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Physical Inactivation of *Toxoplasma gondii* Oocysts in Water[▽]

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Inactivation of *Toxoplasma gondii* oocysts occurred with exposure to pulsed and continuous UV radiation, as evidenced by mouse bioassay. Even at doses of ≥500 mJ/cm^2, some oocysts retained their viability.

Human toxoplasmosis acquired through the consumption of infective *Toxoplasma gondii* oocysts in contaminated drinking water is an increasing public health risk worldwide (1, 5, 17–19). Currently, there are no specific recommendations or approved methods to inactivate *T. gondii* in public water supplies, and limited research indicates that oocysts may be difficult to destroy in water by conventional disinfection methods (5, 8, 15, 19a, 20). Although *T. gondii* oocysts can be inactivated by exposure to boiling, freezing, and gamma irradiation (4, 6, 10, 11), the effect of UV radiation on these oocysts has not been evaluated.

The purpose of this study was to evaluate the efficacy of pulsed and continuous UV radiation (PUV and CUV, respectively) at various doses for inactivation of *T. gondii* oocysts in water intended for public consumption. Posttreatment oocyst viability was assessed by mouse bioassay and by using serology, immunohistochemistry, and in vitro parasite isolation to confirm infection.

T. gondii oocysts of genotype II (16) were obtained as described elsewhere (19a) from feces of two pairs of infected kittens. PUV was administered with an Excimer laser (LPX-200; Lambda Physik, Germany) with 248-nm monochromatic, coherent beams (KrF mode). PUV exposures were calculated from the operating PUV energy fluence (mJ/cm^2/pulse) measured with a 5-cm-diameter (18.1-cm^2 active area) calibrated joule meter (Jmax 43; Molectron Detector Inc., Portland, OR), the pulse repetition rate (pulses/s), and the target area (cm^2). Direct, horizontal laser beams were used to expose all targets contained in quartz tubes (1-cm diameter, 10-cm length). All of the physical and optical devices used in these exposures have been previously reported (14).

In experiment 1, sporulated oocysts were exposed to PUV at 0 (positive control), 100, 200, 500, and 1,000 mJ/cm^2. Oocysts (10^5) were added to 5 ml of double-distilled H_2O (ddH_2O) in a quartz test tube which contained a Teflon-coated magnetic stirring rod and was then sealed with Parafilm. The oocysts were continuously stirred during the entire treatment at all levels of UV exposure. All experiments were conducted in triplicate, with test replicates treated consecutively. Following treatment, replicate samples at each treatment level were pooled, mixed, and then divided into thirds for subcutaneous inoculation into three mice. Positive control mice ($n = 3$) were inoculated with either 10^5 (experiments 1 and 2) or 10^4 (experiments 3 and 4) sporulated, untreated oocysts suspended in 3 to 5 ml of ddH_2O in quartz tubes for 1 h at room temperature prior to inoculation. A similar procedure was repeated for experiment 2, except with PUV doses of 0, 100, 200, 500, and 750 mJ/cm^2. UV exposure in experiments 3 and 4 was determined with a calibrated joule meter (Jmax 43; Molectron Detector Inc., Portland, OR). Oocysts in experiments 3 and 4 were irradiated with UV energy at 0, 45, 106, 196, 408, 498, or 755 mJ/cm^2. To prepare the samples, oocysts (10^4 suspended in 100 μl ddH_2O) were added to 2.9 ml ddH_2O in quartz cuvettes that were thoroughly resuspended by gentle shaking immediately prior to treatment.

A conventional, low-pressure Hg lamp (250-MF; 1.5-cm diameter, 30-cm length; Ultra Dynamics Corporation, Santa Monica, CA) was used in all CUV experiments. The energy fluence (mJ/cm^2) and exposure rates (mJ/cm × s) at a designated perpendicular distance (12.5 cm) from the lamp surface were calibrated prior to the experiments. Different UV radiation exposure levels were achieved by varying the exposure time. At the chosen position and distance, UV source emittance (mJ/cm^{2}s) was calibrated with ferrous sulfate solution (2), whose response to UV energy was standardized and measured with a 5-cm calibrated joule meter. Experiments 1 to 4 were performed in the same manner as for PUV experiments 1 to 4, except for small differences in UV exposure doses within the selected ranges. Exposure doses of 0, 100, 200, 500, and 1,000 mJ/cm^2 were tested in experiment 1, while a narrower range of 0, 20, 40, 80 and 100 mJ/cm^2 was evaluated in experiment 2. In experiments 3 and 4, CUV exposure doses (0, 160, 186, 228, 335, 412, and 731 mJ/cm^2) were determined as previously described for PUV experiments 3 and 4 with a single set

