Inactivation of *Cryptosporidium parvum* oocyst infectivity by disinfection and sterilization processes

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Background: ***Cryptosporidium parvum*** **is a common cause of self-limited gastroenteritis in the normal host but may cause severe disease in immunocompromised persons. Person-to-person transmission has been well documented in households, child care centers, and hospitals. Because contaminated environmental surfaces and medical devices such as endoscopes may play a role in disease transmission, we studied the susceptibility of** ***C parvum*** **to chemical agents commonly used for disinfection and evaluated the efficacy of sterilization processes.**

Methods: **Seven disinfectants were studied at their use dilution using a suspension test. Antimicrobial activity was assessed with the use of a cell infectivity assay.**

Results: **All sterilization processes tested (steam, ethylene oxide, Sterrad 100) inactivated 3 logs or greater of** ***C parvum*****. The only liquid disinfectant/sterilant able to inactivate greater than 3 logs of** ***C parvum*** **was 6% and 7.5% hydrogen peroxide. Agents that did not completely inactivate** ***C parvum*** **included hydrogen peroxide at lower concentrations or exposure times, peracetic acid, sodium hypochlorite, a phenolic, a quaternary ammonium compound, 2% glutaraldehyde, and ortho-phthalaldehyde.**

Conclusions: **Most high-level disinfectants used on endoscopes have limited efficacy against** ***C parvum*****. However, the infectivity of** ***C parvum*** **on dry surfaces decreases rapidly. Therefore, current cleaning and high-level disinfection guidelines are adequate to prevent nosocomial transmission of** ***C parvum*** **by means of endoscopes. (Gastrointest Endosc 1999;49:605-11.)**

Cryptosporidium was first recognized in 1907 by Clarke and Tyzzer[1] and linked to human gastroenteritis in 1976 by Nime et al.[2] It is now a well-recognized cause of gastroenteritis in both immune compromised[3,4] and immune competent[5] persons. There are multiple routes of transmission including person-to-person,[5] potable water,[6-8] swimming in pools,[9-12] food,[13] and zoonotical transmission.[14] Transmission has been documented within households,[5,15-17] child care centers,[5,18-21] and healthcare facilities.[22-29] Transmission via water[30,31] and food[32] and in the child care setting[21] has recently been reviewed.

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Cryptosporidiosis represents an emerging highly infectious risk for the following reasons.[33-35] First, it is a common cause of self-limited gastroenteritis in the normal host but it can cause potentially life-threatening disease in immunocompromised patients. Second, it is a highly infectious enteric pathogen. Based on human volunteer studies, the ID_{50} (amount of oocysts required to infect 50% of volunteers) has been estimated at only 132 oocysts but some infections followed the ingestion of just 30 oocysts.[36] Infection after the ingestion of a single oocyst has been reported.[37] Animal challenge studies have confirmed that 10 or fewer cysts are infectious.[38] Third, it is persistent in water and resistant to chlorine at levels used in potable and swimming pool water.[30-31] Fourth, it is ubiquitous in many feral, domestic, and agricultural animals and represents a major threat to the water supply in the United States. The small dose required for infection, the fecal-oral route of transmission, and environmental survival allow *Cryptosporidium* to spread in households, healthcare facilities, and child care centers.

Person-to-person transmission of *Cryptosporidium* may occur by means of direct contact or indirectly by contamination of the environment or medical equip-

ment. Because of this possibility of indirect transmission, we studied the susceptibility of *Cryptosporidium* to surface disinfectants, high-level disinfectants (used on endoscopes), and sterilization processes.

MATERIAL AND METHODS

Oocyst

Oocysts of *C parvum* (Iowa isolate) which were isolated from the feces of experimentally infected neonatal calves and purified by differential sucrose gradients as previously described[39] were purchased from Pleasant Hill Farm (Troy, Id.). Oocysts were stored in a phosphate-buffered saline solution (PBS), pH 7.5, containing 1000 U/mL penicillin and 1000 μg/mL streptomycin (Gibco, Grand Island, N.Y.). The oocyst suspension was stored at 4° C and used within 4 months of preparation. Before the experiments, the oocysts were washed twice in PBS then resuspended in sterile, distilled water. Oocyst concentrations were determined by hemocytometer enumeration under phase-contrast microscopy.

Cell cultures

Madin-Darby canine kidney (MDCK) cell cultures (ATCC CCL 34, Rockville, Md.) were obtained from the laboratory of M. J. Arrowood (Centers for Disease Control and Prevention, CDC, Atlanta, Ga.). MDCK cells were maintained in Dulbecco's modifed Eagle's (DMEM)/F12 medium (Sigma, St. Louis, Mo.) supplemented with 10% fetal bovine serum (Sigma), 2 mM l-glutamine (Sigma), and 15 mM HEPES buffer (Sigma). Cell cultures for the *Cryptosporidium* oocyst infectivity assay were propagated in double-chambered Lab Tech Chamber Slides (Nunc, Naperville, Ill.) using serum-free Ultraculture Medium (BioWhittaker, Walkersville, Md.) supplemented with 2 mM l-glutamine and antibiotics (kanamycin 250 μg/mL, gentamycin 50 μg/mL, mycostatin 150 μg/mL) at 37° C in a 5% carbon dioxide atmosphere.

