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Sources and Fate of *Giardia* Cysts and *Cryptosporidium* Oocysts in Surface Waters[1]

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ABSTRACT

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A literature review was conducted to evaluate the mechanisms mediating the fate of *Giardia lamblia* cysts and *Cryptosporidium* sp. oocysts in surface waters, particularly in lakes and reservoirs. Emphasis was placed on quantification of source and sink terms as applied in mass balance models. The literature review results indicated that cysts and oocysts [referred to collectively as (oo)cysts] are commonly detected over a wide range of concentrations in a wide variety of aquatic systems. Humans and other animals are considered to be the sources of (oo)cysts introduced to aquatic systems. Most studies included some measure of (oo)cyst viability but not necessarily infectivity. Sedimentation was identified as an important loss mechanism for (oo)cysts in lakes and reservoirs. There were general indications that ambient irradiation or pH levels would have little effect on (oo)cyst viability or infectivity, while temperature, drying, and redox levels may have more varying effects. The (oo)cysts would be expected to remain viable for longer periods than for fecal bacteria in similar circumstances. Kinetic submodels (as associated coefficients) required to quantify these phenomena are generally unavailable.

Key Words: *Cryptosporidium, Giardia,* oocysts, cysts, surface water, reservoir.

An extensive search of the literature was conducted in order to obtain information about the sources and fate of *Giardia lamblia* cysts and *Cryptosporidium* sp. oocysts in surface waters. Interest in modeling, management, and treatment for protection from protozoan pathogens has developed in response to an increased incidence in waterborne disease related to these organisms. The review seeks to obtain quantitative information on the sources and environmentally-mediated fate of cysts and oocysts and to identify values for selected kinetic

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coefficients which may be used in models describing pathogen fate and transport (cf. Auer et al. 1998). As a case study, information on protozoan pathogens in Cannonsville Reservoir (New York) and its watershed is presented and reviewed.

Characteristics of *Giardia* and *Cryptosporidium*

Giardia and *Cryptosporidium* are protozoa carried in the gastrointestinal tract of many animals and are pathogenic for humans, causing gastrointestinal disorders. Several species of *Giardia* and *Cryptosporidium* have been identified, with *Giardia lamblia* (synonym: *duodenalis*) and *Cryptosporidium parvum* appearing to be the species responsible for most cases of disease in humans (Hibler and Hancock 1990, Rose 1990). These protozoa are known to infect a wide variety of other animals, including cattle, dogs, beaver, rats, otter, muskrats, rabbits, deer, and coyotes (e.g., Hansen and Ongerth 1991, Hibler and Hancock 1990, Isaac-Renton et al. 1992, Ong et al. 1996, Ongerth et al. 1995, Roach et al. 1993, Rose 1990, Webster and MacDonald 1995). Cross-transmission of *Giardia* and *Cryptosporidium* between humans and other animals has been reported (as reviewed by Hibler and Hancock 1990, Rose 1990).

Both species produce resistant, reproductive forms that may be transmitted via the fecal-oral route, by direct contact with contaminated fecal material or by ingestion of contaminated food or water. These forms represent the infective stages and are termed cysts (produced due to asexual reproduction) for *Giardia* and oocysts (produced due to sexual reproduction) for *Cryptosporidium*. For purposes of this discussion, the term (oo)cysts will be used for both the *Giardia* cysts and *Cryptosporidium* oocysts. Ingestion of drinking water containing (oo)cysts has been the reported cause of many waterborne outbreaks of giardiasis or cryptosporidiosis (Bridgman et al. 1995, Colbourne 1989, Craun 1988, Fox and Lytle 1993, Pett et al. 1993, and as reviewed by Badenoch 1990, Hibler and Hancock 1990, Lisle and Rose 1995, Roach et al. 1993, Rose 1990, Solo-Gabriele and Neumeister 1997). (Oo)cysts are considered to have high probabilities of infection, whether from exposure to one (oo)cyst or ingestion of one glass of contaminated water (Medema et al. 1995, Rose et al. 1991b, 1995). Dupont et al. (1995) reported the median infective dose for *C. parvum* in healthy adults to be 132 oocysts. The ovoid *Giardia lamblia* cysts are approximately 8 to 14 μm in diameter and the typically spherical *C. parvum* oocysts are approximately 4 to 6 μm in diameter. As will be described in more detail below, the (oo)cysts have relatively high levels of resistance to many environmental parameters as well as to many water disinfection/treatment procedures.

Presence of Cysts and Oocysts in Surface Waters

Many studies have been conducted over the past several decades on the incidence of *Giardia* cysts and *Cryptosporidium* oocysts in surface and drinking waters as well as in sewage. A listing of some of these studies is presented in Table 1, along with the methods used for detection and viability/infectivity determinations.

