Applied and Environmental Microbiology, Nov. 2002, p. 5387–5393 Vol. 68, No. 11
0099-2240/02/$04.00+0 DOI: 10.1128/AEM.68.11.5387–5393.2002
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Efficacy of UV Irradiation in Inactivating *Cryptosporidium parvum* Oocysts

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Received 16 April 2002/Accepted 14 August 2002

To evaluate the effectiveness of UV irradiation in inactivating *Cryptosporidium parvum* oocysts, the animal infectivities and excystation abilities of oocysts that had been exposed to various UV doses were determined. Infectivity decreased exponentially as the UV dose increased, and the required dose for a 2-log_{10} reduction in infectivity (99% inactivation) was approximately 1.0 mWs/cm^2 at 20°C. However, *C. parvum* oocysts exhibited high resistance to UV irradiation, requiring an extremely high dose of 230 mWs/cm^2 for a 2-log_{10} reduction in excystation, which was used to assess viability. Moreover, the excystation ability exhibited only slight decreases at UV doses below 100 mWs/cm^2. Thus, UV treatment resulted in oocysts that were able to excyst but not infect. The effects of temperature and UV intensity on the UV dose requirement were also studied. The results showed that for every 10°C reduction in water temperature, the increase in the UV irradiation dose required for a 2-log_{10} reduction in infectivity was only 7%, and for every 10-fold increase in intensity, the dose increase was only 8%. In addition, the potential of oocysts to recover infectivity and to repair UV-induced injury (pyrimidine dimers) in DNA by photoreactivation and dark repair was investigated. There was no recovery in infectivity following treatment by fluorescent-light irradiation or storage in darkness. In contrast, UV-induced pyrimidine dimers in the DNA were apparently repaired by both photoreactivation and dark repair, as determined by endonuclease-sensitive site assay. However, the recovery rate was different in each process. Given these results, the effects of UV irradiation on *C. parvum* oocysts as determined by animal infectivity can conclusively be considered irreversible.

Widespread low-level contamination of surface waters with *Cryptosporidium parvum* oocysts has been reported in many countries (10, 25). Cryptosporidiosis outbreaks arising from water supplies have been documented (1, 18), and it is believed that *C. parvum* oocysts are widespread in rivers. The concentration of *C. parvum* oocysts in source river waters can be as high as 10^4 oocysts per 100 liters (20, 29). Although coagulation-sedimentation and rapid sand filtration can remove *C. parvum* oocysts and reduce their numbers by 2 to 3 log units (11, 19), the expected rate of removal may not sufficiently reduce the risk of infection to an acceptable level in cases where the source water is highly contaminated. Chlorine has been used as a disinfectant in many water supplies; however, *C. parvum* oocysts are insensitive to the concentrations routinely used (7, 12, 17). Thus, there is interest in developing an alternative, more effective disinfectant for inactivating these recalcitrant microorganisms.

UV disinfection systems produce no hazardous by-products and are easy to maintain. The UV system is considered one of the more effective disinfection techniques for bacteria and viruses in drinking water and wastewater (26). Although there have been many studies on UV inactivation of microorganisms such as *Escherichia coli* (14), relatively few studies on UV inactivation of *C. parvum* have been conducted. This is because, based on assessment by in vitro excystation and vital-dye methods, UV irradiation was considered ineffective in inactivating *C. parvum* (2, 23, 27). However, evidence based on assessment by cell culture techniques suggests that the present practice of UV irradiation at low doses may effectively inactivate *C. parvum* oocysts (4, 21).

A problem with UV disinfection is that some microorganisms have the ability to repair DNA lesions by mechanisms such as photoreactivation and dark repair (8, 9, 22, 33). Photoreactivation is a phenomenon in which UV-inactivated microorganisms recover activity through the repair of lesions in the DNA by enzymes under near-UV light. Photoreactivation may occur in microorganisms in UV-exposed wastewater after its discharge to watersheds, because UV-inactivated microorganisms would normally be exposed to sunlight, including near-UV light. In dark repair, UV-inactivated microorganisms repair the damaged DNA in the absence of light. Dark repair may occur in UV-exposed drinking water after it is distributed by water supply systems. Therefore, in order to achieve an appropriate UV disinfection level, it is necessary to quantitatively evaluate the effects of photoreactivation and dark repair. However, the ability of *C. parvum* to perform photoreactivation and dark repair following UV irradiation has not yet been clarified, despite the importance of these mechanisms in the inactivation of *C. parvum* by UV irradiation technology.

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Inactivation of microorganisms by UV irradiation occurs through the formation of lesions in DNA, and the dominant UV-induced lesion is cyclobutane pyrimidine dimers (8). The presence of UV-induced lesions would prevent normal DNA replication, leading to inactivation. In previous studies, photoreactivation and dark repair in *C. parvum* were investigated by the endonuclease-sensitive site (ESS) assay, which can determine the number of UV-induced cyclobutane pyrimidine dimers in the genomic DNA (24, 31). The results indicated that *C. parvum* has the ability to carry out photoreactivation and dark repair at the genomic level.

In this study, we sought to elucidate the efficacy of UV irradiation at various temperatures and intensities as well as to assess the effects of visible-light irradiation or dark storage after UV irradiation by use of animal infectivity, in vitro excystation, and the ESS assay.

MATERIALS AND METHODS

C. parvum oocysts. The *C. parvum* strain HNJ-1 (a human isolate recovered by M. Iseki, Kanazawa University, Kanazawa, Japan), which was passaged in SCID mice (C.B-17/Icr; CLEA Japan Inc., Tokyo, Japan) at the Research Institute of Biosciences, Azabu University, was used for this study. Fresh feces from several infected mice were placed in 500 ml of purified water, emulsified, and filtered through a 0.1-mm-mesh nylon sieve. A crude oocyst suspension (20 ml) was underlaid with a sucrose solution (specific gravity, 1.10 at 20°C) and centrifuged at 1,500 × *g* at 4°C for 15 min. The interface and upper layer were recovered and diluted with 150 mM phosphate-buffered saline (PBS, pH 7.4, comprising 0.20 g of potassium dihydrogen phosphate, 0.20 g of potassium chloride, 1.15 g of disodium hydrogen phosphate, and 8.0 g of sodium chloride in 1 liter of distilled water) containing 0.1% (vol/vol) Tween 80. The diluted solution was centrifuged at 1,500 × *g* and 4°C for 15 min. The precipitate was diluted to 20 ml with PBS containing 0.1% (vol/vol) Tween 80. The suspension was underlaid with a sucrose solution and centrifuged at 1,500 × *g* and 4°C for 15 min. The interface was recovered. Washing with PBS containing 0.1% (vol/vol) Tween 80 was repeated four times to remove fecal matter. The stock of purified oocysts was stored in PBS at 4°C and used in experiments within 10 days.

UV irradiation. The UV irradiation conditions for each experiment are shown in Table 1. A small portion of a purified suspension of *C. parvum* oocysts was placed in an open plastic petri dish (inner diameter, 56 mm) containing 10 ml of 150 mM PBS to produce a final concentration of 10^6 oocysts per ml. Since the extinction coefficient was 0.050 cm^{-1} at 254 nm and the loss of UV intensity in a 4.0-mm thickness of the oocyst suspension was only 2%, the UV intensity at the surface of the oocyst suspension was regarded as the UV intensity in the entire suspension layer.

The dish was placed under a 5-W low-pressure mercury lamp (QCGL5W-14 97D; Iwasaki Electronic, Co., Ltd., Tokyo, Japan). The intensity of the UV light at a wavelength of 253.7 nm ranged from 0.048 to 0.60 mW/cm^2, as measured by a UV dose rate meter (UTI-150-A; Ushio Inc., Tokyo, Japan). The dose of UV irradiation was altered by controlling the exposure time. The oocyst suspension was gently mixed by using an electromagnetic stirrer during UV irradiation.

Fluorescent-light irradiation and storage in the dark. Table 2 summarizes the experimental conditions of UV irradiation and the subsequent recovery treatments. Immediately after UV irradiation, one group was exposed to fluorescent light by use of a 15-W fluorescent lamp (FL15N white-light fluorescent lamp; Toshiba, Tokyo, Japan). The intensity of the fluorescent light at a wavelength of 360 nm was measured by a UV-A region dosimeter (UVR-2 with UD-36; TOPCON, Tokyo, Japan). Throughout the duration of fluorescent-light irradiation, the oocyst suspension was gently mixed by using an electromagnetic stirrer. Another group was covered with aluminum foil and incubated at 20°C for 4 to 24 h.

Infectivity. Infectivity was determined via animal infectivity tests with SCID mice in a specific-pathogen-free area at the Research Institute of Biosciences, Azabu University. This study was approved by the Animal Research Committee of Azabu University. Five-week-old SCID mice were used for the tests after 1 week of conditioning for adaptation to the new cage. Mice were individually housed in cages and provided autoclave-sterilized food and water. Three to eight mice were used per dilution. Fivefold serial dilutions were made using sterilized tap water. A 0.5-ml aliquot of a selected dilution series was administered orally to the mouse. The mortality of the mice at the early stage of the experiments was 2 out of 789 mice; no mouse died beyond 1 week after oral administration. Four weeks after oral administration, fresh feces were collected, suspended in 50 ml of purified water, and emulsified using a vortex mixer. A 5-ml portion of the suspension was overlaid on 8 ml of a sucrose solution and centrifuged at 1,500 × *g* and 4°C for 15 min. The interface was recovered, and approximately 1 ml of the supernatant was filtered through a 25-mm cellulose acetate membrane disk filter (pore size, 0.8 μm). The filter was then stained using immunofluorescent antibodies against oocyst wall protein (Hydrofluoro Combo Kit; Strategic Diagnostics Inc., Newark, Del.) and observed under an epifluorescent, differential-contrast microscope (BX-60; Olympus, Tokyo, Japan) at a magnification of ×400 to determine the presence or absence of oocysts. The most probable number (MPN) of infection was calculated from oocyst-positive mice by using the MPN program developed by Hurley and Roscoe (15). The relative infectivity of each sample was calculated as MPN_a/MPN_0, where MPN_a is the MPN of oocysts after UV irradiation and MPN_0 is the MPN of oocysts before UV irradiation.

In the animal infectivity test, the dark repair process was monitored closely, as it occurred in the interval between the time immediately after UV irradiation of oocysts and the time at which oocysts reached the infection site. Thus, the results include the effect of the dark repair process.

In vitro excystation. The viability of the oocysts was determined by a modification of Woodmansee's method (35). The original excystation protocol was modified slightly by addition of a 5-min preincubation at 37°C in acidified Hank's balanced salt solution (pH 2.75) prior to incubation for excystation. After the preincubation period, the oocysts were incubated in the excystation medium at 37°C for 60 min.

