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ORIGINAL ARTICLE

# Batch solar disinfection inactivates oocysts of *Cryptosporidium parvum* and cysts of *Giardia muris* in drinking water

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## Abstract

**Aim:** To determine whether batch solar disinfection (SODIS) can be used to inactivate oocysts of *Cryptosporidium parvum* and cysts of *Giardia muris* in experimentally contaminated water.

**Methods and Results:** Suspensions of oocysts and cysts were exposed to simulated global solar irradiation of 830 W $m^{-2}$ for different exposure times at a constant temperature of 40°C. Infectivity tests were carried out using CD-1 suckling mice in the *Cryptosporidium* experiments and newly weaned CD-1 mice in the *Giardia* experiments. Exposure times of ≥10 h (total optical dose *c.* 30 kJ) rendered *C. parvum* oocysts noninfective. *Giardia muris* cysts were rendered completely noninfective within 4 h (total optical dose >12 kJ). Scanning electron microscopy and viability (4′,6-diamidino-2-phenylindole/propidium iodide fluorogenic dyes and excystation) studies on oocysts of *C. parvum* suggest that inactivation is caused by damage to the oocyst wall.

**Conclusions:** Results show that cysts of *G. muris* and oocysts of *C. parvum* are rendered completely noninfective after batch SODIS exposures of 4 and 10 h (respectively) and is also likely to be effective against waterborne cysts of *Giardia lamblia*.

**Significance and Impact of the Study:** These results demonstrate that SODIS is an appropriate household water treatment technology for use as an emergency intervention in aftermath of natural or man-made disasters against not only bacterial but also protozoan pathogens.

## Introduction

*Cryptosporidium parvum* is a protozoan parasite responsible for a number of waterborne outbreaks of human cryptosporidiosis worldwide (Robertson *et al.* 1992; Slifko *et al.* 2000). The oocysts' capability of surviving in the environment for long periods of time makes waterborne transmission of *Cryptosporidium* a serious global issue in drinking water safety (Robertson *et al.* 1992; Freire-Santos *et al.* 2000). Due to the robust oocyst structure, the small size of this parasite and the low sedimentation rate, conventional water treatment is not totally effective, and oocysts may be present and infective in treated water even if no treatment failure has occurred (Solo-Gabrielle and Neumeister 1996). The parasite *C. parvum* is known to be a major cause of human and animal diarrhoea outbreaks as a result of contaminated water supplies. The largest outbreak occurred in 1993 in Milwaukee (WI, USA) when an estimated 403 000 people contracted the disease following contamination of the municipal water supply

 

(MacKenzie *et al.* 1995), and *c.* 2 years after the outbreak, the number of resulting deaths was 54, of whom 85% had acquired immunodeficiency syndrome (AIDS) (Hoxie *et al.* 1997). In addition, cryptosporidiosis is a significant cause of diarrhoea in children in developing countries. The symptoms of the disease are more pronounced in these children leading to malnutrition and impaired physical and cognitive development (Checkley *et al.* 1998; Clarke 1999; Guerrant *et al.* 1999). It is estimated that contaminated drinking water and lack of sanitation leads to the death of *c.* 4500 children each day (WHO/UNICEF 2005).

*Giardia lamblia* (syn. *Giardia duodenalis*, *Giardia intestinalis*) is the aetiological agent for worldwide outbreaks of giardiasis associated with drinking water. This protozoan causes as many as 2·5 million cases of giardiasis in the USA each year (Furness *et al.* 2000), of which appox. 25% are waterborne. The strategic aim of this research was to determine whether batch-process solar disinfection (SODIS) successfully inactivates cysts of *G. lamblia*. In these studies, *Giardia muris* was used as a surrogate for *G. lamblia* because it is an established model (Hayes *et al.* 2003); and has been used successfully in other water disinfection studies (Rice *et al.* 1982; Leahy *et al.* 1987). Cysts of *G. lamblia* have been shown to be equally or less resistant to UV disinfection than cysts of *G. muris* (Mofidi *et al.* 2002).

The SODIS technique involves storing contaminated drinking water in transparent containers (plastic bags, plastic bottles or glass bottles) that are placed in direct sunlight for periods of up to 8 h before consumption (Wegelin *et al.* 1994; Conroy *et al.* 1996). This technique is highly effective against a broad range of bacterial fungal and free-living protozoan pathogens such as *Escherichia coli* (Kehoe *et al.* 2001b), *Vibrio cholerae* (Conroy *et al.* 2001; Kehoe *et al.* 2004), *Salmonella typhimurium* (Smith *et al.* 2000), *Shigella dysenteriae* type I (Kehoe *et al.* 2004), *Pseudomonas aeruginosa*, *Candida albicans*, *Fusarium solani* and the trophozoite stage of *Acanthamoeba polyphaga* (Lonnen *et al.* 2005). Previous studies have reported a reduction in incidence of diarrhoea among those children who drank water exposed to direct sunlight compared with another group that drank water not exposed to sunlight (Conroy *et al.* 1999, 2001). The biocidal effect of sunlight is due to optical and thermal processes, and a strong synergistic effect occurs at temperatures exceeding 45°C (McGuigan *et al.* 1998). In addition to direct UV killing, sunlight is absorbed by photosensitizers present in the water that then react with oxygen producing highly reactive oxygen molecules such as hydrogen peroxide and superoxides, which exert a disinfecting effect (Whitelam and Codd 1986).

