sand and water referred as the '*schmutzdecke'* (Morgan, 1990; Duke *et al.,* 2006). The '*schmutzdecke'* is a complex biological gelatinous layer made up of

micro-organisms such as bacteria, fungi, protozoa, rotifera (microscopic aquatic larvae), and a range of aquatic insect larvae and this layer provides additional filtration (Morgan, 1990).

The standard process that takes place at a water treatment plant includes coagulation, flocculation, sedimentation, filtration and disinfection (Abberszadegan *et al.,* 1997; Sangware, 2005; Kim and Kang, 2008). There are 2 types of sand filters, the slow and the rapid. The slow sand filters have a depth range of 0.5-1.2 m with filtration ranges being 0.1-0.3 m/hr whilst the rapid filters has an increased filtration range of 5-15 m/hr (WHO, 1997; Sangware, 2005). Rapid sand filters need a coagulation stage first but for slow-sand filtration there is no coagulation pre-treatment. Rapid sand filters are usually cleaned on a daily basis, by reversing the flow of water through the entire filter bed, referred to as backwashing, whilst slow sand filters are cleaned less frequently by removing the top layer of the media (Schmitt and Shinault, 1996).

There are a number of sand filters that can be used at a household point-of-use level. A biosand filter (BSF) is a slow-sand filter that is used at a point-of-use water treatment such as a home (Fig 1.6). It was developed by Dr David Manz in the 1990's at the University of Calgary in Alberta, Canada (Duke *et al.,* 2006). The first filters for use were installed in Nicaragua in 1996 and ever since the BSF type of filter has been used in 20 different countries including Honduras, Mexico, India and Uganda (Duke *et al.,* 2006). It is a concrete container that is approximately 0.9 m tall and 0.3 m^2 that is filled with sand (Lantagne *et al.,* 2005). Water is poured from the top, with a flow rate of an average of 30-40 l/hr and water is maintained at 5-6cm above the sand layer (Lantagne *et al.,* 2005; Duke *et al.,* 2006).

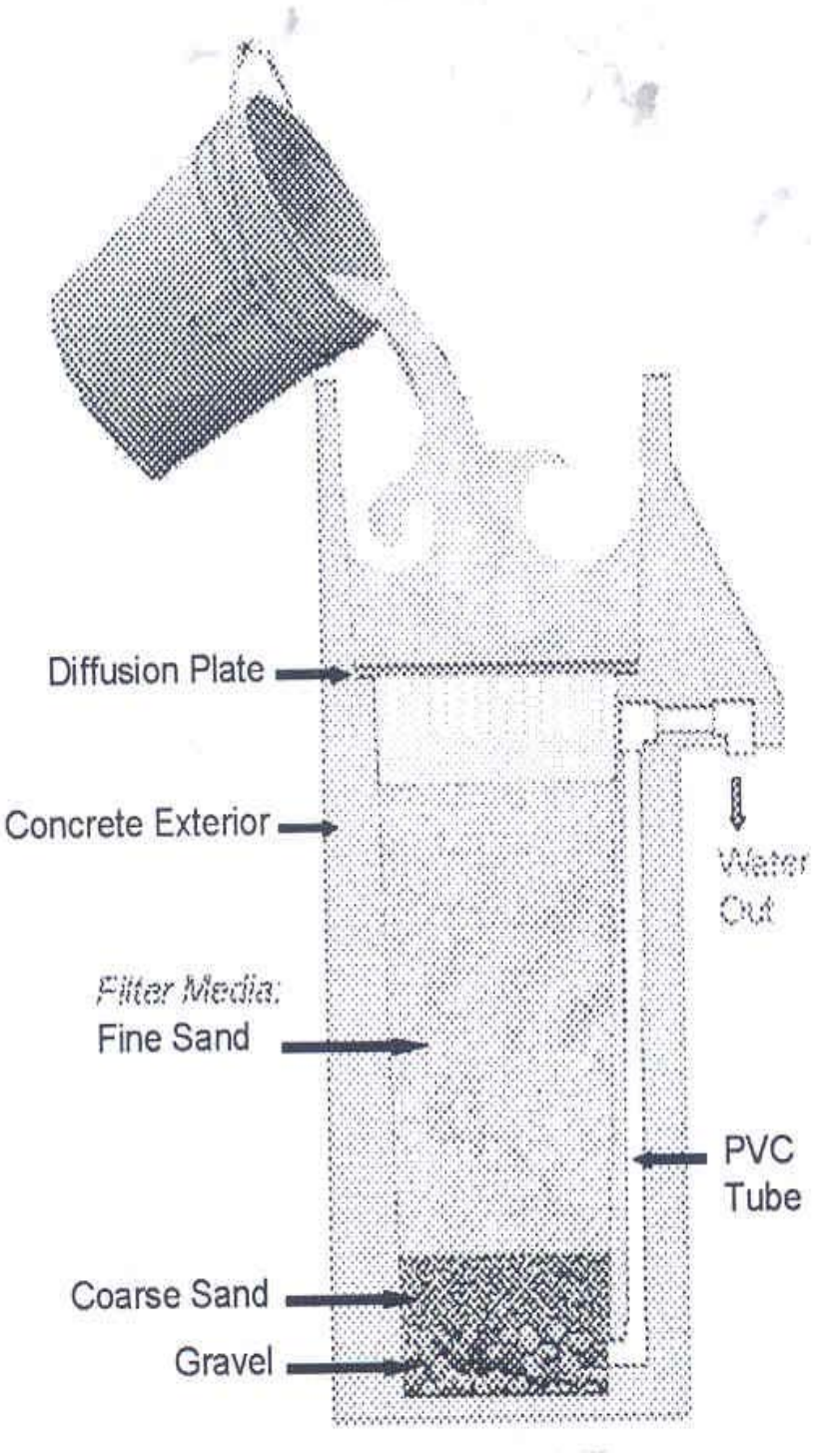

Figure 1.6 : The Biosand filter (adapted from Duke *et al.,* 2006)

Different types of media are used namely fine and coarse sand including gravel.

However studies done in both the laboratory and field indicated that the conventional slow sand filters had a higher microbial reduction of *Escherichia coli* as compared to the biosand filters (Stauber *et al.,* 2006). In another study by Duke *et al.* (2006) in rural Haiti where the majority of people used shallow wells, use of BSF showed that there was a 98.5 % overall removal of bacteria by the biosand filters including a decrease in turbidity of the filtered water from an average of 6.2 NTU to 0.9 NTU.

Other point-of-use water treatments include the metal bucket filters that use 2 buckets. The one on top is perforated with a mesh strainer or cloth at the bottom and gravel is placed at the bottom then about 40-75 cm of sand is placed at the top. This container is then placed on top of a similar bucket with a solid base that receives the filtered water. The media needs to be cleaned regularly after several weeks (Sobsey and Bartram, 2004). Drum or barrel filters use 200 l drums that also contain gravel and sand. These filters can either be down flow or up flow and they also need to be cleaned on a regular basis. Roughing filters are used at both household and community water levels. These consist of rectangular multi-compartment basins constructed of concrete and other materials. They require skills for operation and maintenance and contain filter material in different granular sizes layered in the different compartments whose porosity successively decreases in the direction of water flow. Removal of bacteria by roughing filters has been reported as 90-99 % effective (Sobsey and Bartram, 2004).

In Zimbabwe there are several different types of sand filters that have been constructed for point-of-use treatment processes (Morgan, 1990). These include use of 200 l drums, brick built containers or traditional pots (Fig 1.7).

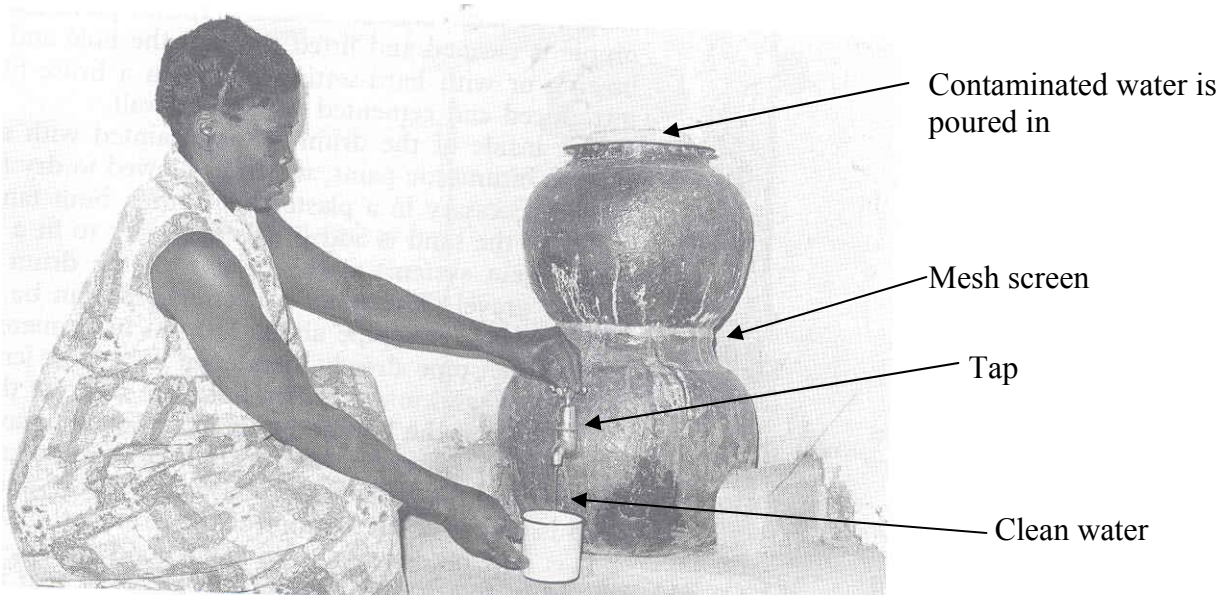

Figure 1.7 - Traditional pots used in sand filtration of water (Adapted from Morgan, 1990)

Raw water is poured in the top pot which contains the sand and a mesh screen at the bottom and clean water is collected from the bottom pot.

1.3.4.1.2 Modifications of sand filters

In recent years, efforts have been made to improve the performance of granular `filter media for removal of microbial contaminants by coating or co-mingling sand, coal and other common negatively charged granular media with metal oxides and hydroxides of iron, aluminium, calcium or magnesium (Chaudhuri and Satter, 1986). Such modified media are positively charged and therefore, more effective for removing and retaining the negatively charged viruses and bacteria by electrostatic adsorption (Chaudhuri and Satter, 1986). Some improved granular media filter-adsorbers have incorporated bacteriostatic agents, such as silver, in order to prevent the development of undesirable biofilms that release excessive levels of bacteria into the product (Ahammed and Chaudhuri, 1999). Production of these more advanced filter media containing charge-modified materials and bacteriostatic agents requires specialized skills and facilities, which are beyond the capabilities of most household users. Such media would have to be

prepared and distributed to communities and households from specialized facilities (Ahammed and Chaudhuri, 1999). The study by Ahammed and Chaudhuri (1999) indicated that coating sand with iron hydroxide or iron and aluminium hydroxide caused a substantial improvement in the aesthetic and microbiological quality of contaminated water.

1.3.4.1.3 Use of activated charcoal as a filtration media

Some adsorbents that have been used for water treatment since ancient times, include charcoal, clay, glass and various types of organic matter such as seeds or rice, *etc.* (Sobsey and Bartram, 2004). These adsorbents can also be used concurrently with filtration and coagulation. Activated charcoal have been used at water treatment plants in order to adsorb toxic organic compounds as well as compounds that cause taste and odor in the water (Morin and Camper, 1997). Other adsorbent materials that have been investigated besides charcoal, include alumina, zeolites, sediment, bentonite, sand, sludge, silica gel, resins and waste tyre rubber. All these have different adsorption mechanisms (Schouten *et al*., 2007).

Activated charcoal is material of very high surface area where a gram of activated charcoal has a surface area of approximately 500 m^2 as determined by nitrogen gas adsorption. It is produced from carbonaceous material such as nutshells, wood or coal. Adsorption is by Van der Waals forces or by London dispersion force. Activated charcoal has been used in different industries for different applications such as gas purification, gold purification, metal extraction, medicine, sewage treatment, air filters, gas masks, filter masks, water purification, filters in compressed air and many other applications (Selvi *et al.,* 2001, Mohan and Singh, 2002).

Activated charcoal has also been incorporated with other technologies for purification of water at point-of-use water treatments. There is the Aquaguard point of use water treatment that consists of a candle prefilter, activated charcoal filter and an ultraviolet irradiation compartment, that has been tested (Grabow *et al.,* 1999). This particular gadget removed all the viruses, bacteria and oocysts of *Cryptosporidium.* Different types of activated charcoal filters have also been used to remove taste, odour, dirt, rust and sand (Tobin *et al.,* 1981). The silver-containing activated charcoal was used to suppress total coliform count.

1.3.4.2 Sedimentation

Sedimentation involves holding or storing water undisturbed and without mixing long enough for larger particles to settle out or sediment by gravity. Care must then be taken to avoid disturbing the sedimented particles when recovering the supernatant water by decanting or other means. Most viruses and bacteria and fine clay materials are too small to be settled out by gravity sedimentation. This process is therefore recommended as a simple pre-treatment of household water prior to application of other treatments to reduce microbes (Sobsey and Bartram, 2004). Sedimentation is ineffective for turbid waters containing non-settable solids therefore an alternative method such as filtration is needed.

1.3.4.3 Use of chemicals for water disinfection

There are a number of chemical methods that are used for water treatment at point-of-use or entry for community water systems. These are grouped into categories according to the purpose and the nature of the technology used. These are:- chemical pre-treatment

coagulation/flocculation or precipitation, adsorption processes, ion exchange processes and chemical disinfection processes (Sobsey and Bartram, 2004).

Chemical precipitation or coagulation and flocculation use various salts of aluminum, iron, lime and other inorganic or organic chemicals for the removal of colloidal particles and microbes. Adsorption processes are usually carried out concurrently with filtration or coagulation. The adsorbents usually are charcoal, clay, glass or various types of organic matter. Adsorption of microbes is variable depending on the adsorbent being used. Ion exchange disinfection involves use of iodine in the form of tri-iodide or penta-iodide exchange resins. Point-of-use iodine resins have been found to extensively inactivate viruses, bacteria and protozoan parasites. The use of resins are limited to some developed countries as they are expensive (Sobsey and Bartram, 2004).

Chemical disinfection of drinking water is widely recognized as safe and effective, and is promoted and practiced at the community level as well as point-of-use treatments (Quick *et al.,* 2002; Stockman *et al.,* 2007). The most widely used chemical disinfectants for drinking water are relatively strong oxidants. These are free chlorine, chlorine dioxide, monochloramines, other halogens and ozone. Acids and bases can also be used as disinfectants as they inactivate microbes by creating either low or high pH levels in water, respectively (Sobsey and Bartram, 2004).

Ozone, an unstable gas is produced when oxygen molecules are dissociated by an energy source to oxygen atoms and subsequently collide with each other and form ozone. It is used to disinfect water. Ozone is unstable in water as it is unable to provide a disinfectant residual (Sobsey and Bartram, 2004). The mechanisms of disinfection using ozone include direct oxidation,

destruction of the bacterial cell wall with leakage of the cellular constituents, reactions with radical by-products of ozone and decomposition and damage of the nucleic acids (purines and pyrimidines). Ozone is capable of inactivating protozoan parasites (Korich *et al.,* 1990). The ability to inactivate waterborne microbes differs among the most commonly used disinfectants as follows, from most to the least potent : ozone > chlorine dioxide > free chlorine > monochloramines (Sobsey and Bartram, 2004). Although ozone disinfection is the most effective, it requires expensive machinery that is usually installed at a water treatment plant for the production of ozone.

Chlorine is the most widely used water disinfectant worldwide as its advantages outweigh disadvantages. It has a residual effect, is germicidal to bacteria, molds and algae, destroys hydrogen sulphide and removes ammonia and other nitrogenous compounds that cause an unpleasant taste (Craun *et al*., 1994). The disadvantages are that it causes production of disinfection by products, one of which are trihalomethanes (THM) (Jun *et al.,* 1996). Continuous intake of THM may cause cancer in some individuals (Driedger and Eyles, 2003; Komulainen, 2004). There are 3 main conditions that affect the effectiveness of chlorine on parasites. These are pH, temperature and chlorine demand or concentration. The cysticidal effect of chlorine is increased at lower rather than higher pH (*i.e.* pH 6 as compared to pH 8). At low pH chlorine exists in an effective hypochlorous acid (HOCl), whilst at high pH chlorine exists in a less effective hypochlorite (OCl^-) form (Jarroll, *et al.,* 1981). Cysts survive at low temperatures, and the concentration of chlorine required in order to make water safe for drinking makes it almost impossible to drink.

1.3.4.4 Ultraviolet lamps

The use of ultraviolet (UV) lamps is also highly effective in inactivating microbes in drinking water. Ultra violet radiation inactivates microbes by chemically altering nucleic acids such as pyrimidine dimers (WHO/AFRO, 2001). Its disinfection effect is usually accomplished with mercury arc lamps containing elemental mercury and an inert gas, such as argon, in a UV transmitting tube such as quartz. There are 2 types that can be used for household water supplies. The lamps are either submerged or mounted above a thin layer of water to be irradiated. The lamps emit nearly monochromatic UV radiation at a wavelength of 254 nm, and its optimum range for UV energy absorption by nucleic acids is about 240-280 nm. These lamps do not introduce chemicals or cause the production of harmful disinfection by-products in water. Disadvantages include the need for a reliable and affordable source of electricity to power the UV lamps. The lamps need to be periodically replaced after every 1-2 years, and the regular cleaning of sleeves of submerged lamps to remove deposits (WHO/AFRO, 2001; Sobsey and Bartram, 2004).