The numbers of intact oocysts (IO) before excystation treatment, partially excysted oocysts (PO), empty oocysts (EO), and free sporozoites (S) were enumerated under a phase-contrast microscope at a magnification of ×400. Excys-

TABLE 1. Summary of experimental conditions

Trial no.	UV intensity (mW/cm²)	Irradiation time (s)	UV dose (mWs/cm²)	Temp (°C)	Evaluation method[a]
1	0.24	5	1.20	5	AI
2	0.24	5	1.20	10	AI
3	0.24	5	1.20	30	AI
4	0.24	7	1.68	5	AI
5	0.24	7	1.68	10	AI
6	0.24	7	1.68	30	AI
7	0.24	10	2.40	5	AI
8	0.24	10	2.40	10	AI
9	0.24	10	2.40	30	AI
10	0.048	25	1.20	20	AI
11	0.12	10	1.20	20	AI
12	0.60	2	1.20	20	AI
13	0.048	38	1.82	20	AI
14	0.12	15	1.80	20	AI
15	0.60	3	1.80	20	AI
16	0.048	50	2.40	20	AI
17	0.12	20	2.40	20	AI
18	0.60	4	2.40	20	AI
38	0.24	167	40.0	20	Ex
39	0.24	167	40.0	20	Ex
40	0.24	333	80.0	20	Ex
41	0.24	333	80.0	20	Ex
42	0.24	500	120	20	Ex
43	0.24	500	120	20	Ex
44	0.24	667	160	20	Ex
45	0.24	667	160	20	Ex
46	0.24	1,000	240	20	Ex
47	0.24	1,000	240	20	Ex
C-1[b]		Not irradiated			AI
C-2		Not irradiated			AI
C-3		Not irradiated			AI
C-4		Not irradiated			AI

[a] AI, animal infectivity; Ex, in vitro excystation.
[b] C, Control samples for MPN_0 in animal infectivity.

TABLE 2. Experimental conditions of UV irradiation and subsequent recovery treatments

Trial no.	UV intensity (mW/cm^2)	Irradiation time (s)	UV dose (mWs/cm^2)	Temp (°C)	Treatment after UV irradiation		Evaluation[b]
					FL[a] dose (mWs/cm^2)	Storage duration (h)	
19	0.10	5	0.50	20	Not treated		AI, ESS
20	0.10	5	0.50	20	150		AI, ESS
21	0.10	5	0.50	20	300		AI, ESS
22	0.10	5	0.50	20		12	AI, ESS
23	0.10	5	0.50	20		24	AI, ESS
24	0.10	10	1.00	20	Not treated		AI, ESS
25	0.10	10	1.00	20	150		AI, ESS
26	0.10	10	1.00	20	300		AI, ESS
27	0.10	10	1.00	20		12	AI, ESS
28	0.10	10	1.00	20		24	AI, ESS
29	0.10	15	1.50	20	Not treated		AI, ESS
30	0.10	15	1.50	20	Not treated		AI, ESS
31	0.10	15	1.50	20	180		AI, ESS
32	0.10	15	1.50	20	360		AI, ESS
33	0.10	15	1.50	20	540		AI, ESS
34	0.10	15	1.50	20	720		AI, ESS
35	0.10	15	1.50	20		4	AI, ESS
36	0.10	15	1.50	20		12	AI, ESS
37	0.10	15	1.50	20		24	AI, ESS

[a] FL, fluorescent light.
[b] AI, animal infectivity.

tation rate (V) was calculated as $\frac{S/4}{IO + PO + EO}$. The survival ratio (S_r) was calculated as V_a/V_0, where V_a is the excystation rate of the UV-irradiated sample and V_0 is the excystation rate of the control sample (before UV irradiation).

ESS assay. A *C. parvum* suspension was frozen in liquid nitrogen for 3 min and then thawed at 95°C for 5 min to break the oocyst walls (P. A. Rochelle, D. M. Ferguson, T. J. Handojo, R. De Leon, M. H. Stewart, and R. L. Wolfe, abstract from the Fourth International Workshops on Opportunitistic Protists, J. Eukaryot. Microbiol. 43:72S, 1996). The DNA was then extracted by the Genomic-tip DNA extraction kit (Qiagen Inc., Valencia, Calif.). UV endonuclease was prepared from *Micrococcus luteus* by using the method for the preparation of fraction II originally described by Carrier and Setlow (3). The DNA solution was incubated with the UV endonuclease at 37°C for 45 min in 30 mM Tris (pH 8.0)–40 mM NaCl–1 mM EDTA in order to incise the nicks at the site of the pyrimidine dimer. The reaction was stopped by addition of concentrated alkaline loading dye to a final concentration of 100 mM NaOH, 1 mM EDTA, 2.5% Ficoll, and 0.05% bromocresol green. The sample was electrophoresed at 0.4 V/cm for 15 h on 0.35% alkaline agarose gels in a solution containing 30 mM NaOH and 1 mM EDTA. 7GT (bacteriophage T4dC+T4dC/*Bgl*I digest mixture; Wako, Tokyo, Japan) and 8GT (bacteriophage T4dC+T4dC/*Bgl*II digest mixture; Wako) were used as molecular-length markers. The gel was neutralized and stained in an ethidium bromide solution overnight to stain the DNA. The stained gel was photographed (Gel Doc 1000; Bio-Rad, Richmond, Calif.) and analyzed (Molecular Analyst software; Bio-Rad). The midpoint of the DNA mass, that is, the median migration distance of each sample, was graphically determined to be the representative migration distance of the sample. The median migration distance was converted to the median molecular length (L_{med}) of the DNA by means of the quadratic regression curve obtained from analysis of the molecular standards. The average molecular length (L_n) of the DNA was calculated as 0.6 $\times L_{med}$ (calculation from Veatch and Okada [32]). The number of ESSs per base was calculated as $[1/L_{n(+UV)}] - [1/L_{n(-UV)}]$, where $L_{n(+UV)}$ and $L_{n(-UV)}$ are the L_ns of the UV-irradiated and nonirradiated samples, respectively (calculation from Freeman et al. [6]).

RESULTS AND DISCUSSION

Efficacy of UV irradiation. The number of orally administered oocysts, infection rate among mice, MPN, 95% confidence interval, and relative infectivity are shown in Table 3. The reduction in infectivity of *C. parvum* oocysts with UV irradiation at 20°C is shown in Fig. 1. Infectivity decreased exponentially as the UV dose increased. The UV doses required for 1-, 2-, and 4-$\log_{10}$ reductions in infectivity were 0.48, 0.97 and 1.92 mWs/cm^2, respectively. The doses resulting in reductions in infectivity in this study were approximately half of the previously reported doses based on assessment by cell culture (4, 21, 30). It has been reported that sensitivity to disinfectants varies among different strains of *C. parvum* (28). The reason that the effective dose of irradiation was about half of the reported values might be related to differences in the experimental conditions, including the use of different strains of *C. parvum* and/or differences between in vitro cell culture methods and in vivo animal infectivity experiments. Our dose of UV irradiation is much lower than the minimum dose of 16 mWs/cm^2 recommended for a 2-$\log_{10}$ reduction of bacteria by the U.S. Public Health Service. Therefore, if the minimum dose is maintained at 16 mWs/cm^2, it can be expected to result in more than 10-$\log_{10}$ inactivation of *C. parvum* oocysts. These results suggest that UV irradiation is a highly effective disinfectant technique for inactivating *C. parvum* oocysts from the perspective of reducing infectivity.

The reduction in the viability of *C. parvum* oocysts by UV treatment as assessed by excystation is shown in Fig. 2. The 6-hit series event model (34) was used as the mathematical model because viability decreased only slightly at the low dose range (<100 mWs/cm^2). The estimated UV dose for a 2-$\log_{10}$ reduction in viability at 20°C was 230 mWs/cm^2, which is approximately 200 times higher than the dose required for an equivalent reduction in infectivity. Hirata et al. reported that the *CT* (product of concentration of disinfectant and contact time) values of chemical disinfectants required for equivalent reductions in the infectivity and the viability of *C. parvum* differed by a factor of 18 for chlorine (12) and by a factor of 3 for ozone (13). The present study showed a marked difference between the effect of UV irradiation on the infectivity of *C.*

