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Efficacy of the solar water disinfection method in turbid waters experimentally contaminated with *Cryptosporidium parvum* oocysts under real field conditions

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Summary

OBJECTIVE To investigate the efficacy of the solar water disinfection (SODIS) method for inactivating *Cryptosporidium parvum* oocysts in turbid waters using 1.5 l polyethylene terephthalate (PET) bottles under natural sunlight.

METHODS All experiments were performed at the Plataforma Solar de Almería, located in the Tabernas Desert (Southern Spain) in July and October 2007. Turbid water samples [5, 100 and 300 nephelometric turbidity units (NTU)] were prepared by addition of red soil to distilled water, and then spiked with purified *C. parvum* oocysts. PET bottles containing the contaminated turbid waters were exposed to full sunlight for 4, 8 and 12 h. The samples were then concentrated by filtration and the oocyst viability was determined by inclusion/exclusion of the fluorogenic vital dye propidium iodide.

RESULTS After an exposure time of 12 h (cumulative global dose of 28.28 MJ/m^2; cumulative UV dose of 1037.06 kJ/m^2) the oocyst viabilities were 11.54%, 25.96%, 41.50% and 52.80% for turbidity levels of 0, 5, 100 and 300 NTU, respectively, being significantly lower than the viability of the initial isolate ($P < 0.01$).

CONCLUSIONS SODIS method significantly reduced the potential viability of *C. parvum* oocysts on increasing the percentage of oocysts that took up the dye PI (indicator of cell wall integrity), although longer exposure periods appear to be required than those established for the bacterial pathogens usually tested in SODIS assays.

keywords SODIS, natural sunlight, PET bottles, turbid waters, *Cryptosporidium*, viability

Introduction

Outbreaks of disease as a result of the consumption of untreated or improperly treated drinking water contaminated with viral, bacterial or parasitic agents that cause diarrhoeal diseases occur on a daily basis worldwide (WHO 2001). In developing countries, global death from diarrhoea of children aged less than 5 years were estimated at 1.87 million, approximately 19% of total child deaths (Boschi-Pinto *et al.* 2008). The WHO and the United Nations Children's Fund (UNICEF) have recently claimed that improvement of drinking water quality and basic sanitation are required to decrease childhood morbidity and mortality and to combat the main transmission route of diarrhoeal diseases (WHO/UNICEF 2007).

One strategy for solving this problem consists of the use of natural sunlight, an abundant source of energy in many developing regions. The bactericidal effect of sunlight has been known for many years (Downes & Blunt 1877). Acra *et al.* (1980) carried out the first practical study of solar disinfection by filling polyethylene bags with water and exposing them to full sunlight and suggested that natural sunlight could destroy pathogenic microorganisms present in the water. Since 1991, the Department of Water and Sanitation in Developing Countries (SANDEC) at the Swiss Federal Institute of Aquatic Science and Technology (EAWAG) has supported the development and application of solar water disinfection, and at present about one million people worldwide use this system for treating drinking water (EAWAG 2008).

Solar water disinfection (SODIS) is a simple, environmentally sustainable and inexpensive point-of-use treatment for drinking water, in which contaminated drinking water is placed in transparent plastic polyethylene terephthalate (PET) bottles and exposed to full sunlight for at least 6 h (or 2 consecutive days if there is more than 50%

cloud cover) during daylight hours when the maximum intensity of radiation occurs. Sunlight disinfects contaminated water via the action of radiation in the spectrum of ultraviolet (UV) light (type A, wavelength 320–400 nm) and the high temperatures reached in the water (EAWAG 2008). Numerous studies in the last 15 years have demonstrated the effectiveness of simulated and natural SODIS on the inactivation of viruses, bacteria, fungi and protozoan parasites (Joyce *et al.* 1996; Smith *et al.* 2000; Conroy *et al.* 2001; Kehoe *et al.* 2004; Lonnen *et al.* 2005; Heaselgrave *et al.* 2006; McGuigan *et al.* 2006; Méndez-Hermida *et al.* 2007; Boyle *et al.* 2008). At present, SODIS is one of the recommended methods for disinfection of household drinking water (WHO/UNICEF 2005).

Cryptosporidium parvum is a protozoan parasite that is often associated with waterborne outbreaks of diarrhoeal disease worldwide; it is one of the major enteric pathogens in children under 5 years in developing countries, and it appears to be endemic in several regions (Ashbolt 2004; Ayalew *et al.* 2008). In developing countries, malnourished children present higher rates of infection than adequately nourished children. There is also evidence that children with cryptosporidiosis are more likely to suffer from malnutrition and to die (Hunter & Nichols 2002).