One of the uncertainties in interpreting the (oo)cyst detection data, particularly for use in risk assessment and modeling efforts, is whether or not the (oo)cysts are viable and, more importantly, still infective. The studies noted in Table 1 used the same general type of sample concentration method (filter cartridge or membrane filter) followed by an immunofluorescence detection technique, similar to "standard" concentration and detection methods (APHA 1994, ASTM 1992). This is the method that has been approved by EPA for the Information Collection Rule (ICR), which is part of the Safe Drinking Water Act regulations (Fout et al. 1996). As reviewed in several recent articles (Hoffman et al. 1997, Jakubowski et al. 1996, Klonicki et al. 1997), studies are being conducted on a wide variety of improvements on methods for (oo)cyst concentration, recovery, and detection, as well as for determinations of viability and infectivity. In general, the presence of (oo)cysts is considered presumptive based on immunofluorescence, size, and shape. It should be noted that detection and confirmation offer little information on the viability or infectivity of the (oo)cysts. However, intuitively, presumed (oo)cysts that can not be confirmed should be less likely to be viable.

The abundance of (oo)cysts in surface waters, treated drinking water, and sewage varies widely, in some cases as much as 100-fold for a single sample source (Table 2). It has been concluded overall that (oo)cysts are quite common in surface waters, albeit often at very low levels (e.g., LeChevallier 1992, LeChevallier and Norton 1995, Rose 1990, Wallis et al. 1996). Significant correlations between presence of cysts and presence of oocysts have typically been reported for the studies listed in Table 2. The studies by LeChevallier et al. (1991a,b) indicated that a high percentage of the detected (oo)cysts were nonviable (based on morphologic observations). Some of the studies have indicated that surface water (oo)cyst

Table 1.–Survey of literature on detection of *Giardia* cysts and *Cryptosporidium* oocysts in water samples.

Reference	Pathogen Studied		Source of Water Sample[a]					Method for		
	Giardia	*Cryptosporidium*	SW	DW	SG	RO	GW	Concentration	Detection	Viability
Glicker and Edwards (1991)	yes	no	yes	yes	no	no	no	PFC; PMF[b]	IF[c]	NC[d]
Hansen and Ongerth (1991)	yes	no	yes	no	no	yes	no	PMF	IF	NC
LeChevallier and Norton (1992,1995)	yes	yes	yes	yes	no	no	no	PFC	IF	morphology
LeChevallier et al. (1991a)	yes	yes	no	yes	no	no	no	PFC	IF	morphology
LeChevallier et al. (1991b)	yes	yes	yes	no	no	no	no	PFC	IF	morphology
LeChevallier et al. (1997)	yes	yes	no	yes	no	no	no	PFC	IF	morphology
Madore et al. (1987)	no	yes	yes	no	yes	no	no	PFC	IF	NC
Ong et al. (1996)	yes	yes	yes	no	no	no	no	PFC	IF	bioassay (*Giardia*)
Ongerth et al. (1995)	yes	no	yes	no	no	no	no	orlon filter cartridge, fecal examination	IF	NC
Roach et al. (1993)	yes	yes	yes	yes	no	no	no	PFC	IF	NC
Rose (1988)	no	yes	yes	yes	yes	no	no	PFC; PMF	IF	bioassay or excystation
Rose et al. (1991a)	yes	yes	yes	yes	no	no	yes	PFC	IF	NC
Stern (1996a)	yes	yes	yes	no	yes	yes	no	PFC	IF	NC
Suk et al. (1987)	yes	no	yes	no	no	no	no	Balston fiber/ epoxy filter	IF	NC
Todd et al. (1991)	yes	yes	yes	no	no	no	no	PFC	IF	NC
Wallis et al. (1996)	yes	yes	yes	yes	yes	no	no	PFC	IF	dye exclusion, bioassay

[a] SW = surface water; DW = drinking water; SG = sewage; RO = agricultural runoff; and GW = groundwater.

[b] PFC - polypropylene filter cartridge; PMF = polycarbonate membrane filter.

[c] IF = immunofluorescence.

[d] NC = not conducted.

Table 2.–Survey of reported levels of *Giardia* cysts and *Cryptosporidium* oocysts detected in water samples.

