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The Low Sedimentation Speed of Cryptosporidium Oocysts: A Further Explanation for Waterborne Outbreaks

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ABSTRACT

The very low sedimentation speed of *Cryptosporidium parvum, C. muris,* and *C. baileyi* oocysts is reported. The regression of the sedimentation speed of oocysts in fecal suspension and tap water could be described by exponential equations. Depending on temperature, sedimentation media, and species, between 0.5 - 3.5 days were needed for the total disappearance of oocysts from the upper layer of water. This characteristic might be attributed to directive selection of *Cryptosporidium* spp. for low density through the way of transmission. The settlement of *C. parvum* was slower than that of *C. baileyi* or *C. muris*. The slow disappearance of Cryptosporidium oocysts from sewage, surface waters, and public water supplies could be one of the major factors responsible for the transmission of this parasites via drinking water.

INTRODUCTION

Cryptosporidium parvum have recently emerged the most prevalent and widespread intestinal parasite in humans in North America (Current and Garcia 1991). It also infects virtually all other mammals causing self-limiting diarrhea (Tzipori and Griffiths 1998). *Cryptosporidium* infections are particularly problematic for immunocompromised individuals since drug therapy to control or eliminate this parasite from human or animal hosts is not available (Griffiths 1998; Tzipori 1998). *Cryptosporidium muris* and *Cryptosporidium baileyi* are prevalent parasites in dairy cattle and poultry, respectively (O'Donoghue 1995; Sréter 1998).

The importance of cryptosporidia as a microbial contaminant of water has only recently been recognized. Much attention is the result of the 1993

waterborne outbreak of cryptosporidiosis occurred in Milwaukee, Wisconsin, which resulted in more than 400,000 people being infected and included at least 70 fatalities (MacKenzie et al. 1994). In recent years, cryptosporidia have been detected in sewage, surface waters, and public water supplies across North America and UK, and several waterborne outbreaks have been reported worldwide (Widmer et al. 1996, Smith and Rose 1998). This is explained by the large number of oocysts excreted by infected hosts (Chappel et al. 1996), the low dosage (30 oocysts) required for infection (DuPont et al. 1995), the lack of, or reduced host specificity (O'Donoghue 1995), the small size of oocysts (Current and Garcia 1991), and the resistance of the oocysts to environmental pressures and water treatments (Korich et al. 1990, Robertson et al. 1992, Fayer 1994, Fayer and Nerad 1996). Herein, we report the very low sedimentation speed of *Cryptosporidium* oocysts, which could also be a significant factor in the transmission of these parasites via drinking water.

MATERIALS AND METHODS

Oocysts of *C. parvum* was isolated from a calf in Hungary and were passaged in specific-pathogen-free C57BL/6N mice immunosuppressed with phosphated dexamethasone (12 μg/ml drinking water) as described elsewhere (Healey et al. 1995). Oocysts of *C. muris* were kindly provided by M. Iseki, (Osaka City University, Medical School, Japan) and passaged in mice (Iseki et al. 1989). Oocysts of *C. baileyi* were originally isolated from an outbreak of cryptosporidiosis in Hungary (Dobos-Kovács et al. 1994) and were maintained by serial passage in chickens. The isolation and storage of oocysts and preparation of inoculum was done as described elsewhere (Sréter et al. 1997).

Cryptosporidia-free bovine and avian feces were collected from a cow and chickens, respectively. Animals were subsequently sampled and examined for the presence of *Cryptosporidium* oocysts by Sheather's sugar flotation (Current 1990) before collection of feces.

Feces were weighed, a 2-fold volume of tap water was added and were broken up with an electric mixer for approximately 2 min. A 15 ml sample (containing 5 g feces) was withdrawn in a graduated cylinder with a wide-bore pipette. The suspensions were transferred into 100-ml beakers, and the volumes were brought to 100 ml with tap water plus formalin to give 4% concentration of the latter. Beakers filled with 100 ml tap water only were also prepared. Samples in the beakers were placed either to refrigerator and stored at 5°C or stored at room temperature (25°C). Feces suspension and tap water samples were inoculated with *C. parvum*, *C. muris*, or *C. baileyi* oocysts (Table 1).

Following the addition of 10^9 oocysts to 100 ml sedimentation medium and stirring for 5 min, four 30-µl aliquots were removed from 1-cm-depth with a digital micropipette immediately after mixing and were transferred to a Fuchs-Rosenthal hemacytometer (Fein-Optik, Bad Blankenburg, Germany). Afterwards, beakers were stored at either 5°C or 25°C (Table 1). The sample was left in the hemacytometers for 4 min, and the oocysts were counted using 640 × magnification. [*Comment*: the sedimentation speed of *Cryptosporidium* oocysts in the hemacytometers was determined by tracing oocysts (n = 60) from the top to the bottom of the chamber. About 4 min proved necessary for the slowest oocysts to settle after filling the chambers and placement of coverslip on the suspension. A shorter period results in underestimation of the number of oocysts (Varga et al. 1995).] The examination was repeated 2, 4, 6, 8, 16, 24, 30, 36, 48, 60, and 72 hours later.