## Materials and methods

### Solar simulator protocol

Suspensions of $1 \times 10^7$ purified *C. parvum* oocysts or $5 \times 10^5$ of *G. muris* cysts were suspended in 10 ml distilled water, in lidded, transparent polystyrene six-well microtitre containers with lid (IWAKI 3810-006, Tokyo, Japan) and placed for varying periods of time, under the light of a solar-filtered (air mass = 1·0) 1000 W xenon arc lamp solar simulator (Oriel, Corp., Stratford, CT, USA) with a sharp cut-off at 320 nm and a maximum optical output irradiance of 870 W $m^{-2}$. Optical irradiances were measured using a calibrated optical power meter (model 200/10+; Coherent, Cambridge, UK). The output spectrum of the solar simulator closely resembles that of natural sunlight at equatorial latitudes at sea level (comparisons of transmission properties are available elsewhere (McGuigan *et al.* 1998). The sample containers were maintained at a constant temperature of 40°C by being immersed horizontally in a water bath such that the lower half of the sample container was in contact with the water bath while the upper half was out of the water and exposed to the simulated sunlight (Fig. 1). All control samples were prepared in the same manner from the same inoculum, and then wrapped in aluminium foil to prevent light falling on the suspensions. Room temperature control samples were stored in the dark at 20°C and SODIS control samples were wrapped in aluminium foil and placed beside the SODIS test samples in the 40°C water bath, but kept out of the collimated illumination. All *C. parvum* and *G. muris* studies were carried out in the simulator system.

### *Escherichia coli* validation of solar simulator protocol

The validity of using transparent polystyrene lidded, six-well tissue culture plates (Sarstedt, Numbrecht, Germany) instead of standard polyethylene terephthalate (PET) soft drink bottles was tested under real sunlight conditions at the Plataforma Solar de Almería solar research facility in Almeria, Spain (latitude 37°05′54″, longitude 2°21′32″). *Escherichia coli* (ATCC 23631) was used as the test organism in these studies, due to its widespread use as a faecal indicator. A single colony was inoculated in 5 ml of sterile Luria broth (L 3522; Sigma, St Louis, Mo, USA) and incubated at 37°C with shaking for 18 h to obtain a stationary-phase culture. Cells were harvested by centrifugation at 2000 ***g*** for 14 min and washed three times with predistilled Milli-Q filtered water. Finally, the pellet was resuspended in sterile predistilled Milli-Q filtered water to a final concentration of $10^6$ colony forming units (CFU $ml^{-1}$). Volumes of 10 ml of this preparation were

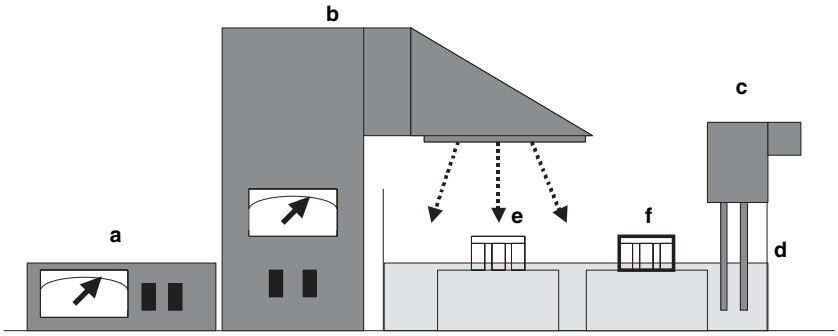


**Figure 1** Schematic diagram of experimental apparatus used for the *Cryptosporidium parvum* and *Giardia muris* simulated solar disinfection (SODIS) studies: (a) lamp power supply; (b) lamp intensity controller; (c) water heater; (d) water bath; (e) SODIS test sample and (f) SODIS control sample wrapped in aluminium foil.

placed in lidded six-well transparent polystyrene tissue culture plates. Three 300-ml volume transparent PET bottles were filled with the same preparation. All samples were placed in basins filled with 2 l of water on the roof of the laboratory, in full sunshine, as shown in Fig. 2. The water-filled basins acted as thermal sinks to remove the effects of differential heating rates that would be caused by the disparity in sample volumes within the PET and polystyrene containers. Consequently, any measured difference in inactivation kinetics between the PET and polystyrene samples must derive from differences in the transmission properties of the container materials. Volumes of 100 $\mu$l were taken from each bottle of the control and irradiated groups at the beginning of each experiment and at each sampling interval. These volumes were diluted in a series of 10-fold dilutions and were then plated in triplicate (0·01 ml per drop) onto Luria agar (L 3147; Sigma) by a modified Miles–Misra technique and the CFU ml$^{-1}$ were calculated by the method of Hoben and Somasegeram (1982) following incubation at 37°C for 18 h. Where fewer than 10 colonies per plate were observed, 250 $\mu$l of the neat sample was transferred to a plate using the spread-plate technique. The colonies were then counted and multiplied by four to convert to CFU ml$^{-1}$. In all cases where *E. coli* samples were replated the following day due to the absence of growth with the selected dilutions, no subsequent growth was observed, suggesting no potential for light/dark repair. The limit of detection of this method is *c.* 4 CFU ml$^{-1}$. All experiments were carried out in triplicate.