Reference	Pathogen Studied		Source of Sample[a]	Levels Detected (cysts or oocysts · L^{-1})	
	Giardia	*Cryptosporidium*		*Giardia*	*Cryptosporidium*
Glicker and Edwards (1991)	yes	no	Raw water supply (69)	0.0034 - 0.0277	NA[b]
Hansen and Ongerth (1991)	no	yes	Watersheds		
			– High use	NA	18.2[c]
			– Low use	NA	0.2 - 2.4[c]
LeChevallier and Norton (1992)	yes	yes	Raw surface water		
			– High turbidity/pollution	1.13 - 31.1 (9.1)[c]	0.82 - 71.9 (4.8)[c]
			– Moderate turbidity/pollution	0.14 - 64.2 (5.8)[c]	0.42 - 5.1 (2.5)[c]
			– Low turbidity/pollution	2.16 - 3.76 (2.9)[c]	0.77 - 8.7 (2.5)[c]
LeChevallier and Norton (1995)	yes	yes	Raw water supply	0.02 - 43.8 (2.0)[c]	0.065 - 65.1 (2.4)[c]
			Finished water	0.98 - 9.0 (2.6)[c]	0.29 - 57 (3.3)[c]
LeChevallier et al. (1991a)	yes	yes	Filtered drinking water (82)	0.0029 - 0.64	0.0013 - 0.48
LeChevallier et al. (1991b)	yes	yes	Surface water (85)	0.04 - 66.0	0.07 - 484.0
LeChevallier et al. (1997)	yes	yes	Finished water reservoirs		
			– Inlet	0.7 - 24 (1.9)[c]	0.7 - 2.4 (1.2)[c]
			– Outlet	1.2 - 107 (6.1)[c]	1.7 - 31 (8.1)[c]
Madore et al. (1987)	no	yes	Raw sewage (4)	NA	850 - 13.700
			Treated effluent (11)	NA	140 - 3,960
			Surface water (6)	NA	2.0 - 5,800
Medema et al. (1995)	no	yes	Surface water (14)	NA	0.31[d]
Ong et al. (1996)	yes	yes	Watersheds[e]		
			– Above cattle ranch (32)	0.0005 - 0.0344	0.0003 - 0.0492
			– Below cattle ranch (30)	0.0006 - 0.0429	0.0014 - 0.300
			– Drinking water intake (27)	0.0046 - 1.880	0.0017 - 0.0443
Ongerth et al. (1995)	yes	no	Watersheds		
			– High use	0.01 - 0.15	NA
			– Low use	0.01 - 0.05	

Table 2.–(Continued)

Reference	Pathogen Studied		Source of Sample[a]	Levels Detected (cysts or oocysts · L^{-1})	
	Giardia	*Cryptosporidium*		*Giardia*	*Cryptosporidium*
Pett et al. (1993)	yes	yes	Surface water (20)	3.19[c]	2.18[c]
Roach et al. 1993)	yes	yes	Drinking water (22)	0.1 - 1.4	0.2 - 0.5
			Raw sewage (8 sites)	26 - 3,022	0 - 74
			Treated effuent (5 sites)	2 - 3,511	0 - 333
Rose (1988)	no	yes	Surface water (90)	NA	0.91 - 0.94[c]
			Raw sewage (11)	NA	28.4[c]
			Treated sewage (22)	NA	17[c]
			Drinking water (14)	NA	0.001 - 0.006[c]
Rose et al. (1991a)	yes	yes	Potable water supplies (257)	0.03[c]	0.43[c]
Stern (1996a)	yes	yes	Surface water (1363)	0.0118[c]	0.0083[c]
			Source water (323)	0.0032[c]	0.0050[c]
			Treated Sewage (206)	3.0758[c]	0.0064[c]
Suk et al. (1987)	yes	no	Surface water (78)	0 - 0.11	NA
Todd et al. (1991)	yes	yes	Surface water (13)	0 - 312	0 - 18.5
Wallis et al. (1996)	yes	yes	Surface water (1,173)	most < 0.002	most < 0.005
			Drinking water (423)	most < 0.002	most < 0.005
			Raw sewage (164)	most < 1,000	1 - 120

[a]Number of samples in parentheses.

[b]NA = not applicable

[c]Mean value

[d]Median value; samples collected, processed, and analyzed using the UK (SCA) standard method.

[e]Partial results from one of two watersheds; entire study included 249 samples analyzed from 12 sites.

abundance, as well as retention of viability, may vary seasonally (Hibler and Hancock 1990, LeChevallier et al. 1991b, Ongerth 1989, Rose et al. 1991a, Wallis et al. 1996). Higher levels and/or greater viabilties are usually, but not always, found from fall to spring. Storm events have also been proposed to affect changes in levels of oocysts, with more land run-off during wet periods (Hansen and Ongerth 1991); related changes in *Giardia* cyst levels might also be expected to occur. The New York City Department of Environmental Protection has reported higher concentrations of (oo) cysts during storm events (Stern 1996c).

LeChevallier and Norton (1995) published a comprehensive review of their North American data base for the incidence of (oo) cysts in surface water samples. From 1988 to 1992, 347 surface water samples from 72 sites were examined for cyst and oocyst levels using essentially the same recovery and detection techniques; individual sites were sampled from 1 to 29 times. The geometric means were 2.0 cysts/L and 2.4 oocysts/L for *Giardia* and *Cryptosporidium*, respectively. (Oo) cyst detections were less frequent in the last two years of the study (included in the 1995 review) than in earlier reports containing the data from the first two years (LeChevallier et al. 1991a, b): 54% vs. 81% for cysts and 60% vs. 87% for oocysts. Either cysts or oocysts were detected in 70% and 97% of the samples included in the 1995 and 1991 reports, respectively. LeChevallier and Norton (1995) noted that the lower detection rates are comparable to those reported from other studies (e.g., Hansen and Ongerth 1991, Ongerth 1989, Rose 1988, Suk et al. 1987, Todd et al. 1991). They suggested that the rate of detection of (oo) cysts could reflect a consistent reduction in incidence, but may also be part of cyclical, multi-year variations in levels.