The aim of the present study was to investigate the efficacy of SODIS procedures in turbid waters experimentally contaminated with oocysts of *C. parvum* contained in 1.5 l PET bottles and exposed to natural sunlight.

Materials and methods

Cryptosporidium oocysts were collected from a naturally infected neonatal Friesian–Holstein calf by rectal sampling. Concentration [phosphate-buffered saline (PBS; pH 7.2)/diethyl ether], purification (discontinuous caesium chloride gradients) and quantification (Neubauer haemocytometer) were performed as reported previously (Kilani & Sekla 1987; Lorenzo-Lorenzo *et al.* 1993). The oocysts were classified as *C. parvum* by analysis of a fragment of the *Cryptosporidium* oocyst wall protein gene (COWP), according to Amar *et al.* (2004).

Red soil, whose composition is very similar to that of soils in tropical areas (Patrick 1980), was collected from close to the Michelin Test Field in Almeria (Spain). Analysis of the soil was performed by the Department of Edaphology of the School of Pharmacy of the University of Santiago de Compostela (DEUSC) (Spain) and the results are shown in Table 1. Turbid water samples were prepared by addition of soil to distilled water to achieve the required turbidity levels of 5, 100 and 300 NTU, as measured with a TN-100 turbidimeter (Eutech Instruments Pte. Ltd., Singapore, Singapore). Briefly, 0.3 g, 7 g and 13 g of soil were added to 500 ml of distilled water to obtain turbidity levels of 5, 100 and 300 NTU respectively. The suspensions were shaken for 30 min and then allowed to settle for 1 h. The supernatants were collected and the turbidity adjusted to the required level with distilled water. The suspensions were then sterilized by autoclaving for 20 min at 120 p.s.i. and stored at 4–8 °C. The properties of the turbid water samples were also analysed by DEUSC, and the results are shown in Table 2.

All experiments were performed under natural solar radiation at the Plataforma Solar de Almería (PSA), located in the Tabernas Desert (Almería, Southern Spain: latitude, 37°05′54″ N; longitude, 2°21′32″ W; altitude, 500 m) in July and October 2007. Measurements of global solar

Table 1 Analysis of the red soil collected in Almeria (Spain)

pH (water)	8.61	Total organic carbon	0.395%
pH (KCl)	7.58	Total nitrogen	0.048%
P-ClH	50.93 mg/kg	C/N ratio	8.2
P-CO_3H^-	14.93 mg/kg	Fe_2O_3	0.060%
$P_{inorganic}$-CO_3H^-	10.62 mg/kg	Al_2O_3	0.162%
$P_{organic}$-CO_3H^-	4.31 mg/kg	Carbonates	8.1%
	Granulometric analysis		
	No destruction of carbonates	Destruction of carbonates	
Sand (500–50 μm)	33%	37%	
Thick silt (50–20 μm)	9%	7%	
Fine silt (20–2 μm)	13%	20%	
Clay (<2 μm)	44%	36%	
Mineralogy of the clay fraction			
Major minerals		Illite, Halloysite	
Minor minerals		Calcite	

Table 2 Analysis of the water samples of different levels of turbidity prepared with the red soil collected in Almeria (Spain)

		DOC	P-CO_3H^-*	$P_{inorganic}$-CO_3H^-*	$P_{organic}$-CO_3H^-*	
	pH*	mg/l				Colour†
5 NTU	7.01	0.7	0.004	0.000	0.004	0.6Y 9.4/1.0
100 NTU	7.41	6.0	0.055	0.013	0.042	6.1YR 6.5/8.3
300 NTU	7.47	12.2	0.254	0.021	0.232	3.8YR 5.3/6.6

*Parameters determined in filtered samples. †Munsell scale.

exposure were made with a pyranometer (model CMP 21, Kipp & Zonen, Delft, The Netherlands) for shortwave global solar radiation measurements in the spectral range from 310 nm to 2800 nm during the experiments. Incoming UV radiation (direct plus diffuse radiation from all directions) was measured at between 295 nm and 385 nm (part of UVA and UVB) with a horizontally placed global UV radiometer (model CUV 3, Kipp & Zonen).