**Figure 2** *Escherichia coli* suspensions contained in transparent polystyrene six-well plates (on the left) and polyethylene terephthalate 300-ml bottles (centre and right) immersed in 3 l water baths, and exposed to strong natural sunlight on the laboratory roof in Southern Spain.

### *Cryptosporidium parvum* studies

*Sample collection and preparation*

*Cryptosporidium parvum* was collected from naturally infected calves by rectal sampling and were stored at 4°C in 0·085 mol l$^{-1}$ potassium dichromate solutions for periods no longer than 1 month. Concentration and purification from faeces was performed using diphasic centrifugation with phosphate-buffered saline (PBS; pH 7·2)/ethyl ether and discontinuous Percoll® (Sigma) gradients, according to Lorenzo *et al.* (1993). These oocysts were classified as *C. parvum* using the COWP gene and genotyping scheme described by Amar *et al.* (2004).

*Viability assays*

Viability was examined using two methods: the first method used fluorogenic dye assays based on the inclusion or exclusion of two fluorogenic stains, 4′,6-diamidino-2-phenylindole (DAPI) (Sigma) and propidium iodide (PI; Sigma) (Campbell *et al.* 1992). The grade of permeability shown by the oocysts to the fluorogenic stains shows the viability status of the parasite. The second viability test used was an oocyst excystation technique developed in one of the authors' laboratories (USC, Spain) (Freire-Santos *et al.* 2000). A high percentage of excystation is related with a high viability.

*DAPI–PI fluorogenic dye assays*

Approximately $2 \times 10^6$ oocysts were taken at each timepoint from test or control samples. These were washed twice in Hank's balanced salt solution (HBSS; Sigma) and suspended in 100 $\mu$l of HBSS containing 10 $\mu$l of DAPI

 

working solution (2 mg $ml^{-1}$ in absolute methanol) and 10 $\mu$l of PI working solution (1 mg $ml^{-1}$ in 0·1 mol PBS) for 2 h at 37°C, the length of time for an optimal dye uptake. Each sample was washed three times with HBSS and concentrated (12 500 ***g***, 30 s) to 100 $\mu$l. The oocysts were then examined by fluorescence microscopy (magnification, ×400). The proportion of DAPI positive/PI positive (DAPI+PI+), DAPI positive/PI negative (DAPI+PI−) and DAPI negative/PI negative (DAPI−PI−) oocysts were quantified by enumerating 100 oocysts in each sample. For each experimental regimen, the percentage of viability was given by [(DAPI+PI−) + (DAPI−PI−)].

*Excystation*

Samples of $2 \times 10^6$ oocysts, suspended in 500 $\mu$l of distilled water, were added to 10 ml of 0·154 mol $l^{-1}$ NaCl solution containing 50 mg of pepsin (62 U $mg^{-1}$ solid) (Sigma) and 70 $\mu$l of concentrated HCl. The solution was incubated at 37°C in agitation. The mixture was neutralized with 0·262 mol $l^{-1}$ $NaHCO_3$ (Sigma) and 22 mg of sodium taurocholate (Sigma) together with 4 mg of bovine trypsin (Sigma) and incubated for a further 120 min at 37°C. The samples were then centrifuged at 1000 ***g*** for 5 min. Aliquots of 10 $\mu$l of the excystation suspension were examined with Normarski interference optics (DIC). The proportion of excysted oocysts and unexcysted oocysts were determined in a succession of random fields. At least 200 oocysts were counted in each sample. The percentage excystation was calculated by:

$$(\text{Number of excysted oocysts/total number of oocysts counted}) \times 100.$$

*Animals and husbandry*

Pregnant Swiss CD-1 mice were purchased from the Central Animal Care Facilities of the University of Santiago de Compostela. Each litter and their mother were maintained individually in plastic cages with wire mesh tops and wood shavings for bedding, at 20°C. Litters received a commercial pelleted feed (Letica, Barcelona, Spain) and water *ad libitum*.

*Experimental infection*

Mice between 2·5 and 3 g (aged 4–6 days) were intragastrically inoculated with 100 $\mu$l of the oocysts suspended in distilled water using a fixed volume 100 $\mu$l pipette. All infectivity assays were performed in duplicate for each exposure time. Each treatment group consisted of the two complete litters. Consequently, the average number of mice per treatment cohort was 15·9 (SD = 2·4). Mice were sacrificed by inhalation of ethyl ether 7 days postinfection. The whole intestine was removed and placed in 5 ml of cold PBS. Intestinal oocyst content was evaluated after homogenization (three times, 10 s each) with an Ultra-homogenizer (Turrax, Staufen, Germany). Oocysts were counted in a haemacytometer using 0·16% malachite green as a counter-stain and expressed as whole intestinal tissue (Lorenzo *et al.* 1993). The infectious dose used for each mouse was $2{\cdot}5 \times 10^4$ oocysts.