The NYC DEP started monitoring for (oo) cysts within its source water in 1992 (Ashendorff et al. 1997). A more comprehensive program that included monitoring 50 sites within its water supply watersheds, including the watershed reservoirs, started in 1993 (Stern 1996a,b). The ranges of cysts detected in 2,806 surface water, source water, and treated sewage samples (Stern 1996a) are reported in Table 2 and are typically at the lower end of the ranges of values presented in this table. The NYC DEP has also obtained information on presumed (oo) cyst presence in urban, agricultural, and undisturbed watershed samples as well as in treated sewage samples (Stern 1996b). Based on 354 samples, cysts were detected in 26% to 41.5% of the samples and oocysts were detected in 9.6% to 37.2% of the samples, with the higher incidence in urban watershed samples. These presumed (oo) cyst detection frequencies are lower than those reported by LeChevallier and Norton (1995).

As part of this extensive study, the NYC DEP has also been monitoring the water entering and leaving Cannonsville Reservoir for (oo) cysts. As shown in Fig. 1, sampling sites are located on the major tributary to the reservoir (the West Branch of the Delaware River, designated as WDBN), an elevation tap at the intake for the West Delaware Outlet Tunnel (designated as CRR2), and the discharge of the West Delaware Outlet Tunnel (designated as WDTO). Sampling occurs on a biweekly to monthly frequency. When the reservoir is online (supplying water to the NYC water supply), sampling of the effluent of the reservoir occurs at the discharge of the West Delaware Outlet Tunnel. Otherwise, the effluent of the reservoir is measured at an elevation tap.

The resulting database for Cannonsville Reservoir includes 95 samples collected over 4 years. Over the sampling period, seasonality has not been observed at these sites. Based on an initial review of the data, there is less detection of *Giardia* cysts leaving the reservoir than entering. The total *Giardia* cyst detection level for water entering the reservoir was 49.2% compared with 38.2% leaving the reservoir. The total *Cryptosporidium* oocyst detection level did not indicate such a reduction. The total *Cryptosporidium* oocyst detection level entering the reservoir was 14.8% compared with 29.4% leaving the reservoir. A similar pattern was observed with the average concentration of (oo) cysts entering and leaving the reservoir. [Note: a less than detection limit value was treated as zero; the average detection limit was 1 (oo) cyst per 100L.] The average total *Giardia* cyst concentration entering the reservoir was 2.577 cysts per 100L compared with 2.359 cysts per 100L leaving the reservoir. The average total *Cryptosporidium* oocyst concentration entering the reservoir was 0.547 oocysts per 100L compared with 0.576 oocysts per 100L leaving the reservoir. However, one should be cautious to draw conclusions on these summary data as the sample values were combined without consideration as to whether the same slug of water was sampled at the outflow as was sampled at the inflow.

In a recent study of six open finished water reservoirs in New Jersey, LeChevallier et al. (1997) reported an increase in the levels of both cysts and oocysts following reservoir storage. (Oo) cysts were detected in 15% of the inlet samples and 25% of the outlet samples; the data reported in Table 2 represent values only for samples found to contain (oo) cysts. Using morphological observations, over 85% of the (oo) cysts were considered to be probably nonviable. The authors speculated that the nonpoint source contamination of the finished water was indirectly or directly related to wild animals in and around the reservoirs.

Many studies have been conducted on possible sources for the (oo) cysts found in surface waters in general. LeChevallier (1992) noted that increases in the abundance of *Giardia* cysts appeared to be related