The cumulative solar global or solar UV exposure is the accumulated global or UV energy per unit of surface area received in the bottle reactors. This was calculated by integrating the average solar UV or global irradiance measured at any time during the different exposure times as it is showed in the following equation:

$$\mathrm{Dose}_{\mathrm{UV;global}} = \int_{t_1}^{t_2} Irradiance(UV; global) \cdot dt$$

where *Irradiance* is given in terms of W/m^2, t in seconds, and Dose in J/m^2.

Clean, 1.5 l transparent PET soft-drink bottles were filled with the turbid water samples (0, 5, 100 and 300 NTU), which were then spiked with 5×10^6 purified oocysts of *C. parvum*. Samples were shaken vigorously by hand for 10–30 s to ensure maximum distribution of the oocysts, and the bottles were then placed on the roof of the laboratory in full sunshine (Figure 1) for 4, 8 and 12 h so that the mid-point of each exposure occurred at noon on each day (approximately 14:00 h, local time) (Figure 2). Bottles containing water samples of 0 NTU were also contaminated with 5×10^6 purified oocysts of *C. parvum*, wrapped in aluminium foil and were placed beside the SODIS test samples to act as dark control samples. During the experiments, the water temperature was monitored every hour with a thermometer (model HI 98509-1, Hanna Instruments, S.L., Eibar, Spain). At the end of the exposure periods the samples were stored overnight in the laboratory (in the dark at 20 °C). All experiments were performed in triplicate.

To evaluate the effect of solar radiation on the viability of *C. parvum* oocysts, but without considering the thermal effect, PET bottles containing water samples of 0 and 300

Figure 1 Exposure to full sunshine of water samples of turbidity 0, 5, 100 and 300 NTU experimentally contaminated with *Cryptosporidium parvum* oocysts and held in 1.5 l transparent PET bottles, and of dark controls, on the roof of the laboratory at the Plataforma Solar de Almería (Spain).

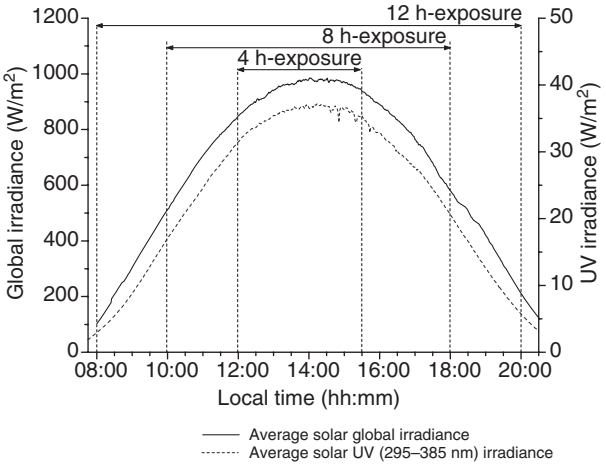

Figure 2 Average global and UV irradiance measurements during the SODIS studies carried out under natural sunlight.

NTU and the dark control samples were contaminated with 5×10^6 purified oocysts of *C. parvum*, placed in a water bath and exposed to full sunshine for 4, 8 and 12 h. The temperature of the water bath was maintained constant at 20–25 °C throughout the experiments by the addition of ice to the water.

After the exposure times, the samples were concentrated by filtration, in a Filta-Max® concentrator unit (IDEXX Laboratories, Inc., Westbrook, ME, USA), to a final volume of 10 ml. One millilitre of each sample was placed in a 1.5 ml microcentrifuge tube and centrifuged at 10 000 ***g***, at 4 °C for 5 min. The supernatant was discarded and the sediment thus obtained was used in the viability assays, as described below.

The potential viability of *C. parvum* oocysts was determined by inclusion /exclusion of the fluorogenic vital dye propidium iodide (PI) (Sigma, Madrid, Spain), in accordance with Campbell *et al.* (1992) and a further modification that includes an immunofluorescence antibody test to verify oocyst identification (Dowd & Pillai 1997). Briefly, sediments obtained from the samples were resuspended in 100 µl of Hanks' balanced salt solution (Sigma, Madrid, Spain) and then incubated with 10 µl of PI working solution (1 mg/ml in 0.1 M PBS, pH 7.2) at 37 °C for 10 min. After PI staining, oocysts were washed twice in PBS at 10 000 ***g***, at 4 °C, for 5 min and incubated with 30 µl of monoclonal antibodies labelled with fluorescein isothiocyanate (FITC) (Aqua-Glo G/C Direct, Waterborne, Inc., New Orleans, LA, USA). Oocysts were first identified under FITC filter (excitation at 450–480 nm; barrier at 515 nm) before being examined for PI inclusion/exclusion under a PI filter (excitation at 510–550 nm; barrier at 590 nm). The proportions of ruptured (ghost), PI positive (dead) and PI negative (viable) oocysts were quantified in an epifluorescence microscope equipped with phase contrast optics, FITC and PI filters (Eclipse 50i Nikon Corporation, Tokyo, Japan). The results are showed as the percentage of oocysts that included/excluded the dye PI obtained for each experiment after triplicate counts of more than 100 oocysts.