TABLE 3. Summary of *C. parvum* infectivity in SCID mice for each trial

Trial no.[a]	Inoculum[b] (no. of positive mice/total no. of mice)	MPN	95% confidence interval	Relative infectivity
C-1	9 (1/5), 49 (3/4), 203 (4/5), 1,010 (5/5)	1.33×10^{-2}	5.56×10^{-3} to 3.19×10^{-2}	1.00×10^{0}
1	149 (1/5), 745 (3/5), 3,725 (5/5), 18,625 (5/5)	1.63×10^{-4}	5.45×10^{-5} to 4.90×10^{-4}	1.23×10^{-2}
2	126 (0/3), 630 (0/3), 3,150 (0/3), 15,750 (2/3), 78,750 (3/3)	5.33×10^{-5}	1.72×10^{-5} to 1.65×10^{-4}	4.01×10^{-3}
3	122 (0/3), 608 (0/3), 3,038 (0/3), 15,188 (1/3), 75,938 (3/3)	3.37×10^{-5}	1.13×10^{-5} to 1.01×10^{-4}	2.53×10^{-3}
8	127 (0/3), 634 (0/3), 3,169 (0/3), 15,844 (0/3), 79,219 (1/3)	3.91×10^{-7}	5.62×10^{-8} to 2.72×10^{-6}	2.94×10^{-5}
10	120 (0/3), 600 (0/3), 3,000 (0/3), 15,000 (2/3), 75,000 (3/3)	5.60×10^{-5}	1.81×10^{-5} to 1.74×10^{-4}	4.21×10^{-3}
11	120 (0/3), 600 (1/3), 3,000 (2/3), 15,000 (2/3), 75,000 (3/3)	1.54×10^{-4}	5.16×10^{-5} to 4.63×10^{-4}	1.16×10^{-2}
12	144 (0/3), 720 (1/3), 3,600 (3/3), 18,000 (3/3), 90,000 (3/3)	7.15×10^{-4}	2.38×10^{-4} to 2.15×10^{-3}	5.38×10^{-2}
17	126 (0/3), 631 (0/3), 3,156 (0/3), 15,781 (0/3), 78,906 (1/3)	3.93×10^{-7}	5.65×10^{-8} to 2.74×10^{-6}	2.95×10^{-5}
18	149 (0/3), 746 (0/3), 3,731 (0/3), 18,656 (3/3), 93,281 (3/3)	8.66×10^{-6}	2.82×10^{-6} to 2.66×10^{-5}	6.51×10^{-4}
C-2	5 (0/8), 24 (1/8), 121 (4/5), 604 (5/5)	9.49×10^{-3}	4.14×10^{-3} to 2.18×10^{-2}	1.00×10^{0}
4	5,625 (0/5), 28,125 (2/5), 140,625 (4/5), 703,125 (5/5)	1.24×10^{-5}	5.22×10^{-6} to 2.97×10^{-5}	1.31×10^{-3}
5	6,850 (0/5), 34,250 (3/5), 171,250 (3/5), 856,250 (5/5)	8.74×10^{-6}	3.62×10^{-6} to 2.11×10^{-5}	9.21×10^{-4}
6	6,088 (0/5), 30,438 (0/5), 152,188 (3/5), 760,940 (5/5)	6.22×10^{-6}	2.56×10^{-6} to 1.51×10^{-5}	6.55×10^{-4}
13	6,700 (2/5), 33,500 (2/5), 167,500 (3/5), 837,500 (5/5)	1.04×10^{-5}	4.34×10^{-6} to 2.48×10^{-5}	1.10×10^{-3}
14	6,700 (1/5), 33,500 (4/5), 167,500 (5/5), 837,500 (5/5)	4.38×10^{-5}	1.77×10^{-5} to 1.09×10^{-4}	4.62×10^{-3}
15	6,750 (0/5), 33,750 (0/5), 168,750 (1/5), 843,750 (5/5)	2.54×10^{-6}	1.08×10^{-6} to 5.96×10^{-6}	2.68×10^{-4}
C-3	9 (1/5), 39 (3/5), 203 (4/5), 1,010 (5/5)	1.29×10^{-2}	5.44×10^{-3} to 3.04×10^{-2}	1.00×10^{0}
19	149 (1/5), 745 (3/5), 3,725 (5/5), 18,625 (5/5)	1.35×10^{-3}	5.44×10^{-4} to 3.34×10^{-3}	1.05×10^{-1}
20	134 (1/5), 670 (3/5), 3,350 (5/5), 16,750 (5/5)	1.50×10^{-3}	6.04×10^{-4} to 3.72×10^{-3}	1.16×10^{-1}
21	153 (1/5), 765 (3/5), 3,825 (5/5), 19,125 (5/5)	1.31×10^{-3}	5.29×10^{-4} to 3.25×10^{-3}	1.02×10^{-1}
22	155 (1/5), 776 (3/5), 3,880 (5/5), 19,400 (5/5)	1.29×10^{-3}	5.22×10^{-4} to 3.21×10^{-3}	1.00×10^{-1}
23	161 (1/5), 805 (3/5), 4,025 (5/5), 20,125 (5/5)	1.25×10^{-3}	5.03×10^{-4} to 3.09×10^{-3}	9.69×10^{-2}
24	269 (1/5), 1,345 (1/5), 6,725 (2/5), 33,625 (5/5)	1.29×10^{-4}	5.35×10^{-5} to 3.12×10^{-4}	1.00×10^{-2}
25	805 (1/5), 4,025 (3/5), 20,125 (4/5), 100,625 (5/5)	1.30×10^{-4}	5.48×10^{-5} to 3.06×10^{-4}	1.01×10^{-2}
26	755 (2/5), 3,775 (2/5), 18,875 (4/5), 94,375 (5/5)	1.33×10^{-4}	5.64×10^{-5} to 3.15×10^{-4}	1.03×10^{-2}
27	759 (1/5), 3,794 (3/5), 18,970 (4/5), 94,850 (5/5)	1.37×10^{-4}	5.81×10^{-5} to 3.25×10^{-4}	1.06×10^{-2}
28	755 (1/5), 3,775 (3/5), 18,875 (4/5), 94,375 (5/5)	1.38×10^{-4}	5.84×10^{-5} to 3.26×10^{-4}	1.07×10^{-2}
C-4	8 (2/5), 40 (3/5), 250 (4/5), 1,350 (5/5)	1.32×10^{-2}	5.29×10^{-3} to 3.30×10^{-2}	1.00×10^{0}
29	3,220 (0/5), 16,100 (0/5), 80,500 (3/5), 402,500 (5/5)	9.45×10^{-6}	3.95×10^{-6} to 2.26×10^{-5}	7.16×10^{-4}
30	3,170 (0/5), 15,850 (0/5), 79,250 (3/5), 396,250 (5/5)	9.60×10^{-6}	4.01×10^{-6} to 2.30×10^{-5}	7.27×10^{-4}
31	2,207 (0/5), 11,033 (1/5), 55,165 (1/5), 275,825 (5/5), 1,379,125 (5/5)	9.74×10^{-6}	4.15×10^{-6} to 2.28×10^{-5}	7.38×10^{-4}
32	2,265 (0/5), 11,325 (0/5), 56,625 (2/5), 283,125 (5/5), 1,415,625 (5/5)	9.99×10^{-6}	4.25×10^{-6} to 2.35×10^{-5}	7.57×10^{-4}
33	2,447 (0/5), 12,233 (0/5), 61,165 (2/5), 305,825 (4/4), 1,529,125 (5/5)	8.87×10^{-6}	3.61×10^{-6} to 2.18×10^{-5}	6.72×10^{-4}
34	2,500 (0/5), 12,500 (0/5), 62,500 (2/5), 312,500 (5/5), 1,562,500 (5/5)	9.05×10^{-6}	3.85×10^{-6} to 2.13×10^{-5}	6.86×10^{-4}
35	2,336 (0/5), 11,680 (0/5), 58,400 (2/5), 292,000 (5/5), 1,460,000 (5/5)	9.69×10^{-6}	4.12×10^{-6} to 2.28×10^{-5}	7.34×10^{-4}
36	2,253 (0/5), 11,266 (1/5), 56,330 (1/5), 281,650 (5/5), 1,408,250 (5/5)	9.75×10^{-6}	4.12×10^{-6} to 2.31×10^{-5}	7.39×10^{-4}
37	2,200 (0/5), 11,000 (1/5), 55,000 (3/5), 275,000 (4/5), 1,375,000 (5/5)	9.32×10^{-6}	3.98×10^{-6} to 2.18×10^{-5}	7.06×10^{-4}

[a] C-1, control for trials 1, 2, 3, 8, 10, 11, 12, 17, and 18; C-2, control for trials 4, 5, 6, 13, 14, and 15; C-3, control for trials 19 through 28; C-4, control for trials 29 through 37.

[b] Number of oocysts per mouse.

parvum and the effect on its viability. This large difference between the UV requirement for reducing infectivity and that for reducing excystation suggests that a large percentage of *C. parvum* oocysts exposed to a low dose of UV irradiation are able to excyst but not infect. Although the excystation assay may prove to be a useful measure of disinfection with respect to oocyst viability, it cannot distinguish between infective and noninfective oocysts (12). Thus, the animal infectivity experiment is the most appropriate evaluation method, given that the primary area of interest is the infectivity of *C. parvum* oocysts. Therefore, in assessing the degree of inactivation by a water treatment process from the perspective of public health, the efficacy of a disinfectant should be evaluated by its effect on infectivity.

Effects of temperature and intensity. The reductions in infectivity observed at various temperatures are shown in Fig. 3. The UV doses resulting in a 2-$\log_{10}$ reduction in infectivity at 5, 10, and 30°C were 1.20, 1.07, and 1.02 mWs/cm^2, respec-

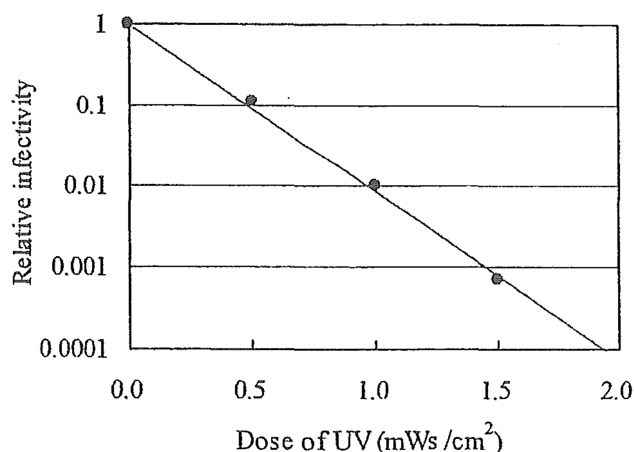

FIG. 1. Relationship between the relative infectivity of *C. parvum* oocysts and the UV irradiation dose.

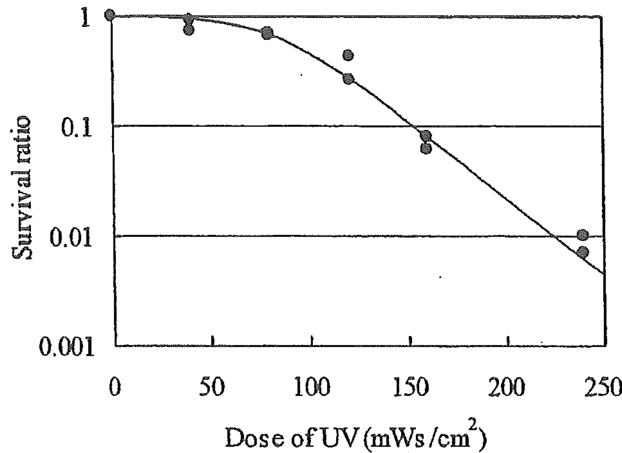

FIG. 2. Relationship between the survival ratio as assessed by in vitro excystation of *C. parvum* oocysts and the UV irradiation dose.

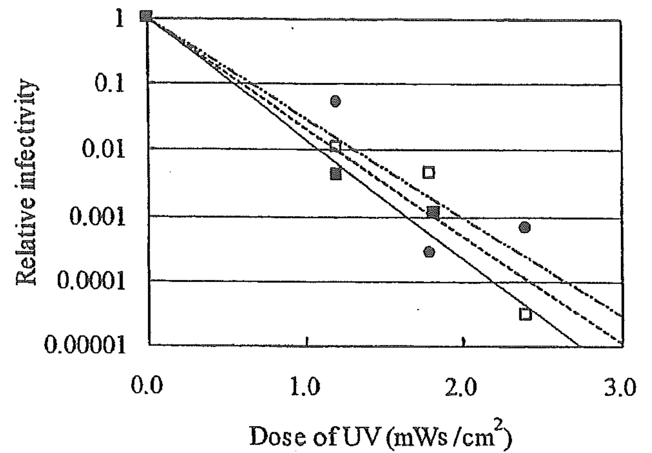

FIG. 4. Effect of irradiation intensity on the relative infectivities of *C. parvum* oocysts exposed to increasing UV dosages. Symbols: ■, 0.048 mW/cm^2; □, 0.12 mW/cm^2; ●, 0.60 mW/cm^2.

tively. The observed increase in the dose required for a 2-$\log_{10}$ reduction in infectivity was only 7% for every 10°C reduction in water temperature. The *CT* requirement of chemical disinfectants generally depends on both the temperature and the concentration of the disinfectant (5, 13). For instance, the temperature factor of ozone has been reported to be as high as 4.09 (16) or 4.20 (13) for every 10°C reduction in temperature. However, temperature did not significantly affect the efficacy of UV irradiation in reducing the infectivity of oocysts in this study.

The reductions in infectivity observed at various irradiation intensities are shown in Fig. 4. The UV doses required for a 2-$\log_{10}$ reduction in infectivity at intensities of 0.048, 0.12, and 0.60 mW/cm^2 were 1.15, 1.20, and 1.34 mWs/cm^2, respectively, indicating that only an 8% increase in the UV dose was required with a 10-fold increase in intensity. These results showed that the disinfecting effect of UV on *C. parvum* oocysts was dependent on the actual irradiation dose only.

Thus, in practice, UV irradiation can be used to inactivate *C. parvum* oocysts in water without considering the effects of either water temperature or irradiation intensity.

Photoreactivation and dark repair in *C. parvum* following UV inactivation. The reduction in infectivity of *C. parvum* oocysts induced by UV irradiation might not be permanent, because the DNA lesions of some microorganisms may be repaired by photoreactivation or dark repair (9). Figure 5 shows the number of ESSs per base in UV-inactivated oocysts after exposure to fluorescent light. The UV-induced pyrimidine dimers were gradually repaired as the time of exposure to fluorescent light increased. The number of ESSs decreased by approximately 30 to 50% after fluorescent-light irradiation at a dose of 720 mWs/cm^2. Figure 6 shows the number of ESSs in UV-inactivated oocysts after dark storage for various durations. The UV-induced pyrimidine dimers were gradually repaired during storage in the dark. Nonetheless, the number of ESSs decreased more slowly during dark storage than during the photoreactivation process. Upon storage in the dark for 24 h, the number of ESSs decreased by approximately 60%,

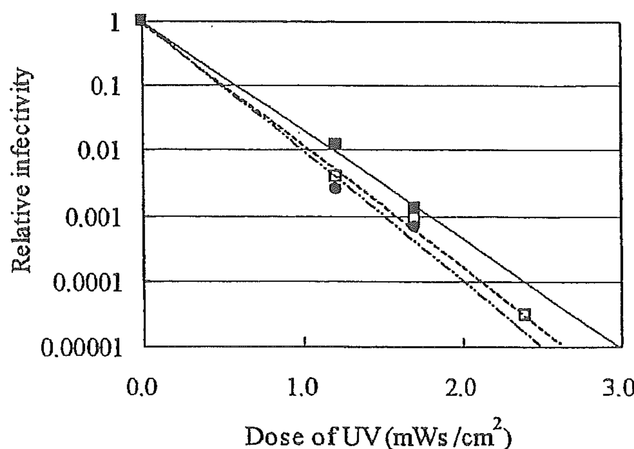

FIG. 3. Effect of water temperature on the relative infectivities of *C. parvum* oocysts exposed to increasing UV dosages. Symbols: ■, 5°C; □, 10°C; ●, 30°C.