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TABLE 1. Results of mouse bioassays at 44 to 48 days postinoculation to assess the viability of *T. gondii* oocysts after exposure to PUV

Oocyst dose	No. of mice	PUV exposure dose (mJ/cm^2)	IFAT[c]	Immunohistochemistry		Parasite isolation
				Reader 1	Reader 2	
Preliminary tests						
Expt 1						
10^5	3	0	3	3	3	2[a]
10^5	3	100	3	2	2	2
10^5	3	200	2	1	1	2
10^5	3	500	0	1	1	0
10^5	3	1,000	0	0	0	0
Expt 2						
10^5	3	0[b]	2	3	3	2
10^5	3	100	1	0	0	0
10^5	3	200	0	0	0	0
10^5	3	500	1	1	1	1
10^5	3	750	0	0	0	0
Confirmation tests						
Expt 3						
10^4	3	0	3	3	3	3
10^4	3	45	3	1	1	3
10^4	3	106	0	0	0	0
10^4	3	196	0	0	0	0
10^4	3	408	0	0	0	0
10^4	3	498	0	0	0	0
10^4	3	755	1	0	0	0
Expt 4						
10^4	3	45	1	0	0	1
10^4	3	106	0	0	0	0
10^4	3	196	0	0	0	0
10^4	3	408	0	0	0	0
10^4	3	498	0	0	0	0
10^4	3	755	0	0	0	0

[a] One culture became contaminated with bacteria and was discarded.
[b] One mouse was found dead (PID 12) and cannibalized; only the spleen was recoverable for immunohistochemical testing.
[c] IFAT with a positive cutoff value of 1:40.

of positive controls because both experiments were conducted on the same day.

For all inactivation experiments, UV-treated oocysts were inoculated subcutaneously into seronegative 20- to 22-g female SW mice (Charles River Laboratories, Wilmington, MA). To determine if mice inoculated with treated oocysts became infected with *T. gondii*, each mouse was evaluated 43 to 48 days postinoculation by serology, immunohistochemistry, and in vitro cultivation of brain tissue for parasite isolation as described elsewhere (19a).

Results of PUV experiments are shown in Table 1. In experiment 1, infections were not detected in any of the mice inoculated with oocysts exposed to 1,000 mJ/cm^2 but at 500 mJ/cm^2, a single mouse became infected. In experiment 2, a PUV exposure dose of 750 mJ/cm^2 appeared to inactivate all oocysts; however, *T. gondii* infection was detected in one of three mice inoculated with oocysts treated with UV radiation at 500 mJ/cm^2. In experiments 3 and 4, all oocysts appeared to be inactivated by PUV doses of $\geq$106 mJ/cm^2, with the exception of a single mouse inoculated with oocysts treated with UV radiation at 755 mJ/cm^2 (experiment 3). This mouse was seropositive for *T. gondii* (1:80) but was negative by immunohistochemistry and parasite isolation.

Results of the CUV experiments are shown in Table 2. In experiment 1, all of the CUV exposure doses tested from 100 to 1,000 mJ/cm^2 effectively inactivated oocysts. In experiment 2, oocysts appeared to be inactivated by CUV doses of 40, 80, and 100 mJ/cm^2 but not by 20 mJ/cm^2. In experiment 3, infections were not detected in any mice inoculated with oocysts treated with CUV doses of 160 to 731 mJ/cm^2, with the exception of one mouse inoculated with oocysts exposed to CUV radiation at 731 mJ/cm^2. This mouse was positive for *T. gondii* by serology and in vitro parasite isolation from brain tissue. Similar results were evident in experiment 4; all of the mice inoculated with oocysts treated with CUV doses of 160 to 731 mJ/cm^2 were negative for *T. gondii* infection, with the exception of one mouse inoculated with oocysts exposed to the 160-mJ/cm^2 CUV dose and one mouse exposed to the 335-mJ/cm^2 dose. Both mice were positive by all three detection methods. *T. gondii* infections were detected in all positive control mice. One control mouse (experiment 1) died at postinfection day (PID) 12 before an antibody response was detectable with the indirect fluorescent-antibody test (IFAT).

