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Effect of Batch-Process Solar Disinfection on Survival of *Cryptosporidium parvum* Oocysts in Drinking Water

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The results of batch-process solar disinfection (SODIS) of *Cryptosporidium parvum* oocysts in water are reported. Oocyst suspensions were exposed to simulated sunlight (830 W m^{-2}) at 40°C. Viability assays (4′,6′-diamidino-2-phenylindole [DAPI]/propidium iodide and excystation) and infectivity tests (Swiss CD-1 suckling mice) were performed. SODIS exposures of 6 and 12 h reduced oocyst infectivity from 100% to 7.5% (standard deviation = 2.3) and 0% (standard deviation = 0.0), respectively.

Solar disinfection (SODIS) involves storing contaminated drinking water in transparent containers that are placed in direct sunlight for periods of up to 8 h before consumption (3, 16). This technique is highly effective against a broad range of pathogens (4, 8, 9, 15, 17). Previous studies report reductions in the incidence of diarrhea in children who used SODIS compared with children who did not (3, 4). The biocidal effect of sunlight is due to optical and thermal processes, and a strong synergistic effect occurs at temperatures exceeding 45°C (11, 12). The aim of our research was to test if inactivation of *Cryptosporidium parvum* oocysts could be achieved by batch-process SODIS. Viability reduction after SODIS was tested by fluorogenic dyes and excystation techniques, while oocyst infectivity was determined in neonatal Swiss CD-1 mice. Ten coccidian-free litters of mice weighing from 2.5 to 3.0 g were used for this study. Each exposure time was assayed in duplicate with from 13 to 22 mice.

C. parvum was collected from naturally infected calves by rectal sampling. Storage, concentration, and purification from feces were performed as reported previously (10). Oocysts were classified as of the bovine genotype (1). Suspensions of 10^7 purified oocysts were resuspended in 10 ml of distilled water in transparent polystyrene six-well microtiter containers with lids (IWAKI 3810-006; Tokyo, Japan) and irradiated with 830 W of simulated sunlight m^{-2} at a temperature of 40°C, using the system described previously (9). Viability and infectivity assays were performed after SODIS durations of 0, 6, and 12 h. Unexposed control samples were wrapped in aluminum and kept at room temperature or at 40°C in the water bath beside the test samples, as required. The viability and infectivity assays have been described by Freire et al. (5) and Lorenzo et al. (10). The data obtained in the studies were analyzed by a test of comparison of proportions and analysis of variance (ANOVA) (Sigmastat for Windows, version 1.0, 1994). Differences in infection intensities were compared by pair-wise multiple-comparison procedures (Student-Newman-Keuls method) and one-way ANOVA.

Results are displayed in Table 1.

Viability assays. Assays with the *C. parvum* isolate used for the experiments shows 93% of the oocysts excysted and 98% of oocysts were potentially viable by the fluorogenic dye test. The viability test using fluorogenic dyes showed that the oocysts stored at 40°C in the absence of optical irradiation remained relatively constant over 12 h at 89% (standard deviation [SD] = 1.4). The number of oocysts only positive to the fluorogenic dye DAPI (4′,6′-diamidino-2-phenylindole) increased from 2.5% at 6 h (SD = 1.5) to 55.5% at 12 h (SD = 4.5) (data not shown). We observed a corresponding decrease of approximately 30% in excystation of these oocysts over this period. Viability decreases after 6 h of exposure compared to room temperature and 40°C control oocyst samples. The percentages of viability after 12 h of exposure were 6% for excystation and 0% for the vital dye assay.

Infectivity assays. The prevalence of the infection in the 0-h control litters 7 days after inoculation was 100%, with an average intensity of $(17.6 \pm 6.7) \times 10^5$ oocysts/homogenized intestine. Litters inoculated with nonirradiated *C. parvum* maintained at a constant temperature of 40°C for various lengths of time showed a prevalence of infection of 100%. No significant statistical differences were observed between the infection intensities of the 40°C control samples obtained at different exposure times, and the intensity was observed at 0 h with control litters. Infectivity prevalence after 6 h was reduced to 7.5% ± 2.3% with infectivity intensities of $(0.6 \pm 0.0) \times 10^5$ oocysts/homogenized intestine, significantly lower ($P < 0.05$) than the intensities obtained from the control litters. Oocysts exposed to SODIS for 12 h were rendered completely noninfectious.

The expected solar irradiance for a cloud-free day at equatorial latitudes would be approximately 1,000 W m^{-2} (14). The 830-W-m^{-2} irradiance produced by our solar simulator corresponds to sunlight conditions at higher temperate latitudes or under hazy clouds (7). Consequently, we maintained the samples at 40°C, nearer the lower end of the water temperature spectrum. Nevertheless, complete inactivation of *C. parvum*

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TABLE 1. Comparison of assay results for solar disinfection of *C. parvum*

Time (h)	DAPI/propidium iodide (%)		Excystation (%)		Infectivity (%)		Infection intensity[a]	
	SODIS	Control	SODIS	Control	SODIS	Control	SODIS	Control
0	98.0 ± 1.4	98.0 ± 1.4	93.0 ± 2.8	93.0 ± 2.8	100.0 ± 0.0	100.0 ± 0.0	17.6 ± 6.7	17.6 ± 6.7
6	5.3 ± 1.5	99.0 ± 1.4	27.0 ± 2.0	96.7 ± 2.1	7.5 ± 2.3	100.0 ± 0.0	0.6 ± 0.0	15.6 ± 4.8
12	0.0 ± 0.0	89.0 ± 1.4	6.0 ± 1.0	60.2 ± 4.9	0.0 ± 0.0	100.0 ± 0.0	0.0 ± 0.0	13.9 ± 7.5

[a] Mean number of oocysts (10^5) per homogenized intestine.

oocysts was observed under these conditions. Previous batch-process SODIS studies in developing countries usually used poly(ethylene terephthalate) (PET) containers (11). However, the optical transmission properties of polystyrene used in our study are very similar to those of PET (7). Previous field studies have shown that 1,000-ml batch-process SODIS reactors can achieve maximum temperatures of anywhere between 40 and 55°C (7, 16). The higher temperatures are achieved if the reactor is fitted with reflectors or the rear surface is coated in a matt black substance. We hypothesize that the increased permeability of the oocyst walls produced by the elevated water temperature 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 (2).

Excystation levels decrease with increasing duration of SODIS exposure. After 6 h of SODIS exposure, 27% (SD = 2.0) of oocysts were capable of excystation; however, only 7.5% (SD = 2.3) of neonatal mice were infected. 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 previous observations (6). The SODIS-induced reduction in infectivity of *C. parvum* oocysts 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 (13).

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