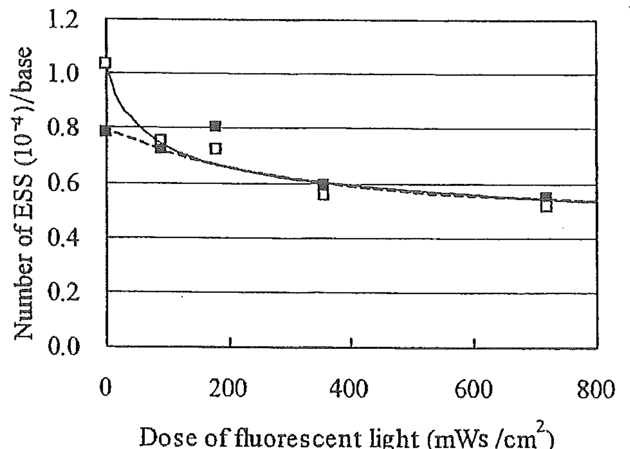

FIG. 5. Relationship between the number of ESSs and the dosage of fluorescent-light irradiation in UV-irradiated (at 0.50 or 1.00 mWs/cm^2) *C. parvum* oocysts. Symbols: ■, 0.50 mWs/cm^2; □, 1.00 mWs/cm^2.

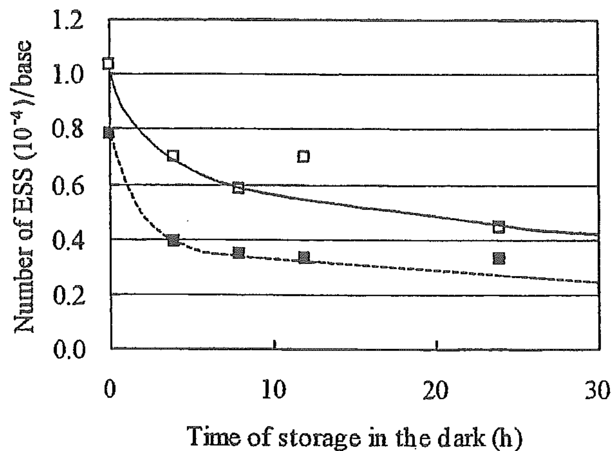

FIG. 6. Relationship between the number of ESSs and the duration of dark storage in UV-irradiated (at 0.50 or 1.00 mWs/cm^2) *C. parvum* oocysts. Symbols: ■, 0.50 mWs/cm^2; □, 1.00 mWs/cm^2.

indicating that *C. parvum* has the ability to repair the pyrimidine dimers in the genomic DNA during either exposure to fluorescent light or storage in the dark.

Table 4 shows the results of the animal infectivity test for oocysts that had been exposed to fluorescent light after UV inactivation. Exposure to UV irradiation of 0.50, 1.00, or 1.50 mWs/cm^2 alone led to a 0.98-, 2.00-, or 3.15-$\log_{10}$ reduction in infectivity, respectively. The degree of reduction in infectivity following photoreactivation in samples that had been exposed to fluorescent light at a dose of 150 to 720 mWs/cm^2 after UV irradiation did not change.

Table 5 shows the results of the animal infectivity test for oocysts that had been stored in the dark after inactivation by UV irradiation. A dark repair period of 4 to 24 h did not change the degree of inactivation of *C. parvum* which had been UV irradiated at 0.5, 1.0, or 1.5 mWs/cm^2, based on the animal infectivity experiments.

Even though UV-inactivated *C. parvum* oocysts underwent photoreactivation or dark repair upon exposure to fluorescent light or storage in the dark, respectively, as observed in the ESS study, the infectivity of *C. parvum* oocysts did not change after either treatment. These results indicate that *C. parvum* did not recover its infectivity following either photoreactivation or dark repair, although recovery was seen at the DNA level.

TABLE 4. Infectivity in SCID mice of *C. parvum* oocysts exposed to fluorescent light after UV inactivation

UV irradiation dose (mWs/cm^2)	Fluorescent-light dose (mWs/cm^2)	$\log_{10}$ reduction
0.50	0	0.98
0.50	150	0.93
0.50	300	0.99
1.00	0	2.00
1.00	150	2.00
1.00	300	1.98
1.50	0	3.15
1.50	180	3.13
1.50	360	3.12
1.50	540	3.17
1.50	720	3.16

TABLE 5. Infectivity in SCID mice of *C. parvum* oocysts stored in the dark after UV inactivation

UV irradiation dose (mWs/cm^2)	Storage duration (h)	$\log_{10}$ reduction
0.50	0	0.98
0.50	12	1.00
0.50	24	1.01
1.00	0	2.00
1.00	12	1.97
1.00	24	1.97
1.50	0	3.15
1.50	4	3.13
1.50	12	3.13
1.50	24	3.15

The reasons for this are as follows. (i) Immediately after UV irradiation of oocysts, the dark repair process begins. Therefore, the results of the photoreactivation experiment also include the effects of the dark repair process. (ii) Although considerable recovery at the DNA level may occur during photoreactivation or dark repair, some lesions at the DNA level remain, and those lesions may inhibit the progression of the life cycle. (iii) Other damage induced by UV irradiation aside from pyrimidine dimers could not be repaired by photoreactivation and dark repair and might play an important role in infection. From these results, we conclude that UV-irradiated oocysts cannot proceed with their life cycle even if they are placed in the presence or absence of visible light.

Conclusions. The results of the present study can be summarized as follows.

(i) The infectivity of *C. parvum* HNJ-1 oocysts decreased exponentially as the UV irradiation dose increased. The UV dose required for a 2-$\log_{10}$ reduction in infectivity was only 1.0 mWs/cm^2.

(ii) The dose required for a 2-$\log_{10}$ reduction in viability as assessed by in vitro excystation was approximately 200 times higher than that required for a 2-$\log_{10}$ reduction in infectivity, suggesting that *C. parvum* oocysts exposed to low-dose UV irradiation are able to excyst but not infect.

(iii) Neither water temperature nor UV intensity significantly affected the reduction in the infectivity of UV-irradiated *C. parvum* oocysts.

(iv) The ESS study revealed that when UV-inactivated *C. parvum* oocysts were exposed to fluorescent light or stored in the dark, photoreactivation or dark repair, respectively, occurred; however, the infectivity of *C. parvum* oocysts was not restored. These results indicate that the life cycle of UV-irradiated oocysts cannot proceed even when they are placed in the presence or absence of visible light.

ACKNOWLEDGMENTS

This study was supported by a 2001 Grant-in-Aid for Scientific Research category (B)(2) (grant 13450217) and, in part, by a 2000 and 2001 Grant-in-Aid for Encouragement of Young Scientists (grant 12750501) from the Ministry of Education, Science, Sports and Culture of Japan.

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PHOTOREACTIVATION OF ENTEROHEMORRHAGIC E. COLI, VRE AND P. AERUGINOSA FOLLOWING UV DISINFECTION

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ABSTRACT

UV disinfection is noted to have some problems, one of which is photoreactivation. Photoreactivation allows inactivated microorganisms to regain viability following UV disinfection. The objective of this study is to determine the susceptibility of enterohemorrhagic *Escherichia coli* (EHEC) O26, vancomycin resistant *Enterococcus* (VRE), and *Pseudomonas aeruginosa* to UV radiation and photoreactivation. The conclusions obtained in this study can be summarized as follows. EHEC O26 exhibited apparent inactivation under sunlight after photoreactivation following UV inactivation. VRE exhibited apparent photoreactivation. The dose of UV light required for 90% inactivation of VRE with and without photoreactivation was 10.9 and 24.2 mW sec/cm^2, respectively. *P. aeruginosa* exhibited apparent photoreactivation under fluorescent lamp and weak regrowth under dark conditions following UV inactivation. The dose of UV light required for 90% inactivation of *P. aeruginosa* with and without photoreactivation was 4.1 and 5.2 mW sec/cm^2, respectively.

KEYWORDS

enterohemorrhagic *Escherichia coli*; photoreactivation; *Pseudomonas aeruginosa*; UV disinfection; VRE

INTRODUCTION

Chlorination has been used for most wastewater disinfection operations in Japan for many years, but alternative wastewater disinfection methods have been developed due to growing concerns regarding the toxicity of chlorine residuals (Water Environment Federation, 1996). UV irradiation has become one of the most important alternatives to chlorination for wastewater disinfection throughout the world. Recently, reevaluation of UV dose required for *Cryptosporidium* inactivation showed that UV is far more effective than it had been thought to be (Clancy *et al.*, 2000). One problem of UV disinfection is photoreactivation. Photoreactivation is the repair of the photochemical damage to DNA in organisms under visible light irradiation (Water Environment Federation, 1996). This repair mechanism allows inactivated microorganisms to regain viability following UV disinfection.

Many researchers have studied the photoreactivation of indicator and non-human pathogenic microorganisms following UV disinfection (Harris *et al.*, 1987; Schonene and Kolch *et al.*, 1992; Lindenauer and Darby *et al.*, 1994; Chang *et al.*,1995; Kashimada *et al.*, 1996). However, there has been little research on the photoreactivation of pathogenic microorganisms. The question remains, to what extent should photoreactivation be taken into consideration during the design of the disinfection process? Photoreactivation of enterohemorrhagic *Escherichia coli* was already studied under luminescent lump (Tosa and Hirata, 1999), but not under sunlight yet. One of the objectives of this study is to determine the photoreactivation of enterohemorrhagic *Escherichia coli* (EHEC) O26 under sunlight following UV disinfection. Secondly, we determined the susceptibility to UV and photoreactivation of *Pseudomonas aeruginosa* and vancomycin resistant *Enterococcus* (VRE). *Pseudomonas aeruginosa* is known as an opportunistic pathogenic bacterium and an indicator bacterium of water treatment. *Enterococcus* is also known as an opportunistic pathogenic bacterium and an indicator bacterium of water pollution.

MATERIALS AND METHODS

Bacterial Strains

One strain of enterohemorrhagic *Escherichia coli* O26 was provided by Prof. M. Fukuyama, College of Environmental Health, Azabu University. One strain of vancomycin resistant *Enterococcus* was provided by Public Health Research Center of Chiba Pharmaceutical Association. One strain of *Pseudomonas aeruginosa* was isolated from river water by using NAC agar method and identified by API20E system (BIOMerieux).

EHEC O26 was spread on tryptic soy agar (Difco Laboratories, Detroit) and incubated at 36 °C for 24 hours. *P. aeruginosa* and VRE were spread on plate count agar and incubated at 36 °C for 24 hours. Several colonies that formed on the plate were suspended in 10 ml of 6 mM phosphate buffer (pH 7.0) and homogenized using a mixer. The suspension was diluted to the bacterial density of about 10^5 CFU/ml in 500ml of 6 mM phosphate buffer (pH 7.0) in a glass beaker held at 20 °C.

Ultraviolet Light Disinfection

UV treatment was carried out in a batch disinfection device. A beaker containing the bacterial suspension mixed with a magnet bar was placed on a magnetic stirrer. A 25W UV lamp (GL-25, NEC, Tokyo) was horizontally suspended 60 cm above the liquid surface. After the appropriate time the irradiation was stopped and a sample was taken for plating out. Incident UV intensity at the liquid surface was measured at 254 nm with a dosimeter (UVR-254, TOPKON, Tokyo).