Statistical analysis was performed by SPSS 7.5 for Windows 95 (SPSS Inc., Chicago, Illinois) statistical package. The relationship between time and oocyst concentration was found to be curved, therefore, nonlinear regression analysis was used to fit a curve to data and find values of variables that make the equation best fit the data.

Table 1. Equations describing the sedimentation speed of oocysts in samples seeded with oocysts of *Cryptosporidium parvum*, *C. muris*, and *C. baileyi*.

Sedimentation medium	Storage temperature (°C)	Equations describing the sedimentation of oocysts [a] in samples seeded with		
		C. parvum	*C. muris*	*C. baileyi*
Fecal suspension	5	$y = 123.8 \times e^{-0.43 \times x}$	$y = 338.3 \times e^{-0.75 \times x}$	$y = 170.3 \times e^{-0.78 \times x}$
	25	$y = 100.2 \times e^{-0.05 \times x}$	$y = 225.5 \times e^{-0.24 \times x}$	$y = 228.3 \times e^{-0.23 \times x}$
Tap water	5	$y = 101.3 \times e^{-0.07 \times x}$	$y = 150.1 \times e^{-0.07 \times x}$	$y = 113.8 \times e^{-0.06 \times x}$
	25	$y = 150.4 \times e^{-0.08 \times x}$	$y = 159.1 \times e^{-0.10 \times x}$	$y = 162.3 \times e^{-0.10 x}$

[a] $r^2 \geq 0.85$, $P < 0.005$, y is the oocyst concentration in percent, x is the time in hours; e = 2.7183.

RESULTS AND DISCUSSION

The sedimentation speed of oocysts of *C. parvum* was very low as illustrated in Figure 1. The regression of the sedimentation speed of oocystsin fecal suspension and tap water could be described by exponential equations (Table 1). The settlement of *C. parvum* was slower than that of *C. baileyi* or *C. muris* (Table 1). It might be attributed possibly to the smaller size (*C. parvum*: 4.5 µm vs. *C. baileyi*: 6.5 µm or *C. muris*: 7.5 µm) and the lower

density of *C. parvum* oocysts ($1.05 < \delta < 1.09$; Current 1990). Because of the less intensive thermal agitation, the disappearance rate of oocysts was slightly lower in tap water at 5°C (Table 1). The sedimentation speed of oocysts was slower in fecal suspension at 25°C. As the density of the majority of fecal particles are higher than that of oocysts, their settlement result the co-settlement of oocysts. The disappearance rate of oocysts was considerably higher in fecal suspension at 5°C because of the less intensive bacterial movement (Table 1).

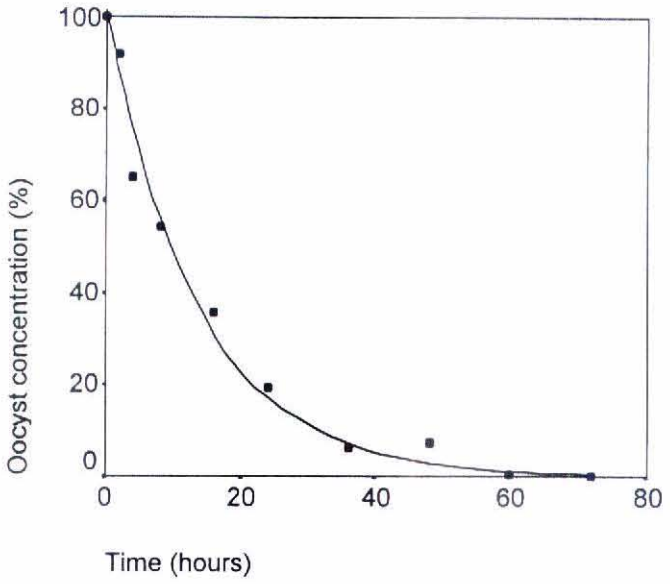

Figure 1. Typical disappearance rate (%) of oocysts of *Cryptosporidium parvum* at 1-cm depth from tap water (storage temperature: 5°C).

As illustrated, the sedimentation speed of *Cryptosporidium* oocysts is very low in aqueous medium as compared to other coccidian oocysts or helminth eggs. Depending on temperature, sedimentation media, and species, between 0.5-3.5 days are needed for the total disappearance of oocysts of at 1-cm depth of water. Deeper from the surface, oocysts can be detected even after several days (data not shown). Moreover, depending on flow rate of water, the oocyst concentration in sewage, surface waters, and public water supplies might decrease more slowly than found in this study. This characteristic was not described earlier (Widmer et al. 1996, Smith and Rose 1998) and might be attributed to the directive selection of cryptosporidia for low density

through the way of transmission (i.e. oocysts with lower sedimentation speed have a greater chance for infecting a new host). Testing of this hypothesis is in progress. Since *Cryptosporidium* spp. survive for months in the environment (Robertson et al. 1992) and disappear very slowly from water, there is a need to explore better water treatment methods (Fricker and Crabb 1998), which may result a more efficient *Cryptosporidium* removal rates so as to ensure public and animal health protection and prevent further waterborne outbreaks caused by these widespread parasites.

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