1.3.4.5 Solar disinfection (SODIS)

Sunlight has germicidal effects as it provides both ultraviolet (UV) radiation and heat. The combined effects of temperatures of 50-60 °C and UV radiation in the near ultra violet (UV-A) range (320-400 nm) of the solar disinfection system (SODIS) is germicidal to many enteric micro-organisms (Sommer *et al.,* 1997; Sobsey and Bartram, 2004). This combination of UV plus heat has a synergistic effect on microbial inactivation greater than predicted by comparable levels of exposure to one of the two agents alone (McGuigan *et al.,* 2006).

1.3.4.5.1 History of SODIS

The use of solar radiation to treat water was practiced in ancient India more than 2000 yrs BC (Sobsey and Bartram, 2004). The use of sunlight as a disinfectant on bacteria was published by Downes in 1886, then later sunlight was proposed to disinfect oral rehydration solutions (Acra *et al.,* 1984; Berney *et al.,* 2006). In 1988, a workshop on solar water disinfection was held in Montreal, and in 1991 a SODIS project was started by Swiss Federal Institute for Environmental Science and Technology (EAWAG). Collaborative work between Switzerland and Instituto de Investigacion y Desarollo en Agua Potable, Saneamiento Basico y Conservacion del Recurso Hidrico (CINARA) in Cali Colombia began in 1993. Further field tests on the inactivation of faecal coliforms were conducted with partners in Costa Rica, Jordan and Thailand, motivated by the interesting results obtained in inactivation of bacteria. In 1995, CINARA did experiments on assessing the inactivation rates and correlation of *Vibrio cholerae* and faecal coliforms (Sommer *et al.,* 1997). They discovered that UV-A dose of 54 Wh/m^2 together with temperatures of at least 50 °C inactivated these bacteria.

EAWAG developed the SODIS reactor in 1994 which uses both radiation and thermal effects and this was tested in CINARA in 1994 (Sobsey and Bartram 2004). The plant consists of a raw water tank in which water is heated to 50 °C, causing the thermovalve to open (Figure 1.8). The water then flows to the irradiation reactor which has a surface area of 1.78 m × 0.20 m and 0.10 m water depth and volume of 36.5 l. It is made of copper covered by glass. After irradiation the disinfected hot water is then cooled in a heat exchanger and the heat produced preheats the raw water (Fig 1.8).

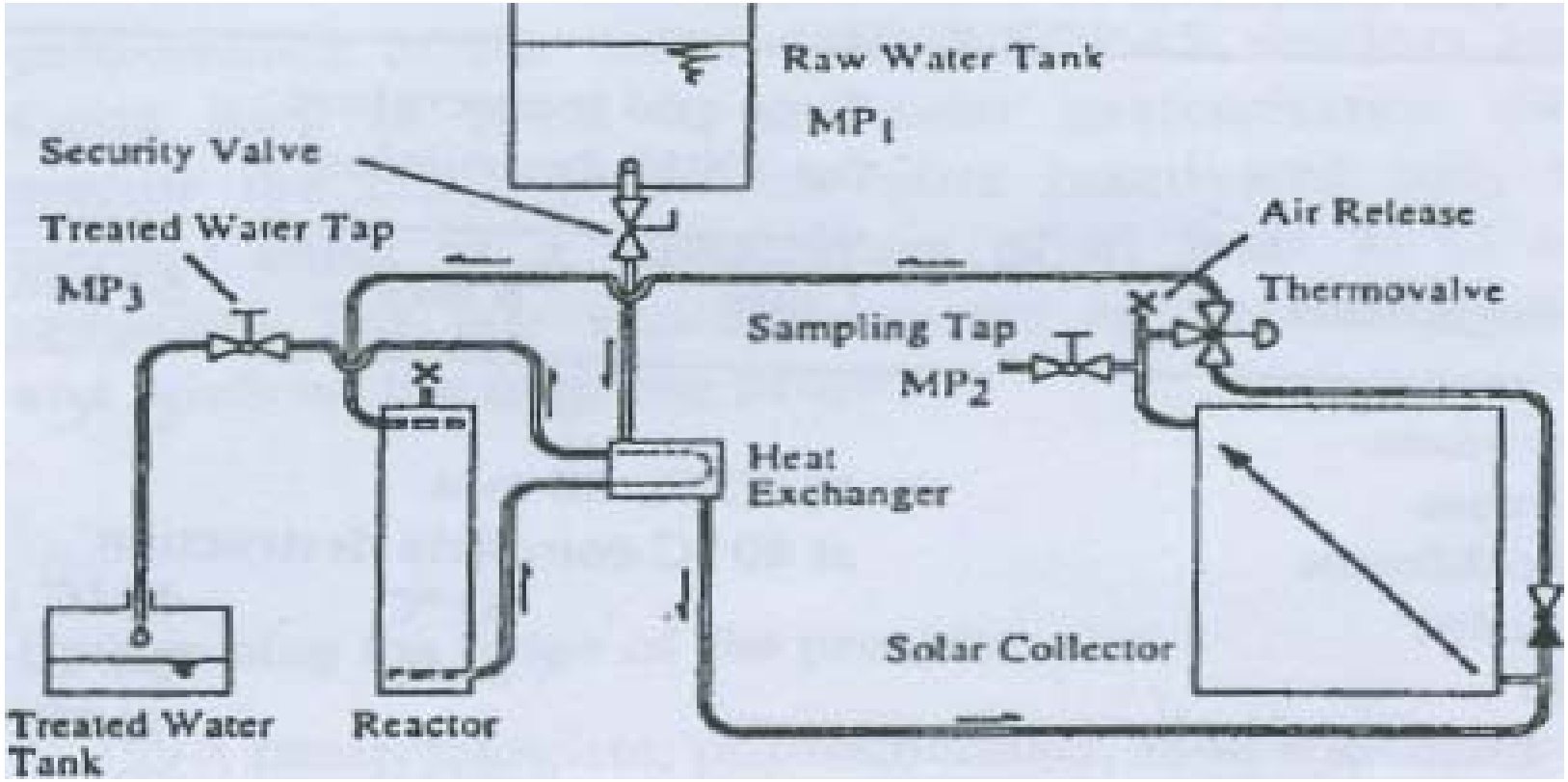

Figure 1.8 : Solar disinfection plant scheme (Adapted from Sommer *et al.,* 1997)

This type of plant can be used at institutions such as schools or hospitals.

This plant worked well only when temperatures were above or at 50 °C on a sunny day. On a cloudy day the waterflow was unsteady as it was difficult to reach the required temperature causing the thermovalve to shut down (Sommer *et al.,* 1997).

Some workers have done experiments in order to modify or accelerate SODIS. One of these methods is the addition of the non-toxic photocatalyst titanium IV oxide (TiO_2) in a process called solar photocatalytic disinfection (SPCDIS) (Mendez-Hermida *et al.,* 2007). This was done in order to enhance and accelerate the inactivation rate of bacterial pathogens and also of oocysts of *Cryptosporidium parvum* in both batch processes or in SODIS reactors. However, this process is not feasible at household level as the photocatalyst particles have to be removed after solar exposure and before consumption. Other workers have improved this method using a photocatalytic insert to accelerate the inactivation kinetics of microbes in drinking water (Lonnen *et al.,* 2005).

Either painting half the water container black or laying it on a black surface has been supported by many workers in that it improved heat absorption (Sommer *et al.,* 1997, McGuigan *et al.,* 2006). It has also been shown that covering the rear surface with aluminium foil improved the microbial inactivation efficacy of SODIS (Kehoe *et al.,* 2001). Implementation of partially painted black containers and those that were not painted into a rural community in Mexico was done in 2005 (Martin-Domingues *et al.,* 2005). Disinfection efficacy for the partially painted containers was reduced to 2 hours. Temperatures of up to 65 °C were experienced compared to unpainted containers where temperatures rose only to 50 °C.

1.3.4.5.2 Kinetics of SODIS

The biocidal effects of UV radiation are due to the fact that sunlight is absorbed by photosensitizers present in the water that react with oxygen producing hydrogen peroxide and superoxide ions in the interior of *Cryptosporidium parvum cysts* and *Giardia muris* cysts (McGuigan *et al.,* 2006). It is important that turbidity does not exceed 30 NTU to facilitate adequate penetration of UV-A throughout the sample. Shallow wells usually have turbid waters, such that in these circumstances other measures like, sedimentation or filtration through a folded cloth need to be undertaken to reduce turbidity (Sobsey and Bartram, 2004). Experiments concerning cloudiness indicated that 3 times more energy was available for heating and irradiation on a day with a clear sky than on a completely overcast day (Sommer *et al.,* 1997).

There are many viability tests for testing the effectiveness of SODIS on protozoan parasite cysts. The use of fluorogenic dyes has widely been implemented whilst other methods include excystation techniques (Mendez-Hermida *et al.,* 2005, Mendez-Hermida *et al.,* 2007). The fluorogenic dyes that are usually used are 4′, 6′-diamidino-2-phenylindole (DAPI) and propidium iodide (PI) (Mendez-Hermida *et al.,* 2005). When using DAPI (2mg ml^{-1} in absolute methanol) the nuclei of viable cysts fluoresce sky blue at 365 nm and propidium iodide (PI) (1mg ml^{-1} PBS 0.1 mol l^{-1}) stains non viable cysts red at 510-560 nm, after incubation for 2 hr at 37 ºC (Thriat *et al.,* 1998). Cysts that are considered to be viable are either (DAPI + PI-) or (DAPI-PI-). Propidium iodide does not penetrate an intact viable cyst (Wallis *et al.,* 1996; Mendez-Hermida *et al.,* 2007). Another fluorogenic dye that can be used is fluorescein diacetate (FDA). The combination of FDA/PI tends to overestimate viability and therefore this method has been taken over by use of DAPI/PI (Thriat *et al.,* 1998). The vital dye eosin has also been

shown to overestimate viability when comparing with the flurogenic dyes DAPI/PI (Thriat *et al.,* 1998).

1.3.4.5.3 Modifications of SODIS

In recent years, there are a number of solar systems that have been developed for cooking, pasteurization of milk and treatment of contaminated water. These include the solar box cooker which was used for cooking then later developed by other workers to pasteurize water (Ciochetti and Metclaff, 1984). The 'Family Sol Saver System' also showed effective disinfection of water using sunlight (Rijal and Fujioka, 2001).

1.3.4.5.4 Applications of SODIS

Several intervention methods have been applied to ascertain the effectiveness of SODIS. Studies on Masaai children of Kenya showed that over a period of one year those that drank solar disinfected water had a significantly lower risk of severe diarrhoea as compared to those who did not use this method by (Conroy *et al.,* 1999). In yet another study by the same researcher in 2001, during a cholera outbreak in Kenya, 3 out of the 155 subjects who used solar disinfection had cholera, compared to 20 of the 144 controls (Conroy *et al.,* 2001). A report by Maeusezahl and Tanner (2003), concerning a SODIS intervention by a non-governmental organization (NGO) in Bolivia, also proved that it was able to reduce the risk of diarrhoea in children below 5 yr by 40 %, and that through the child's improved health, the mother gained 6 extra productive working days over a year.

2.1 SUMMARY

Parasitic infections are a public health problem especially in developing countries. Prevalence of parasitic infections in 1 260 school pupils living in an urban, rural tribal trust land and commercial farming environments of Zimbabwe was investigated. Stool specimens were collected on 3 alternate days and processed for parasite identification and a questionnaire was administered to ascertain the risk factors for infection. The assays carried out on individual specimens were wet preparations. The specimens were pooled and preserved in sodium acetate formaldehyde and the formal ether concentration method was performed on the pooled specimens. The staining methods carried out were the cold Ziehl Neelsen stain and Gomori/trichrome staining. *Giardia duodenalis* and *Cyclospora cayetanensis* were prevalent in the rural tribal trust land (TTL). The risk factors for being infected with *G. duodenalis* being, not washing fruits or vegetables before eating and drinking contaminated water from deep protected wells. Parasites most prevalent in the commercial farming area were *Entamoeba histolytica/dispar, Cryptosporidium parvum* and the helminthes. The risk factor of being infected with *E. histolytica/dispar* was not taking measures to make water safe for drinking. Rare protozoan parasites such as *Entamoeba polecki* and *Enteromonas hominis* were also identified in the rural TTL and commercial farming environments respectively. *Blastocystis hominis* was prevalent in the urban area. Measures such as health education and provision of safe water and provision of good sanitation may play a pivotal role in the reduction of these infections.

2.2 INTRODUCTION

World Health Organization (WHO) data on the burden of disease suggest that approximately 3.2 % of deaths (1.8 million) and 4.2 % of disability-adjusted-life years (61.9 million) worldwide are attributable to unsafe water and poor sanitation and hygiene leading to 99.8 % deaths in developing countries. About 88 % of diarrhoeal disease is attributed to unsafe water supply, inadequate sanitation and poor hygiene (WHO, 2004). Previous data for Zimbabwe indicated that in some parts of the country, there is quite a notable portion of diarrhoeal cases due to parasites (NMRL, 2004). Studies done in Brazil and Zimbabwe in urban and rural settings showed that *G. duodenalis* was more prevalent in an urban area compared to a rural environment (Mason *et al.,* 1986; Giraldi, 2001). The latter study attributed this to overcrowding as implicated in a study done in Turkey (Okyay, 2004).

Communities do not stay the same after a period of time due to migration and other social and economic factors. The previous studies in Zimbabwe were done 21 years ago by Mason *et al.,* in 1986 and Simango and Dindiwe also in 1986, hence the need to ascertain the current status of protozoan parasitic infections in urban, rural and commercial farming environments. Epidemiological research carried out in different countries has shown that the social and economic status of individuals are an important factor in the prevalence of intestinal parasites and these factors tend to change over time due to termination of contracts of employment (Okyay, 2004). The objective of the present study was to ascertain the current status of gastrointestinal parasitic infections in 3 different environments. Additional parameters that were added to this current study were the questionnaire survey and health education.

2.3 MATERIALS AND METHODS

2.3.1 Study areas and design

The study was a cross-sectional comparative study of 3 different communities, from urban, rural TTL and commercial farming community (Appendix A). The study sites were Tafara 1 Primary School in a high density urban setting 21 km away from the City centre of Harare having a longitude of 31° 11', latitude 17°49' and altitude of 1 483 m above sea level. The Chiweshe rural TTL was headed by a chief who had several headman below him then the villagers. Musarara Primary School was in Chiweshe rural TTL, having a longitude of 31° 00', latitude of 17° 14' and altitude of 1 250 m above sea level. The commercial farming community was Bvumba in the Eastern Highlands of Zimbabwe having a longitude of 32° 48' latitude of 19° 50'and altitude of 4 200 m above sea level. The commercial farming community had banana and coffee plantations and the people lived in compounds. Study participants from the commercial farming community were from 2 schools namely Bvumba and Crake Valley Primary Schools in order to obtain the required sample size. The sample size for each community was calculated to be 431 at 95 % confidence interval (CI) considering 75 % compliance rate.

2.3.2 Study participants

The study participants were school children from grades 1 to 7, aged 4 - 15 yr with an average age of 8 yr. Five hundred and ninety were males (590) and 626 were females. The total number of pupils that enrolled after written consent from parents or guardians and verbal agreement of all the pupils was 491 (39 %) pupils from the urban area, 401 (32 %) from the rural area and 368 (29 %) from the farming area making, a total of 1 260 pupils.

2.3.3 Stool parasitology

Stool specimens were collected on 3 alternate days to allow a 80 % recovery rate of parasites (Kang, 1998). The questionnaire was carried out on the other 4 alternate days of the week. On each day of collection, specimen description and wet faecal smears in saline were carried out. Each specimen was preserved in sodium ammonium formaldehyde (SAF). To increase parasite detection rates the 3 stool specimens taken from each individual on alternate days was pooled into a single specimen (Garcia, 1999). The formol ether concentration method and the Gomori/Trichrome staining technique were then perfomed on the SAF preserved specimens as described by Cheeseborough, (2005). The wet preparations these specimens were examined using the × 10, × 40 and × 100 objective lens of a light microscope and × 100 for the stained slides.

2.3.4 Chemotherapy

Participants who were found to be infected by the pathogenic protozoan parasites, *G. duodenalis* and *E. histolytica* were treated with metronidazole tablets (100 mg/day taken orally for 7 days). Those infected with the soil transmitted helminths were given one single dose of 400 mg albendazole orally and those that had schistosomiasis were given a single dose of prazinquantel at 40 mg/kg body weight. All drugs were administered by a qualified nurse who clearly explained all the side effects. A pamphlet was given to the parents of the pupils that had to take metronidazole as this drug had to be taken over several days and therefore required parental or guardian monitoring to ensure compliance. The drugs with single doses were administered by the nurse.

2.3.5 Questionnaire and health education

The questionnaire which was administered by the research assistants under the researcher's guidance, dealt with the subjects; socio demographic data, water sources and type of storage containers and the type of sanitation they used. Their knowledge on causes of diarrhoea and water disinfection and lastly hygienic practices was also probed (Appendix B).