Visible Light Irradiation

Visible light irradiation was carried out in a batch irradiation device. A 15W fluorescent lamp (Lumicrystal-15N, Mitsubishi, Tokyo) was horizontally suspended about 15 cm above the liquid surface. A beaker containing the UV-treated bacterial suspension mixed with a magnet bar was placed on a magnetic stirrer. Samples were taken after timed intervals for plating out. Incident visible light intensity at the liquid surface was measured at 360 nm with a dosimeter (UVR-1 and UVR-36, TOPKON, Tokyo).

Sunlight irradiation was also carried out in a batch irradiation system. UV irradiated samples are carried outside from the laboratory and irradiated to sunlight. The beaker was covered by quartz glass for inhibiting contamination during sunlight irradiation.

Bacterial Assay

Most samples were diluted with 6 mM phosphate buffer solution (pH 7.0) and poured with the same agar as cultured before inoculation to water. Some samples with low bacterial density were concentrated by the membrane filtration technique and the filter was placed on the agar. After a 24-hour incubation at 36 °C in a dark place, the colonies that formed on the plates were counted.

Modeling

Kashimada *et al.* (1996) assumed that photoreactivation follows a saturation-type first order reaction. However, this assumption cannot be applied to the photoreactivation process with the higher UV doses used in this study, because a shoulder was seen at the start of photoreactivation. Consequently, data from this shoulder was omitted in modeling. Survival data were treated according to Chick-Watson's law. However, the relationship between UV dose and survival ratio of bacteria does not always follow Chick-Watson's law. Convex curves were analyzed using the series-event model in this study (Severin *et al.*, 1983). UV doses required for 90% inactivation were then computed from these models.

RESULTS AND DISCUSSION

Photoreactivation of Pseudomonas aeruginosa

The relationship between the survival ratio of VRE and visible light dose is shown in Figure 1. Apparent photoreactivation was observed in *P. aeruginosa*, while a weak increase in the survival ratio occurred under dark conditions following UV disinfection. Photoreactivation in *P. aeruginosa*(S21) was significant but not significant in *P. aeruginosa*(ATCC 15442) and *P. aeruginosa*(ATCC 15442 mutant ml) (Hassen *et al.*, 2000). Variation in the photoreactivation of *P. aeruginosa* exists.

Dukan *et al.* (1997) suggested that recovery in phosphate buffer of an HOCl-stressed population is in large part due to growth of a few cells at the expense of damaged cells. Moreover, dark repair may occur in the growing medium during incubation under dark conditions. In this study increases in survival ratio occurred under dark conditions and the survival ratios reached to over 1.0 after low UV doses. Thus, increases in survival ratio of *P. aeruginosa* includes regrowth of surviving cells.

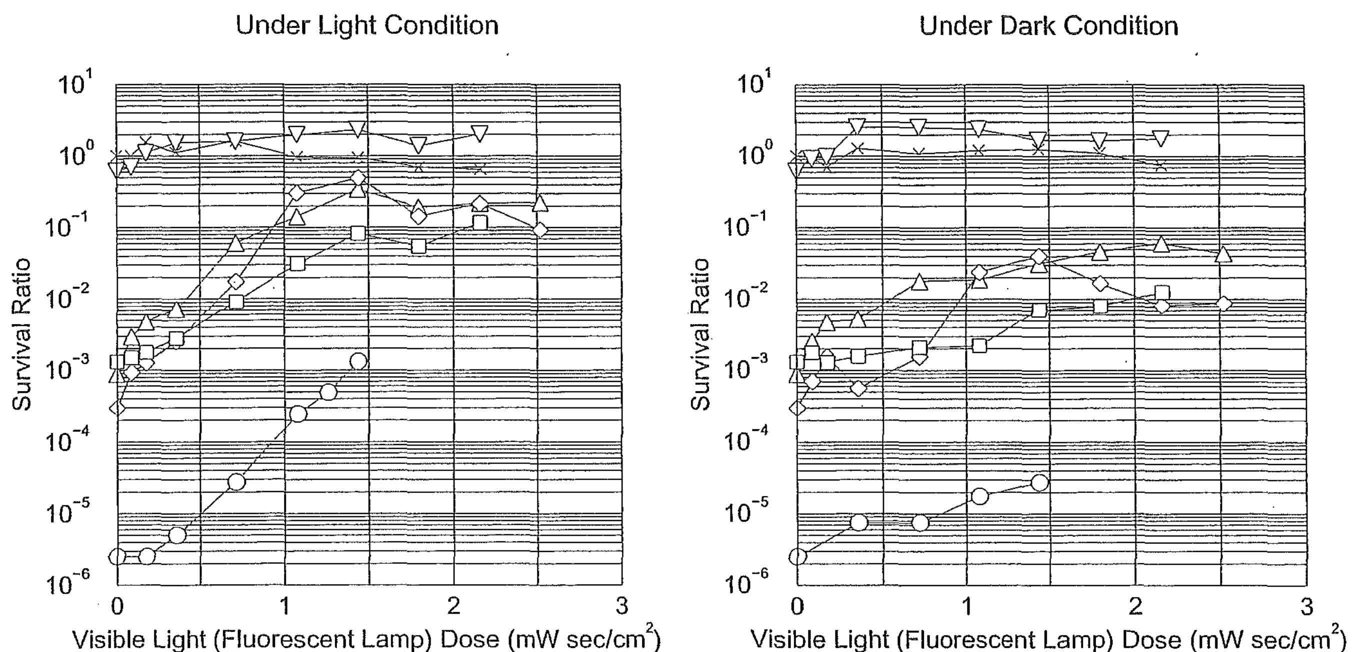

Figure 1: Photoreactivation of *Pseudomonas aeruginosa*

Photoreactivation of EHEC O26 under Sunlight

The relationship between the survival ratio of EHEC O26 and visible light (sunlight) dose is shown in Figure 2. Apparent photoreactivation was observed in EHEC O26 under sunlight, while a decrease in the survival ratio occurred under intense sunlight following UV disinfection. No increase in the survival ratio was observed in EHEC O26 not irradiated to UV, while a decrease in the survival ratio of EHEC O26 was observed under intense sunlight. These decreases in the survival ratio were observed after about 100 mW·min/cm^2 irradiation of sunlight (visible light).

Photoreactivation of EHEC O26 under luminescent light was already reported (Tosa and Hirata, 1999). This study shows photoreactivation following UV disinfection may occur under sunlight, but inactivation may also occur under sunlight following photoreactivation. No repair for *Salmonella* was observed after a 60 mW sec/cm^2 irradiation and a 24-hour incubation (Baron, 1997). That result may be due to sunlight inactivation as observed in this study.

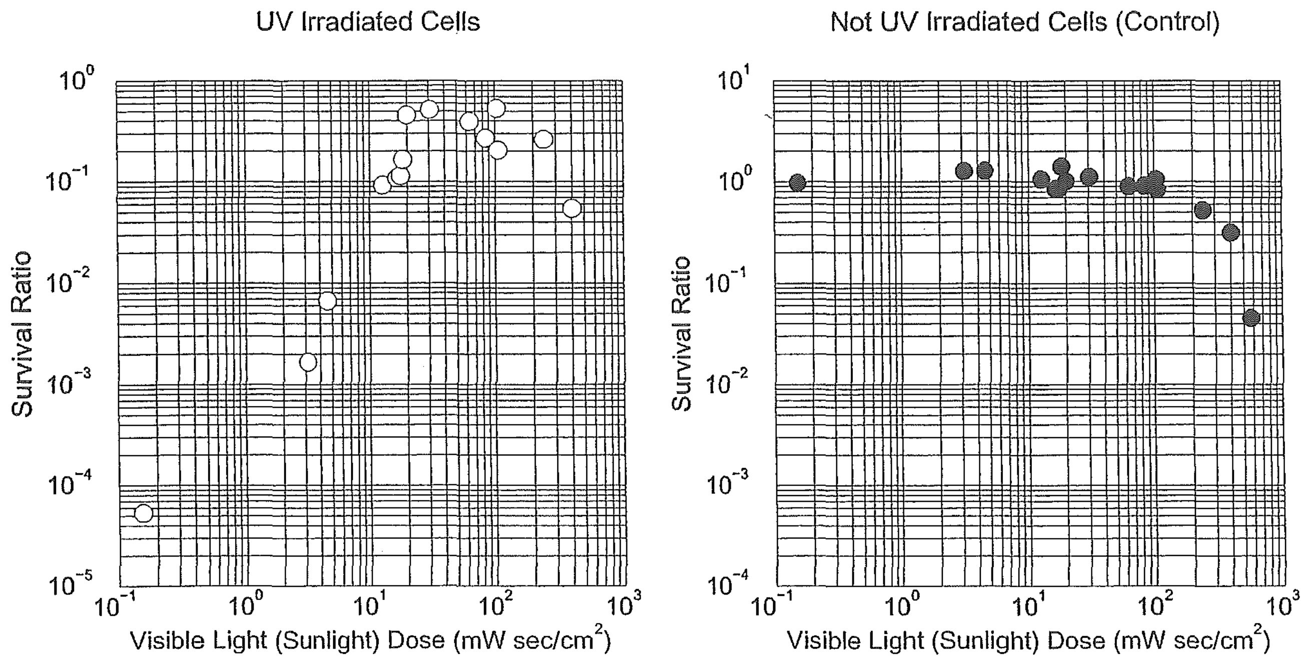

Figure 2: Photoreactivation of EHEC O26 under Sunlight

Photoreactivation of Vancomycin Resistant Enterococcus

The relationship between the survival ratio of VRE and visible light dose is shown in Figure 3. Apparent photoreactivation was observed in VRE, while no increase in survival ratio in VRE was observed following UV disinfection under dark condition. Even decreases in the survival ratio were observed after some UV doses.

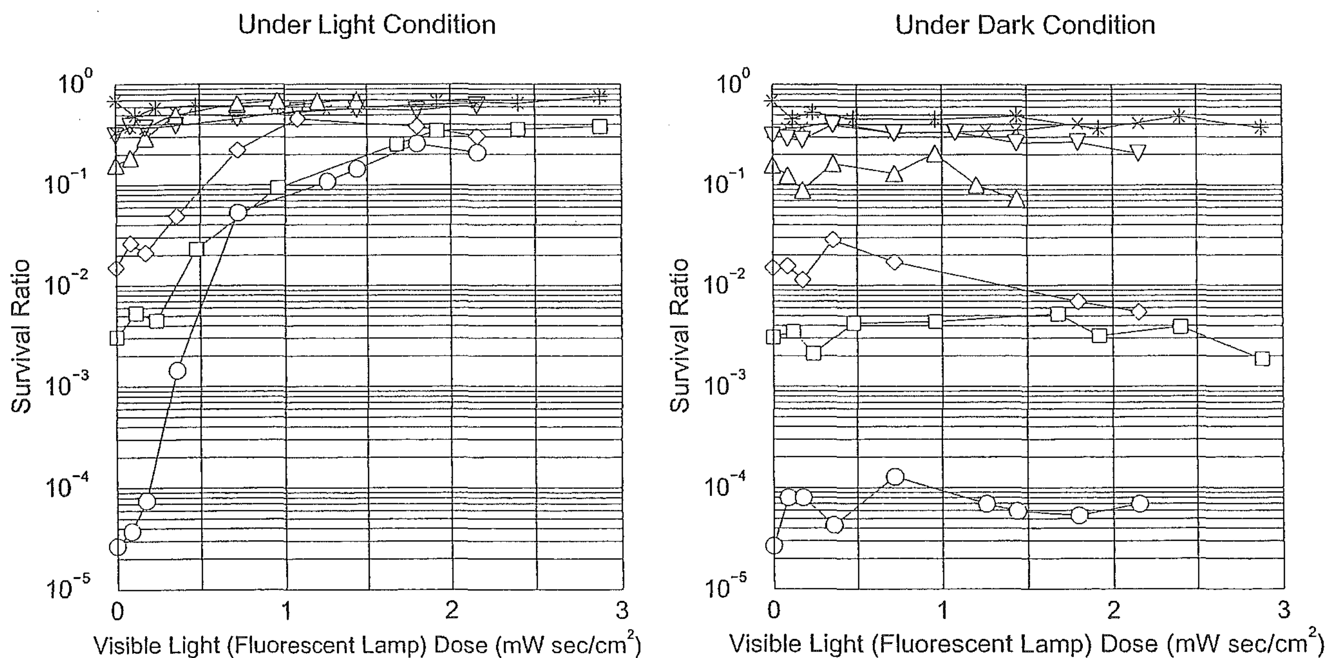

Figure 3: Photoreactivation of VRE

Comparison of UV Dose Required for 90% Inactivation of Tested Bacteria

The UV doses required for 90% inactivation of tested bacteria are shown in Figure 4 (Tosa and Hirata, 1999). VRE was the most UV resistant bacteria tested, while *P. aeruginosa* and EHEC were weaker.