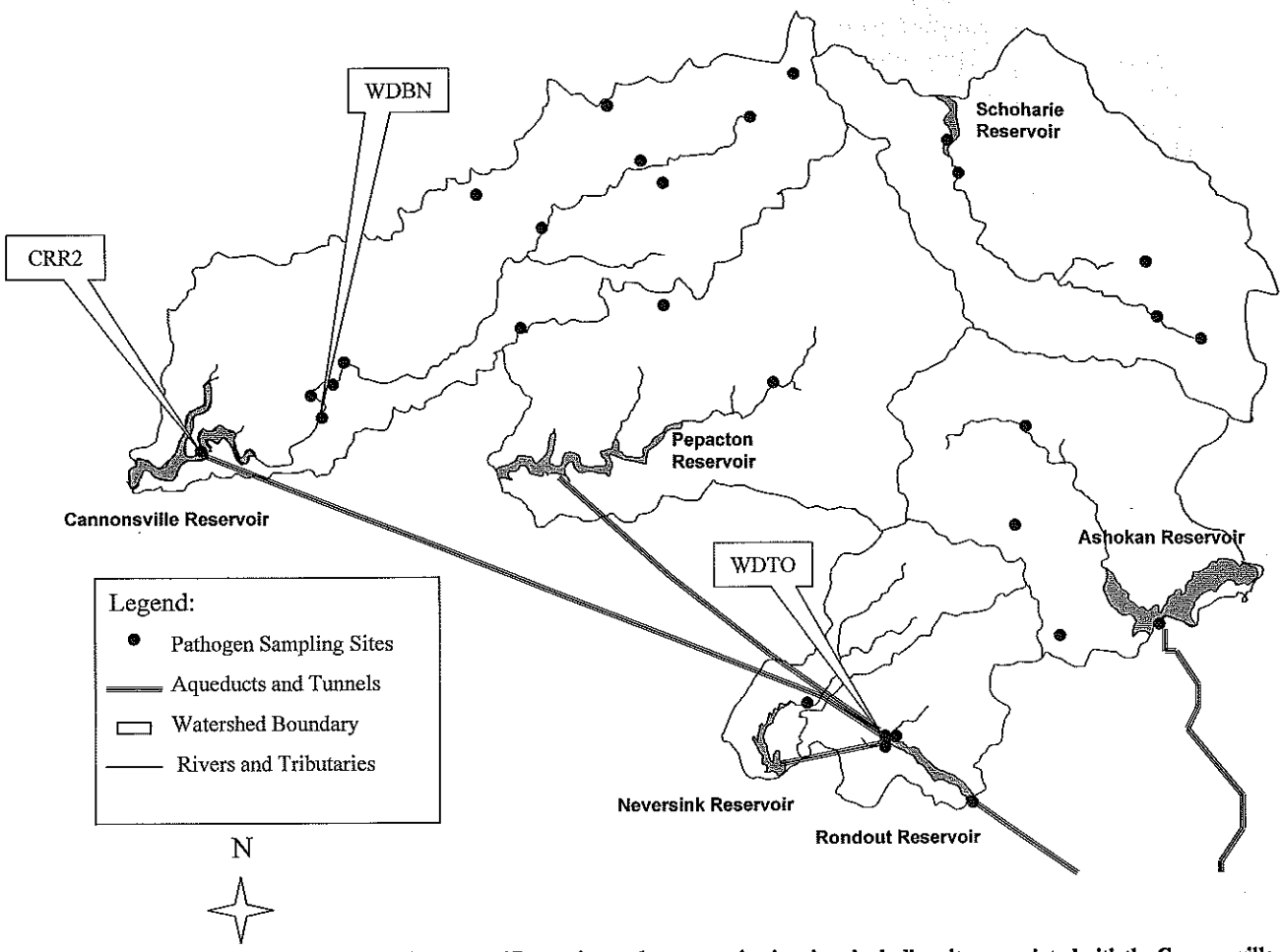

Figure 1.–New York City Department of Environmental Protection pathogen monitoring sites, including sites associated with the Cannonsville Reservoir and watershed.

to inputs of sewage or human fecal material, while increases in the abundance of *Cryptosporidium* oocysts appeared to be associated with nonpoint source inputs, such as land runoff and animal fecal material. NYC DEP has also reported that (oo)cysts are most commonly detected in effluent from wastewater treatment plants, followed in frequency by discharges from urban, agricultural, and undisturbed watersheds (Stern 1996c).

A possible relationship between various indicators of surface water quality and the presence of (oo)cysts has been discussed in several studies. In some cases, no relationship has been noted between levels of total coliform (TC) bacteria, fecal coliform (fc) bacteria, or turbidity and (oo)cyst levels (Chauret et al. 1995a, Rose 1988, Rose et al. 1991a, Willey et al. 1986). However, others (LeChevallier et al. 1991b, LeChevallier and Norton 1992) have reported significant relationships between TC bacteria, FC bacteria, and turbidity and (oo)cyst levels. They attributed the difference in findings to the fact that their studies examined a wider variety of source waters with more samples having higher turbidity and TC and FC bacteria levels, indicating increased pollution and an increased probability of (oo)cyst presence. Using multiple linear regression techniques, LeChevallier et al. (1991b) were able to assign 49.1% of the variation in *Giardia* cyst levels to variations in TC bacteria and turbidity levels and the level of watershed protection (an estimate of the amount of pollution in the watershed). In a like manner, 51.9% of the variation in *Cryptosporidium* oocyst levels could be explained by the type of surface water, TC bacteria levels, water temperature and pH, turbidity, and level of watershed protection.

Environmental Fate of Cysts and Oocysts

From a water supply perspective, the key difficulty in dealing with the *Giardia* cysts and *Cryptosporidium* oocysts is that they are, by nature, much more resistant to changes in environmental conditions than are the

vegetative forms. This, coupled with uncertainty in determining (oo)cyst viability and infectivity, makes prediction of the fate of these protozoan pathogens in natural systems difficult. Factors potentially lowering the levels of viable (oo)cysts in surface waters include temperature, pH, solar radiation, and predation.