Health education and hygiene promotion was also administered to pupils at the school visited. This was carried out on the last day of the visit after the sampling and questionnaire survey had been done. It was administered at assembly where all the pupils of that school were present and a down up approach was applied. This involved discussing hygienic practices that can prevent acquisition of diarrhoeal diseases and how to disinfect water. Charts with illustrations were also shown and each teacher was given a chart to place on the wall in their classroom (Appendix C).

2.3.6 Statistical analysis

The statistical package used to analyze the data was stata version 9 (StataCorp LP, College Station TX, USA). The Chi square test was used to compare the percentage distribution of parasitic infections in the different communities, whilst the Fischer's exact test was used to calculate the prevalence of helminths in the different schools because of the low number of these particular parasites. Logistic regression was used on the stool parasitology results and questionnaire in order to identify the risk factors associated with infection by a particular parasite. The inclusion and exclusion criteria were applied for this analysis. A total of 1 260 pupils had agreed to be in the study and only 967 fulfilled the inclusion criteria. Those who did

not bring a sample, submitted only one sample or did not participate in the questionnaire survey were excluded.

2.4 RESULTS

Of the 1 260 pupils that had enrolled, only 1 057 (85 %) submitted 2 or 3 stool samples but 90 did not submit a questionnaire therefore were not included leaving 967 participants satisfying. the inclusion criteria. There were some study participants who submitted only 1 stool sample while others were unable to submit a specimen, the highest rate of defaulters being from the urban environment (25 %) (Table 2.1).

The most prevalent parasites in the rural TTL were *G. duodenalis, C. cayetanensis, E. coli, I. butschlii, C. mesnelli* whilst in the commercial farming environment the parasites were *E. histolytica, C. parvum, I. butschlii* and the helminths (Fig 2.1, Table 2.2). *Blastocystis hominis* was most prevalent in the urban community (Table 2.2). Rare parasites such as *Entamoeba polecki* and *Enteromonas hominis* were identified in the rural and farming environments respectively. The logistic regression analysis was on data from 967 participants who satisfied the inclusion criteria of providing 2 or 3 stool samples and a completed questionnaire. Significant associations were noted between parasitic infections and questionnaire data (Table 2.3). The related factors of infection by *G. duodenalis* were not washing fruits or vegetables, the type of drinking water source and not knowing that water may cause diarrhoea (Odds Ratio: 1.64, 1.08 and 1.71 respectively). If an individual did not take any measures to make water safe for drinking then there was a high chance of being infected by *E. histolytica/dispar* (Odds Ratio: 3.02).

Prevalence of *G. duodenalis, E. histolytica/dispar* and *C. parvum* was higher in the 6 - 15 yr olds while *C. cayetanensis* and *E. coli* were more prevalent in the ≤ 5 yrs (Table 2.4).

Table 2.1 : Compliance of study recruits from the different communities

Type of community	**Non compliant**		**Compliant**		**Total recruits**
	Number	%	Number	%	
Urban	121	25	370	75	491
Rural TTL	14	3	387	97	401
Commercial farm	68	18	300	82	368
Total	**203**	**16**	**1 057**	**84**	**1 260**

The majority of individuals were compliant in all the 3 communities.

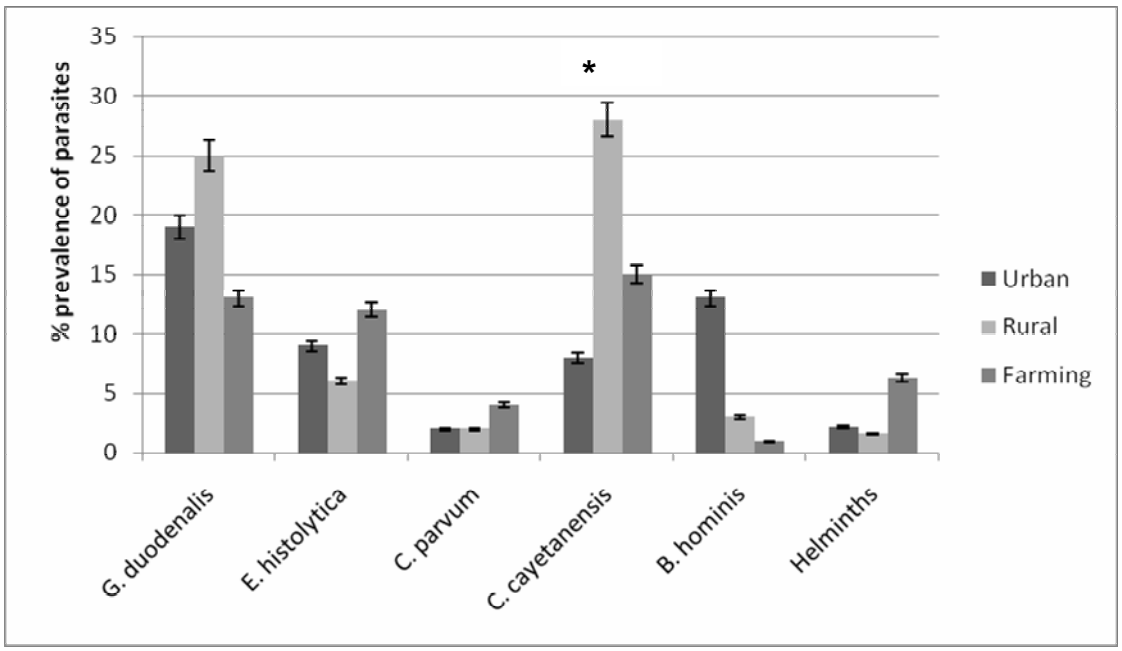

Figure 2.1: Percentage prevalence of the potentially pathogenic parasites in the 3 different communities

(*G. duodenalis* - *Giardia duodenalis, E. histolytica* - *Entamoeba histolytica, C. parvum* - *Cryptosporidium parvum, C. cayetanensis* - *Cyclospoara cayetanensis, B. hominis* - *Blastocytis hominis*) *$p<0.05$

The distribution of the parasitic infections were such that *G. duodenalis* was most prevalent in the rural community, *E. histolytica* in the farming community and *Blastocystis hominis* in the urban community.

Table 2.2 : Percentage distribution of parasitic infections by environment

Parasite	**Urban**		**Rural**		**Farming**	
	n	**%**	**n**	**%**	**n**	**%**
Giardia duodenalis	94	19	100	**25**	49	13
Entamoeba histolytica	45	9	22	6	45	**12**
Cryptosporidium parvum	9	2	9	2	16	**4**
Cyclospora cayetanensis	38	8	114	**28**	55	15
Entamoeba coli	137	28	261	**65**	158	43
Entamoeba hartmanni	33	**7**	17	4	15	4
Iodamoeba butschlii	41	9	98	**24**	87	**24**
Chilomastix mesnelli	38	8	68	**17**	15	4
Blastocystis hominis	63	**13**	12	3	2	1
Enteromonas hominis	0	0	0	0	1	**0.3**
Entamoeba polecki	0	0	1	**0.3**	0	0
Hookworms	0	0	0	0	6	**2**
Ascaris lumbricoides	11	2	1	0.3	12	**3**
Schistosoma mansoni	0	0	0	0	5	**1**
Schistosoma haematobium	1	0.2	1	**0.3**	0	0
Enterobius vermicularis	0	0	0	0	1	**0.3**
Hymenolepsis nana	0	0	3	**1**	0	0

n = number of study participants that were positive. Percentages that are in bold indicate the highest prevalence of that parasite in comparison to the other 2 communities. Rare parasites such as *Enteromonas hominis* and *Entamoeba polecki* were also identified.

Table 2.3 : Associations between parasitic infections and questionnaire data

Parasite	Parameter	OR	P value
G. duodenalis	Fruits or vegetables washed before eating	1.64	0.02
	Drinking water source	1.08	0.04
	Water may cause diarrhea	1.71	0.01
E. histolytica/dispar	No water disinfection methods undertaken	3.02	0.04
C. parvum	Ethnic group	1.99	0.001
	An open water storage container	4.32	0.006
C. cayetanensis	Residing in a rural TTL	9.52	0.00
	Residing in a commercial farming community	24.94	0.006
	Type of toilet used	1.44	0.037
	Communal hand washing before eating	2.05	0.024
E. coli	Residing in a rural TTL or commercial farming area	3.33	0.00
E. nana	School attended	7.66	0.00
	Type of toilet	1.49	0.02
E. hartmanni	Type of toilet	1.72	0.04
I. butschlii	Gender of participant	1.42	0.06
C. mesnelli	Drinking water source	1.14	0.00
B. hominis	School attended	1.17e	0.00
A. lumbricoides	School attended	9.46e	0.00
	Females	4.73	0.03

OR is the odds ratio which if greater than 1 correlates to an association of the identified parameter to the parasite.

Table 2.4 : Prevalence of parasitic infections according to age and gender

Parasite	**≤5yr T=120**		**6-15yr T=1196**		**Males T=590**		**Females T=626**		**P values**
	n	%	n	%	N	%	n	%	
G. duodenalis	18	15	235	20	123	21	121	19.3	0.025
E. histolytica	4	3	109	9	53	9	58	9.2	0.05
C. parvum	0	0	35	3	20	3	15	2.4	0.132
C. cayetanensis	25	21	197	16	96	16	107	17	0.849
E. coli	57	48	532	45	251	43	293	47	0.319
E. hartmanni	1	1	64	5	23	4	42	7	0.005
I. butschlii	23	19	214	18	92	16	130	21	0.049
C. mesnelli	15	13	116	10	56	10	65	10	0.871
B. hominis	3	3	74	6	42	7	34	5	0.048
Enteromonas	0	0	1	0.1	1	0.2	0	0	0.541
E. polecki	0	0	1	0.1	1	0.2	0	0	0.541
Hookworms	0	0	6	1	4	0.7	2	0.3	0.511
A. lumbricoides	1	1	23	2	8	1	15	2	0.33
S. mansoni	0	0	5	0.4	2	0.3	3	1	0.755
S. haematobium	0	0	2	0.2	1	0.2	1	0.2	0.922
E. vermicularis	0	0	1	0.1	0	0	1	0.2	0.577
H. nana	1	1	1	0.1	1	0.2	2	0.3	0.762

Note : T = total number tested. *Cyclospora cayetanensis* and *E. coli* were more prevalent in children aged ≤ 5 yr.

The population of those ≤ 5 yr old was 9 % compared to 6 - 15 yr age group that made up 91 % of the study population. There was not much difference in being infected by parasites taking in to account gender and the number of species infecting an individual (Table 2.4 and Table 2.5).

Concerning sanitary habits in the 3 environments, there were 3 types of toilets that were used, *i.e.* the water closet type was most common in the urban community, the Blair toilet was most common in the commercial farming community, whilst the pit latrines were most common in the rural TTL (Table 2.6). The rural TTL had the highest number of people that did not possess a toilet (66 %) (Table 2.6). Concerning toilet habits of school children, Crake Valley was found to have the highest number of pupils that defaecated in the bush [43 % (82/191) ($p<0.05$)], followed by Tafara with 35 % (123/352), Musarara, 32 % (127/396) and lastly Bvumba 23 % (35/151). Crake Valley also had the highest number of pupils that had helminthic parasitic infections with *A. lumbricoides* being the most prevalent (Table 2.7).

The most common water source in the rural TTL were deep protected wells. *Giardia duodenalis* was the most prevalent parasite in that community. In the commercial farming community the majority of people used lakes or dams where as piped water systems were most common in the urban community (Table 2.8).

Table 2.5 : Number of parasite species infecting an individual according to age and gender

No of parasite species	≤ 5 yr T = 120 n	%	6 – 15 yr T = 1 196 n	%	Males T = 590 n	%	Females T = 626 n	%
1	15	13	312	26	156	26	158	25
2	19	16	245	21	127	22	125	20
3	11	9	156	13	76	13	83	13
4	11	9	71	6	28	5	49	8
5	4	3	28	2.3	11	1.8	18	3
6	0	0	3	0.3	1	0.2	2	0.3
7	0	0	2	0.2	1	0.2	1	0.2

Two people harboured 7 different species of parasites, regardless of gender.

Table 2.6 : Type of toilets used in the 3 environments

Sanitation	Total number	Urban 353 N	%	Rural 396 n	%	TTL Commercial 342 n	Farm %
Water closet type toilet	**256**	241	94	4	2	11	4
Blair toilet	491	49	10	213	43	229	46
Pit latrine	293	49	17	147	50	97	33
No toilet	48	11	23	32	66	5	10

Regardless of the environment some people did not possess a toilet at their homesteads although the rural area had the highest percentage (66 %).

Table 2.7 : Percentage distribution of helminths in the 4 schools

Parasite	**Tafara**		**Musarara**		**Bvumba**		**Crake Valley**		**Fischer's Exact**
	n	**%**	**N**	**%**	**N**	**%**	**n**	**%**	
Hookworms	0	0	0	0	1	0.6	5	**2**	0.000
Ascaris lumbricoides	11	2	1	0.3	0	0	12	**6**	0.000
Schistosoma mansoni	0	0	0	0	2	1	3	**2**	0.003
Enterobius vermicularis	0	0	0	0	0	0	1	**1**	0.129

Ascaris lumbricoides was most prevalent at Crake Valley School. The Fischer's exact is the same as the p value, but is only used whenever numbers are small.

Table 2.8 : Association of *Giardia duodenalis* with the different water sources in relation to the type of community

Water source	No. of people that use source	No. infected with parasites		People that use that water Source					
		n	%	Urban N	%	Rural n	%	Farming n	%
Unprotected wells	123	12	10	33	27	24	20	66	53
Protected wells	80	15	19	11	14	41	51	28	35
Springs	11	2	18	3	27	4	36	4	36
River or stream	23	6	26	0	0	4	17	19	82
Lake or dam	27	5	19	0	0	1	4	26	96
Boreholes	184	43	23	19	10	133	72	32	17
Unprotected deep wells	53	10	19	14	26	38	71	1	2
Protected deep wells	159	40	25	16	10	131	82	12	8
Dug well in a river	176	30	17	0	0	19	11	157	89
Piped water	259	53	20	257	99	0	0	1	0.4

Ninety-nine percent of urban people had a piped water system, but other sources of water were still being used by this community.

2.5 DISCUSSION

Intestinal parasites are prevalent worldwide although high prevalences are usually in the developing countries due to their low socio-economic status, and poor living conditions such as overcrowding, poor sanitation, unsafe water and unhygienic personal habits (Noor Azian, 2007). These risk factors vary from one community to another. The present study was to ascertain the prevalence pattern of parasitic infections in 3 different environments and the probable reasons behind the prevalence rates observed. This is unique in that the behaviour pattern, knowledge and source of drinking water all affect the infection status of the participants in the given environments. Access to information on hygienic practice, provision of proper sanitation and knowledge of source of infection help to reduce unnecessary exposure to parasitic infection.

Giardia duodenalis and *C. cayetanensis* were most prevalent in the rural TTL, *E. histolytica/dispar*, *C. parvum* and helminthic infection in the commercial farming environment, while in the urban environment *Blastocystis hominis* was most prevalent. Previously, in Zimbabwe *G. duodenalis* had been more prevalent in urban areas as compared to rural areas (Mason *et al.,* 1986). According to the findings of the current study, *G. duodenalis* is more prevalent in the rural areas. Gender did not pose a risk factor for infection by a parasite.

The risk factors associated with *G. duodenalis* included not washing fruits before eating and the type of water source. Unwashed vegetables have been found contaminated with parasite cysts and ova. In a study done in Gaza, where certain vegetables that are eaten raw, were washed the water was concentrated by centrifugation and the resultant pellet examined. They found out that

G. intestinalis, E. histolytica and *A. lumbricoides* were the most commonly found parasites (Al-Shawa and Mwafy, 2007). Water bodies are known to be contaminated with *G. duodenalis,* and in this study the type of drinking water source was a risk factor for infection with *G. duodenalis.* In the USA ground water has been shown to contribute to a significant fraction of all waterborne disease outbreaks as an estimated 750 000 to 5.9 million illnesses result from it, causing 1 400-9 400 deaths annually (Macler and Merkle, 2000). Deep protected wells were being used by 82 % of people in the rural TTL where *G. duodenalis* was most prevalent. They were most likely a risk factor associated with infection with the parasite. These deep protected wells probably provide a favourable environment for the survival of the parasites.

In this study *C. cayetanensis* occurrence was associated with type of community one resided in and the type of toilet used. Fifty percent of the study subjects who used Blair toilets were infected with *C. cayetanensis* and these were the most common type of toilets in the rural and farming communities. Infection with *C. cayetannensis* was also common amongst people who used a communal dish for washing hands before eating food. In Zimbabwe the staple food is eaten using hands. It therefore follows that if people that wash their hands contaminated with faeces, after using the toilet and then go on to use a communal water dish, they may contaminate the water used for washing hands before eating. In a study done in India, they found out that food and vegetable vendors who washed their hands in water storage containers contaminated this water with pathogenic parasites, as the original water was free of these parasites (Jonnalagadda and Bhat, 1995). Furthermore, according to WHO (2004), the simple act of washing hands at critical times has been found to reduce the number of diarrhoeal cases by up to 35 %. Another contributory factor to the high parasitic prevalence was the absence of a toilet at a homestead and

such cases were in the rural area (66 %). These people either defaecated in the bush, further contaminating the environment or used the toilet next door. *Cyclospora cayetanensis* was also high amongst those that were ≤ 5 yrs age group. This conforms with results from another study, whereby this parasite was more prevalent in children 22.5 % (16/71), although these children were below 10 yr of age (Lopez *et al.,* 2003). The risk factor of being infected in that particular study was drinking well water from the northern region of that community as compared to the southern region. In this study the risk factor was living in a rural TTL or commercial farming environment, rather than an urban environment.

