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ORIGINAL ARTICLE

Impact of solar radiation in disinfecting drinking water contaminated with *Giardia duodenalis* and *Entamoeba histolytica/dispar* at a point-of-use water treatment

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Abstract

Aims: To determine the impact of natural sunlight in disinfecting water contaminated with cysts of *Giardia duodenalis* and *Entamoeba histolytica/dispar* using plastic containers.

Methods and Results: Known quantities of *Giardia duodenalis* and *Entamoeba histolytica/dispar* cysts in sterile water were exposed to the sun. Containers were made of polyethylene terephthalate, eight painted black on one side, one not painted and another cut open at the top and the last was a high density polypropylene container. Viability testing was performed using vital and fluorescent dyes. The same assays were conducted under cloudy conditions. Thermal control tests were also performed using heat without ultra violet light from the sun. Results show that 99·9% of parasites was inactivated when water temperatures reached 56°C after sunlight exposure.

Conclusion: Both solar radiation and heat produced by the sun have a synergistic effect in killing cysts of *Giardia duodenalis* and *Entamoeba histolytica/dispar* when temperatures rise above 50°C, with complete death at 56°C, using painted 2-l PET containers.

Significance and Impact of the Study: Solar disinfection system using PET containers painted black on one side can be used to disinfect water against *Giardia duodenalis* and *Entamoeba histolytica/dispar* using natural sunlight.

Introduction

Approximately, one-sixth of the world's population lacks access to safe water sources. An estimated 3·4 million deaths a year are attributable to waterborne diseases. *Giardia duodenalis* and *Entamoeba histolytica* are common intestinal protozoan parasites infecting humans' worldwide (Wang *et al.* 2004). Infection with *G. duodenalis* is prevalent among institutionalized people and lower socioeconomic community settings causing acute self-limiting diarrhoea, chronic diarrhoea, malabsorption and weight loss (Marshall *et al.* 1997; Thriat *et al.* 1998). About 10% of the world's population are carriers of *E. histotytica* (Mandell *et al.* 2000). 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 the invasion of extraintestinal organs such as the liver and lungs, where marked tissue destruction may occur. Approximately, 34–50 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). Chlorine, a widely used disinfectant at water treatment plants, does not kill some protozoan parasites if its concentration and contact time with water are reduced (Korich *et al.*1990; Wallis *et al.* 1996; Liberti *et al.* 2002). This treated tap water, which is usually regarded as 'safe', has caused several outbreaks of

cryptosporidiosis, giardiasis and amoebiasis in developed countries such as the United States, Canada, United Kingdom and Sweden, due to either failures in the water treatment process or other means (Ljungstrom and Castor 1992; Sinclair *et al.* 1998; Isaac-Renton *et al.*1999; Gardner and Hill 2001; Barwick *et al.* 2002). In Africa, such outbreaks may be occurring, but are not being reported due to the lack of resources for detection of these parasites in water sources. Therefore, as treated and untreated drinking water is regarded as unsafe, there is a need to develop point-of-use water treatment technologies that will help in the reduction of diarrhoeal diseases. Rural people, the majority of whom drink untreated raw water, will benefit more as household treatment can often provide these benefits much more quickly than it will take to design, install and deliver piped community water supplies. Moreover, morbidity and mortality due to waterborne pathogens would be reduced in the vulnerable groups of people such as HIV/AIDs positive people, the young and the old who are all prone to opportunistic infections.

The solar disinfection system (SODIS) system involves storage of water in 1–2 l polyethylene terephthalate (PET) plastic containers for a period of up to 8 h in direct sunlight before consumption (Mendez-Hermida *et al.* 2005). Many experiments have been conducted on different species of bacteria, some viruses and a few protozoan parasites such as *Cryptosporidium parvum, Giardia muris* and *Acanthamoeba polyphaga* (Lonnen *et al.* 2004; Mendez-Hermida *et al.* 2005; Heaselgrave *et al.* 2006; McGuigan *et al.* 2006). We present an analysis of solar disinfection on *Giardia duodenalis* and *Entamoeba histolytica/dispar* using different types of containers.