Differences in the percentage of PI negative oocysts and temperatures reached within the PET bottles for different turbidity levels and exposure times were compared by pairwise multiple comparison procedures (Student-Newman–Keuls method) and one-way ANOVA, with GraphPad Instat®, version 3.05, statistical software (©1992–2000 GraphPad Software, La Jolla, CA, USA). Differences were considered significant at a probability level of $P < 0.05$.

Results

The isolate of *C. parvum* used in the experiments showed a potential viability of 91.60% (only 8.40% of oocysts were PI positive). The weather conditions during the SODIS studies were sunny and cloudless; the average daily (from 08:00 h to 20:00 h local time) accumulated global and UV irradiances were 28.28 MJ/m^2 (standard deviation, 0.17 mJ/m^2) and 1037 kJ/m^2 (standard deviation, 21 kJ/m^2), respectively. The maximum local noon global and UV irradiances recorded within this period were 997.70 and 37.70 W/m^2, respectively (Figure 2).

The ambient temperature recorded during the experimental period varied from 19 to 38 °C. The mean temperature profiles for the water samples contained in the PET bottles and exposure times of 4, 8 and 12 h are shown in Figure 3. The maximum water temperatures recorded within the PET bottles were 49.7 °C, 48.5 °C, 46.3 °C and 45.9 °C for turbidity levels of 300, 100, 5 and 0 NTU respectively. The maximum temperature of the water in the dark control samples was 44.5 °C. The only significant differences in the temperature occurred between the most turbid water samples and the dark control ($P < 0.05$).

The inactivation kinetics for *C. parvum* oocysts suspended in water of different levels of turbidity and exposed to natural sunlight are shown in Figure 4. After an exposure time of 4 h and a cumulative dose of global radiation of 13.55 MJ/m^2 (509.25 kJ/m^2 of cumulative UV radiation dose), the oocyst viability decreased from 91.60% to 30.75%, 46.05%, 63.05%, 74.04% and 83.30% for samples of 0, 5, 100, 300 NTU and the dark

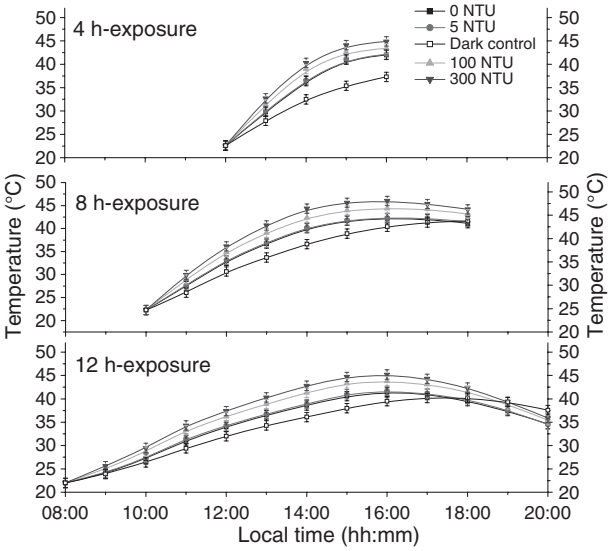

Figure 3 Profiles of the mean of the water temperatures recorded within the 1.5 l PET bottles containing water samples of different levels of turbidity and exposed to full sunlight for 4, 8 and 12 h.

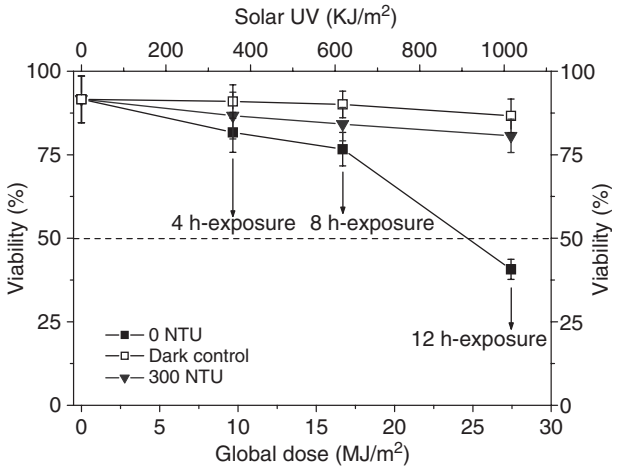

Figure 4 Inactivation kinetics for *Cryptosporidium parvum* oocysts suspended in water of different levels of turbidity and exposed to natural sunlight for 4, 8 and 12 h in 1.5 PET bottles.