Infection with *E. histolytica/dispar* was associated with lack of measures to make water safe for drinking. These measures were boiling water, adding a disinfectant chemical such as Jik™ (Sodium hypochlorite solution), covering the water storage container or filtering the water. The highest prevalence of this parasite was in a farming area. It is therefore important that some form of water disinfection is undertaken in order to avoid infection with *E. histolytica/dispar.* In a study done in Nigeria, prevalence of *E. histolytica* was high among those who ate from the same plate, used their fingers to eat, ate away from home, including how household water supplies were stored in the home (Oyerinde *et al.,* 1978). The first 3 parameters were not investigated in this study while the last one was, although the Nigerian results contradicted with these results as storage of water did not influence presence of *E. histolytica.*

Cryptosporidium parvum was also prevalent in the farming area. An interesting finding was the association of this parasite with an ethnic group of people who were the Mozambicans. The farming area under study was at the border of Zimbabwe and Mozambique. A few of the school

children who attended school in this area would walk from Mozambique to attend school. Therefore *Cryptosporidium* could be more prevalent amongst these individuals as is indicated by the results, although further studies need to be conducted in that country. In general *Cryptosporidium* is mostly associated with HIV/AIDS patients as it is usually prevalent amongst those infected (Escobedo and Nu´n˜ez, 1999).

Blastocystis hominis prevalence was highest amongst those that lived in an urban area. In a study done in Malaysia in an aborigine community, *B. hominis* was the most prevalent parasite (52.3 %) (Noor *et al.,* 2007). Previously, this parasite was considered to be non pathogenic, but recent case studies have proven it to be a potential pathogen. A case report done, on a 10 year old girl who had watery diarrhoea, anorexia, vomiting and weight loss demonstrated *Blastocystis* from her stool sample, and she improved after metronidazole treatment (Antonelli *et al.,* 1996).

Other interesting findings in this study include identification of *Entamoeba polecki* from a rural area and *Enteromonas hominis* from a farming area. The low prevalence of *E. polecki* is conforming to other studies conducted in rural areas such as those done in Malaysia, in which the prevalence was 1.5% and the study in Cambodia, in which the prevalence was 1.1 % (Park *et al.,* 2004; Noor *et al.,* 2007).

The highest prevalence of all helminthic infections was among those that attended Crake Valley School in a farming environment. This school had the highest number of students who defaecated in the bush followed by Tafara in the urban environment. These results are conforming to another study done by Ulukanligil and Seyrek, (2003) in Turkey that showed that

high helminthic infections were associated with poor sanitary conditions. Sanitation needs attention as even in an urban area there were people without toilets at their residences. According to WHO (2004) improved sanitation reduces diarrhoea morbidity by 37.5 %.

One of the limitations of this study is that effect of the imparted health education was not assessed due to lack of time. There is therefore a need to do further studies to assess the effects of that health education.

2.6 CONCLUSION

Giardia duodenalis and *C. cayetanensis* were the most prevalent parasites in the rural area. The risk factors associated with *G. duodenalis* infection were, not washing fruits or vegetables and drinking water from deep protected wells. Whilst that of *C. cayetanensis* infection was being ≤ 5 yr in age including washing hands in a communal dish before eating a meal. Parasites that were prevalent in the farming area were *E. histolytica/dispar, C. parvum* and helminths. The risk factor of being infected with *E. histolytica/dispar* was drinking contaminated water. *Cryptosporidium parvum* was associated with a particular ethnic group of children, whilst helminths prevalent in one school associated with bad sanitary habits of defaecation in the bush. *Blastocystis hominis* was most prevalent at Tafara School in the urban community. This parasite is one of the pathogenic protozoan parasites that is not routinely identified in diagnostic laboratories as more sensitive tests such as Gomori/trichrome staining is required for its identification. Rare parasites such as *Entamoeba polecki* and *Enteromonas hominis* were also found in some of the participants.

3.1 SUMMARY

Drinking water is a potential source of parasitic infections whether treated or untreated. Parasites have been identified in both treated and untreated water in the developed countries where resources for parasite identification are available. In order to establish that water maybe one of the sources of parasite infection in Zimbabwe, a study was carried out in Chiweshe TTL. Permission, consent or ascent was sought from the relevant authorities and 113 participants whose age range was 2 – 89 yr. Stool samples and water samples from their drinking water sources were collected and analyzed for parasites. HIV testing was also carried out after counseling. Five participants had similar parasites identified in both their stool and water samples regardless of their HIV status. The drinking of contaminated water plays a pivotal role in the acquisition of parasitic infections.

3.2 INTRODUCTION

The United Nations named the decade 2005-2015 "Water for Life", declared it the International Decade for Action and set the world agenda on a greater focus on water-related issues. Coverage for both improved water supply and sanitation lags behind in the poorest communities in rural areas and urban/peri-urban slums of the developing countries (WHO, 2004). It is also reported that 80 % of all illness in these countries of the developing world is related to water and sanitation (Pritchard *et al.,* 2007).

Micro-organisms that cause diarrhoea due to the drinking of contaminated water are predominantly of faecal origin and the indicator organism for bacterial contamination is *Escherichia coli* (Ashbolt, 2004). Parasites and viruses do not have indicator organisms. Specific tests have to be done for their identification. Intestinal parasites cause diarrhoeal diseases, increased morbidity and mortality in children and/or including individuals that are immunocompromised such as HIV positive individuals (Saidi *et al.,* 1997, Zali *et al.,* 2004).

Disease outbreaks caused by protozoan cysts in drinking water sources have been recorded in several developed countries such as the United States, United Kingdom, Canada and Sweden as chlorine that is commonly used to disinfect water does not kill them (Korich *et al.,* 1990; Ljungstrom and Castor, 1992; Wallis *et al.,* 1996; Sinclair *et al.,* 1998; Isaac-Renton *et al.,* 1999; Barwick *et al.,* 2002). Such outbreaks are definitely occurring unchecked in the developing world as most countries do not have the resources to carry out the required tests. An outbreak of *Cryptosporidiosis* occurred in Nevada, USA, in 1996, that was traced back to the drinking water source. There were no abnormalities detected in the treatment operations, of both the raw and treated water (Goldstein *et al.,* 1996).

In a developing country such as Zimbabwe, there are many water sources that are used by different communities. These include boreholes, shallow or deep wells or tap water, the latter being predominately used by the urban population. In the last few years in an urban setting such as Harare, the local City Council authorities have had challenges in municipal water treatment due to lack of foreign currency for the purchasing of treatment chemicals. An estimated 70 % of the national population of Zimbabwe lives in rural areas where ground water is consumed

without treatment. Analysis of water from boreholes and shallow wells in Kamangira village in Marondera district indicated that 22 % of these were not safe for human consumption as the faecal coliform count was more than the WHO recommendation of 0 CFU/100/ml (Dzvairo *et al.*, 2006). Studies in the developed world have reported the potential presence of intestinal protozoan parasites in treated tap water following outbreaks (Korich *et al.,* 1990; Ljungstrom and Castor, 1992; Wallis *et al.,* 1996; Sinclair *et al.,* 1998; Isaac-Renton *et al.,* 1999; Barwick *et al.,* 2002). The present study was conducted to ascertain the presence of protozoan parasites in a variety of water bodies in a rural setting. To achieve this goal, more sensitive tests could have been used, but due to lack of resources, available tests were used.

3.3 MATERIALS AND METHODS

3.3.1 Study site

The study site was Chiweshe rural tribal trust land. It is located 70 km from Harare, on longitude of 31° 00 mins, latitude of 17° 14 mins and an altitude of 1 250 m. The study population was 113 people, comprising of 34 males and 79 females and age range being 2 to 89 yr.

3.3.2 Consent and permission

Approval to carry out the research was obtained from the Medical Research Council of Zimbabwe. Consent was also obtained from the stakeholders of Mashonaland Central, namely the Provincial Medical Director, the District Medical Officer, the District Administrator, the 2 Chiefs of that area, the village headman and the villagers themselves. Permission to work together with the Hospital was also obtained from the Chief Executive Officer of Howard

Mission Hospital. At the hospital the research team worked together with the counselors at Tariro HIV/AIDS Care Clinic who did the pre and post counseling services for this study population that was going to go through HIV testing.

The area chief organized the meeting where all the village headmen and their people were asked to assemble at their meeting place in Musarara village (Appendix E). The objectives of the study were explained to the people. A down up approach technique of addressing the people was employed so that the villagers would speak more freely about their concerns, on gastrointestinal infections and hygiene practices. This is a technique whereby questions were asked and villagers gave their perceptions concerning the subject under discussion. Village mapping was then done where each headman was asked to form a group with the people in his village. Thereafter a map of each particular village was drawn showing the main features such as the main road, shops, church or a school. Each individual participant's home was clearly marked on the map and names of those consenting to participate in the study were recorded followed by home visits.

3.3.3 Specimen collection

Home visits involved administration of the questionnaire and conducting HIV testing using 2 rapid tests kits namely SD BIOLONE HIV 1/2 (Standard Diagnostics, Inc, Korea) and Determine HIV-1/2 kit (Abbot Laboratories, Tokyo, Japan). Pre-HIV test counseling was also done at the meeting by counselors from Howard Mission Hospital. Rapid HIV testing using the 2 kits mentioned above was done at home. Disclosure of their status was done by counselors at Tariro Clinic at the Howard Mission Hospital. Stool samples were also collected as well as water samples from their drinking water source.

3.3.4 Processing of stool specimens

Stool specimens were processed as previously described. Laboratory tests carried out were the formalin ether technique and the Gomori/trichrome staining. The cold Ziehl Neelsen method was carried out to identify the intestinal protozoan parasites such as oocysts of *Cryptosporidium* in faeces (Cheeseborough, 2005).

3.3.5 Processing of water samples

The physicochemical analysis of water was done during the home visits. This is the measurement of the temperature, pH, turbidity and conductivity. Measurement of turbidity was conducted using the Hach Portable 2100P Turbidometer, pH and temperature was measured using the Boeco Germany PT370 pH meter and conductivity was measured using the HI98311 conductivity meter from Hanna Instruments, Mauritius. Specimens were collected separately in wide mouthed plastic bottles for different tests such as for physico-chemical tests and 1 000 ml was for parasitology analysis. The samples for parasitological analysis were placed in cooler boxes, and rushed to the laboratory for processing within 4 to 6 hours.

At the laboratory, the water was centrifuged at 500 *g* for 3 minutes. Wet preparations were done on the sediments and examined under the microscope using the × 10 then × 40 objective lens for parasite presence. The sediments were also preserved in formalin. The Zinc sulphate floatation technique was then carried out on the formalin preserved samples as described by Cheeseborough (2005), where the sediments were used in the technique. Dried preparations were stained using the cold Ziehl Neelsen technique then examined under oil immersion at × 100

magnification for oocyst identification. Frequencies using Stata Version 9 statistical package were used to calculate results of the prevalence of parasites in both stool and water samples.

3.4 RESULTS

There were 113 participants, of these 29 (25.7 %) of the study subjects were HIV positive and 84 (74.3 %) were negative by the 2 rapid serology tests. One hundred and one participants were included in the analyses as they submitted both water and stool samples whilst 12 were unabe. Seventeen participants (18.3 %) produced watery, diarrhoeic stool samples, of these 13(76 %) had parasitic infections and 6(26 %) of the 13 were HIV seropositive.

The pathogenic protozoan parasites identified in stool were *Cyclospora cayetanensis* 23(22.1 %), *Entamoeba histolytica/dispar* 19(18.2 %), *Cryptosporidium parvum* 8(7.6 %) and *Giardia duodenalis* 6(5.7 %). The non-pathogenic species found were *Entamoeba coli* 45(43.3 %), *Iodamoeba butschlii* 15(14.4 %), *Chilomastix mesnelli* 11(10.6 %), *Endolimax nana* 4(3.9 %), *Blastocystis hominis* 3(2.9 %) and *Trichomonas hominis* 1(0.9 %) (Table 3.1). The number of participants that did not harbour any parasites were 25(24 %). Only one person was infected with a helminth, *Schistosoma mansoni.* Polyparasitism was evident with 20(19.2 %) harbouring 2 different species of protozoan parasites, 15(14.4 %) harbouring 3 and 7(6.7 %) harbouring 4 species all in different combinations (Table 3.1).

Table 3.1 : Parasitic species identified in humans in Chiweshe Rural community separated according to HIV status and ± diarrhoea (D)

Parasites identified	No. of individuals infected		HIV positive		HIV negative	
	n	%	D	No D	D	No D
Entamoeba coli	45	43.3	0	6	7	32
No parasites	25	24	2	6	1	16
Cyclospora cayetanensis	23	22.1	0	1	1	1
Entamoeba histolytica	19	18.2	1	2	3	13
Iodamoeba butschlii	15	14.4	0	1	3	11
Chilomastix mesnelli	11	10.6	0	0	2	9
Cryptosporidium parvum	8	7.6	2	3	2	1
Giardia duodenalis	6	5.7	0	2	0	4
Endolimax nana	4	3.8	1	0	0	3
Blastocystis hominis	3	2.8	1	0	1	1
Trichomonas hominis	1	0.9	0	0	0	1
Schistosoma mansoni	1	0.9	0	1	0	0

Entamoeba coli although being the most prevalent did not cause diarrhoea in HIV positive people. (No D – No diarrhoea). Polyparasitism was evident with 42 participants harbouring 2 to 4 different species of parasites.

One of the important findings was that five participants had similar parasites identified in both their stool and water samples with 4 of them being HIV positive and having diarrhoea. The participant who was HIV negative did not have diarrhoea. Four of these drank water from deep protected wells with 2 being infected with *E. histolytica*, 1 with *C. parvum* and the other with both *G. duodenalis* and *C. cayetanensis.* These parasites were also identified in their water bodies. The fifth person drank water from an open unprotected shallow well and *E. histolytica* was identified in both stool and water source (Table 3.2).

Water was collected from 30 different water bodies, the majority of the bodies were deep protected wells and 2 were small water treatment plants. At Howard SWTP *C. parvum* and *C. cayetannensis* were identified before water treatment only, whilst for Nzvimbo SWTP the same parasites were identified before and during treatment. In both cases no parasites were identified after water treatment.

The physico-chemical results of the drinking water sources shown in Table 3.4 involved measurement of temperature, conductivity, pH and turbidity with the first 2 parameters not having WHO normal values although the recommended temperature for drinking water is 25 ºC according to UK standards (Pritchard *et al.,* 2007). Water from taps had the highest temperatures whilst all water sources were within normal pH range. The shallow protected well had the lowest conductivity but highest turbidity value whilst boreholes had the highest conductivity values. The well with a bush pump had the lowest turbidity value (Table 3.4).

Table 3.2 : Identical parasites being identified in a participant's stool and drinking water source

Parasite	Drinking water source	HIV Positive		HIV Negative	
		Diarrhoea	No diarrhea	Diarrhoea	No diarrhea
Entamoeba histolytica	Open unprotected shallow well	1	0	-	-
	Deep protected well	-	-	0	1
Giardia duodenalis	Deep protected well	1	0	-	-
Cryptosporidium parvum	Deep protected well	1	0	-	-
Cyclospora cayetanensis	Deep protected well	1	0	-	-

Deep protected wells were associated with parasite cyst contamination. The 4 HIV positive people had diarrhoea and these parasitic infections could have been the cause.

Table 3.3 : Water samples analyzed and number of participants that use them

Type of water body	Water Body		Participants that use it	
	n	%	N	%
Shallow unprotected well	4	14.3	4	0.04
Shallow protected well	1	3.57	6	0.06
Shallow well with a bush pump	1	3.57	1	0.01
Deep unprotected well	5	17.8	3	0.003
Deep protected well	14	50	34	33.4
Boreholes	2	7.14	49	48.5
Communal tap water	1	3.57	4	0.04

The 2 boreholes in that community were being used by the majority of individuals (48.5 %). The 2 small water treatment plants were not included on this table as the participants that use them were those that used communal tap water.

Table 3.4 The mean physico-chemical analysis results for different water sources

Water source	Temp		pH		Conductivity		Turbidity	
	ºC	**Range**	**pH**	**Range**	**µS/cm**	**Range**	**NTU**	**Range**
Shallow unprotected wells	22.25	21.5-23	7	6.8-7.2	162.5	130-195	0.28	0.2-0.35
Shallow protected wells	24.5	24.5-24.5	6.7	6.7-6.7	35	35-35	43.2	43.2-43.2
Shallow well with a bush pump	26	26-26	6.6	6.6-6.6	165	165-165	0.03	0.03-0.03
Deep unprotected wells	23	21-24	7.1	6.9-7.5	160.6	117-217	0.58	0.53-0.6
Deep protected wells	24.5	22.8-27.5	6.8	5.7-7.2	148.7	0.09-285	7.5	0.78-7.94
Boreholes	24.5	24.5-25	6.7	6.7-6.8	278.5	255-280	7.5	0.78-7.94
Communal tap water	27	27-27	6.85	6.7-6.9	205.3	200-207	1.58	0.1-6.03

WHO guidelines:- pH – 6.5-8.5 Conductivity – No normal range Turbidity – 1-5NTU

Temperature – There is no WHO normal range. UK normal value is 25 °C (Pritchard *et al.,* 2007).