Materials and methods

Stool samples were collected from 100 rural school children aged between 5 and 15 from Shangure Primary school in Goromonzi, Zimbabwe, having a longitude of 31°21′, latitude 17°52′ and altitude of 1465 m. The participants were selected by convenience sampling in which interested students volunteered to participate. The participants were screened for the presence of *Giardia duodenalis* and *Entamoeba histolytica/dispar* by performing wet preparations using physiological (0·85%) saline. Specimens found to be positive for both parasites were purified using the discontinuous percoll gradient centrifugation as described by Ekert *et al.* (1992). These were then quantified using a haemocytometer (American Optical Corporation, NY, USA) then stored in 5-ml aliquots in distilled water at 8°C as they remain viable at this temperature for up to 77 days (DeRegnier *et al.* 1989). Identification of *Entamoeba histolytica/dispar* was based upon the morphological characteristics after Gomori/trichrome staining of the presence of one to four nuclei, ±rounded chromatoid bars and the size of the cyst of 5–20 μm.

One millilitre (ml) aliquots of $3{\cdot}52 \times 10^5$ cysts ml^{-1} of *Giardia duodenalis* and $4{\cdot}36 \times 10^5$ cysts ml^{-1} of *Entamoeba histolytica* parasite suspensions were added separately to 10 different 2-l containers that had 1800 ml of tap water and the contents were mixed thoroughly then exposed to the sun. Viability testing of cysts was performed before addition of these parasites to the test containers to ensure 100% viability of all cysts before testing. Containers used were 8 × 2 l 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 up of high density polypropylene (HDPP) material. The initial temperature of the contents was measured, then temperatures were recorded hourly for 7 h, with one PET container painted black on one side being taken hourly for viability assays and the rest of the containers were analysed after the 7 h. One of the PET containers was a control, which was kept in the cupboard for 7 h, without being exposed to the sun. The water from the containers was spun in 50-ml sample batches at a centrifuge speed of 500 ***g*** for 3 min. The sediments were pooled to make a 1-ml suspension. Viability testing was performed by using vital and fluorescent dyes. Fifty microlitres of the sediment was stained with the vital dyes namely, 0·4% trypan blue, 0·3% Congo red as described by John and John (1997). Viable cysts do not take up the dye and a haemocytometer (American Optical Corporation) was used to count the viable and nonviable cysts. The fluorescent dyes 4,6 diamidino-2-phenylindole dihydrochloride (DAPI) (2 mg ml^{-1} in absolute methanol) and also propidium iodide (PI) (1 mg ml^{-1} PBS 0·1 mol l^{-1}) were used all being incubated for 2 h at 37°C (Thriat *et al.* 1998. 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 nonviable (Wallis *et al.* 1996). The same assays were also run under cloudy conditions.

Assays were also run in the laboratory to detect the effect of temperature alone without the ultra violet rays from the sun. One-hundred millilitres of distilled water was placed in a conical flask. One millilitre of 1.42×10^5 cysts ml^{-1} of *G. duodenalis* and $8{\cdot}6 \times 10^4$ cysts ml^{-1} of *E. histolytica/dispar* was added. The initial temperature was recorded. This was placed in a water-bath for the equivalent times at the average temperatures that were attained when solar radiation was being used. These were 34°C for 1 h, 44°C for 2 h, 46°C for 3 h, 49°C for 4 h, 52°C for 5 h, 55°C for 6 h and 56°C for 7 h. After incubation at the set times, the water was spun at 500 ***g*** for 3 min. The supernatant was decanted and 1 ml of

sediment was left. Viability assays as described above were then carried out on the average final concentration of $8{\cdot}67 \times 10^4$ cysts ml^{-1} *G. duodenalis* and of $7{\cdot}15 \times 10^4$ cysts ml^{-1} *E. histolytica/dispar*.