*Electron microscopy*

*Cryptosporidium parvum* oocysts from test and control exposure experiments were observed under scanning electron microscopy (SEM). The microscope used was a SEM LEICA 440 (Wetzlar, Germany). Samples of the parasite suspensions that were subjected to the following different treatments were filtered through polycarbonate membrane with a pore size of 2 $\mu$m (Whatman, Maidstone, UK):

1. Starting sample at $t = 0$ h.
2. Sample at 40°C at $t = 10$ h with no light.
3. Sample exposed to 870 W $m^{-2}$ at 40°C at $t = 10$ h.

Using 2% glutaraldehyde fixative filters were incubated overnight. Samples were then filtered through and dehydrated in ethanol, critical point dried and sputter-coated with gold (Reduker *et al.* 1985). Measurements were made from a succession of random fields at magnifications ranging from 2000× to 47 000× and represented in $\mu$m.

**Statistical analysis**

Data obtained in the studies *in vitro* were analysed by a test of comparison of proportions and ANOVA (SIGMASTAT for Windows Ver. 1·0, 1994; Systat Software, Inc. (SSI), Richmond, WA, USA). Differences in infection intensities were compared by a pair-wise multiple comparison procedure (Student–Newman–Keuls method) and one-way ANOVA.

***Giardia muris* studies**

*Sample collection and preparation*

The Roberts-Thomson strain (Roberts-Thomson *et al.* 1976) of G. muris was used in this study and was obtained from Dr Frank Schaefer of the US Environmental Protection Agency. The procedure for the collection, preparation and inoculation of the cysts, the infectivity assay and the MPN software used, is described elsewhere (Mofidi *et al.* 2002). Cyst numbers in the stock $5 \times 10^5$ $ml^{-1}$ cyst suspension were quantified by counting microscopically on a haemocytometer. Cyst concentration was adjusted, using deionized water, to $5 \times 10^4$ per ml by adding 1 ml of stock suspension to 9 ml of deionized water in the test well.

*Animals and husbandry*

All of the mice used in this study were 3·5 week old, weaned, CD-1 (Charles River Laboratory, Wilmington,

MA, USA) females. Mice were housed in the Animal Care Facility of Oregon Health and Science University. Before use, the authors verified that the infectivity model accurately predicted MPN values published in *Standard Methods* (American Public Health Association 1998). The infectious dose of *G. muris* for the CD-1 mouse in this model is of the order of one to five cysts.

Previous SODIS studies of *C. parvum* oocysts (Mendez-Hermida *et al.* 2005) and *A. polyphaga* cyst inactivation (Lonnen *et al.* 2005) indicated that it was unlikely that any observable effect would take place within the first 4 h. Consequently, in order to minimize the number of animals used, this was the first time point sampled after the start of the experiment.

Approval was obtained from the ethics committee of the host institutions where the *C. parvum* (USC) and *G. muris* (OHSU) infectivity assays were carried out, prior to the start of the studies.

## Results

### Validation of the solar simulation model

Statistical analysis was performed using one-way analysis of variance (ANOVA). No significant difference ($P > 1{\cdot}0$) in viable bacterial population was observed for *E. coli* samples contained in polystyrene (six-well plates) and PET containers and exposed to strong natural sunlight, for any of the time points up to 120 min (Fig. 3). The average UVA irradiance level recorded during these real solar exposures was 48 W $m^{-2}$, which compares favourably with the value of 45 W $m^{-2}$ provided by the Xenon arc lamp-based solar simulator apparatus.

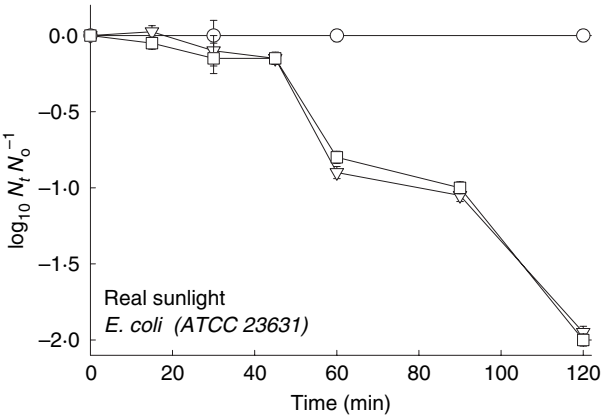


**Figure 3** Comparison of population dynamics for *Escherichia coli* suspensions contained in transparent polystyrene six-well plates and polyethylene terephthalate (PET) 300-ml bottles, immersed in 3 l water baths and exposed to strong natural sunlight on the laboratory roof in Southern Spain. (○) 20°C dark control; (▽) polystyrene six-well plate solar disinfection (SODIS); (□) PET bottle SODIS. Error bars indicate SE limits.

### *Giardia muris* studies

*Infectivity assays*

Infectivity of the samples at the start of the exposure was 100% and no reduction in infectivity occurred over the 12-h duration in the room temperature (20°C) control samples (Table 1). Litters inoculated with cysts that were maintained at a constant 40°C, but not exposed to the simulated solar irradiance experienced a steady reduction in infectivity to almost zero (0·2% infectivity, 95% CI 0·1–1%) over the 12-h duration of the experiment. First-order regression analysis of this data yields a thermally induced reduction in infectivity of *c.* 8% per hour ($R^2 = 0{\cdot}945$). Cysts subjected to full SODIS conditions (40°C and 870 W $m^{-2}$) were rendered completely noninfective (<0·02% infectivity, 95% CI 0·0–7%) after 4 h exposure.