Temperature was low in the unprotected wells whilst, conductivity was high in water sources that are used by the majority of people such as boreholes and communal tap water. This is an indication of an increased amount of ions in those water sources.

The total number of deep protected wells were 14 and the open unprotected wells were 4 out of a total of 30 water bodies analyzed. Other free living protozoa that were present in water bodies included the *Paramecium* ciliate found in 6(20 %) of the water bodies, 12(40 %) had unidentified water flagellates. Eleven out of 30 (36.6 %) water bodies had protozoan parasites (Table 3.5). Two (6.6 %) of the water bodies had *G. duodenalis,* 4(13.3 %) had *E. histolytica*, 1(3.3 %) had *C. parvum*, 3(10 %) had *C. cayetanensis* and 1(3.3 %) had *E. coli* (Table 3.5). Water samples from deep protected wells used by 34(33.6 %) of the people, yielded a significant association ($p < 0.05$) with *G. duodenalis, E. histolytica/dispar, C. cayetanensis* and the unidentified water flagellates 12(40 %).

Table 3.5 : Summary of parasite cysts detected in drinking water sources

Name of parasite	**Water source**	**No. of water sources contaminated (Total-11)**
Giardia duodenalis	Deep protected wells	2
Entamoeba histolytica	Deep protected wells	3
	Open unprotected well	1
Cryptosporidium parvum	Deep protected wells	1
Cyclospora cayetanensis	Deep protected wells	3
Entamoeba coli	Deep protected wells	1

Parasite cysts were identified from deep protected wells which are assumed to be 'safe' since they are 'protected.'

3.5 DISCUSSION

Developed countries occasionally experience outbreaks associated with intestinal protozoan parasites in treated tap water despite very high water treatment standards. Much needs to be done at water treatment plants in the developing countries where resources for water treatment and identification of these parasites are limited. Furthermore parasite and HIV prevalence is high amongst the people living in developing countries.

In the current study stool parasitology results indicated that there was a high prevalence of parasitic infections in the study area as 83 % of participants harboured intestinal parasites irrespective of their HIV status. However this is probably an underestimation as a minimum of 3 stool samples need to be analyzed in order to maximize the chances of detection as parasites tend to be excreted intermittently. A study done in a rural community in Saudi Arabia indicated that the sole source of domestic water was the factor most significantly associated with the high prevalence rates of parasitic infections in that community (Omar *et al.,* 2005). In this current study water is one of the sources of infection since 11 (36.6 %) of the water bodies had parasites identified in them. The present study detected a high prevalence of *C. cayetanensis* in both the individuals sampled (22.1 %) and their drinking water sources (10 %). The findings of a study by Nimri, (2003) in rural Jordan also indicated that the risk factors of acquiring cryptosporidiosis and cyclosporiosis were source of drinking water, contact with animals, and eating unwashed vegetables. These risk factors could also apply to the current study. *Cyclosporiosis* is an emerging disease also known to cause prolonged diarrhoea as does *Cryptosporidium* in immunocompromised individuals (Sancak *et al.,* 2006).

Physico-chemical results showed that tap water had the highest temperature value of 27 °C probably due to heating of the pipes by the sun. The unprotected wells, whether deep or shallow had neutral pH value of 7 whilst the rest of the water sources had pH < 7 although in the normal range making the water slightly acidic giving the water a corrosive nature on materials that are used in wells such as casings and screens. Conductivity is a measurement of the ability of water to conduct an electrical current and is related to the amount of dissolved ions in the water, but this does not give an indication of which minerals are present (Dzvairo *et al.,* 2006). The shallow protected well and 2 deep protected wells had the lowest conductivity, an indication of less dissolved minerals, whilst the rest of the water sources had average conductivity values of >100 µS/cm a likely signal of the presence of contaminants in the water. A study in Kamangara village in Marondera also showed variability of conductivity values of water from boreholes in that environment which was attributed to closeness to pit latrines (Dzwairo *et al.,* 2006). Those that had high conductivity values also had high ammonium and nitrate ions, probable contamination from the nearby pit latrines from which these ions leached, hence the increase in conductivity. In this study distances to pit latrines were not taken into account but this effect could be the cause of variability of results. Turbidity is one of the indicators of the aesthetic quality of the water as turbid or cloudy water is unappealing and may represent a health concern as colloidal particles may harbour pathogenic microorganisms (Pritchard *et al.,* 2007). Normal turbidity, according to WHO guidelines, is 1-5 nephelometric units (NTU). The shallow protected well had a high turbidity of 43 and this could be due to disturbance of soil whilst drawing the water. Some deep protected wells including boreholes also had turbidities of 7.5 which were slightly high. In a study done in Malawi, high turbidity values were obtained during the rainy season, similar to the

findings of the current study which was also conducted during the rainy season, hence the increased turbidity values of the water bodies (Pritchard *et al.,* 2007).

One of the findings was that 5 participants had similar parasites identified in both their stool and water source samples regardless of their HIV status and 4 of these had diarrhoea. It is a well known fact that acquisition of intestinal parasitic infection could be due to ingestion of contaminated food or water, chances are very high that the people acquired parasites from their drinking water source. *Giardia duodenalis* cysts were identified in ground water used by some Mexico city dwellers (Cifuentes *et al.,* 2004). A review on microorganism water outbreaks showed that at least 325 water-associated outbreaks of parasitic protozoan diseases have been reported worldwide (Karanis *et al.,* 2007). *Giardia duodenalis* and *Cryptosporidium parvum* accounted for the majority of outbreaks (132; 40.6 % and 165; 50.8 %, respectively) while *Entamoeba histolytica* and *Cyclospora cayetanensis* have been the aetiological agents in nine (2.8 %) and six (1.8 %) outbreaks, respectively, all from drinking water sources.

Water is essential for life. People living with HIV are vulnerable to infections if they use contaminated water. In addition, the same water is used to take tablets if they are on anti-retroviral drugs (ARVs) or medication for other illnesses related to their HIV status. There are high chances that disinfected water from the SWTPs is contaminated with *Cryptosporidium parvum* and *Cyclospora cayetanensis* as these were identified before and during treatment although not after treatment as methods used for analysis were not very sensitive. The method used to identify these was the cold Ziehl Neelsen method, and yet there are other methods such as immunomagnetic separation and flow cytometry with activated cell sorting which impart high

capture efficiency and selective separation of oocysts (Quintero-Betancourt *et al.,* 2002. There is also 'Method 1623' developed by Environmental Protection Agency of the US government, which was not accessible for this study (US EPA, 2005).

3.6 CONCLUSION

Drinking water from deep protected wells was shown to pose a risk factor in acquisition of diarrhoeagenic protozoan parasites by individuals irrespective of HIV status. Further research needs to be carried out in order to ascertain the relationship of deep protected wells and parasitic contamination. Some people are consuming non treated water from water bodies that are contaminated with protozoan parasites which can cause diarrhoea. There is a need to identify a cost effective disinfection method for the less privileged rural people (who are the majority 70 % of the population in the country), that is able to eliminate infections by protozoan parasites. This should be accompanied by an improvement in hygienic practices.

4.1 SUMMARY

There are several physical methods that can be employed in order to make water safe for drinking. These include, boiling, sedimentation, filtration, solar disinfection and ultra violet radiation. Sand and activated charcoal filtration were analyzed for their capability in the removal of protozoan parasite cysts from contaminated drinking water. Equal amounts of sand were placed a plastic or metal buckets that had a mesh screen at the bottom. Contaminated water was

poured in and the resultant filtrate was collected in another bucket. This was centrifuged and the sediments were analyzed for parasite cysts. A suggested point-of-use technology that uses sand and local wood charcoal was also designed. Novel activated charcoals were also tested for their capability of adsorbing parasite cysts. Sand filtration using a narrow bucket was capable of capturing more parasite cysts as compared to a wider bucket. There was a 99.9 % reduction of parasites when using the point-of-use technology including activated charcoal from baobab shells and macadamia nut shells. Filtration therefore can be used at both a point-of-use and water treatment plant for water disinfection against parasitic contamination.

4.2 INTRODUCTION

WHO and UNICEF estimate that up to 1.1 billion people worldwide still do not have access to 'improved' sources of drinking water such as a protected well or a piped connection. Approximately 2.2 million die of waterborne diseases each year (Mintz *et al.,* 2001; Nath *et al.,* 2006). In many developing countries, even municipal piped water is regarded as unsafe due to inadequate maintenance of pipes, low pressure, intermittent delivery and lack of chlorination (Mintz *et al.,* 1995). Studies have shown that improving the microbiological quality of household water by on-site point-of-use treatment and safe storage in improved vessels reduces diarrhoeal and other waterborne diseases in communities and households of both developing and developed countries (Mintz *et al.,* 1995, Quick *et al.,* 1997).

There are a number of physical methods for water treatment that can be used at household level. These include boiling, solar disinfection, ultra violet radiation, plain sedimentation, aeration and

filtration. Filtration is a physical process that involves the removal of suspended solids from water by passing it through a porous medium such as sand (Sangware, 2005).

There are also a variety of filters and filtration processes available for household point-of-use treatment of water. These include; granular media, rapid rate depth filters that use sand, gravel, diatomaceous earth, coal, other minerals and vegetable and animal derived depth filters that use coal, sponge, charcoal and cotton. Fabric, paper, membrane canvas filters use cloth, other woven fabrics, synthetic polymers or wick siphons whilst ceramic and other porous cast filters, use clay and other minerals and septum and body feed filters use diatomaceous earth and other fine media. Slow and rapid sand filter use sand (Sobsey and Bartram, 2004).

In 1892 outbreaks of cholera occurred in London whereby individuals that drank filtered water did not acquire the disease whilst among the individuals that drank unfiltered water even death occurred (Huisman and Wood, 1974). These events clearly indicated the importance of water filtration in improving the physical, chemical and microbiological quality of water (WHO, 2001).

Both sand and granular activated charcoal have been used for decades in water treatment plants. The purpose of the activated charcoal was and still is to adsorb a wide range of organic and inorganic compounds, and to improve the taste and remove odor (Morin and Camper, 1997). Different types of activated charcoal filters have also been used in order to remove the unpleasant taste, odor, dirt, rust and sand. The silver-containing activated charcoal was used to suppress total coliforms but not bacterial growth (Tobin *et al.,* 1981). The present study is an

analysis of the effect of sand and activated charcoal from different Zimbabwean indigenous trees in the removal of protozoan parasites from contaminated drinking water.

4.3 MATERIALS AND METHODS

4.3.1 Collection of faeces and river sand

One hundred stool specimens for the isolation of protozoan parasite cysts were collected from pupils randomly selected from grade one to five at Shangure Primary School located in Goromonzi. The age range was 5 to 12 yr. Permission to conduct the study and collect specimens from students was granted by the Medical Research Council of Zimbabwe, the Ministry of Education, the headmasters and teachers at the school. The parents or guardians of the pupils agreed by signing consent forms and we also obtained ascent from the participants themselves. River sand used for the experiments was collected from Nora River in Goromonzi, 50 km away from Harare.

4.3.2 Parasite screening and quantification

Specimens were screened for the presence of protozoan parasites cysts using the formol ether concentration technique. The cysts in sediments which were preserved in 10 % formalin were then quantified using a haemocytometer chamber [Optical Corporation, Buffalo NY] (Isaac-Renton *et al.,* 1999). Each formalized parasitic suspension was thoroughly mixed. A cleaned haemocytometer chamber was covered with a precision ground coverslip. Loading of the chamber with this suspension was by capillary action after introduction with a Pasteur pipette. The charged chamber was allowed to equilibrate for 5 - 10 min after which the parasites were

counted using a light microscope under × 40 objective. All parasites within 5 of the 9 large squares of the chamber were counted and an average was calculated. The total volume of fluid under each of the large squares was calculated by multiplying the depth of the chamber (0.1 mm) by the width (1 mm) and the length (1 mm) of the square. The total volume of fluid under each large square is 0.0001 cm^3, which is equivalent to 0.0001 ml. Each parasite count was expressed as cysts/ml.

4.3.3 Analysis of course sand using different types of buckets

The raw river sand was placed in a bucket that had a mesh screen (2 mm × 2 mm holes) at the bottom. Water was made to run through for several minutes in order to remove the silt. The sand was then placed in a closed bucket and treated with 4 % sodium hydroxide (Merck Laboratory Suppliers (PTY) Ltd) overnight in order to digest the organic matter. It was rewashed thoroughly in small amounts under running tap water using a mesh strainer for more that 5 min and then left to dry. Two metal buckets (height 37.8 cm and diameter 30 cm) were placed one on top of each other supported by 2 metal rods in between with the one with a double mesh strainer (2 mm×2 mm square holes) at the bottom being on top. Nine cups (4.04 kg) of this treated coarse river sand (0.38×0.34 mm) were placed in the top bucket that had a mesh strainer and this made a 5 cm sand bed. The average particle size of the sand was measured using a graticule (Tonbridge, Kent). Five milliliters of known, counted protozoan parasite cysts were put in 10 l of tap water in a plastic container. The water was poured evenly and slowly over the sand bed. The water that had collected in the second bucket was then spun at 100 *g* for 3 min in 50 ml centrifuge tubes

that had tapered ends. The sediments were pooled, then centrifuged again and the supernatant removed using a plastic pipette leaving 1 ml of the final sediment. This was analyzed using a light microscope and the parasites were quantified using a haemocytometer.

The whole process was repeated using 8,08 kg sand that had a 10 cm height and 12.12 kg sand that had a 15 cm height on these metal buckets. The same assays using the same quantities of sand were carried out using a V-necked plastic bucket (height 32 cm, bottom diameter 24 cm, top diameter 28 cm).

4.3.4 Combination of fine sand and activated local wood charcoal

A mug of activated coarse local wood charcoal (114.16 g) was mixed with a 10 cm fine sand (average particle size 0.24 × 0.15 mm) bed in a metal bucket that had a mesh screen at the bottom. Approximately 260.4 g of the local charcoal was placed evenly into a round, flat cotton bag (diameter-30 cm making a 2 cm charcoal bag). This was sewn and was placed on top of the fine sand bed. Ten litres of parasite contaminated water was poured through this layer. The resultant filtrate was re-poured into a 2 l container that was tied with a cotton cloth at the open end, with 1 cup of coarse charcoal at the bottom followed by a mixture of 4 cups of fine sand with 1 cup of coarse charcoal (Fig 4.1).

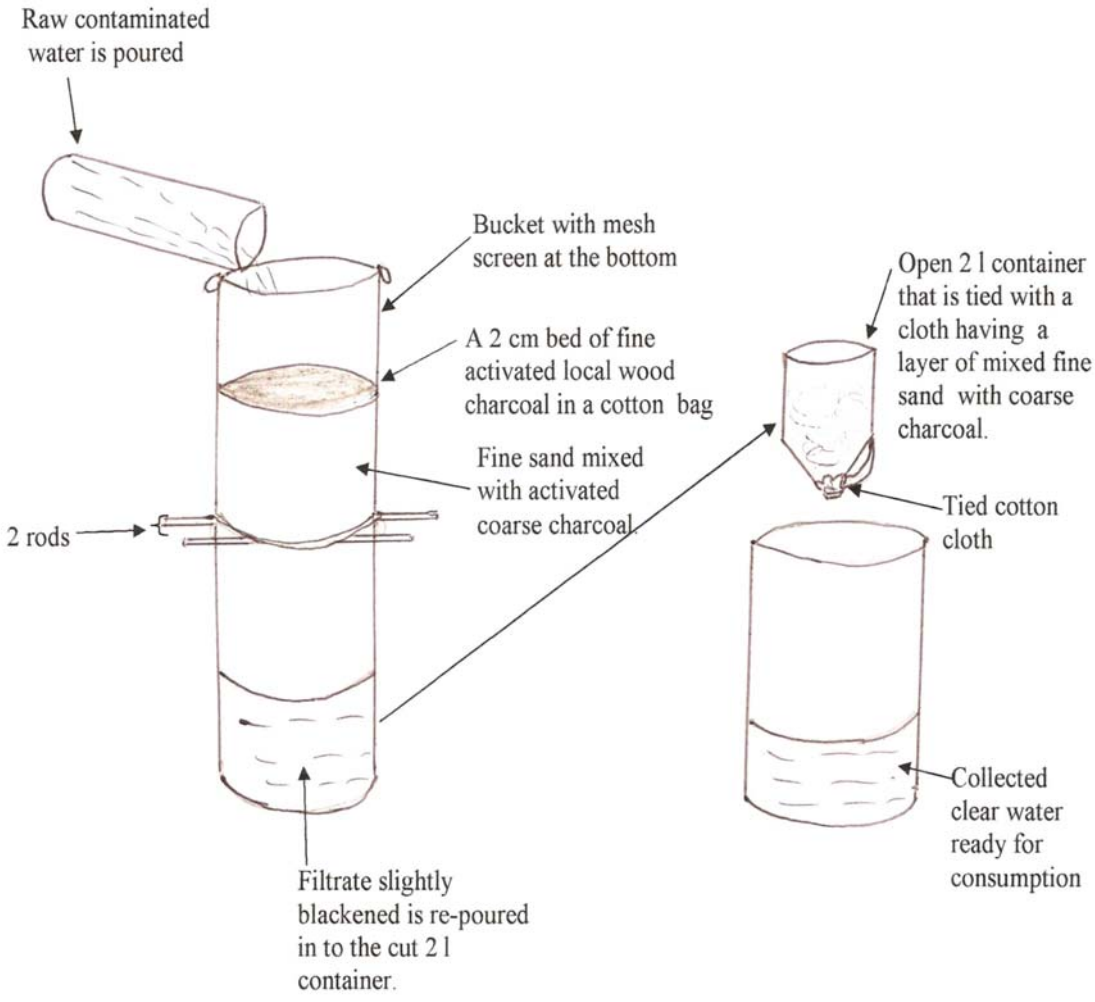

Figure 4.1 : A suggested combined sand and charcoal point-of-use water filter

At least 3 buckets are needed and a funnel for this technology to be used.