The UV doses required for 90% inactivation of VRE with and without photoreactivation was 10.9 and 24.2 mW sec/cm^2, respectively. The UV doses required for 90% inactivation of *E. coli* (ATCC 11229) without photoreactivation was 2.5 and 7.0 mW sec/cm^2,respectively (Harris et al., 1987). VRE may be significantly more resistant to UV disinfection than *E. coli* (ATCC 11229). From the results of Kashimada *et al.* (1996), the UV dose required for 90% inactivation of fecal coliforms, with and without photoreactivation, is computed to be 24 and 5.2 mW sec/cm^2, respectively. These values indicate that fecal coliforms are not more resistant without photoreactivation to UV disinfection than VRE but as resistant without photoreactivation as VRE. Therefore, fecal coliforms may not be useful as an indicator of VRE in the UV disinfection process for non-photoreactivating conditions. However, photoreactivation improved the survival of the investigated VRE to more effectively than that found in *Escherichia coli* (ATCC 11229) (Harris *et al.*, 1997), but as effectively as has been observed in fecal coliforms (Kashimada *et al.*, 1996). These findings suggest that fecal coliforms could be used as a removal indicator of VRE during the UV disinfection process for photoreactivating conditions.

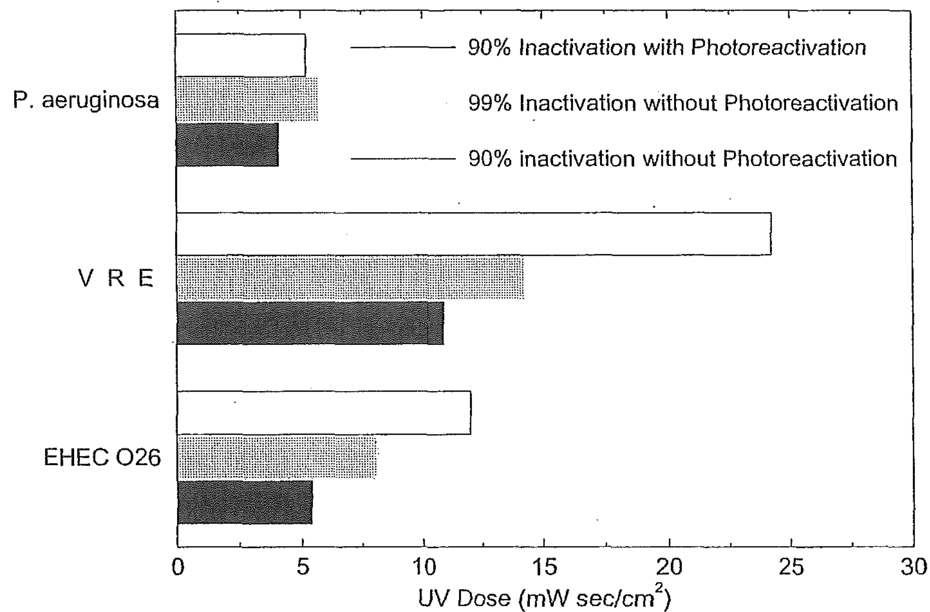

Figure 4: Comparison of UV Dose Required for 90% Inactivation of Tested Bacteria

Many researchers have reported the effect of water transparency on UV disinfection efficiency. This minus effect is usually estimated by considering UV dose decreases in the target water. UV dose decreases are estimated by determining UV absorbance at 254 nm in target water (Kamiko and Ohgaki, 1989). This estimation method is being widely used and our data will be useful after UV dose is calibrated by this estimation. Photoreactivation may also be affected by water transparency, and the effect may be estimated by similar method used for estimation of UV dose decreases in water. The difference between inactivation and photoreactivation will be the wavelength of water transparency to determine. For now we have presented only basic data on photoreactivation of three bacteria, but our data is useful for further photoreactivation studies or design of UV disinfection processes if combined with some UV/Visible light decrease estimation methods mentioned above.

CONCLUSIONS

The conclusions obtained in this study can be summarized as follows. EHEC O26 exhibited apparent photoreactivation under sunlight following UV inactivation. VRE exhibited apparent photoreactivation. The dose of UV light required for 90% inactivation of VRE with and without photoreactivation was 10.9 and 24.2 mW sec/cm^2, respectively. *P. aeruginosa* exhibited apparent photoreactivation under fluorescent lamp and weak regrowth under dark conditions following UV inactivation. The dose of UV light required for 90% inactivation of *P. aeruginosa* with and without photoreactivation was 4.1 and 5.2 mW sec/cm^2, respectively.

ACKNOWLEDGMENT

We would like to thank Public Health Research Center of Chiba Pharmaceutical Association for providing *VRE* cultures. We also would like to thank Miss. R. Tahara for her assistance.

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「特集：クリプトスポリジウムと濁度管理強化対策」

クリプトスポリジウム汚染と濁質管理の重要性

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キーワード：クリプトスポリジウム（208）、濁度（459）リスク対策、沪過、懸濁物質（239）
分 類 項 目：水質管理一般（120101）、凝集・沈澱（120702）

1.　はじめに

河川水、湖沼水などの表流水は表面流出や排水による汚染を受けやすいので、表流水を水源とする水道では浄水処理が必須である。浄水処理は通常、原水中の懸濁粒子を物理的に取り除いて清澄水にする沪過プロセスと、病原微生物を殺滅（あるいは不活化）する消毒プロセスから構成される。わが国の水道で用いられる沪過プロセスには、緩速砂沪過法、急速砂沪過法、膜沪過法があり、このうち急速砂沪過法が最も多用されている。病原微生物も粒子状なのでこれらの沪過プロセスで相当程度除去されるが、後述する膜沪過法を除いて、処理水に少量の病原微生物が残存することは避けられない。このため、水道水の微生物的安全性の確保には、沪過プロセスを通過した病原微生物が消毒プロセスでどの程度殺滅あるいは不活化されるかが重要となる。

塩素には強い殺菌力があり、水道水の微生物的安全性の高揚と維持に大きく貢献してきたが、その主たる対象は塩素感受性の高い病原細菌であった。しかし近年になって水道水を原因とする微生物感染症の集団発生が米国、英国などで多発、その原因病原微生物がクリプトスポリジウムとジアルジアに代表される原虫であることが明らかになった[1~3]。クリプトスポリジウムについてはわが国でもこれまでに2件の報告があり[4,5]、そのうち1996年に発生した埼玉県越生町の集団感染事例は、凝集沈澱・急速砂沪過・塩素消毒からなる処理システムを有している水道によるもので、終息後に行われたアンケート調査によると感染者数8,800余人にものぼる大規模なものであった[5]。

水系感染する原虫類には赤痢アメーバ、クリプトスポリジウム、ジアルジア、サイクロスポーラなどがある。これらの原虫類の感染形であるシストやオーシストは消毒剤に対する抵抗性や環境における生残性が著しく高く、現在水道界で使用されている塩素消毒ではジアルジアを除いてほとんど不活化されないため、既存の浄水処理システムは沪過の物理的除去機能のみの１段階バリヤーとしてしか機能しない。

クリプトスポリジウムのオーシスト（以下、単にクリプトスポリジウムと表記）は病原性原虫類のなかでサイズが最も小さく、消毒剤に対する抵抗性が最も高い。したがって、クリプトスポリジウムに十分対応できるシステムを構築すれば、そのシステムは同時に他の原虫類に対しても十分機能すると見なすことができよう。そこで本稿では、クリプトスポリジウムのみを取り上げて論じることとしたい。

2.　我が国の水源汚染の現状

水道水源を含む表流水から様々な濃度でクリプトスポリジウムが検出されている[6~10]。観測された最大値は10^4個/100lレベルで、水道原水取水地点における最高レベルは10^3個/100lに達する。検出率は試験水量によって当然異なるが、水量100lレベルで試験するとクリプトスポリジウムは広範囲な表流水源から検出されるものと推測される。

汚染源としては、人、産業動物、野生動物が考えられる。下水処理場67箇所で行われた全国調査によると、流入下水約0.2lの試験で陽性率9.6%、濃度範囲は8～50個/l、処理水で陽性率12.2%、

濃度範囲は0.05～1.6個/lと報告されている[11]。平均回収率が流入下水で約14％とあるので、これで補正すると流入下水約60～360個/lとなる。その中の最高濃度360個/lのクリプトスポリジウムがすべてヒト由来とし、1人1日平均下水量を350l/（人・日）、排出量を10^9個/（人・日）とすると、罹患率は1.3人/10,000人となる。ピーク排出期間を1週間とし、それ以外には排出しないとして1年当たりの患者発生率を単純計算すると70人/10,000人・年（感染者発生率は平均で1日当たり100,000人に2人に相当）となるが、新感染症法でクリプトスポリジウムが第4類に指定されたにもかかわらず、クリプトスポリジウム患者発生の報告はほとんどない。また、処理水では回収率（約46％）で補正すると濃度は0.1～3.5個/lとなる。通常の水道水源における処理排水の環境水による希釈率を100倍とすると環境水中の濃度は最高で0.001～0.035個/l、10倍としても0.01～0.35個/lの増加にすぎず、水道水源で観測された高い値を説明できない。現状では、水道水源のクリプトスポリジウムは、ヒト由来というよりは、家畜などの産業動物や野生動物由来の可能性が高いのかもしれない。しかしながら、クリプトスポリジウムは人獣共通感染病原体なので、ヒト由来でなくとも、ヒト由来の場合と同様の対策が必須となる。