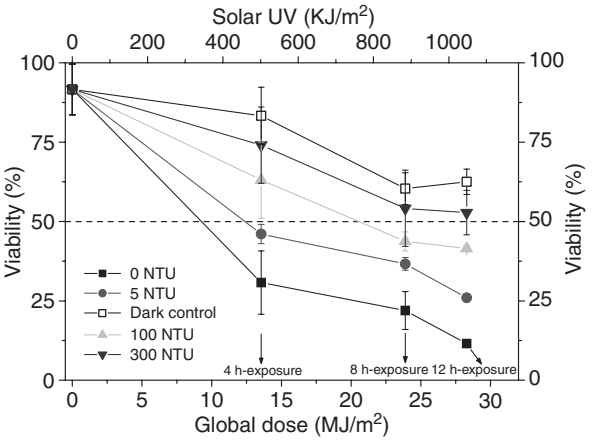

Figure 5 Inactivation kinetics of *Cryptosporidium parvum* oocysts suspended in water samples of different turbidity levels and exposed to real sunlight for 4, 8 and 12 h in 1.5 l PET bottles at constant temperature of 20–25 °C.

control samples, respectively. The oocyst viability decreased further after an exposure time of 8 h, and after an exposure time of 12 h, the oocyst viabilities were 11.54%, 25.96%, 41.50%, 52.80% and 62.50% for turbidity levels of 0, 5, 100, 300 NTU and dark control samples; the viability of oocysts in turbid water samples was significantly different from the viability of the initial isolate ($P < 0.01$). The cumulative global radiation dose received by these samples was 28.28 MJ/m^2 (cumulative UV dose of 1037.06 kJ/m^2).

The results obtained when *C. parvum* oocysts were suspended in water samples of 0 and 300 NTU and exposed to natural sunlight at a constant temperature of 20–25 °C are shown in Figure 5. The cumulative global radiation dose during this study was 27.44 MJ/m^2 (cumulative UV dose of 1011.78 kJ/m^2) and under these conditions, the differences in the oocyst viability for 300 NTU and dark control samples were minimal, whereas in clear water samples (0 NTU), a decrease from 91.60% to 40.70% was observed at the end of the 12 h exposure period.

Discussion

This is the first study that evaluates the efficacy of SODIS for the inactivation of *C. parvum* oocysts in turbid waters under natural conditions using 1.5 l PET bottles. The results show that after 12 h of exposure to full sunlight, the number of PI positive oocysts increased significantly from 8.40 to 88.46% in the clear water samples (0 NTU), whereas in highly turbid water samples (300 NTU) the percentage of PI positive oocysts increased only 47.20%.

The effectiveness of natural SODIS for inactivation of *C. parvum* oocysts has been previously evaluated. Gaafar (2007) exposed samples of contaminated drinking water in plastic and glass tubes to sunlight and observed that the exposure of the samples during 24 h inactivated *C. parvum*, and no parasitic forms were observed in mouse intestinal sections. Similarly, Méndez-Hermida *et al.* (2007) observed a reduction in oocyst viability from 98% to 0.3% after 12 h exposure of experimentally contaminated drinking water samples in glass tubes. In a recent study involving the use of methylacrylate cuvettes, King *et al.* (2008) concluded that solar UV radiation may rapidly inactivate *C. parvum* in environmental water samples.

In this work, the effectiveness of SODIS procedure against *Cryptosporidium* was evaluated determining the potential oocyst viability by inclusion/exclusion of fluorogenic vital dye PI, because there are no animal husbandry or cell culture facilities available at the PSA. According to Robertson and Gjerde (2007), this method, which is relatively cheap and readily implemented, provides useful initial data for investigating the effect of environmental pressures on *Cryptosporidium*, although it overestimates the oocyst infectivity, in comparison with the neonatal murine model. Therefore, the obtained values in oocyst viability may correspond to smaller values in infectivity.