A survey of studies examining the effects of solar radiation, pH, and temperature on (oo)cyst inactivation, along with the methods applied in these studies for enumeration and viability determinations, is presented in Table 3. More detailed results relating environmental factors and (oo)cyst viability are detailed in Table 4. Unlike bacteria, such as the TC and FC studied by Auer and Niehaus (1993), solar radiation effects on (oo)cysts may be minimal to nonexistent. Carrington and Ransome (1994) reported that continuous exposure to daylight conditions (pH 7.0; 10 °C) for up to 2 months had virtually no effect on oocyst viability. Lorenzo-Lorenzo et al. (1993) determined that 150 minutes of exposure to ultraviolet (UV) radiation (15,000 $mW \cdot sec^{-1}$) was required to completely eliminate oocyst infectivity. Chauret et al. (1995b) also found no difference in oocyst viability with exposure to sunlight (as compared to dark conditions); black light was found to be more effective than UV radiation in affecting survival (Table 4). In general, a pH of less than 4 (and close to 2.0) is optimum for (oo)cyst excystation (Meyer 1979, Carrington and Ransome 1994). Studies on the effect of pH alone indicated no relationship between pH (≥4) and changes in excystation levels (Carrington and Ransome 1994, Chauret et al. 1995b, deRegnier et al. 1989, Fayer and Leek 1994). However, pH may be a significant factor in combination with other environmental parameters (Chauret et al. 1995b, LeChevallier et al. 1991b). In their review of factors influencing TC and FC mortality, Chamberlin and Mitchell (1978) classified predation as a minor factor. Badenoch (1990) suggested that predation would not be expected to be a significant removal mechanism for (oo)cysts.

Temperature is the major environmental factor affecting (oo)cyst viability in surface waters. Numerous lab and field studies (see Tables 3 and 4) have shown that viability decreases with increasing temperature. As indicated in Table 4, (oo)cysts retain viability longer at temperatures from 0 to 10°C than at 20 to 30°C; (oo)cysts most readily excyst, producing more vulnerable vegetative forms, at 37°C. Temperatures above 50 to 60°C caused rapid decreases in (oo)cyst viability and infectivity. Freezing (-13 to -70 °C) typically produced the most rapid loss of viability and infectivity, although Robertson et al. (1992) reported that some oocysts remained viable up to 775 hours after slow, rather than snap, freezing (i.e., freezing in a refrigerator set at -22 °C rather than immersion in liquid nitrogen). Fayer and Nerad (1996) also found that oocysts retained infectivity longer at higher freezing temperatures (e.g., -10 °C). Repeated freezing and thawing, as might occur in many surface water systems, reduced viability faster than freezing alone for both cysts and oocysts (Carrington and Ransome 1994, Meyer 1979). (Oo)cyst levels and viability and surface water temperatures appear to be highly correlated, with higher (oo)cyst levels and viabilities found at lower temperatures (deRegnier et al. 1989, LeChevallier et al. 1991b, LeChevallier and Norton 1992). Temperatures nearer 37°C appear to increase (oo)cyst permeability and make them not only more likely to excyst but also to be more sensitive to other environmental factors.

Several studies have examined the *in situ* survival of (oo)cysts. Hansen and Ongerth (1991) reported that *Giardia* cysts suspended in a lake (at two depths) or a river retained viability for 1 to 2 months compared to only 14 days in tap water. Of all the water quality data examined, including dissolved oxygen, ammonia, nitrate, phosphorus, turbidity, and pH, only lower temperature (<10 °C) was significantly correlated with longer viability. In a series of studies conducted over a nearly 1-year period, Carrington and Ransome (1994) observed the levels and retention of viability of *Cryptosporidium* oocysts when suspended in ponds; environmental parameters such as light, temperature, pH, and dissolved oxygen content were monitored over the course of the study. Oocyst levels and viability were higher for studies extending from November to April (22 week study) than for those extending from April to January (six studies varying from 9 to 31 weeks). The authors attributed the longer winter survival to lower temperatures and/or light intensities, although differences in pH and dissolved oxygen levels were also noted. In a study of a more limited scope, Robertson et al. (1992) found that oocysts from one *Cryptosporidium* isolate retained viability longer in river water than in tap water (89% vs. 96% inactivated after 176 days, respectively), while there was no difference in viability for a second isolate (99% inactivated after 176 days). No measurements were made of any environmental parameters, but the results do indicate the potential for different oocyst responses to environmental conditions.

The effects of factors such as desiccation, redox levels, and association with feces from warm-blooded animals on (oo)cysts have also been examined. Kasprzak et al. (1980) reported that about 80% and 100% of detected *Giardia* cysts were apparently inactivated after 1 and 24 hours, respectively, of air-drying at 24 °C. In a similar study with *Cryptosporidium* oocysts, Robertson *et al.* (1992) found that 97% and 100% of the oocysts were inactivated after 2 and 4 hours, respectively, of air-drying at 24 °C. Fayer and Leek (1984) tested for the

Table 3.–Survey of literature on environmental factors affecting *Giardia* cysts and *Cryptosporidium* oocysts.

Reference	Pathogen Studied		Environmental Factor			Viability Test Method	Test Site	
	Giardia	*Cryptosporidium*	Light	Temp	pH		Field	Lab
Carrington and Ransome (1994)	no	yes	yes	yes	yes	Dye exclusion/ inclusion	yes	yes
Chauret et al. (1995)	No	yes	yes	yes	yes	Excystation	yes	no
deRegnier et al. (1989)	yes	no	no	yes	yes	NC[a]	yes	yes
Fayer (1994)	no	yes	no	yes	no	Bioassay	no	yes
Fayer and Leek (1984)	no	yes	no	yes	yes	Excystation	no	yes
Fayer and Nerad (1996)	no	yes	no	yes	no	Bioassay	no	yes
Kasprzak et al. (1980)	yes	no	no	yes	no	Dye exclusion	no	yes
Meyer (1979)	yes	no	no	yes	yes	Excystation	no	yes
Robertson et al. (1992)	no	yes	no	yes	no	Excystation, dye exclusion	yes	yes
Yozwiak et al. (1993)	no	yes	no	yes	no	Excystation, mouse infectivity	no	yes

[a] NC = not conducted.