Results

Viability testing was performed using the dyes before all the experiments were carried out. For the vital dye-PI, the viable cyst did not take up any fluorescence, whereas for DAPI the nuclei of viable cysts exhibited blue fluorescence at 365 nm and nonviable cysts exhibited no blue fluorescence. These nonviable cysts also fluoresced red at 510–560 nm when using PI. Experiments were carried out at the end of the rainy season of 2007 in January and February, when both full sunshine and cloudy conditions were experienced. The results that were considered were those when there was a clear sky or slightly overcast for the sunshine results, and cloudy days were those considered to either be very cloudy or completely overcast. When there was 50% cloudiness, the results were not considered. These experiments were repeated 15 times and the average results with their standard deviations were used for analysis.

After sunshine exposure when peak temperatures were above 50°C, 99·9% of parasites was inactivated after 7 h when using 2-l painted PET containers (Fig. 1). For the PET bottle that had an open top, after 7 h of sunlight exposure 93·2% of *G. duodenalis* and 92·6% of *E. histolytica/dispar* were inactivated. For the PET bottles not painted, 95% of *G. duodenalis* and 81·3% of *E. histolytica* were inactivated. For the HDPP bottle, 93·2% of *G. duodenalis* and 85·1% of *E. histolytica/dispar* were also

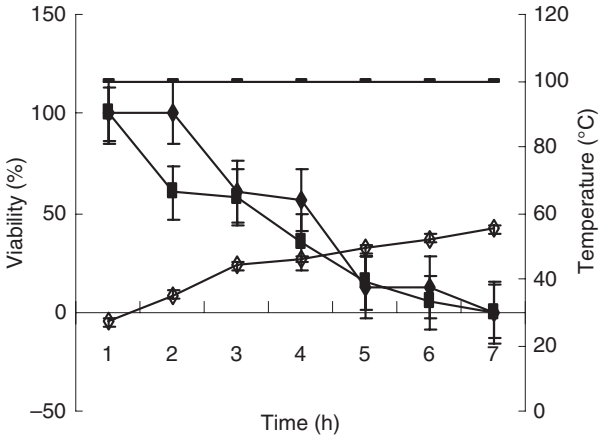

Figure 1 Comparison of *Giardia duodenalis* and *Entamoeba histolytica/dispar* when using painted PET bottles. Error bars represent SD. (–■–), % *G. duodenalis*; (– – –), Control; (–♦–), % *E. histolytical/dispar*; (–◊–) Temperature.

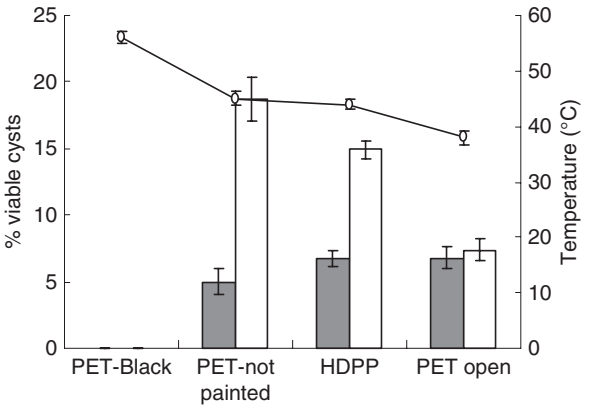

Figure 2 Comparison of different types of containers after 7 h. Error bars represent SD. (■), % Gd viable; (□), % Eh viable; (–o–) Temp.

inactivated (Fig. 2). More absorption of heat was found in the PET plastic containers painted black on one side than in those that were not painted, that were opened and that contained HDPP (Fig. 2).

Under cloudy conditions, the 2-l PET bottle painted black on one side attained the highest temperature of 38°C after 7 h in the open, 31% of *G. duodenalis* was still alive compared to 58% of *E. histolytica/dispar* (Table 1). Exposure of the parasites to heat alone did not inactivate parasites (Table 2).