A geographical area is considered suitable for SODIS when it receives 3–5 h of global solar radiation above 500 W/m^2. Moreover, it is known that ambient air temperature can enhance SODIS when it is higher than 20 °C (EAWAG 2008) and a strong synergistic effect between the optical and thermal processes at temperatures above 45 °C has been described (Wegelin *et al.* 1994; McGuigan *et al.* 1998). This study performed at the PSA involved carrying out the different tests under the required conditions: the global solar radiation surpassed 500 W/m^2 during the minimum time recommended, and even at the shortest exposure time tested (4 h), the intensity of radiation was higher than 800 W/m^2 during the entire assay (see Figure 2); the environmental temperature registered during the days that the assays were carried out ranged between 19 and 38 °C, and a temperature of more than 45 °C was reached in all the bottles. Thus, decreases in the oocyst viability of 80, 65, 50 and 40 percentage points were detected for water with turbidity levels of 0, 5, 100 and 300 NTU respectively (see Figure 4).

However, SODIS field trials in different geographical regions, carried out by SANDEC, have shown that temperatures above 45 °C are rarely reached. For example, mountainous areas are subject to high cloud cover because of orographic lifting, and to cooler temperatures than occur at lower elevations (EAWAG 2008). The tests performed at controlled temperatures (20–25 °C) and with clear and turbid waters (of 0 and 300 NTU respectively) enabled us to show the effect that solar radiation alone has on the potential oocyst viability. A reduction in oocyst viability of approximately 50% points (from 91.60% to 40.70%) was detected in samples with a turbidity level of 0 NTU, whereas for highly turbid water samples (300 NTU) the reduction was very low (from 91.60% to 80.64%).

On the other hand, it is known that temperature is a key abiotic factor that affects oocyst survival in the environment (King *et al.* 2005). The decrease in oocyst viability detected in the dark control samples was probably because of the high temperatures reached inside the bottles during the experiments (maximum 44.9 °C) as they were in contact with the laboratory roof. In this way, a reduction in oocyst viability was not observed in the dark control samples when the assays were performed at controlled temperature.

In those geographical regions where the WHO recommends the use of SODIS as an alternative method of disinfection to guarantee the safety of water destined for human consumption, the levels of water turbidity varied widely. Depending on weather conditions and time of collection, the turbidity of water in some areas of Kenya can vary between 5 and 2000 NTU daily (Joyce *et al.* 1996). Laboratory experiments have shown that in water samples of turbidity higher than 200 NTU, less than 1% of the total incident ultraviolet light (UV) penetrates further than a depth of 2 cm from the surface (Joyce *et al.* 1996). It is therefore recommended that water destined for treatment by SODIS do not display turbidity levels above 30 NTU (EAWAG 2008). However, unfortunately in many regions in which this method is used to disinfect water, the turbidity levels are higher. Anyway, Conroy *et al.* (1999) reported a reduction in the risk of diarrhoeal diseases in Massai children who drank water exceeding levels of 200 NTU that was exposed to sunlight. The results obtained in the present study showed a decrease in the efficacy of SODIS with increasing turbidity. Furthermore, when the temperature of the water did not surpass 25 °C, the decrease in the oocyst viability in water of 300 NTU was very low, independently of the time of exposure.

Like *Cryptosporidium*, viral and bacterial pathogens can be transmitted via drinking water in developing areas (see Ashbolt 2004). Recently, Boyle *et al.* (2008) concluded that vegetative stages of bacterial species are easily inactivated by SODIS, whereas microbial species that form spores are less sensitive to the harsh optical, thermal and oxidative conditions established within the water container during SODIS, and can survive the process. Moreover, after SODIS procedures, some bacteria may regrow, and therefore the EAWAG recommends that SODIS treated water should be consumed within 24 h after the treatment (EAWAG 2008). However, protozoan parasites including *Cryptosporidium* can only survive in the environment as a transmission stage and only reproduce inside susceptible hosts (Fayer 2008).

Moreover, it appears that *C. parvum* oocysts survive as infectious agents after longer periods of exposure in SODIS procedures than other indicator microorganisms of faecal contamination, and therefore these organisms may be appropriate for studying the efficacy of this water disinfection system.

Conclusions

The SODIS method, carried out under field conditions with PET bottles containing turbid waters contaminated with *C. parvum* oocysts, significantly reduced the potential viability of *C. parvum* oocysts on increasing the percentage of oocysts that took up the dye PI (indicator of cell wall integrity), although longer exposure periods appear to be required than those established for the bacterial pathogens usually tested in SODIS assays. Further research is required to evaluate the effects of solar radiation and the high temperatures reached during SODIS procedures on oocyst infectivity.

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