Table 4.–Survey of reported effects of environmental factors on *Giardia* cysts and *Cryptosporidium* oocysts.

Reference	Pathogen Studied		Environmental Factor Studied			Effect
	Giardia	*Cryptosporidium*	Light	pH	Temperature (°C)	
Carrington and Ransome (1994)[a]	no	yes	950 lux (continuous daylight)	4, 7, 10	-25, 4, 10, 20, 30	No effect of light Little effect of pH (≥ 14 wks) Viability greatest at 4 and 10 °C; fastest inactivation at -25°C
Chauret et al. (1995b)	No	yes	sunlight, black light/UV (~500 ($W cm^{-2}$)	5, 7, 9	4, 20	No effect of temperature or pH Little effect of sunlight 20% (UV) and 0% (black light) excystation after 54 days
deRegnier et al. (1989)	yes	no	NA[b]	5.0 - 8.4	8 - 37	Viability greater at lower temperatures No effect of pH
Fayer (1994)	no	yes	NA	NA	72.4 (for 1 min.) 64.2 (for 5 min.)	Infectivity lost Infectivity lost
Fayer and Leek (1984)	no	yes	NA	6.0, 7.6	20, 37	38% (at pH 6.0) and 54% (at pH 7.6) excystation 8.0% (at 20 °C) and 91.3% (at 37°C) excystation
Fayer and Nerad (1996)	no	yes	NA	NA	5, -10, -15, -20, -70	Infectivity highest at 5 and -10 °C no infectivity loss after 168 hrs) Infectivity lowest at -70 °C (all infectivity lost after 1 hr)
Kasprzak et al. (1980)	yes	no	NA	NA	-20, 4, 24, 37, 50	50% viable at 4 °C (after 18 wks) and 24 °C (after 7 wks) 0% viable at -20 °C (after 24 hrs), 37 °C (after 3 wks), and 50 °C (after 12 hrs)
Meyer (1979)[d]	yes	no	NA	0.5, 2, 4, 6.2	-13, 8, 21, 37	Optimum excystation - pHs 0.5 and 2; little at pHs 4 and 6.2 Cyst viability retained up to 77 (8 °C), 24 (21 °C), 4 (37 °C), and 1 (-13 °C) days
Robertson et al (1992)[c]	no	yes	NA	NA	-22	100% inviable - snap-freeze 79% inviable (114 hr) - slow-freeze
Yozwiak et al. (1993)	no	yes	NA	NA	5 15 25	Viability reduced 4.9% (10 wks) Infectivity decreased 99.6% (21 wks) Infectivity decreased 98.5% (4 wks)

[a]Repeated freezing and thawing (cycle of -22 °C for 30 min. and 19 -20 °C for 30 min) reduced viability from 88.9% to 23.3%.
[b]NA = not applicable.
[c]Also examined the effects of air drying (10 viable but deformed cysts after 24 hours).
[d]Repeated freezing and thawing reduced cyst viabilities to near zero.
[e]Also examined the effects o air drying (97% and 100% inviable cysts after 2 and 4 hours, respectively) and survival in river water (increase from 22.3% to 57.0% inviable cysts by day 47).

effects of reducing conditions (50% CO_2 at 20 or 37 °C for 18 hours) on oocyst excystation; excystation was greater after incubation at 37 °C (56% to 91% compared to about 8% to 13% at 20 °C). However, they did not report results for exposure to air alone due to "inconsistent results." Anaerobic conditions produced greater reductions in oocyst survival in water (pH 7.0) at 4 and 20 °C (compared to aerobic incubation), with the greater reduction found at the higher temperature. In studies with an anaerobic sludge digester, Van Praagh et al. (1993) found that the time required to achieve 99.9% *Giardia* cyst inactivation varied with temperature, i.e., about 15 days, 21 hours, and 11 minutes at 21.5, 37, and 50 °C, respectively. Studies conducted with *Cryptosporidium* reported high levels of oocyst inactivation after 24 to 48 hours at 37 °C in an anaerobic sludge digestion system (Stadterman et al. 1996, Whitmore and Robertson 1995). Several studies have also been conducted on (oo)cyst survival in association with warm-blooded animal fecal material (Carrington and Ransome 1994, Kasprzak et al. 1980, Robertson et al. 1992). It is possible that survival may be greater when in association with fecal material because feces could reduce cyst or oocyst permeability, making them less susceptible to other environmental factors and thus prolonging their viability. In a study simulating environmental conditions in a drinking water distribution system, Rogers et al. (1996) found that oocysts associated with biofilms retained viability and infectivity for several weeks.