T. gondii is gaining recognition as a waterborne pathogen (1, 5, 17, 18), but how to effectively eliminate this parasite from drinking water remains undetermined. The physical methods (PUV and CUV) tested in this study were more effective at oocyst inactivation than chemical treatments previously tested (19a).

TABLE 2. Results of mouse bioassays at 45 to 48 days postinoculation to assess viability of *T. gondii* oocysts after exposure to CUV

Oocyst dose	No. of mice	CUV exposure dose (mJ/cm^2)	IFAT[c]	Immunohistochemistry		Parasite isolation
				Reader 1	Reader 2	
Preliminary tests						
Expt 1						
10^5	3	0	2[a]	3	3	2[b]
10^5	3	100	0	0	0	0
10^5	3	200	0	0	0	0
10^5	3	500	0	0	0	0[b]
10^5	3	1,000	0	0	0	0[b]
Expt 2						
10^5	3	0	3	3	3	3
10^5	3	20	2	2	2	2
10^5	3	40	0	0	0	0
10^5	3	80	0	0	0	0
10^5	3	100	0	0	0	0
Confirmation tests						
Expt 3						
10^4	3	0	3	3	3	3
10^4	3	160	0	0	0	0
10^4	3	186	0	0	0	0
10^4	3	228	0	0	0	0
10^4	3	335	0	0	0	0
10^4	3	412	0	0	0	0
10^4	3	731	1	0	0	1
Expt 4						
10^4	3	0	3	3	3	3
10^4	3	160	1	0	1	1
10^4	3	186	0	0	0	0
10^4	3	228	0	0	0	0
10^4	3	335	1	0	1	1
10^4	3	412	0	0	0	0
10^4	3	731	0	0	0	0

[a] One mouse died at PID 12; antibody immune response was not detectable.
[b] One culture became contaminated with bacteria and was discarded.
[c] IFAT with a positive cutoff value of 1:40.

Oocyst inactivation occurred at PUV doses as low as 40 mJ/cm^2 and CUV doses as low as 45 mJ/cm^2. However, oocyst inactivation was not 100% reliable at higher doses of UV administered both by pulsed and continuous sources. Unexpectedly, infections were detected in mice inoculated with oocysts treated with PUV doses as great as 755 mJ/cm^2 and CUV doses of 731 mJ/cm^2. The infection detected in the mouse inoculated with oocysts treated with a PUV dose of 755 mJ/cm^2 was supported via seroconversion to a 1:80 titer for *T. gondii* in the IFAT. However, tissues from this mouse were negative for infection by immunohistochemistry and parasite isolation. In all other cases, infection was confirmed by identification of *T. gondii* tachyzoites in mouse tissues at 45 days postinfection by immunohistochemistry and/or parasite isolation. Mouse bioassay is commonly used for detecting *T. gondii* oocyst viability (12, 13, 19a). By using serology, immunohistochemistry, and in vitro isolation to detect infection, the sensitivity was increased so that inoculation with a single oocyst was potentially detectable (19a).

In this study, similar ranges of UV radiation were tested in both PUV and CUV experiments 3 and 4 to facilitate comparisons between the two UV delivery systems and to confirm the reproducibility of results between experiments. Lower levels of CUV radiation consistently inactivated oocysts compared with PUV. These results were unexpected, and the reasons for this difference are unknown. We had hypothesized that greater inactivation would occur with a lower dose of pulse-derived UV energy because PUV delivers the same amount of UV energy as CUV but in shorter periods of time (seconds versus minutes). Thus, we expected that this greater concentration of UV energy delivered in a shorter time period should result in more irreversible DNA damage.

The goal of our study was to evaluate the efficacy of UV treatments for complete inactivation of oocysts under defined conditions with the hope of formulating recommendations for the treatment of drinking water. Partial oocyst inactivation appears to have occurred with UV treatment since some oocysts survived the treatment and remained infectious. The infective dose of *T. gondii* oocysts for humans is not known, but animal infectivity studies indicate that an infectious dose could be as low as a single oocyst (3, 7, 9, 19a).

Overall, though, our results suggest that a minimum UV exposure dose of 1,000 mJ/cm^2 may be required for either PUV or CUV treatment of water to increase the probability of consistent and complete oocyst inactivation.

Further investigations of UV treatment of *T. gondii* oocysts

are warranted to evaluate the effectiveness of UV radiation in water with different levels of turbidity. Combining chemical disinfection treatments with UV treatments might also provide additional benefits of residual disinfection.

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