### *Cryptosporidium parvum* studies

*Viability assays*

The results obtained from the assays with the *C. parvum* isolate used for the experiments shows 93% of the oocysts excysted and 98% of oocysts potentially viable by fluorogenic dye test. The viability of the oocysts stored at 40°C in the absence of optical irradiation remained relatively constant over 12 h at 96% (SD = 4·1; SE = 1·8%) (Fig. 4a). There was, however, a progressive increase in permeability of the oocyst walls to DAPI from 4% at 0 h to 60% at 12 h. In addition, we observe a corresponding decrease of *c.* 11% in excystation of these oocysts over this period (Fig. 4b). After 10 h of optical exposure all the oocysts were permeable to the fluorogenic dye PI. Results obtained after 6 and 8 h of exposure, confirm a drastically lower viability (40% and 2% respectively) than those obtained from both the room temperature and 40°C control oocysts samples (*c.* 96% and 98% respectively). On the other hand, 6% of the oocysts were still capable of excystation after 12 h of SODIS exposure.

*Infectivity assays*

The prevalence of *C. parvum* infection in the 0 h control litters, 7 days after inoculation was 100% with an average intensity of 17·6 (SD ± 6·5) × $10^5$ oocysts/homogenized intestine. Litters inoculated with *C. parvum* maintained at a constant temperature of 40°C for varying lengths of time, but not optically irradiated also showed a prevalence of infection of 100% (SD = 2·0; SE = 1·1%). No significant statistical differences ($P > 1{\cdot}0$) were observed between the infection intensities of the 40°C dark control samples obtained at different exposure times and the

 

**Table 1** Summary of the *Giardia muris* infectivity tests

| Experiment | Water temperature (°C) | 870 W m$^{-2}$ light (Y/N) | Time (h) | Number of infected mice per dilution cohort of five mice | | | | | % Infectivity* | 95% Confidence limit |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Undiluted | 1/10 | 1/100 | 1/1000 | 1/10 000 | | |
| Thermal control | 20 | N | 0 | – | – | 5 | 5 | 1 | 100 | 110–1100% |
| | | | 6 | – | – | 5 | 5 | 2 | 100 | 150–1700% |
| | | | 12 | – | 5 | 5 | 4 | 1 | 100 | 58–400% |
| SODIS control | 40 | N | 0 | – | – | 5 | 5 | 1 | 100 | 110–1100% |
| | | | 4 | – | 5 | 5 | 2 | 0 | 49 | 15–150% |
| | | | 8 | – | – | 5 | 0 | 0 | 23 | 6·8–70% |
| | | | 12 | – | – | 1 | 0 | 0 | 0·2 | 0·01–1·0% |
| SODIS | 40 | Y | 0 | – | – | 5 | 5 | 1 | 100 | 110–1100% |
| | | | 4 | 0 | 0 | 0 | 0 | 0 | <0·02 | 0·0–0·7% |
| | | | 8 | 0 | 0 | 0 | 0 | 0 | <0·02 | 0·0–0·7% |
| | | | 12 | 0 | 0 | 0 | 0 | 0 | <0·02 | 0·0–0·7% |

SODIS, solar disinfection.
*Calculated from the most probable number determination of infective cysts per 1000 cysts.

intensity observed at 0 h with control litters. Oocysts exposed to SODIS for 10 h were rendered completely noninfectious (Fig. 5a). Following exposure of 6 and 8 h, infectivity prevalence reduced to 7·5% and 6·5% respectively. In both these cases, the infectivity intensities were 0·625 ($\pm$0·00) $\times$ $10^5$ oocysts/homogenized intestine, significantly lower ($P < 0·05$) than the intensities obtained from the control litters (Fig. 5b).

### Scanning electron microscopy

The SEM micrographs show the deterioration in the *C. parvum* oocyst walls for SODIS exposures of 10 h (Fig. 6). Oocysts that have been immersed in water at 40°C in the absence of simulated sunshine for 10 h show almost no deterioration of cell membrane.

## Discussion

Previous studies have reported that the ability of waterborne *S. typhimurium* to induce infection in a BALB/c mouse model, decreases at a faster rate than the viability of the organism during SODIS (Smith *et al.* 2000). Culturable cells that were subjected to incomplete irradiations of only 1·5-h duration were found to be less infective than their nonirradiated counterparts. Consequently, there is a significant risk that viability assays in the absence of infectivity data will under-estimate the effectiveness of the SODIS process. *In vitro* excystation, live-dead vital dyes (DAPI and PI), and infectivity in CD-1 mice were used in this study as indicators of oocyst viability and infectivity. Viability assays by DAPI/PI vital staining have been reported to demonstrate a very good correlation with the maximized *in vitro* excystation assay