Besides inactivation losses, (oo)cysts may be removed from the water column by settling or sedimentation. Loss of (oo)cysts due to sedimentation in reservoirs or lakes has been suggested to have occurred in several watershed studies (Hibler and Hancock 1990, Ongerth 1989). The NYC DEP reported that (oo)cyst detection was greater for waters entering reservoirs than for water leaving the system (Stern 1996c). Settling velocities have been calculated for individual (oo)cysts (Badenoch 1990) yielding values of 0.18 and 0.35 $cm \cdot h^{-1}$ (0.04 and 0.08 $m \cdot d^{-1}$) for *Cryptosporidium* (different assumptions regarding cyst diameter and density and water temperatures) and 1.98 $cm \cdot h^{-1}$ (0.48 $m \cdot d^{-1}$) for the larger *Giardia* cysts. Chapra (1997) calculated similar values based on Stokes' law. These estimated velocities are significantly less than those estimated for FC bacteria (~1.4 $m \cdot d^{-1}$) by Niehaus and Auer (1993) using sediment traps. This disparity is understandable given the fact that (oo)cyst velocities were calculated for individual, discrete particles, while FC velocities were measured in the field on large aggregates of sedimenting material which contained bacterial cells. Such aggregation may be expected for (oo)cysts in lakes and thus sedimentation loss may be significant. A 10-oocyst *Cryptosporidium* aggregate, containing no other material, would settle at a calculated rate of 0.58 $cm \cdot h^{-1}$ (0.14 $m \cdot d^{-1}$) (Badenoch 1990). This is still considerably less than the rate observed for FC bacteria in the field.

(Oo)cysts lost from the water column by settling may later be resuspended and returned to the overlying water. Thus the fate of (oo)cysts in the sediments is of importance as well. Possible factors influencing (oo)cyst fate in the sediments include: temperature, pH, redox conditions, and desiccation (during periods of drawdown). Although no published studies were found on (oo)cyst survival in sediments, the persistence of viable FC and other bacteria of warm-blooded animal fecal origin in sediments has been well-documented (Howell et al. 1995, LaLiberte and Grimes 1982, Matson et al. 1978, Sherer et al. 1988, 1992, Stephenson and Rychert 1982). In general, fecal bacteria were present in higher levels in the sediments than in the water column and resuspension of viable bacteria could be demonstrated. Matson et al. (1987) suggested several mechanisms for resuspension, including increased river discharge, wind-induced turbulence (for shallow systems), activity of aquatic macroorganisms, and human-related water resource use. Resuspension was demonstrated to occur in Cannonsville Reservoir in 1995, a major drawdown year (Effler et al. 1998). This phenomenon probably occurs widely in other reservoirs that experience periods of fluctuating water levels.

Giardia cysts and *Cryptosporidium* oocysts are relatively resistant to the effects of disinfection procedures as well as to environmental factors. Traditional chlorination procedures are considered to be relatively ineffective against the oocysts, in particular. Both Hoff (1990) and Hibler and Hancock (1990) found that *Giardia* cysts were relatively resistant to chlorination at water temperatures between 0.5 and 5 °C and free chlorine levels of up to 4 $mg \cdot L^{-1}$. Chlorine has been found to be even less effective against oocysts (e.g., Korich et al. 1990, Carrington and Ransome 1994), with 0.5 to 80 $mg \cdot L^{-1}$ chlorine residual levels and 30-minute contact times resulting in less than 50% oocyst inactivation levels. Ozonation (typically applied at from 1 to 3 $mg \cdot L^{-1}$) has been found to render nearly 100% of the (oo)cysts non-viable and/or non-infective with contact times of two to five minutes, with the oocysts appearing to require higher ozone residuals or contact times than the cysts (Carrington and Ransome 1994, Finch et al. 1993, Korich et al. 1990, Labatiuk et al. 1992, Parker et al. 1993, Wallis et al. 1990). A variety of factors have been found to influence ozone's effectiveness, including temperature, pH, and turbidity (Labatiuk et al. 1992, Parker et al. 1993, Wallis et al. 1990).

Summary

A review of the literature pertaining to the environmental fate and transport of *Giardia* cysts and *Cryptosporidium* oocysts in freshwater systems, including lakes and reservoirs, has demonstrated the embryonic nature of this topic area. A wide range of (oo)cyst levels has been reported for a wide variety of surface waters, although relatively little information is available on the actual viability or infectivity of the detected (oo)cysts. In contrast, much less published information is available on (oo)cyst loading levels, sources, and sinks within watersheds, with the NYC DEP having one of the most extensive ongoing programs to obtain such information. Knowledge of the environmental dynamics of protozoan (oo)cysts is generally poorly developed, in contrast to that for FC bacteria. The effects of low temperatures (0 to 10 °C) on prolonging (oo)cyst viability have been well documented. Effects of other environmental parameters have generally not been conclusively determined; however, parameters such as pH and redox level could play roles in affecting (oo)cyst viability and infectivity either alone or in combination with other parameters. Any loss of (oo)cysts from the water column due to settling would likely occur in association with larger aggregates of particles, rather than by individual (oo)cysts or aggregates of (oo)cysts. No specific kinetic coefficients for use in modeling studies were found in any of the studies described in this literature review.

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