3. 塩素抵抗性

常温における遊離塩素のクリプトスポリジウム不活化能力（マウス感染性を低下させる能力）は濃度80mg/l、接触90分（CT値＝7,200mg min/l）で2 log（＝99％）以上[12]、同濃度、接触120分（CT値＝約9,600mg min/l）で3 log（＝99.9％）以上[13]とされ、水道で使用される塩素消毒レベルでは有意な不活化がまったく期待できないと考えられてきた。しかし、これらの値はいずれも実際の水道で使用されることのない非常に高い塩素濃度での知見であり、濃度依存性に関する検討がなされていない。そこで筆者の研究室では、実際の水道で用いられる濃度としての遊離塩素濃度1 mg/lを含む広範な塩素濃度での不活化試験を行った。オーシストは *Cryptosporidium parvum* HNJ-1株、不活化の評価はマウス感染性（投与したマウスの糞にオーシストが出現するかどうかを判断基準とする方法）とした。その結果、不活化CT値は塩素濃度に大きく依存し、2 log不活化CT値はpH 7、水温20℃、塩素濃度1 mg/l付近のとき約1,600mg min/lとなり、前述の2つの既報値の約1/3～1/4に相当する値であった[14]。平均遊離塩素濃度を1 mg/lとし、浄水池で1時間、配水池で12時間（合計で13時間）の滞留時間が保証されたときの単純CT値は780mg min/lとなる。これに前塩素処理や中間塩素処理が行われている場合はさらにCT値が加わることになるので、現行の塩素消毒システムでまったく不活化されないわけではない。しかし、期待できたとしても不活化レベルはおそらく最大でも1 log未満であり、クリプトスポリジウム対策としては極めて不十分である。したがって、クリプトスポリジウムによる感染を防止するには浄水処理システムにおける沪過等での物理的除去が極めて重要となる。

4. 各種浄水処理方法の物理的除去能力

4-1 緩速沪過法

水の微生物衛生が沪過によって著しく改善されるという、いわゆるMills-Reinke現象の発見と沪過の普及をもたらした浄水方法であり、生物沪過膜の性状からみて高度のクリプトスポリジウム除去が期待しうる。実際、本法におけるクリプトスポリジウム除去率について4.0log（＝99.99％）以上[15]、4.5log（＝99.997％）以上[16]との報告がある。しかし一方で、沪過成績が水温、沪過速度、沪材径、沪層厚等に大きく左右されやすいという欠点もあり、清浄な原水にしか適用できないため実態調査や実験研究が極めて少ない。このため、現時点では緩速砂沪過法におけるクリプトスポリジウム除去の平均的な期待値は必ずしも明らかではない。充分に成熟した生物沪過膜を常時確実に維持できるのであれば、次項の急速沪過法を凌ぐ4 logレベルのクリプトスポリジウム除去が期待できるとみてよいのかもしれない。

4-2 急速沪過法

「凝集沈澱＋急速沪過」が基本である急速砂沪過法は世界で最も多用されている浄水処理方法であり、クリプトスポリジウム除去に関するいくつかの調査・研究が行われている。米国及びカナダ

の表流水を水源とする浄水場66箇所で行われた調査によると、SWTR（Surface Water Treatment Rule；表流水処理規則）の濁度基準（1ヵ月間の検査試料数の95％が0.5NTU以下）の達成率が78.1％、沪過水の濁度が平均0.19NTUのとき、原水、沪過水ともにクリプトスポリジウム陽性データセットから算出したクリプトスポリジウムの平均除去率は2.38log（＝99.6％）であった[17,18]。河川原水に加熱処理済みクリプトスポリジウムを添加した急速沪過パイロットプラント実験では、沪過水濁度を常時低レベル（0.1～0.2NTU）に維持したときのクリプトスポリジウム除去は1.9～3 log（＝98.7～99.9％）であり、運転方式が直接沪過方式であっても除去率に差異は生じないとの報告もある[19]。筆者等も我が国の浄水場で調査したが、クリプトスポリジウム等暫定対策指針[20]に基づいて濁度0.1度未満を常時達成している急速砂沪過池の沪過水（浄水場のデータによればほとんど常時0.01度程度）からもクリプトスポリジウムが検出され、原水のクリプトスポリジウム陽性率は100％、幾何平均値は40個/100lで、この原水の沪過水について検出下限値1～2個/2,000lで試験したときの陽性率は35％、陽性試料の幾何平均値は0.12個/100lであった（表-1）。また、原水も沪過水もともに陽性のデータセットから計算した除去率は1.9～3.0logの範囲にあって、平均値は2.5log（＝99.7％）であった（表-2）。この調査では滞留時間の考慮なしにサンプリングしているので必ずしも正確でない可能性もあるが、LeChevallier et al[17]の調査結果やNieminski and Ongerth[19]の調査・実験結果とほぼ一致していることからみて、除去実態をおおむね把握できていると考えられる。

このように、濁度管理を徹底して沪過水の濁度を確実に0.1度未満にしたとしても、標準的な凝集沈澱と砂沪過を組み合わせた急速沪過法では、平均的なクリプトスポリジウム除去は2.5log程度にとどまり、常時3 logを超える高い除去率の維持には、新たな運転管理方法の開発を待たなければならないのかもしれない。逆にいえば、汚染された原水の場合、沪過水の濁度管理が不十分になると、クリプトスポリジウム感染症の集団発生をもたらす危険性が高いことになる。

表-1 原水と沪過水のクリプトスポリジウム濃度

	原水（個/100l）	沪過水（個/100l）
陽性試料/検査試料数（陽性率）	13/13（100％）	9/26（35％）
陽性試料の幾何平均（濃度範囲）	40（16～150）	0.12（0.05～0.8）

表-2 急速砂沪過法におけるクリプトスポリジウムと濁度の除去率

	除去率(log)	除去率の範囲(log)	データ組数
オーシスト	2.47±0.36	1.88～3.00	8
濁　度	3.11±0.19	2.66～3.32	12

4-3 膜沪過法

近年、新たに開発・導入された精密沪過膜（MF膜）あるいは限外沪過膜（UF膜）を用いた膜沪過法は、その除濁の原理から見て、ほとんど完全なクリプトスポリジウム除去が期待できる処理技術と考えられている。実際、Jacangero et al[21]は水質の異なる3種類の表流水原水にジアルジアとクリプトスポリジウムを10^4～10^5個/l添加して数種類の精密沪過膜（公称孔径0.1～0.2 μm）と限外沪過膜（分画分子量100,000～500,000Da）の性能試験を行い、沪過水1 lからジアルジアもクリプトスポリジウムも検出されず、膜の種類や形状、原水の水質に関係なく6 log（＝99.9999％）の除去が期待できると報告している。筆者らも水道水にクリプトスポリジウムを添加してMF膜とUF膜のクリプトスポリジウム除去能を評価したが、MF膜（公称孔径0.25 μm）及びUF膜（分画分子量13,000Da）の両者とも7 log（＝99.99999％）かそれ以上の高率で除去できた[22]。一方、Drozd and Schwartzbrod[23]はクリプトスポリジウムを10^5個/l添加した河川水で管型セラミックMF膜（孔径0.2 μm）のクリプトスポリジウム除去能を調べ、平均で4.8log（＝99.998％）とよく除去できるものの、常時沪過水にクリプトスポリジウムが出現し、特に沪過の終期に高濃度でリークすることを認めており、リークの原因の可能性として、膜沪過装置の欠陥とオーシストの変形を挙げている。

MF膜でみられた極少量のリークの原因が膜そのものにあるのか、それとも膜の破断やシーリング部分からの漏れ等によるものかは不明で、なお議論の余地があるものの、現在水道で使用されているMF膜沪過やUF膜沪過による浄水処理システムは、完全ではないにしても、いずれも十分に高度なクリプトスポリジウム除去が期待できると見てよいであろう。U. S. EPA は、これらの問題点も含めた総合的な判断に基づいて膜沪過法によるクリプトスポリジウムオーシストの除去率を5 log程度とみなしている。膜や装置の工学的な信頼性を一層確実なものとすることができれば、実質上クリプトスポリジウムの完全除去が期待できる技術であることは間違いない。

つい最近になって、特殊なMF膜として大孔径膜（Micron-pore membrane）が開発された[24]。この膜は従来のMF膜に比べると孔径が2.0 μmとかなり大きいため超微細な懸濁粒子の除去はあまり期待できないが、濁度が0.5～1.0度程度以下であれば膜間差圧0.1～0.2kPa程度で5 m/dの沪過が可能で、しかも大孔径側の孔径分布が極めてシャープで3.2 μmポリスチレン標準粒子を実質上完全に（＞8 log（＝99.999999％））排除し、クリプトスポリジウムも6 logを超える高率で除去できるという特徴がある。除濁済みあるいは高度の除濁を必要としない原水に対するクリプトスポリジウム対策技術の一つとして期待される。なお、この大孔径膜の開発段階で、最大径が比較的大きい膜では Drozd and Schwartzbrod[23]の場合と同様、明らかなリークが生じた。しかし、膜を通過したクリプトスポリジウムは通常の遠心濃縮で壊れやすくなっていた。また、遠心後にも完全な形態を保持しているクリプトスポリジウムの脱嚢率が50％以下に低下していた例も少なくなかった。このことは、膜沪過プロセスでのクリプトスポリジウムのリークがあったとしてもその多くが膜を通過する過程で致命的な損傷を受けている可能性が高いことを示しており、興味深い。

5. 浄水処理におけるクリプトスポリジウムの制御

5-1 浄水のクリプトスポリジウム許容レベル

クリプトスポリジウム1個の感染確率が0.4％と非常に高く、ピーク排出量はヒトで10^9個/（人・日）、ウシで10^{10}個/（頭・日）にも達する[2]。そのうえ、塩素消毒では高率の殺滅や不活化が期待できない。浄水処理でクリプトスポリジウムはかなり除去できるものの完全ではないので、原水中に存在すればその極一部が浄水にリークすることは避けられない。となれば、浄水のクリプトスポリジウム許容濃度が必要となる。そこで、クリプトスポリジウムの Dose response model として次式(1)[25]が適用できるとし、生の水道水の飲水量を2 l、検出されたクリプトスポリジウムのすべてに感染性があると仮定して許容感染リスクと許容濃度の関係を計算した。結果を表-3に示す。いずれの場合も現実的には計測不能なレベルである。これとは別に、筆者らは、現時点までの科学的知見や食中毒の発生等の社会的背景などを考慮して許容感染リスクを10^{-2}/年、飲水量を1 l（飲水量実態調査に基づく95％値）とし、水道水の最大許容濃度（いかなるときにも超えてはならない濃度）として1個/100lを提案している[26]。

$$P_d = 1 - \exp(-rN) \quad \cdots\cdots(1)$$

$$P_y = 1 - (1 - P_d)^{365} \quad \cdots\cdots(2)$$

N：クリプトスポリジウム経口摂取量、r：パラメータ（238.604）、

P_d：1回（＝1日）当たりの感染確率、P_y：1年当たりの感染確率

表-3 水道水のクリプトスポリジウム許容濃度計算値

許容感染リスク P_y	許容濃度
10^{-4}/年	3.3個/100m³
10^{-3}/年	3.3個/10m³
10^{-2}/年	3.3個/m³

水道水に関する許容感染リスクの値としてどの値を選択すべきかは今後の議論に待たなければならないが、最も高い濃度である筆者等の試算結果を採用したとしても現行の試験方法ではほとんど検出されないレベルであり、それを常時定量的に把握することは不可能に近い。それゆえ、日常的には、浄水のクリプトスポリジウムを直接試験して管理するのではなく、何らかの代替的な手法で