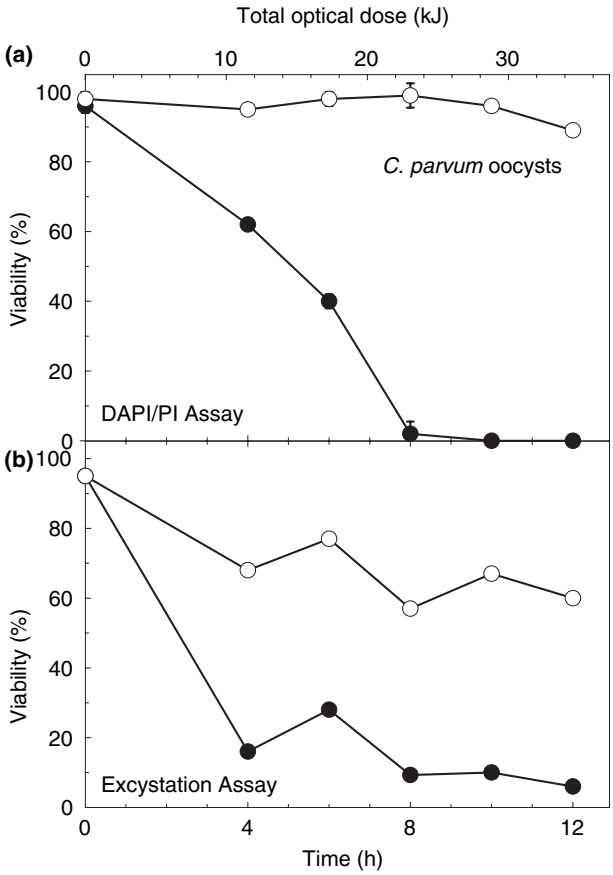


**Figure 4** (a) 4′,6-diamidino-2-phenylindole/propidium iodide assay and (b) excystation assays of solar disinfected oocysts of *Cryptosporidium parvum* exposed to a total irradiance of 830 W m$^{-2}$ and maintained at 40°C as a function of both time and total optical dose. (○) 40°C dark control; (●) 40°C SODIS. Error bars indicate SE limits.


 

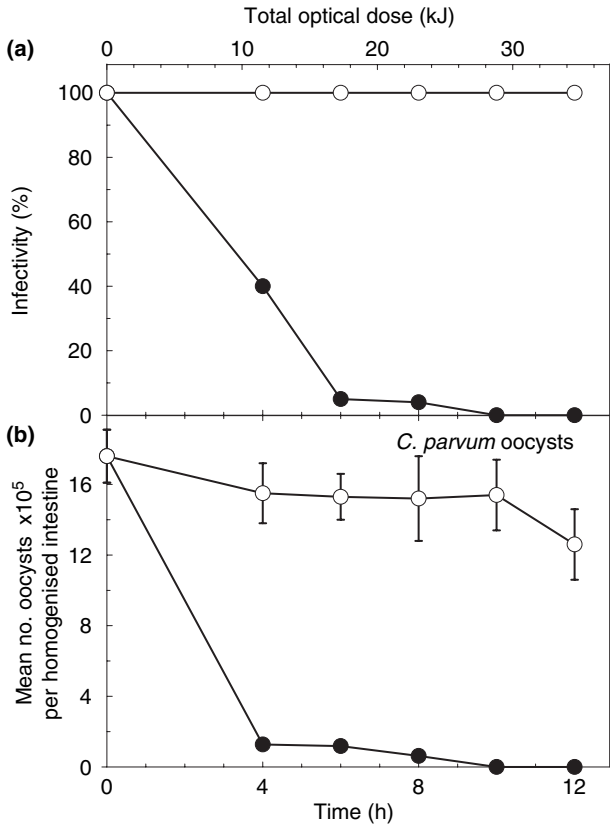


**Figure 5** (a) Infectivity and (b) infection intensity (mean number of oocysts $\times 10^5$ per homogenized intestine) assays of solar disinfected oocysts of *Cryptosporidium parvum* exposed to a total irradiance of 830 W $m^{-2}$ and maintained at 40°C as a function of both time and total optical dose using a newborn Swiss CD- 1 mice infectivity assay. (○) 40°C dark control; (●) 40°C SODIS. Error bars indicate SE limits.

(Robertson *et al.* 1993; Black *et al.* 1996), although evidence exists to suggest that *in vitro* excystation assays may overestimate viability in comparison with animal infectivity assays (Finch *et al.* 1993). Our findings show that the dye permeability assay indicated a higher survival rate than the *in vitro* excystation assay did, as previously observed (Jenkins *et al.* 1997), although both methods underestimated the inactivation of *C. parvum* oocysts in comparison with the animal infectivity methods. These results are in agreement with earlier studies reported for oocysts treated with ozone, UV light or chlorine compounds, which showed neonatal mouse infectivity as the most sensitive indicator of oocyst inactivation (Korich *et al.* 1990; Finch *et al.* 1993; Black *et al.* 1996; Bukhari *et al.* 2000; Quilez *et al.* 2005).

At this time, there are no successful protocols for the *in vitro* excystation of *G. muris* cysts. Consequently, the ability to infect the murine host is universally accepted as the most accurate protocol for determining whether cysts remain infective.