Liquid disinfectants/sterilants

The disinfectants studied were by chemical class, use-dilution (trade name, if applicable; manufacturer): hydrogen peroxide, 6% and 3% (Mallinckrodt [30% hydrogen peroxide], McGaw Park, Ill.); Sporox, hydrogen peroxide, 7.5% (Reckitt-Colman, Montvale, N.J.); alkaline glutaraldehyde, 2.4% (CIDEX, Johnson & Johnson, Arlington, Tex.); phenolic (active ingredient, 9.65% sodium o-phenolphenate, 8.34% sodium p-tertiary-amylphenate), 1:128 (Vesphene IIse, Calgon-Vestal, St. Louis, Mo.); quaternary ammonium (active ingredients, 8% alkyl [50% C_{14}, 40% C_{12}, 10% C_{16} dimethyl benzyl ammonium chloride]), 1:128 (TBQ, Calgon-Vestal, St. Louis, Mo.); sodium hypochlorite, 5.25% (Clorox, Oakland, Calif.); ethyl alcohol, 70% (Fisher Scientific, Norcross, Ga.); iodophor (1% available iodine), manufacturers recommended concentration, undiluted (Betadine, Purdue Fredrick Co., Norwalk, Conn.); peracetic acid, 0.35% and 0.2% (Alrich Chemical Co., Milwaukee, Wis.); peracetic acid, 0.2% (Steris Corp.[35% peracetic acid], Mentor, Ohio); and, ortho-phthalaldehyde, manufacturer's recommended concentration-undiluted (CIDEX-OPA, Johnson & Johnson). All products were stored in the dark at room temperature (23° to 25° C) and tested within their specified use-life. For evaluation of the Steris System disinfectant, the contents of the Steris carton were carefully diluted into 6.1 L of sterile, distilled water. The mixture was stirred until all contents were completely dissolved as has previously been described,[40] then the sterilant was immediately used in the assay.

Products were analyzed before use when applicable to determine the current disinfectant concentration and test efficacy of the neutralizer chosen to terminate the reaction at the end of exposure. All disinfectants requiring dilution were diluted in sterile, distilled water (hardness ≤ 0.6 ppm dissolved solids) according to the manufacturer's instructions. The hypochlorite was tested using a pHydrion Testuff Sanitizer Kit (Fisher Scientific, Norcross, Ga.). The chlorine concentration was approximately 5.25%, which was completely neutralized by the addition of an equal volume of 2% sodium thiosulfate. The hydrogen peroxide and Sporox were analyzed with a hydrogen peroxide test kit (Hach, Loveland, Colo., with a sensitivity of 0.2 mg/L [ppm]). The initial concentration of the diluted product (Mallinckrodt) was 6% to 7% or 2% to 3% which was neutralized by an equal volume of 2% sodium thiosulfate. The glutaraldehyde product was tested using glutaraldehyde monitor strips (PyMaH Corp., Flemington, N.J.). Strips are semi-quantitative indicators that allow detection above or below 1% only. The initial concentration of the product was greater than 1% and was neutralized to less than 1% with the addition of an equal volume of 2% glycine. Betadine, an iodophor, was tested using a pHydrion Micro and Lo iodine test kit (Micro Essential Laboratory, Brooklyn, N.Y.) with a sensitivity of greater than 0 mg/L. The available iodine concentration was approximately 1%, which was neutralized by an equal volume of 2% sodium thiosulfate. No assay method was available for the ethyl alcohol, peracetic acid, CIDEX-OPA, Vesphene IIse, or TBQ. An equal volume of double-strength Letheen Broth (Difco, Detroit, Mich.) was adopted as a neutralizer for these products, except peracetic acid which was neutralized with 1% sodium thiosulfate.

Sterilization processes

The ethylene oxide sterilizer (2057 Eagle Series ETO sterilizer/aerator, AMSCO, Erie, Pa.) was a standard sterilizer which used a hydrochlorofluorocarbon carrier gas (Oxyfume 2002, Allied Signal, Morristown, N.J., 10% ethylene oxide, 90% hydrochlorofluorocarbons [27% chlorodifluoromethane, 63% chlorotetrafluorethane]). This sterilizer was operated at 54° C with a 2-hour exposure to ETO followed by overnight aeration (~12 to 14 hours) at 55° C.