4.3.5 Single use of different types of fine activated charcoal

The activated charcoal used singly for the assays included imported commercial charcoal (Hopkin and Williams Ltd, England), local wood charcoal, activated charcoal from the Zimbabwe National Water Authority (ZINWA), and the rest was charcoal derived from macadamia nut shells, baobab shells and amarula stones provided by the Department of Biochemistry, University of Zimbabwe. In the control test, no charcoal was added. One hundred milliliters of distilled water was placed in 7 separate volumetric flasks. One milliliter known quantity of protozoan parasite cyst was added in each including 0.2 g of activated charcoal. This was incubated at room temperature with constant automatic agitation by this incubator/shaker for 24 hr. The water was then poured through a cotton cloth that had been tested for passage of parasites. (All parasites had passed through cloth when tested.) The filtered water was then centrifuged and the 1ml sediment analyzed microscopically. A haemocytometer was used to count the number of cysts in the final filtrate.

4.4 RESULTS

4.4.1 Parasites in human stool specimens

Out of the 100 stool specimens examined, 60 % harboured protozoan parasite cysts with some showing polyparasitism. Parasites isolated included *Giardia duodenalis, Entamoeba histolytica, Entamoeba coli, Endolimax nana, Chilomastix mesnelli, Iodamoeba butschlii,* and *Entamoeba hartmanni.* Out of the 60 pupils that harboured parasites, 63 % had a single infection, 32 % had double infections and 5 % had triple parasitic infections.

4.4.2 Effects of different types of buckets on parasite load

The Chi square test was used to compare the same sand beds in different buckets. There was no significant difference in the parasites that passed through the sand when compared, the two different buckets having the same mass of sand but different heights showed that a height of 20 cm captured more parasites (95 %) than the 15 cm height (93%), although these results were not statistically significantly different using the Chi square test. The 5 cm height sand bed captured 80 % of the parasites.

4.4.3 Combination of fine sand and charcoal

A 10 cm bed of fine sand was used with a layer of fine activated local wood charcoal which resulted in the complete removal of parasites although there was slight blackening of the water. The use of another 10 cm fine sand bed that was mixed with coarse local wood charcoal in a 2 l plastic container, removed the blackness. However removal of inherent aquatic microorganisms such as *Paramecium* was at 98 %.

4.4.4 Different types of charcoal

Baobab shells and macadamia nut shells were capable (99.9 %) of adsorbing protozoan parasite cysts while the commercial charcoal from Hopkins in England completely adsorbed all the other intestinal protozoan parasite cysts except for *Entamoeba coli,* where there was a 98 % reduction of these parasites. The amarula stones had a 33.5 % reduction of parasite cysts, whilst the local wood charcoal only had a 17 % reduction (Table 4.1). All the activated charcoal had a 99.9 % adsorption of *G. duodenalis* except for the ZINWA activated charcoal.

Table 4.1 : Use of activated charcoal in the removal of protozoan parasites

Name of Parasite	**Control [ ] cells/ml**	**Local wood [ ] cells/ml**	**Amarula stones [ ] cells/ml**	**Zinwa [ ] cells/ml**	**Hopkins Williams [ ] cells/ml**	**Baobab shells [ ] cells/ml**	**Macadamia nutshells [ ] cells/ml**
Entamoeba histolytica	6×10^6	2×10^3	2×10^3	0	0	0	0
Entamoeba coli	7.8×10^6	4×10^3	0	0	6×10^3	0	0
Giardia duodenalis	3.4×10^6	0	0	2×10^3	0	0	0
Chilomastix mesnelli	8×10^6	8×10^3	2×10^3	0	0	0	0
Endolimax nana	8×10^6	3.6×10^4	4×10^3	2×10^4	0	0	0
Entamoeba hartmanni	1.2×10^6	1×10^4	1×10^4	2×10^3	0	0	0
Average % reduction of parasites	**0**	**17**	**33.5**	**99.6**	**83.8**	**99.9**	**99.9**

[ ] = concentration

The 0 indicates that after following the adsorption procedure as described in the methods section, no parasites were identified in the resultant.

4.5 DISCUSSION

Additional improvement of drinking-water quality, for instance such as point-of-use disinfection, is likely to lead to reduced episodes of diarrhoea by 45 % (WHO, 2004). The findings of this study confirm that sand filtration does effectively reduce parasite cysts in contaminated drinking water. If the same amount of sand is placed in 2 different vessels *i.e.* a narrow and a wide vessel, then a narrow vessel will capture more parasites due to the increased height of the sand. Morgan, (1990) indicated that sand has a purifying effect on water if the water is constantly in contact with it as it adsorbs bacteria. Sand at the bottom of a container will have less organic matter making conditions for the pathogenic bacteria unsuitable for their survival.

The smaller pore size produced by fine sand reduces the porosity of the media making fine sand advantageous over course sand in the passage of any material (Kang *et al.,* 2006). It was considered worthwhile to test the performance of a combination of fine sand and the local wood charcoal. Although there was a slight tint of black in the collected water after filtering, the combination yielded positive results, showing removal of parasite cysts. A filter of second charcoal together with the fine sand was able to reduce that black tint. There is no health threat in ingesting activated charcoal. It is used in the treatment of poisonings and overdoses by oral ingestion as it prevents absorption of toxic substances by the gastrointestinal mucosa. Most rural communities and even some urban communities do not have electricity and use wood for cooking purposes, hence charcoal from wood is therefore readily available for this technology.

Previous experiments have indicated the effectiveness of activated charcoal in adsorbing *Staphylococcus albus* (Tichonova *et al.,* 1989). Experiments were then carried to find out the activated charcoal that was specifically capable to remove protozoan cysts on its own. The available activated charcoal were then tested, and it was noted that charcoal from baobab fruit shells and macadamia nut shells were capable of adsorbing all protozoan parasite cysts. These trees are locally available in the Southern African region. These activated charcoals were only available in their fine form, and further tests need to be conducted in their coarse form.

The protozoan cysts that were of interest in this particular study are, *G. duodenalis* and *E. histolytica*. All the activated charcoal tested were 99.9 % capable of removing cysts of *G. duodenalis* from the contaminated water except the Zimbabwe National Water Authority activated charcoal which was unable to remove *G. duodenalis*. Further research needs to be done in order to assess the surface proteins of this parasite that facilitates its adsorption to different charcoals and not others. *E. histolytica* and the rest of the other tested cysts could only be completely adsorbed by charcoal from baobab fruit shells and macadamia nut shells (99.9 %).

The use of very fine sand mixed with activated coarse charcoal is recommended for point of use treatment of water especially in rural areas where it can be used initially for the removal of protozoan cysts. It can be combined with chlorination for disinfection against bacteria. Water treatment plants can use activated charcoal from baobab fruit shells and macadamia

nut shells for the removal of parasite cysts in the same manner as activated charcoal for the adsorption of odours.

4.6 CONCLUSION

This study confirmed that using the same amount of sand, in a narrower vessel is more effective in capturing more parasites as compared to a wider vessel. A combination of fine sand with activated charcoal from local wood charcoal, reduced parasites by 99.9 % and the additional stage of re-pouring the water through a smaller filter with fine sand mixed with course activated local wood charcoal resulted in production of cleaner water that did not have that slight black tint caused by the fine charcoal in the first stage of filtration. Testing of different types of charcoals from different trees showed that activated charcoal from baobab fruit shells and macadamia nut shells were capable of adsorbing protozoan parasite cysts by 99.9 % and all the activated charcoal also adsorbed *G. duodenalis* by 99.9 % except for the ZINWA charcoal which adsorbed by 99.6 %. The identified activated charcoals can be used at water treatment plants in order to remove protozoan parasite cysts.

5.1 SUMMARY

It is estimated that about 875 million cases of diarrhoea in developing countries and 4.6 million deaths that occur each year are due to lack of safe water. Conventional water treatment such as use of chlorine is not effective in the disinfection of parasite cysts, hence the reported outbreaks from treated tap water. Solar disinfection (SODIS) was used experimentally in order to ascertain its effectiveness against *G. duodenalis* and *E. histolytica/dispar*. Stool samples from 100 pupils that were positive for *G. duodenalis* and *E. histolytica/dispar* were purified using the discontinuous percoll gradient centrifugation. Known aliquots of parasites were placed in different types of 2 l plastic containers that contained 1 800 ml of water and exposed for 7 hr to sunshine with viability of parasites being tested hourly. After 7 hr of sunshine with temperatures raising up to 56 ºC, both species were inactivated. Solar disinfection of drinking water was then implemented in the community. There was a significant reduction of *G. duodenalis, E. coli* and *I. butschlii* ($p < 0.05$) parasitic infection detected in the faeces of those that drank from containers exposed out in the sun whilst those that did not expose their containers, showed no significant reduction in any of the parasite cysts. Solar disinfection can be used in developing countries whilst the implementation of a piped water system is under progress.

5.2 INTRODUCTION

Solar disinfection is a technique that involves exposing raw water in plastic containers or glass bottles to sunshine for several hours to achieve microbial decontamination (Mendez-Hermida *et al.,* 2007). This can be done either as a batch process or using continuous water flow systems that use SODIS reactors. A batch process involves either bottles or plastic bags at a point-of-use home water treatment, while continuous flow systems are designed to provide disinfected water to institutions such as schools or hospitals (Sommer *et al.,* 1997).

This technique has been tested under laboratory conditions on a number of organisms in order to ascertain its effectiveness. It has been shown to be effective against a broad range of bacterial pathogens, namely, *Escherichia coli* (Joyce *et al.,* 1996, McGuigan *et al.,* 1998; Berney *et al.,* 2006), *Salmonella typhimurium* (Smith *et al*. 2000), *Vibrio cholerae* (Conroy *et al.,* 2001) and *Shigella dysenteriae* type 1 (Kehoe *et al.,* 2004). The virus that has been investigated is the polio virus (Heaselgrave *et al.,* 2006) and parasites include, *Acanthamoeba polyphaga,* (Heaselgrave *et al.,* 2006), *Cryptosporidium* (McGuigan *et al.,* 2006, Mendez-Hermida *et al.,* 2005 and 2007) and *Giardia muris* (McGuigan *et al.,* 2006).

The effectiveness of SODIS on reducing parasitic infections in a community has not been investigated in Zimbabwe before. Solar disinfection has been implemented in Kenya in a Masaai community in order to ascertain whether there was a reduction in diarrhoeal cases (Conroy *et al.,* 1999). Three hundred and forty nine children were recruited for the study, where half drank water from plastic bottles exposed to sunlight on the roof of the house and

the other half drank from the water bottles that were kept indoors. The Masaai community elders did home visits every 2 weeks for a year. There was a modest reduction of severe diarrhoeal episodes (48.8 %) in those children that drank solar disinfected water compared to those that did not (58.1 %). When a cholera epidemic occurred after this study, only 3 (1.9 %) children out of 155 who drank solar disinfected water had cholera compared to 20 (13.9 %) out of 144 who did not drink solar disinfected water (Conroy, 2001). The present study investigated the effectiveness of solar disinfection on parasites both in the laboratory and a selected community.

5.3 MATERIALS AND METHODS

5.3.1 Screening for parasites in faeces

Stool samples were collected from 100 rural school children aged between 5 and 15, from the Shangure Primary School in Goromonzi, Zimbabwe. Goromonzi has a longitude of 31° 21', latitude of 17° 51' and altitude of 1 465 m. The participants were selected by convenience sampling in which interested students volunteered to participate and the male to female ratio was 48:52. The participants were screened for the presence of *Giardia duodenalis* and *Entamoeba histolytica/dispar* by performing wet preparations of faeces in physiological (0.85 %) saline (Garcia, 1999; Cheesebrough, 2005). One drop of saline was placed on a microscope slide. A small amount of each faecal specimen was thoroughly emulsified in the saline using an applicator, then covered by a 22 mm long coverslip. These suspensions were systematically scanned using the × 40 magnification for parasites. D'Antoni's Iodine was added through the side of the coverslip when the slide was positive

for protozoan parasite cyst and the slide reexamined in order to visualize the finer details of the cysts (Garcia, 1999).

Confirmatory identification of *Entamoeba histolytica/dispar* and *Giardia duodenalis* was based upon the morphological characteristics after Gomori/trichrome staining was carried out. The noted features for *E. histolytica/dispar* were the presence of 1 - 4 nuclei, ± rounded chromatoid bars and a cyst measuring 5-20 µm. *Giardia duodenalis* were either in the form of an oval or ellipsoid cyst that contained 2-4 nuclei with up to 4 pairs of median bodies running in the middle and the whole cyst measuring 8 to 12 µm long by 7 to 10 µm wide (Desowitz, 1980).

5.3.2 Purification of protozoan parasite cysts

Specimens found to be positive for *Giardia duodenalis* and *Entamoeba histolytica/dispar* only were purified using the discontinuous percoll gradient centrifugation (Ekert *et al.,* 1992). This procedure involved initially washing the stool specimen containing cysts twice in 10 ml of phosphate buffered saline (PBS) by spinning at 500 *g* (2000 rpm) for 10 min. The supernatant was decanted and 1 ml of the suspension was layered on a discontinuous Percoll gradient. The gradient consisted of 4, 2 ml layers with percoll densities of 1.13 g/ml (undiluted percoll), 1.09 g/ml (1.5 ml percoll + 0.5 ml H_2O), 1.05 g/ml (0.7 ml percoll + 1.5 ml H_2O) and 1.01 g/ml (0.1 ml percoll + 1.9 ml H_2O) which were carefully layered with a pipette in a transparent 15 ml conical glass tube (critical Ø of 17 mm) and centrifuged at 650 g (2600 rpm) for 15 min. The band containing purified cysts which lay between the 1.09 and 1.05 layers was transferred to a clean tube, washed 3 times in PBS at 1500 *g* (6000

rpm) for 10 min and then the sediment was resuspended in distilled water and stored at 4 ºC to maintain viability of cysts (Ekert *et al.* 1992). (Fig 5.1). The parasites were quantified using a haemocytometer chamber, as described previously and stored in 5 ml aliquots in distilled water at 4 ºC until use.

Viability testing of cysts using vital and fluorescent dyes was done before addition of these parasites to the test containers. Viable cysts do not take up the dye. Fifty microlitres of the sediment was stained with the vital dyes, namely, 0.4 % Trypan blue and 0.3 % Congo red as described by John and John (1997). A haemocytometer was used to count the viable cysts and estimate the percentage viability. The fluorescent dyes 4,6 diamidino-2-phenylindole dihydrochloride (DAPI) (2mg ml^{-1} in absolute methanol) and Propidium iodide (PI) (1mg ml^{-1} PBS 0.1 mol l^{-1}) were added to the parasite aliquot and incubated for 2 hr at 37 ºC (Thriat *et al.,* 1998). A viable cyst does not take up the vital dyes while for DAPI viable cysts fluoresce blue at 365 nm and non viable cysts do not. The non viable cysts also fluorescenced red at 510-560 nm when using PI. Cysts that were considered to be viable were either (DAPI + PI-) or (DAPI-PI-). PI does not penetrate an intact viable cyst, therefore a stained cyst indicates that it is non viable (Wallis *et al.,* 1996).

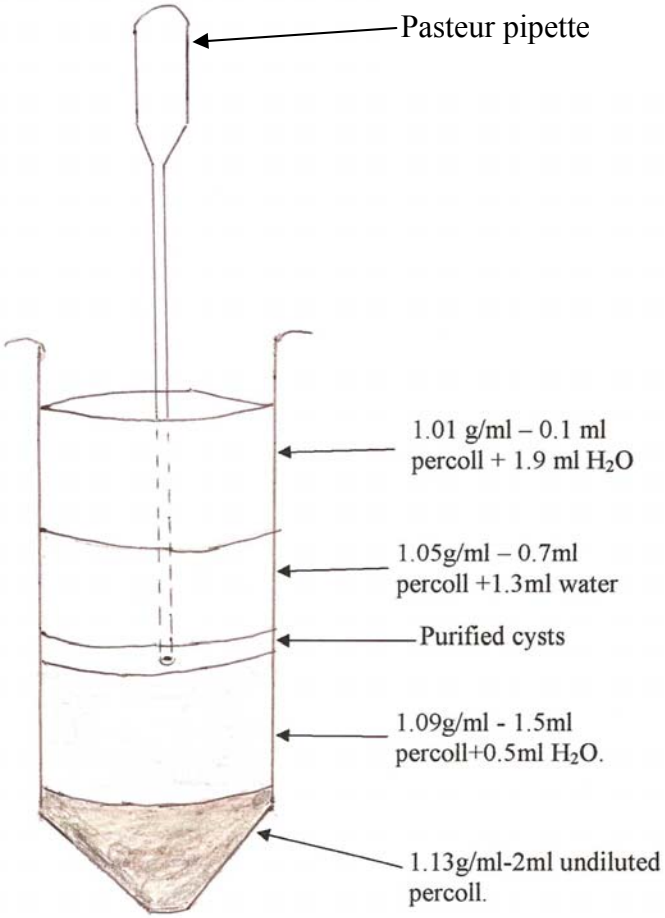

Figure 5.1 : The discontinuous percoll gradient centrifugation

A Pasteur pipette is used in order to remove the layer of purified cysts.