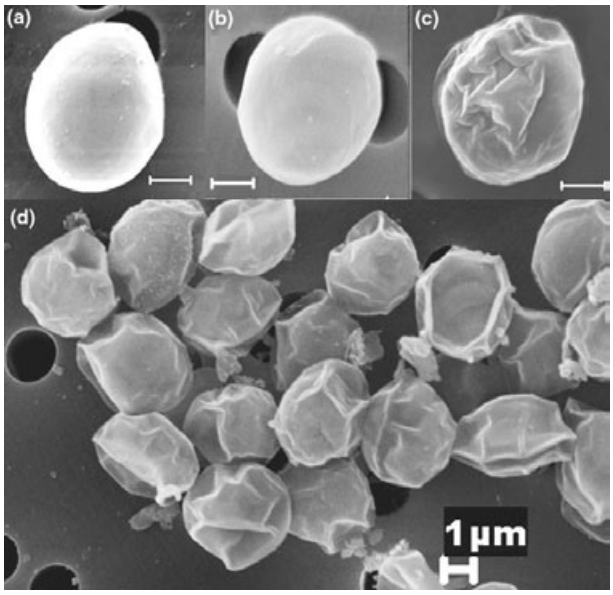


**Figure 6** Scanning electron micrograph of oocysts of *Cryptosporidium parvum*. (a) *Cryptosporidium parvum* at 40°C at time = 0 h; (b) *C. parvum* at 40°C at time = 10 h; (c) *C. parvum* at 40°C + 870 W $m^{-2}$ at time = 10 h and (d) Wide-field view of *C. parvum* at 40°C + 870 W $m^{-2}$ at time = 10 h. Magnification is ×30 000. In each case, the scale bar represents 1 $\mu$m.

We find that *G. muris* cysts are much more amenable to batch process SODIS than oocysts of *C. parvum* or cysts of *A. polyphaga* (Lonnen *et al.* 2005). *Giardia muris* cysts are rendered completely noninfective after 4 h of simulated sunshine while 7·5% of oocysts of *C. parvum* retained infectivity after 6 h of exposure under similar conditions, and *A. polyphaga* cysts remained viable after 12 h exposure at 40°C. It is unfortunate that the number of infective *Giardia* cysts at the 4-h time point on the graph is 0 and no intermediate results were obtained. The infectivity studies were designed so that the number of mice required would be as low as possible. Both sets of previous protozoan studies conducted by the authors (*C. parvum* and *A. polyphaga*) indicated that at least 8 h of SODIS exposure was required before significant inactivation would be observed. It was for this reason that 4 h was chosen to be the first time point. Nevertheless, the strategic objective of the study has been attained as it is clear that *G. muris* cysts are rendered noninfective after at least 4 h of SODIS.

The results of this study indicate that cysts of *G. muris* are highly sensitive to batch-process SODIS. As cysts of *G. lamblia* have been reported to have equal or less resistance to UV disinfection than those of *G. muris* (Mofidi *et al.* 2002; Hayes *et al.* 2003) it is likely that *G. lamblia* will also be amenable to SODIS. It is generally recommended that water stored in SODIS containers be subjected to at least

6 h of full sunshine (Conroy *et al.* 1996, 2001). Previous field studies have shown that 1000-ml batch-process SODIS reactors can achieve maximum temperatures of anywhere between 40°C and 55°C. Higher temperatures are usually only achieved if the reactor is fitted with either rear-foil reflectors or the rear surface is coated in a matt black substance (paint, mud, etc.). The maximum optical output irradiance of 870 W $m^{-2}$ from the solar simulator used during our experiments was *c.* 13% lower than the standard 1000 W $m^{-2}$ irradiance one would expect at equatorial latitudes, but still corresponds to intermediate to strong solar irradiance for temperate latitudes (Joyce *et al.* 1996). Consequently, we decided to maintain the samples at 40°C, nearer the lower end of the water temperature spectrum. Even though the thermal and optical conditions used in our studies were at the low end of the normal range, *G. muris* cysts and *C. parvum* oocysts were rendered completely noninfective within 4 and 10 h, respectively, of SODIS exposure. It is reasonable to expect that they would be rendered noninfectious after shorter periods under the stronger solar irradiance that is available in tropical or equatorial latitudes. Standard SODIS operational guidelines (WHO 2005) should be sufficient to render drinking water contaminated with infective cysts of *G. lamblia* safe and potable.

It is unrealistic to expect 10 h of unbroken sunshine in many parts of the globe. However, if sufficient SODIS bottles are available such that water samples can be exposed for at least two consecutive days, SODIS becomes feasible as an appropriate domestic level water intervention technology outside tropical and equatorial latitudes. If it is not possible to expose the water samples for two consecutive days, our studies show that exposures to only 6 or 8 h of sunlight still produce a decrease of more than 90% in both infectivity and intensity of *C. parvum* infection. Inactivation of the oocysts can be accelerated if steps are taken to increase the maximum water temperature achieved within the SODIS reactor during exposure by darkening the rear-side of the container or placing it in a sheltered location on a dark background (Fayer 1994; Sommer *et al.* 1997).

