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# Comparison of viability and infectivity of *Cryptosporidium parvum* oocysts stored in potassium dichromate solution and chlorinated tap water

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**Abstract**

The present study was undertaken to compare the viability and infectivity of *Cryptosporidium parvum* oocysts that had been stored for 1, 4, 7, 10, 13, 16, 20, 25 and 30 months at 4 °C in 2.5% potassium dichromate (Cr) or chlorinated tap water, respectively. An excystation protocol was performed *in vitro* to evaluate viability. One hundred and eighty female BABL/c mice were used to evaluate the infectivity of oocysts by investigating the prepatent period of *C. parvum* infection, the quantity of oocysts excreted, and the number of parasites that colonized the villi of the ileum. The results showed that *C. parvum* oocysts preserved in Cr for 1–16 months or in water for 1–13 months were capable of excystation *in vitro* and infection of mice. The excystation rates of oocysts and the prepatent periods in mice infected by oocysts stored in Cr and water were not significantly different ($p > 0.05$), and there was a strong correlation between prepatent period and duration of oocyst storage (Cr: $R^2 = 0.92$; water: $R^2 = 0.98$). There were no significant differences in oocyst shedding from feces or parasitism of the terminal ilea of mice by *Cryptosporidia* between the two storage media ($p > 0.05$). In conclusion, *C. parvum* oocysts may be stored at 4 °C in water instead of Cr for the purposes of laboratory research. However, the presence of viable *C. parvum* oocysts in water is a severe challenge to the drinking water treatment industry.


*Keywords:* *C. parvum* oocyst; Mice; Viability; Infectivity; Water; Potassium dichromate

## 1. Introduction

*Cryptosporidium parvum* causes chronic, severe life-threatening gastroenteritis in immunocompromised patients, and acute but self-limiting infection in immunocompetent people, throughout the world (O'Donoghue, 1990). Despite a large number of studies that have demonstrated the efficacy of passive immunotherapy or chemotherapeutic agents against cryptosporidiosis, no significant clinical benefit has been demonstrated (Castro-Hermida and Ares-Mazás, 2003; Zardi et al., 2005). The infective stage of *C. parvum* is the oocyst, 4–6 μm in diameter, which is excreted and fully sporulated in the feces of the host animal (Taylor and Webster, 1998). *C. parvum* oocysts can resist a range of environmental conditions and remain viable for a long period, which represents a challenge to the water industry (Freire-Santos et al., 1999, 2000; Fayer et al., 2000). Currently, *C. parvum* is considered to be a significant cause of waterborne enteric disease and to be responsible for recent waterborne outbreaks of human

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cryptosporidiosis (Dillingham et al., 2002). The acknowledged importance of *C. parvum* as a zoonotic enteropathogen has stimulated research on the transmission of cryptosporidiosis, and the viability *in vitro* and infectivity *in vivo* of *C. parvum* oocysts. The viability and animal infectivity of *C. parvum* oocysts maintained in water have been assessed (Neumann et al., 2000). It is generally accepted by most investigators that *C. parvum* oocysts should be stored at 4 °C in 2.5% potassium dichromate solution (Cr) and used within 3–6 months of isolation and purification to ensure high viability *in vitro* and adequate infectivity rates (Yang et al., 1996; Koudela et al., 1998; Surl et al., 2003). However, there have been few evaluations of the differences in viability and animal infectivity of *C. parvum* oocysts stored in Cr and water. I*n vitro* assays for the viability of oocysts frequently employ vital dyes (Neumann et al., 2000; Castro-Hermida and Ares-Mazás, 2003), but the ability to produce patent infection in susceptible animals is necessary to demonstrate unequivocally the infectivity of stored oocysts *in vivo*. The present study was undertaken to compare the viability *in vitro* and infectivity of *C. parvum* oocysts in immunosuppressed BABL/c mice after oocysts had been stored for l–30 months in Cr or water.

## 2. Materials and methods

### 2.1. Oocyst preparation

*C. parvum* oocysts were originally isolated from the feces of a naturally infected piglet. BABL/c mice were used to reproduce oocysts to maintain their viability. Oocysts were isolated by the sucrose flotation technique (Scott et al., 1995), and two equal quantities of oocysts ($1.0 \times 10^7$ ml$^{-1}$) were stored for different times in Cr and water at 4 °C, respectively. Oocysts were stored for 1, 4, 7, 10, 13, 16, 20, 25 and 30 months in Cr and water before viability and infectivity of *C. parvum* oocysts were assayed.

### 2.2. Viability assay in vitro—excystation technique

An excystation protocol (Vergara-Castiblanco et al., 2000; Castro-Hermida et al., 2003) was performed as described previously. Briefly, *C. parvum* oocysts ($2.0 \times 10^6$) were resuspended in 10 ml of 0.9% NaCl, then 0.5% of pepsin (Sigma, America) and 70 μl of concentrated HCl were added. The reactants were mixed thoroughly prior to incubation at 37 °C for 30 min. After this preincubation treatment, the mixture was neutralized with 2.2% (w/v) sodium bicarbonate before adding 22 mg of sodium taurocholate and 4 mg bovine trypsin. The mixture was incubated at 37 °C for 2 h, and then fixed by addition of 2.5% glutaraldehyde. The percentage excystation was determined by scanning 10 μl aliquots of suspension under a Nikon optics microscope and counting all the intact and empty oocysts in 20 random fields. The excystation percentage was calculated using the equation: (number of empty oocysts/total oocysts, intact and empty) ×100. All samples were performed three times, and the average values were then calculated.

### 2.3. Viability assay in vivo

The BABL/c mouse model was used to evaluate the infectivity of oocysts by detection of the prepatent period of *C. parvum* infection, the quantity of oocysts excreted, and the number of parasites that colonized the villi of the ileum.

#### 2.3.1. Animal infection

One hundred and eighty 25-day-old female BABL/c mice, weighing 14–16 g, were assigned to 18 groups (10 mice per group), numbered individually and immunosuppressed with dexamethasone (Sigma, America) (Huang and Yang, 2002). All groups were maintained separately in plastic cages with wire mesh tops, and wood shavings were supplied for bedding. On the fourth day post-immunosuppression, each mouse in groups 1–9 was orally infected by stomach tube with $1.0 \times 10^4$ oocysts that had been stored in Cr for 1, 4, 7, 10, 13, 16, 20, 25 or 30 months. Mice in groups 10–18 were infected in the same way with $1.0 \times 10^4$ oocysts that had been stored in water for the same periods. Before mice were infected, oocysts were washed three times with PBS containing penicillin (60 μg/ml) and streptomycin (190 μg/ml) (Amresco, America).

#### 2.3.2. Determination of prepatent period

Fecal pellets were collected from the rectum of all mice daily. One or two fecal pellets from each mouse were smeared in a circular pattern (2 cm in diameter) onto a microscope slide, stained by the modified acid-fast staining technique (MAFS) (Martinez and Belda Neto, 2001), and observed under a microscope using a 100× objective to verify the presence of oocysts. The prepatent period of *C. parvum* infection was determined as the time from the day of infection to the first day when oocysts were detected in the feces. In each group, the average values were then calculated as the average prepatent period.