5.3.3 Effectiveness of different containers and conditions in inactivating protozoan parasite cysts

Containers used were 8 × 2 l polyethelene terephthalate (PET) plastic containers painted black on one side, 1 × 2 l PET container not painted, 1 × 2 l PET container cut open at the top and 1 × 2 l container made of high density polypropelene (HDPP) material. One milliliter (ml) aliquots of 3.52×10^5 cysts ml^{-1} of *Giardia duodenalis* and 4.36×10^5 cysts ml^{-1} of *Entamoeba histolytica* parasite suspensions were added separately to each of the 11 different 2 l containers with 1 800 ml of distilled water. The contents were mixed thoroughly and then exposed to the sun. The initial temperature of the contents was measured, then temperatures were recorded hourly for 7 hr, with one PET container painted black on one side being taken hourly for viability assays and the rest of the containers were analyzed after the 7 hr. One of the PET containers was a control, which was kept in the cupboard for 7 hr, without being exposed to the sun. The water from the containers was centrifuged in 50 ml sample batches at centrifuge speed of 500 *g* for 3 min. The sediments from each container was pooled to make a 1 ml suspension. Viability testing using both the vital and fluorescent dyes was done. These assays were run 15 times. The same assays were also run under cloudy conditions.

5.3.4 Effect of temperature in inactivating protozoan parasite cysts

Assays were also run in the laboratory to detect the effect of temperature alone without the UVA from the sun. One hundred milliliters of distilled water was placed in a conical flask. One milliliter of 14.2×10^4 viable cysts ml^{-1} of *G. duodenalis* and 8.6×10^4 cysts ml^{-1} of *E. histolytica/dispar* was added and the initial temperatures were recorded. These conical flasks

were placed in a water-bath for the equivalent times and average temperatures that were attained when solar radiation was used. These were 34 ºC for 1hr, 44 ºC for 2 hr, 46 ºC for 3 hr, 49 ºC for 4 hr, 52 ºC for 5 hr, 55 ºC for 6 hr and 56 ºC for 7 hr. Each flask was held at each temperature for the time indicated. After incubation at the set times, the water was centrifuged at 500 *g* for 3 min. The supernatant was decanted and 1 ml of sediment was left.

5.3.5 Implementation of SODIS in a rural community.

The study site for this intervention study was Musarara Primary School in Chiweshe rural area (longitude, 31º00'; latitude, 17º 49' and altitude 1 465 m). Two hundred and sixty-eight school children from grades 3 to 6 aged 7 to 14 years, (average age 11) were recruited for the study after giving their consent as previously described. There were 171 (64 %) females and 97 (36 %) males. Stool samples were collected on 3 alternate days and analysed for parasitic infections, as described previously. All the pupils that were positive for parasitic infections were treated with metronidazole for protozoan parasites and albendazole for helminths. This was done so that at baseline level, everyone would be at zero level for parasitic infections. The pupils were made to stand in a queue and each was given a new 2 l polyethelyne terephthalate plastic container that was painted black on one side and these were numbered from 1 to 268. The pupils were instructed to fill their bottles with drinking water to below the brim from their home drinking water sources and shake the bottles so that they would be well aerated. Pupils that had been given odd numbers were told to expose their containers to sunlight with the non painted side facing the sun. Those with even numbers were the control group, whose containers were to be kept indoors. Each pupil was then given a chart (Appendix F) to record, the days that were cloudy and when they had

imbibed water from elsewhere besides the water from the container provided. However drinking water from other sources was discouraged.

Follow up was done monthly for 2 months, *i.e.* at the end of each month. Stool samples were once again collected on 3 alternate days and processed according to parasitological standard operating procedures as described previously. At the end of the 1st month random home visits were done for 12 of the participants without prior warning. This was done to ascertain whether the containers were really being used as directed.

Statistical analysis was done using Stata Version 9. The paired t test was used to compare the changes in parasite levels at the end of the 1st and 2nd months.

5.4 RESULTS

5.4.1 Solar disinfection in the laboratory

The initial average concentration of cyst after purification was 8.67×10^4 cells ml^{-1} of *G. duodenalis* and 7.15×10^4 cells ml^{-1} of *E. histolytica/dispar*. Experiments were carried out at end of the rainy season of January and February, when both full sunshine and cloudy conditions were experienced. The results considered were those obtained when there was a clear sky or slightly overcast (sunny day) and those that were either cloudy or completely overcast (cloudy day) (Table 5.1). When there was 50 % cloudiness, the results were not considered. During exposure to sunshine, peak water temperatures were above 50 °C, 99.9 % in the 2 litre painted PET containers, parasite cysts were inactivated after 7 hr (Fig 5.2).

Table 5.1 : Effect of cloud cover to viability of parasite cysts in contaminated water stored in a variety of plastic containers

Type of container	**Highest temperature Attained**	**% viable *G. duodenalis***	**% viable *E. histolytica***
2 l open container	31 ºC	56	75
2 l PET container not painted.	34 ºC	100	75
2 l HDPP	36 ºC	70	76
2 l PET container painted black on one side	38 ºC	31	58

Cloud cover prevented UVA penetration from sunlight and also decreased the heat production hence the continual viability of some cysts.

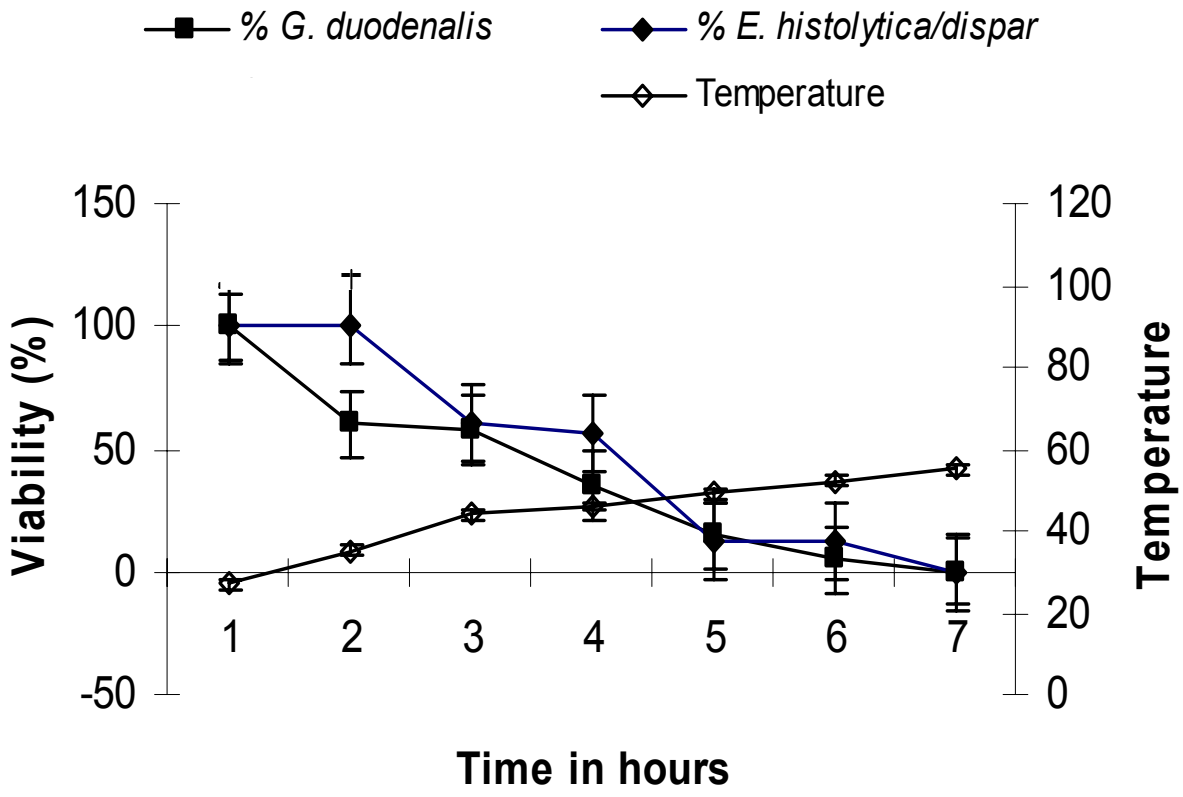

Figure 5.2 : Viability of *G. duodenalis* and *E. histolytica/dispar* in contaminated water contained in partially painted black PET bottles exposed to solar radiation. Error bars represent SD.

There was a gradual decrease of viability of cysts after 2 hr of sunlight exposure and after 7 hr all cysts were non viable when temperatures were > 50 ºC.

After 7 hr of sunlight exposure 93.2 % of *G. duodenalis* and 92.6 % of *E. histolytica/dispar* cysts were inactivated in PET bottles that had an open top. For the PET bottles not painted

95 % of *G. duodenalis* and 81.3 % of *E. histolytica* was inactivated. When the HDPP bottle, 93.2 % and 85.1 % of *G. duodenalis* and *E. histolytica/dispar,* respectively, was also inactivated (Fig 5.2). PET plastic containers painted black on one side absorbed more heat than those not painted, those open and the high density polypropylene (HDPP) containers.

Under cloudy conditions the 2 l PET bottle painted black on one side attained the highest temperature of 38 °C after 7 hr in the open and 31 % of *G. duodenalis* were still alive compared to 58 % of *E. histolytica/dispar* cysts (Table 5.1). Heat alone without sunshine, was also not capable of inactivating cysts of *G. duodenalis* and *E. histolytica/dispar* (Table 5.2). Parasites remained viable after being exposed to direct heating alone even at 56 °C for 7 hr.

5.4.2 Implementation of SODIS in a rural community.

Stool samples were described as either formed, semi formed or watery. The former first two descriptions were considered to be normal stool while that which was watery was considered to be diarrhoeic. There was a minor difference in diarrhoea episodes between the 2 groups, as 8 % of those that exposed water containers to sunlight had diarrhoea as compared to 9 % of those that did not. The rates of parasitic re-infection were also similar at 70/268 (26 %) for the group that exposed water containers to sunlight and 71/268 (26 %) for those that did not.

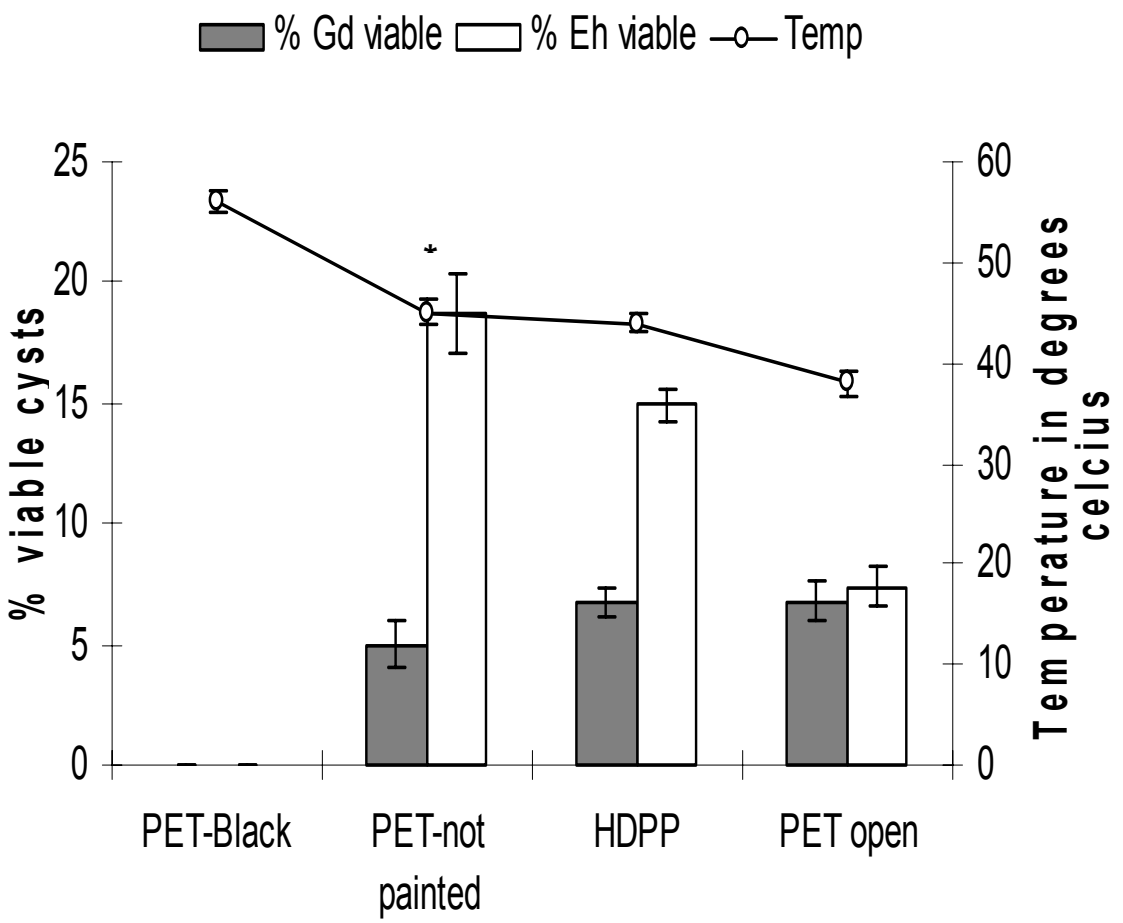

Figure 5.3 : Parasite viability in contaminated water in a variety of containers after 7 hr of exposure to natural sunlight.

(Gd- *Giardia duodenalis,* Eh- *Entamoeba histolytica/dispar.* PET – polyethylene terephthalete, HDPP – high density polypropelene) (*-$p<0.05$)

The efficiency of PET containers painted black on one side can clearly be noted as other containers still had viable cysts after 7 hr of sunlight exposure.

Table 5.2 : Effect of different levels of directly applied heat on viability of *Giardia duodenalis* and *Entamoeba histolytica* in water

Time (hr)	Temperature (ºC)	% viable *G. duodenalis*	% viable *E. histolytica*
1	35	48.9	56.4
2	44	48.2	59.3
3	46	25.7	60
4	49	25.3	53.4
5	52	13.2	44.7
6	55	10.6	29
7	56	9.1	5

Exposure of the parasites to direct heating alone of water to temperatures between 35 °C and 56 °C did not inactivate all parasite cysts.

There was a significant reduction of *G. duodenalis, E. coli* and *I. butschlii* ($p < 0.05$) parasitic infection of those that drank from containers painted black on one side, while for those that did not expose their containers, there was no significant reduction in any of the parasites (Table 5.3). Out of all the 268 participants, only 47 brought back the charts that indicated the days they had drunk water from elsewhere, the days that were cloudy, and days they had diarrhea. On analysis of the charts, all of them indicated there had been some cloudy days and all of them except for 1 had occasionally drank water from elsewhere besides their containers. One of them on 50 occasions out of the 61 (82 %) of the study period had drunk water from elsewhere. Observational results of 12 participants that were visited indicated that the containers were placed on the stand for drying plates 75 % (9/12) or on top of chicken run houses. One of the pupils had their container in the kitchen next to other water storage containers although this pupil was in the exposure group, another said that their container had disappeared and the other said their container had been eaten by a rat.

Table 5.3 : Comparison of parasitic infections of the 1st and 2nd months for pupils that exposed 2 l PET containers painted black on one side to sunlight

Parasite	P-value	SD (±)	95% confidence interval	t-test
G. duodenalis	**0.002**	0.33	0.03 - 0.15	3.09
E. histolytica/dispar	0.842	0.43	-0.07 - 0.08	0.19
E. coli	**0.035**	0.61	0.01 - 0.22	2.12
E. nana	0.202	0.40	0.02 - 0.11	1.28
I. butschlii	**0.003**	0.39	0.04 - 0.17	3.07
C. mesnelli	1.000	0.27	-0.05 - 0.05	0.00
E. hartmanni	0.132	0.29	-0.86 - 0.11	-1.54
C. cayetanensis	0.134	0.34	-0.10 - 0.01	-1.50
S. matheei	0.319	0.09	-0.01 - 0.02	1.00
T. hominis	0.319	0.09	-0.02 - 0.01	-1.00

P-values that are in bold are significant (*i.e* < 0.05).