Table 1 SODIS under cloudy conditions

Type of container	Highest temperature attained (°C)	% viable *Giardia duodenalis*	% viable *Entamoeba histolytica*
2-l container	31	56	75
2-l PET container not painted	34	100	75
2-l HDPP	36	70	76
2-l PET container painted black on one side	38	31	58

Table 2 Use of heat in killing parasites

Time (h)	Temperature (°C)	% Viable *Giardia duodenalis*	% Viable *Entamoeba histolytica/dispar*
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

Discussion

The biocidal effect caused by sunlight is due to the optical and thermal processes that occur at temperatures above 45°C (McGuigan *et al.* 1998, 2006). 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 nonpainted PET containers were not as efficient in capturing heat from the sun as compared to the painted container. Painting the container black on one side caused the increase in temperature. This agrees with the study of Martin-Dominguez *et al.* (2005) who inactivated total coliforms and *Escherichia coli* using PET bottles partially blackened on one side and others not blackened. In another study by Kehoe *et al.* (2001), covering the rear surface of the solar disinfection container with aluminium foil improved the inactivation efficiency of the system.

Sunlight has germicidal effects as it provides both ultraviolet (UV) radiation and heat. The combined effects of temperatures of 50–60°C and UV radiations in the UVA range (320–400 nm) and UVB range (280–315 nm) of the SODIS are germicidal to inactivate extensively many enteric micro-organisms (Sobsey and Bartram 2002). This 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 according to our results of the low temperatures when there was cloud cover and when parasites were heated to the high temperatures of 56°C with the exclusion of sunlight, there was no complete inactivation of both *G. duodenalis* and *E. histolytica.* Therefore, we conclude that the synergistic effect of UV light from the sun and of the heat produced by the sun, that occurs for some bacterial cells, also takes place for these particular parasites under study. However, other studies have indicated that under cloudy conditions containers should be placed outside for 2 days for effective disinfection by this process. These results concerning cloudiness are conforming to other studies cited by Sommer *et al.* (1997), that showed different percentages of UVA available on days with different levels of cloudiness. Therefore, less UVA and less heat decrease the biocidal effects of SODIS. Further studies measuring the UV intensity should be carried out in our settings, as this was not performed in this study.

Many documented studies concerning SODIS have been carried out involving *Giardia* and *Cryptosporidium,* and none have 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 this study clearly indicates that loss of viability of the latter organism is slower as compared to the former (Fig. 1). 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 where 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 after 7 h of full sunshine exposure when temperatures reach 56°C of *E. histolytica/dispar.*

Plastics that are exposed to the sun produce photoproducts at the outer surface of the PET bottles. Increased use of these products also leads their higher availability. Experiments carried out by Kohler and Wolfensberger (2003) and Wegelin *et al.* (2000), concluded that these substances including the plasticizers, di(2-ethylhexyl)adipate and the chemical di(2-ethylhexly)phthalate, are at low concentrations well below the limits of safe drinking water and there was no significant difference observed between new and used bottles. Therefore, PET plastic containers have no risk of toxicity when used for this technology.

Conclusion

Solar radiation may offer a method of disinfection of drinking water that requires few resources and no expertise as:

1. PET bottles painted black on one side are capable of inactivating cysts of *G. lamblia* and *E. histolytica/dispar.*
2. The inactivation rate of *E. histolytica/dispar* is slower than that of *G. lamblia.*
3. There is a synergistic effect between the heat and the UV light produced by the sun in the inactivation of these parasites.

To achieve the WHO and United Nations Millennium Development Goals of halving the number of people without clean safe water by the year 2015, we recommend the PET plastic containers painted black on one side to be used as one of the point-of-use water treatments as these can inactivate some micro-organisms that cause diarrhoea especially in developing countries.

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Conflicts of interest

None declared.

Ethics approval

This study protocol was approved by Higher Degrees Committee of the University of Zimbabwe, the Medical Research Council of Zimbabwe (MRCZ), Approval Number: MRCZ/A/993b.

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