The sample containers used in this study were made of polystyrene. Batch-process SODIS studies in developing countries usually use PET containers (Joyce *et al.* 1996). However, the optical transmission properties of polystyrene are very similar to those of PET in that they both are opaque to wavelengths below UVB with a sharp cut-off at <320 nm. Above this threshold wavelength, UVA (320–400 nm) is readily transmitted by both the materials (Kehoe 2001a). It is not envisaged that polystyrene containers would be used in the field, but for the purposes of these studies polystyrene can be taken as a suitable substitute for PET.

We observe that after 6 h of SODIS exposure 60% of infective forms of *C. parvum* were permeable to DAPI and from 10 h all oocysts were PI+ and rendered noninfectious in Swiss CD-1 mice. This shift may be a consequence of the change in the oocyst wall permeability due to exposure to the light. Oocysts exposed to the simulator light and counter-stained with malachite green solution, when monitored through DIC microscopy, did take up more dye after longer exposures. Control samples at 0 h and 40°C temperature did not display visible changes in dye uptake as viewed under DIC microscopy. SEM micrographs showed damage on the oocyst wall after light exposure. We hypothesize that the increased permeability of the oocyst walls produced by the elevated water temperature and the deterioration of the oocyst surface observed in our SEM studies after 10 h of SODIS exposure, may facilitate the transport of UV radiation products such as hydrogen peroxide and superoxide ions into the interior of the oocysts where they can have a greater biocidal effect (Blewett 1988).

Data shows a decrease in excystation levels with increasing duration of SODIS exposure. After 10 h of SODIS exposure only 10% of oocysts were capable of excystation, however, infectivity assays showed these oocysts to be noninfectious. This suggests that SODIS exposure may produce oocysts that are capable of excysting, but whose sporozoites are incapable of producing an infection, which is consistent with the observations of others (Hirata *et al.* 2000; Smith *et al.* 2000; Hayes *et al.* 2003).

It should be noted that the reduced infectivity of *C. parvum* oocysts or *G. muris* cysts induced by incomplete solar radiation may not be permanent, and DNA repair processes may start after exposure. However, recent studies show that DNA repair after UV exposure of oocysts may not be sufficient to allow them to recover infectivity (Morita *et al.* 2002). Some authors have described the effective inactivation of *C. parvum* by UV light. Their findings show nucleotide excision repair genes in the *C. parvum* and *Cryptosporidium hominis* DNA that may have an effect in the inability of UV-exposed oocysts to regain infectivity (Rochelle *et al.* 2005). Furthermore, the serious damage as viewed under SEM on the oocyst wall should confirm this hypothesis as all *C. parvum* oocysts after 10 h of SODIS exposure were PI+, suggesting membrane damage to the sporozoites. We conclude that a recovery in the oocyst infectivity status is unlikely after SODIS disinfection.

The current disinfection studies were carried out in distilled water. The efficacy of SODIS using natural waters is likely to be influenced by turbidity. The authors have previously reported enhanced solar inactivation of *E. coli* in heavily turbid waters (200 NTU) (Joyce *et al.* 1996). However, this was caused by accelerated heating of the

water due its dark, cloudy nature. It is unlikely that SODIS would be as successful in turbid water contaminated with oocysts as they are more thermotolerant. Studies carried out by the Swiss Federal Institute of Aquatic Science and Technology (EAWAG 2005) have indicated that if the water temperature is unlikely to exceed 45°C during solar exposure (as might be expected outside the tropics or at altitude) it is important that turbidity does not exceed 30 NTU to facilitate adequate penetration of UVA throughout the sample. In these circumstances, a variety of household water treatment and storage techniques can be used to reduce the turbidity such as storage and sedimentation, flocculation (with alum or the crushed seeds of the *Moringa olifeira* tree) and sedimentation or filtration through a folded cloth (Sobsey 2004).

*Cryptosporidium parvum* infection in immunocompetent individuals can result in diarrhoea, abdominal cramps, fever, nausea and vomiting. The disease is self-limiting. However, immunocompromised patients such as people with HIV/AIDS, the very young and elderly are susceptible to opportunistic infections of cryptosporidiosis, which manifests as a severe, persistent diarrhoeal illness that may be fatal (Ramratnam and Flanigan 1997; Clarke 1999; Xiao *et al.* 2000; Hunter and Nichols 2002; Mele *et al.* 2003). HIV individuals treated with highly active antiretroviral therapy (HAART) have lower rates of morbidity and mortality (LeMoing *et al.* 1998). However, with the increasing AIDS epidemic in developing countries and the lack of access to HAART, both the morbidity and mortality due to this infection is problematic in these areas. It is widely accepted that three main factors are important contributors to the dynamics of cryptosporidiosis in developing countries (Casemore *et al.* 1997):

1. The immune status of the host.
2. The volume of water consumed by the host.
3. The concentration of infective oocysts in the drinking water.

Our studies show conclusively that SODIS provides protection against giardiasis and cryptosporidiosis. Even greater protection is available if SODIS exposure of duration longer than 6 h is possible. This study demonstrates that SODIS is an appropriate household water treatment technology at domestic level for water contaminated with oocysts of *C. parvum* or cysts of *G. lamblia* in both the developed and developing world.

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