± SD – Standard deviation CI – Confidence Interval

(*G. duodenalis-Giardia duodenalis, E. histolytica/dispar-Entamoeba histolytica/dispar, E. coli-Entamoeba coli, E. nana-Endolimax nana, I. butschlii-Iodamoeba butschlii, C. mesnelli-Chilomastix mesnelli, E. hartmanni-Entamoeba hartmanni, C. cayetanensis-Cyclospora cayetanensis, S. matheei-Schistosoma matheei, T. hominis-Trichomonas hominis)*

5.5 DISCUSSION

The biocidal effect caused by sunlight is due to the optical and thermal processes that occur at temperatures above 45 ºC (McGuigan *et al.,* 1998, 2006). In order to increase the absorption rate of heat, techniques such as laying the container on a black surface or painting the containers black on one side can be employed. In our experiments an open PET container and non-painted PET containers were not as efficient in capturing heat from the sun as compared to the black painted container. Painting the container black on one side caused the increase in temperature. This agrees with the Mexican study where there was inactivation of total coliforms and *Escherichia coli* using PET bottles partially blackened on one side and others that were not (Martin-Dominguez *et al.,* 2005). In another study carried out in 2001, covering the rear surface of the solar disinfection container with aluminium foil improved the inactivation efficiency of the system (Kehoe *et al.,* 2001).

Sunlight has germicidal effects as it provides both ultraviolet (UV) radiation and heat. The combined effects of temperatures of 50-60 °C and UV radiation in the UV-A range (320-400 nm) of the solar disinfection (SODIS) system is germicidal enough to extensively inactivate many enteric micro-organisms (Sobsey and Bartram, 2004). The combination of UV and heat, has a synergistic effect on microbial inactivation. Evidence of this synergistic effect has been documented for vegetative bacteria, but it has not been studied for viruses or parasites (Sobsey and Bartram, 2004). It is interesting to note that results of the present study indicated that only heating the parasite cysts without UVA radiation from the sun did not completely inactivate both *G. duodenalis* and *E. histolytica.* Therefore it is concluded that the synergistic effect that occurs for some bacterial cells also takes places for these

particular parasites under study. Other studies cited indicated that less UVA translated to less heat and therefore decreased biocidal effect (Sommer *et al.,* 1997).

Many documented studies concerning SODIS have involved *Giardia* and *Cryptosporidium,* and none had tested effects of SODIS on cysts of *E. histolytica/dispar* (Mendez-Hermida *et al.,* 2005; McGuigan *et al.,* 2006,). Comparison of *G. duodenalis* and *E. histolytica/dispar* in the current study clearly indicates that loss of viability of the latter organism is slower as compared to the former (Fig 5.3). The low heat produced by PET bottles that were not painted and also HDPP containers also had decreased effect of loss of viability of *E. histolytica/dispar.* About 10 % of the world's population are carriers of this particular organism, and these parasitic infections are prevalent in developing countries were usage of unsafe water is high (Kang *et al.,* 1998; Mandell *et al.,* 2000). This study has now indicated for the first time the effectiveness of painted PET bottles on complete loss of viability of *E. histolytica/dispar,* after 7 hr of full sunshine exposure when temperatures reach 56 °C.

Many have wondered whether the photoproducts produced by sunlight exposed PET containers have a toxic effect as increased use also causes an increase of these products. Experiments carried out by Wegelin *et al.* (2000) and Kohler and Wolfensberger, (2003), concluded that these substances including the plasticizers, di(2-ethylhexyl)adipate (DEHA) and the chemical di(2-ethylhexly)phthalate (DEHP), are at low concentrations well below the limits of safe drinking water. There was no significant difference between new and used bottles. Polyethelyne terephthalate plastic containers therefore pose no risk of toxicity when used for this technology.

Several intervention studies for point-of-use water treatment have produced positive results. In a study done in Madagascar after a cholera outbreak, the individuals that used sodium hypochlorite solution, or drank heated rice water and those that had a household tap (as this eliminated the need for storage where contamination can take place) were protected against waterborne illnesses (Reller *et al.,* 2001). In Kenya the charity orgnisation CARE, introduced clay pots and 1 % sodium hypochlorite solution packaged in 500 ml bottles, with a 8 ml cap suitable for point-of-use water disinfection and the intervention succeeded because of the intensive marketing strategies undertaken (Makutsa *et al.,* 2001). The implementation of SODIS in a Maasai community also saw a reduction of diarrhoeal cases in individuals that used SODIS as compared to those that did not (Conroy *et al.,* 1999).

In the present study, it has been demonstrated that there was a significant reduction of *G. duodenalis, E. coli* and *I. butschlii* infection in participants that exposed black painted containers to the sun, as compared to those that did not. These results are unique in that they are the first to demonstrate SODIS effect on parasitic infections. Under field settings, *E. histolytica/dispar* was not reduced significantly as *G. duodenalis* suggesting that perhaps the rate of disinfection for *E. histolytica/dispar* using SODIS was slower as compared to *G. duodenalis*. The fact that some study participants indicated presence of cloudy days, suggested that the parasite is not adequately disinfected because of the low temperatures achieved on these days.

The rate of recurrence of parasitic infections in both groups was almost the same and so were the diarrhoea episodes. This could be due to the fact that parasitic infections are not acquired only by ingestion of water, but also by consuming contaminated food. In a study by Al-Shawa and Mwafy, (2007) in Gaza, they found some vegetables to be contaminated with *G. intestinalis, E. histolytica* and *A. lumbricoides*. According to the submitted charts the majority of pupils occasionally drank water from other sources. An intensive health education program with the guardians of these pupils prior to the intervention study was lacking in order to emphasize the importance of consuming water from the supplied containers.

5.6 CONCLUSION

Solar radiation may offer affordable method of disinfection of drinking water that requires few resources and no expertise as, PET bottles painted black on one side are capable of inactivating cysts of *G. duodenalis* and *E. histolytica/dispar.* The inactivation rate of *E. histolytica/dispar* is slower than that of *G. duodenalis.* There is a synergistic effect between the heat and the UVA produced by the sun in the inactivation of these parasites. SODIS is effective in reducing parasitic infections caused by *G. duodenalis, E. coli* and *I. butschlii* in a field setting although this should be accompanied by health education and motivational training that would aid behavioural change in order for proper use of this technique.

In order to achieve the WHO and United Nations MDGs of halving the number of people without clean safe water by the year 2015, we recommend use of PET plastic containers

painted black on one side to be used as one of the point-of-use water treatments as they inactivate some micro-organisms that cause diarrhoea especially in developing countries.

6.1 OVERALL DISCUSSION

Intestinal parasitic infections are a public health problem worldwide. Prevalence studies of parasitic infections in an urban, rural tribal trust land and commercial farming environments, established that *G. duodenalis* and *C. cayetanensis* were more prevalent in the rural areas. The former was associated with individuals that did not wash their fruits/vegetables before eating, and the type of water source they used. Deep protected wells were associated with the presence of parasite cysts and this type of water body was most common in the rural areas, suggesting that the important determining factor for infection were the deep protected wells. *Cyclospora cayetanensis* was associated with children ≤ 5 yr of age and practicing of unhygienic habits such communal handwashing in a dish before eating a meal. This is a common customary practice in the rural environment. The parasite was also associated with the use of a Blair toilet and since there is no tap water to wash hands after use, there are high chances that people do not wash their hands after using the toilet, thereby contaminating the communal hand washing dish before taking meals.

Parasites that were identified in Bvumba commercial farming area were *E. histolytica/dispar, C. parvum* and helminth infections. *E. histolytica/dispar* was associated with lack of measures intended to make water safe for drinking, hence the need to identify a simple practical method of disinfecting water. *C. parvum* was associated with people of Mozambican origin. The farming area in this study was at the border of Zimbabwe and Mozambique. Some pupils from Mozambique attended school in this area and the pupils that came from Mozambique were found to have a high prevalence of the parasite.

Helminths were associated in particular with Crake Valley Primary School where the majority of students sometimes defeacated in the bush. There is need for provision of good sanitary conditions including health education in order to reduce helminthic infections.

Blastocystis hominis was more prevalent in the urban environment. This is a pathogenic parasite that causes diarrhoea. It was identified by using the Gomori/Trichrome staining which is not routinely used in diagnostic laboratories. The parasite could be prevalent in an urban area causing diarrhea but is not being diagnosed leading to mismanagement of the patients infected as they might be treated of other diseases.

The prevalence study had a bias in that it was carried out amongst school children. This group in the population is accessible at schools as compared to the general population. They also have the advantage of being at the same place at the same time. School children within the age range of 0 to 15 yr are also known to have a higher parasitic burden as compared to adults. Primary school children cooperate even more as compared to high school pupils as the latter would be reluctant to submit a stool specimen. During the community based study, when there was collection of water and stool from those that used that water source, adults had many excuses including that they had already defaecated early in the morning and so could not do that again. We would wait for nature to take its course, or return back the following day to collect the specimen, *etc.* Despite these problems, there were others that did cooperate.

Water is essential for life. The United Nations' International Drinking Water Supply and Sanitation Decade *i.e.* 1981-1990, failed to achieve its goal of universal access to safe drinking water and sanitation by 1990 as about 1.1 billion people still lacked access to improved water supplies and 2.4 billion people were without adequate sanitation, in 1990 (Lantagne *et al.,* 2005). Then in September 2000, the Millennium Development Goals (MDGs) were adopted and signed by heads of states and governments, at the United Nations Millennium Summit. One of the goals that was agreed upon was to halve the people without access to safe water by 2015 (Pritchard *et al.,* 2007). How can this be best achieved in a developing country such as Zimbabwe?

Deep protected wells were found to be associated with parasitic contamination. In rural areas this type of water source is the most common and actually thought to be 'safe' since it is a 'protected' well. These type of wells actually seem to provide a favourable environment for parasites to thrive. Furthermore, 4 out of the 5 study subjects who had similar parasites being identified in both their stool and water samples drank water from deep protected wells. A point-of-use water treatment would be an ideal solution in order to disinfect the water. At small water treatment plants, *Cryptosporidium parvum* and *Cyclospora cayetanensis* were found to contaminate water before and during treatment. After treatment there were no parasites identified. These coccidian parasites are opportunistic pathogens in the immunocompromised, increasing morbidity and mortality, as they cause persistent and/or chronic diarrhoeas. The fact that we were unable to identify them after water treatment does not rule out their presence in the treated tap water. The assays conducted are not as sensitive as the recommended tests but were however used due to lack of resources. The

recommended methods require amongst other equipment, an immunomagnetic separation apparatus and a differential interference contrast microscope, which were beyond the project funds to purchase. This equipment is also not available in the local water analysis laboratories.

Water in Zimbabwe is not tested for parasitic contamination although bacteriological and chemical analysis is done. Two methods of water disinfection against parasitic infections were then tested in the laboratory. One of the techniques was implemented in the community. Tests using sand filters and different activated charcoals were carried out. The type and amount of sand that was ideal for capturing parasites was identified and an ideal point-of-use sand filter was also postulated which used a combination of both the sand and the charcoal. Potential activated charcoals that could be used at water treatment plants were also identified namely baobab fruit shells and macadamia nut shells. These were capable of adsorbing protozoan parasite cysts in contaminated water. The ZINWA activated charcoal that is used at water treatment plants was tested and observed to be unable to adsorb *G. duodenalis.*

Sunlight was tested for its capacity to disinfect water contaminated with *G. duodenalis* and *E. histolytica/dispar.* There was a synergistic effect between the sun and the heat produced by the sun that caused inactivation of cysts. Complete inactivation was attained at 56 ºC after 7 hours of sunlight exposure. Solar disnfection was then implemented in one of the communities in order to test its effectiveness. There was a significant reduction of infection

with *G. duodenalis, E. coli* and *I. butschlii* for the participants that exposed their containers that were painted black on one side to the sun.

6. 2 OVERALL CONCLUSION

The first objective was to determine the prevalence of protozoan parasites cysts in individuals residing in an urban, rural tribal trust land and commercial farming environments. Our hypothesis was true in that protozoan parasites were prevalent in these 3 different communities. *Giardia duodenalis* and *C. cayetanensis* were more prevalent in the rural TTL. The risk factors of being infected by *G. duodenalis* was eating fruits or vegetables that are not washed and drinking water from deep protected wells. Hence it is important that fruits are washed before eating them and the importance of disinfecting water especially from deep protected wells should be carried out. The risk factor of being infected by *C. cayetanensis* was being ≤ 5 yr of age and washing hands in a communal dish. This parasite is associated with unhygienic practices that are also common in children ≤ 5 yr. The simple act of using the pouring system when washing hands before eating a meal may reduce this parasite.

E. histolytica/dispar, C. parvum and helminth infections were prevalent in the farming area. To reduce infection with *E. histolytica/dispar* water disinfection is an important factor. *Cryptosporidium parvum* had a significant association with an ethnic group. Helminth infections were more common at those schools where pupils defaected in the bush.

The provision of toilets will prevent such a habit from persisting to continue to take place.

Blastocystis hominis was more prevalent in the urban area. This parasite may cause diarrhoea in some individuals. It is important that diagnostic tests are improved in routine laboratory practice as it was identified only after carrying out the complicated Gomori/trichrome staining technique.

Water is one of the sources of parasitic infections as some individuals had the same parasites in both their stool specimens and their drinking water sources. The deep protected wells had the highest association of parasitic contamination. These findings confirmed the second objective of the study of the probable correlation of common intestinal protozoan parasite cysts and their drinking water sources. Hence the hypothesis was true in that drinking water is contaminated with protozoan parasite cysts.

An ideal sand filter was postulated as a point-of-use water treatment in a rural area, while activated charcoals from baobab fruit shells and macadamia nut shells were also identified that were capable of completely adsorbing parasite cysts. The hypothesis of the third objective was true in that sand filtration and some activated charcoal are capable of removing protozoan cysts from contaminated drinking water.

SODIS was found to inactivate *G. duodenalis* and *E. histolytica/dispar* completely when temperatures rose above 56 °C after 7 hr of sunlight exposure. SODIS can be used in a community to reduce infection by *G. duodenalis, E. coli* and *I. butschlii.* The hypothesis of

the fourth objective was fulfilled in that solar radiation did inactivate protozoan parasites in contaminated water enough to render it safe for human consumption.

6.3 RECOMMENDATION

In order to reduce intestinal parasitic infections in the community; health and hygiene education, deworming programs should be carried out in schools, provision of sanitary facilities and water disinfection methods should be implemented. Since water bodies have been found to be contaminated with parasite cysts, cost effective disinfection methods need to be applied to prevent ingestion of contaminated water in both the rural TTL and urban environments.

It is suggested that people in rural TTL either use the recommended sand filter or SODIS. All these techniques need health education, community awareness and participation, for them to be accepted. Activated charcoals from baobab fruit shells and macadamia nut shells are recommended to be used by ZINWA so that those that consume tap water will not be at risk of acquiring protozoan parasitic infections. These are indigenous trees in Zimbabwe and the Department of Biochemistry, University of Zimbabwe has a blast furnace that makes activated charcoal from these trees. There will be no importation costs for ZINWA.

Water analysis for parasitic contamination should be carried out by all that do water analysis. Therefore there is need for training of personnel and acquisition of resources for this to be achieved. For all that to be achieved there is need for commitment by the City

Councils, resource mobilization and finances. Whilst trying to put in place all these logistics, it is important that other methods of water disinfection are carried out. These include the boiling of water, use of the suggested sand filter, use of baobab fruit shells and macadamia nut shells at water treatment plants and solar disinfection. If the above suggestions are implemented, the nation would at least be able to provide safe drinking water to the communities.

6.4 FUTURE WORK

In this study prevalence of parasitic infections was carried out amongst school children. Future work should involve the general population so that a realistic overview of parasitic infections amongst different age group ranges can be evident. The effectiveness of health education and deworming programmes need to be assessed further using two groups of people whereby one group is given health education and the other is not. Follow up over a period of a year can be done quarterly to assess the effectiveness of health education. Another important factor that came out of this prevalence study was the high prevalence of *C. parvum* amongst pupils of a certain ethnic group. Further studies need to be carried out in that community to ascertain why this particular ethnic group had an increased risk of being infected with *C. parvum*.

Water analysis of different water bodies using method 1623 which is specific for parasitic water analysis should be carried out on different water bodies in Zimbabwe including the drinking tap water (US EPA, 2005). This will help assess how much our drinking water is contaminated. This exercise should also be done in the different seasons to assess if there is seasonal contamination pattern. There is also a need to find out why deep protected wells support the presence of *Giardia duodenalis*. These studies will involve detailed physicochemical analysis, microbiological analysis including conductivity studies that will clearly indicate those appropriate water conditions that favour the survival of *Giardia*. Polymerase chain reaction and genetic characterization needs to be carried out on those isolates that would have been identified from individuals who would have the same parasites in their stool specimens and their drinking water sources. This will confirm whether the source of infection of the individual was really their drinking water source.

The feasibility and effectiveness of charcoals from baobab fruit shells and macadamia nut shells in the removal of parasites at water treatment plants needs to be assessed. There is a need to assess the surface proteins of *Giardia* to find out what makes it able to attach to these particular particles and not others. It is also important to find out the properties of these particles that gives them the ability to adsorb *G. lamblia.* Electron micrograph pictures of these charcoals need to also be studied.

Implementation of anti-protozoan methods for water disinfection using solar radiation in a community needs to be carried out. Whole households should be involved not just children. Follow up should be done for more than a year to cover the 4 seasons and see its

effectiveness throughout. The Millennium Development Goals of clean water for all by the year 2015 in Zimbabwe might be achieved through this study.

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