評価・管理するのが妥当であろう。

5-2　浄水の濁度管理の必要性

水道によるクリプトスポリジウム感染症の集団発生の多くが、不幸にして原水の高度の汚染と浄水処理上の何らかの欠陥-特に除濁機能の低下-が重なったときに生じている。クリプトスポリジウム暫定対策指針[20]では、クリプトスポリジウム汚染のおそれのある原水を取水している水道に対して沪過水濁度0.1度以下を維持できるように施設や運転方法を改善するように求めている。この濁度0.1度以下という値は、クリプトスポリジウムやジアルジアなどの原虫汚染対策として沪過水濁度の管理を強化して0.5NTU以下としたがなお不十分であったため、これを0.1NTU以下としたという米国等の経験に基づくものである。濁度を除去すればするほど原虫のリスクが低下するという知見は、水道による多数の原虫感染症の集団発生という大きな犠牲を払って得た貴重な経験に裏打ちされており、高い信頼性があると考えてよいであろう。したがって、クリプトスポリジウムによる汚染のおそれの高い水源から原水を取水している水道では、可能な限り浄水処理施設の運転管理を注意深く行って当該浄水処理施設の除去能力を最大限発揮させることによって濁質の除去を図り、濁度0.1度以下を常時維持するように努めることがまずは肝要である。

5-3　代替指標による除去能力の評価

しかしながら、浄水の濁度を常時0.1度未満に維持しさえすればいついかなるときも絶対に安全であるとの保証はないのかもしれないという危惧が残る。特に原水が低濁度であってクリプトスポリジウム汚染レベルが高いような場合は特にそうである。仮に沪過水濁度を常時0.05度に維持できたとしても、たとえば原水濁度が5度の場合の濁度除去は2 log（=99%）にすぎないし、原水濁度1度の場合はわずか1 log（=90%）にしかならないので、原水が高度の汚染を受けている場合は結果として除去不十分となり、水道水の微生物的安全性が脅かされるおそれがあるからである。したがって、クリプトスポリジウムによる汚染のおそれの高い水源を利用する水道にあっては、濁度管理と同時に、原水の汚染状況と浄水システムの除去能力の双方を評価しておくのが望ましい。

(1)　原水の汚染レベル

最も単純かつ信頼性のある方法は、原水の水質検査の際にクリプトスポリジウム試験を行って定量的な濃度データを蓄積することであろう。試験水量は、クリプトスポリジウム濃度の定量値が得られる量であればよいが、汚染レベルの比較的低いところでは100lかそれ以上の水量が必要である。一定数（少なくとも10個）のデータが蓄積されたら、図-1に例示したような累積出現確率分布

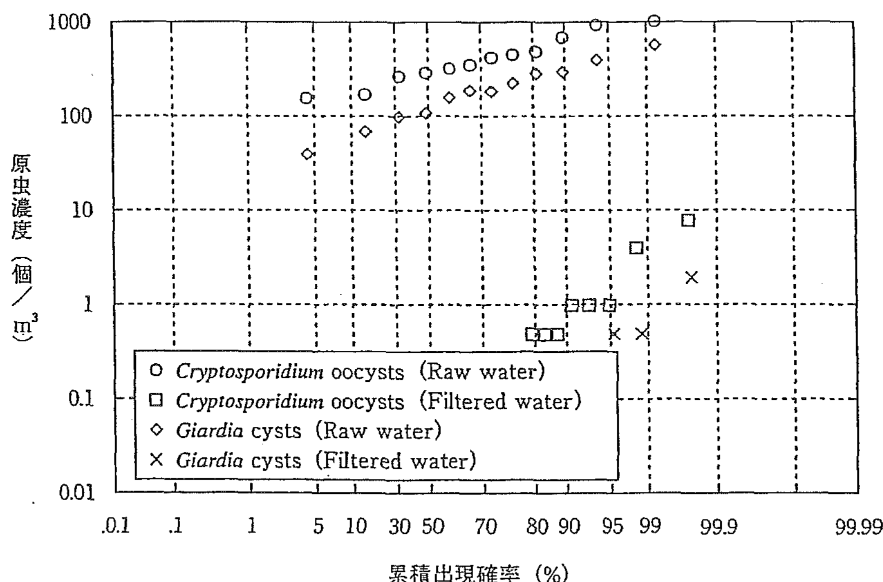

図-1　原水と沪過水におけるクリプトスポリジウムとジアルジアの出現濃度分布

図を描くとよい。このような図があると、たとえば累積濃度出現確率50％値、90％値、95％値などを容易に読み取ることができる。

(2)　実測値に基づく除去能力の推定

原水の場合と同様、浄水についても頻回にクリプトスポリジウムを実測して定量的な濃度データを入手し、原水と処理水の結果から除去能力を計算する方法が考えられる。しかし、たとえば急速砂沪過法であれば、前述したように2～3log レベルの除去が期待されるので、除去の定量値を算出するには、原水中の濃度が著しく高い場合を除いて、原水の試験水量の100倍ないし1,000倍もの水量で試験することが必要になる。比較的高度に汚染された原水であっても、沪過水の試験水量は m^3 レベル以上になる。不可能ではないが、余りにも多大の労力を必要とし高頻度の試験は困難である。となれば、日常的には代替指標で評価する以外にない。

(3)　代替除去指標による推定

クリプトスポリジウムは直径4～6 μm の懸濁粒子なので、沪過等によるクリプトスポリジウムの除去と、濁度や粒子の除去との間に一定の相関関係があることが予想される。そこで、原水と沪過水についてクリプトスポリジウムと濁質のデータを同時に取得してクリプトスポリジウム除去と濁質除去の関係を明らかにし、濁質除去データからクリプトスポリジウム除去を推定する方法が考えられる。この関係式については、LeChevallier and Norton[27]が急速沪過法を基本とする３ヵ所の浄水場で原虫の除去と濁度及び５ μm 以上の粒子の除去との関係を調べ、それぞれ次式を得ている（寄与率 r^2 は文献中の相関係数 r から筆者が計算）。

$$\log_{10}(Cr_{in}/Cr_{tr}) = 0.886 \cdot \log_{10}(T_{in}/T_{tr}) + 0.494 \quad \cdots(1)$$

$(r^2 = 0.59)$

$$\log_{10}(Cr_{in}/Cr_{tr}) = 0.660 \cdot \log_{10}(P_{>5\,in}/P_{>5\,tr}) + 0.488 \quad \cdots(2)$$

$(r^2 = 0.69)$

Cr：クリプトスポリジウム濃度、T：濁度、$P_{>5}$：５ μm 粒子濃度。

添字 in は原水、tr は処理水を表す。

Nieminski and Ongerth[19]は不活化したクリプトスポリジウムを河川原水に添加してパイロットプラント実験を行い、クリプトスポリジウムの除去と濁度の除去、クリプトスポリジウム除去と4～7 μm 径粒子の除去との間に有意な相関を認め、回帰式として次式を得ている。

$$\log_{10}(Cr_{in}/Cr_{tr}) = 0.9631 \cdot \log_{10}(T_{in}/T_{tr}) + 1.1009 \quad \cdots(3)$$

$(r^2 = 0.55)$

$$\log_{10}(Cr_{in}/Cr_{tr}) = 0.8856 \cdot \log_{10}(P_{4\text{-}7\,in}/P_{4\text{-}7\,tr}) + 0.4647 \quad \cdots(4)$$

$(r^2 = 0.79)$

$P_{4\text{-}7}$：径4－7 μm の粒子数。

また、Li et al[28]は、特殊な沪過方法ではあるが、Bag filter で沪過したときクリプトスポリジウム除去と4～6 μm ポリスチレン粒子の除去との間に傾きが１の完全な直線関係が得られたとし、クリプトスポリジウムの除去性を4～6 μm ポリスチレン粒子を用いて代替評価できるとしている。

このように、クリプトスポリジウムは浄水処理過程において水中の他の懸濁粒子とよく似た挙動を示すことから、クリプトスポリジウム除去の代替評価指標としては濁質除去と粒子除去が有力な候補である。

a）濁度除去による評価

原水と浄水の濁度を測定して濁度の除去率を計算し、その値からクリプトスポリジウム除去率を推定する方法である。水道は長年にわたる原水や浄水の濁度計測の経験があり、取り入れやすい。また、クリプトスポリジウム問題が発生して以来、超低レベルの濁度を測定できる濁度計が開発・実用化され、濁度の連続監視も可能な点からも有効な代替法といえよう。ただ、超低濁度では計測方式によって濁度の値が異なり、特に0.1度程度以下ではかなり異なるという問題がある。このため、どの計測方式の濁度計を採用すれば精度の高いクリプトスポリジウム除去の推定ができるのかは必ずしも明らかになっていない。

概算としては、濁度除去とクリプトスポリジウ

ム除去が同じとするか、あるいは安全を見込んでクリプトスポリジウム除去は濁度除去の0.8〜0.9掛け程度と見るのが妥当であろう。ただし、超低濁度のデータから計算する場合は推定値が過大になっていないかどうか、慎重なチェックが望まれる。

b）粒子除去による評価

これまでの知見では、クリプトスポリジウム除去は濁度除去よりも粒子除去との間により高い相関が認められているので、粒子除去から推定する方法がより有力な候補といえる。代替指標とする粒子のサイズは、Nieminski and Ongerth[19]のやLi et al[28]の結果に基づいて、とりあえずは4〜7μm辺りの粒子径範囲を選択し、粒子除去からクリプトスポリジウム除去への換算は、濁度の場合と同様、粒子除去とクリプトスポリジウム除去が同じとするか、あるいは安全を見込んで粒子除去の0.8〜0.9掛け程度と見ておくのが無難であろう。

(4) 水道水の安全性の評価

原水の推定最大汚染レベルのクリプトスポリジウムが常時原水に含まれているとし、現行の浄水システムにおける濁度あるいは粒子の除去成績から推定されるクリプトスポリジウム除去率を適用して、沪過水中の濃度を推定し、所定のレベルを超えないかどうかを判定する。許容値として認知された具体的な数値はまだないので判定に困るが、私見としては社会的に検知しうる集団感染は生じないと思われる常時1個/100l未満が一応の目安であろう。

a）浄水のクリプトスポリジウム濃度が所定のレベルを超えないと判定された場合

当該水道の浄水方法並びに管理方法はおおむね妥当であり、供給する水道水は安全と判断することができよう。したがって、ミスの発生防止に努め、濁度あるいは粒子の管理を継続的に注意深く行えばよい。

b）浄水のクリプトスポリジウム濃度が所定のレベルを超えると判定された場合

この場合、2つのケースが考えられる。1つは、濁度除去あるいは粒子除去が不十分な場合である。この場合は、浄水プロセスの運転管理方法を改良・改善するなどして濁質除去能力を高める努力が必要である。改善が達成できれば、あとはそれを常時維持するための努力を払えばよい。

もう一つは、濁質除去は十分なされているが、原水の推定最大汚染レベルが高いために十分な安全性が保証できない場合である。この場合、放置すれば社会的責務を果たせないので、水源の変更、汚染源対策の実施、浄水プロセスの付加あるいは更新などを含む恒久的対策が必要となろう。

7. おわりに

先人たちの努力によって明治時代に始まった水道施設の布設が飲料水を介した水系感染症の防止に大きく貢献してきた。特に、従来型の緩速沪過法や急速沪過法は、塩素消毒と相俟って、細菌感染症の防止に多大な貢献をしてきた。しかし残念なことに、塩素の効かない病原体―特に、原虫クリプトスポリジウム―が今、水道水の安全性を大きく揺るがし、克服しなければならない新たな難問を水道界に課している。本稿では急速砂沪過法に代表される既存の浄水システムにおけるクリプトスポリジウム問題への対応策として濁度と粒子数の管理について述べたが、もう一つの観点として、不活化による対応も重要である。現在、紫外線がクリプトスポリジウムやジアルジアの不活化に極めて有効であることを示す証拠が次々と出てきており、大きな期待が寄せられている。わが国の水道の歴史も既に100年を超え、水道施設の更新時期に来ている今、クリプトスポリジウム問題を水道界が新たな展開を図る好機と捕らえ、21世紀の主要社会基盤施設として更に発展することを期待する。

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