The Sterrad 100 system (Advanced Sterilization Products [ASP], Irvine, Calif.), uses a single diffusion stage (hydrogen peroxide vapor) and a plasma stage per sterilization cycle. Approximately 6 mg/L of hydrogen peroxide is delivered to the evacuated chamber and allowed

to diffuse for a fixed period of time. The vapor is excited by a radiofrequency energy of 13.6 MHz creating a secondary, low temperature plasma that forms around the products being sterilized. Hydrogen peroxide is converted into a variety of species, including the free radicals hydroxyl and hydroperoxyl which recombine during the process to form primary oxygen and water by-products, eliminating the need for aeration.[41]

The steam autoclave system (Vernitron Steam Sterilizer, Carlstadt, N.J.) was operated at 121° C for 18 minutes on a liquid cycle.

Liquid disinfection/sterilization exposure procedure

For each experimental treatment 100 μL of a suspension containing 10^5 or 10^6 purified oocysts were aliquoted to 1.5 mL polypropylene, sterile, siliconized micro centrifuge tubes. Oocysts were pelleted, washed twice in sterile, distilled water then resuspended in 0.5 mL of disinfectant pre-equilibrated to the target temperature. Samples were incubated in a thermostatically controlled waterbath at the target temperature for the specified time intervals. At completion of the required contact time with the disinfectant, 0.5 mL of the disinfectant neutralizer was added to stop the reaction. The oocysts were then pelleted by centrifugation, washed a second time with neutralizer, then washed with PBS to remove any residual disinfectant. After being washed, the oocysts were resuspended in 100 μL of Ultraculture medium before inoculation into MDCK cell cultures. In each experiment, all disinfection treatments were performed in triplicate at each concentration. A positive infectivity control consisting of 10-fold serial dilutions of *C parvum* oocysts resuspended in PBS was processed as part of each experiment, along with a neutralizer control consisting of neutralized disinfectant and a negative control of sterile, distilled water.

Sterilization exposure procedure

Ethylene oxide and Sterrad 100. Aliquots of oocyst suspension containing 10^7 oocysts were dispensed into 1.5 mL sterile, siliconize polypropylene microtubes. Oocysts were pelleted by centrifugation then resuspended in 20 μL 70% ethanol. The suspension was aseptically transferred to the carriers, number 10 Bard-Parker (Becton Dickinson Acute Care, Franklin Lakes, N.J.) stainless steel surgical blades. Carriers were placed under a laminar flow hood for approximately 15 minutes to allow for evaporation of all ethanol. After the suspension dried, the carriers were placed in either a sterile petri dish (uncovered) or the center of 40 cm long stainless steel lumen units of either 1 or 3 mm diameter. The lumen test units consisted of a removable 5 cm center piece (1.2 cm diameter) of stainless steel sealed to the narrow tubing by hard rubber septums. Four test units (two 1 mm in diameter and two 3 mm in diameter) and one petri dish were placed into carrier trays. The lumen test units were placed on the extreme periphery of the carrier tray while the petri dish was placed in the center of the carrier tray. The tray was then wrapped in Kimguard (Kimberly Clark, Roswell, Ga.). Samples were placed in the sterilizer along with other medical devices and supplies and processed according to manufacturer's recommendations. After sterilization, the carriers were removed, transferred to 1.5 mL sterile, siliconized micro centrifuge tubes containing PBS/0.1% Tween 80 and then incubated at room temperature for 30 minutes. Oocyst recovery was facilitated by vortexing and scraping of the carrier. Recovery of oocysts was quantitated before sterilization using the same procedures and estimates based on hemocytometer enumeration. Suspensions containing oocysts were diluted 10-fold to a final concentration of 10^6 and 10^5 per 100 μL, washed twice in PBS, then resuspended in 100 μL of Ultraculture medium before addition to cell cultures. An oocyst infectivity control was prepared as part of each experiment along with a negative control consisting of oocyst suspension inoculated onto carriers and left at room temperature in a laminar flow hood for the duration of the sterilization process (Sterrad 2 to 3 hours, ETO 18 to 24 hours).

Heat (steam sterilization). Suspensions containing 10^6 and 10^5 purified oocysts were dispensed to 1.5 mL polypropylene, sterile, siliconized microtubes. Tubes were wrapped in Kimguard (Kimberly Clark, Roswell, Ga.), then placed into the autoclave with caps open and processed according to standard operational procedure. At the completion of an autoclave run, sample tubes were capped, then immediately transferred to ice to cool down. Tubes were centrifuged and the oocysts were washed twice in PBS. Control oocyst suspensions were kept at room temperature for the duration of the autoclave cycle. Oocysts were resuspended in 100 μL Ultraculture medium and then added to cell cultures.

In vitro infectivity assay

Four-day old MDCK cell monolayers were inoculated with *C parvum* oocysts from the disinfection and sterilization experiments. The cell cultures were incubated for 48 hours at 37° C. Infected cell monolayers were washed with PBS then fixed with Bouin's solution (Sigma). The fixed cells were blocked by exposure to 1% bovine serum albumin in PBS for 30 minutes. Cell culture–associated infective stages of *C parvum* were reacted with a rhodamine-labeled monoclonal antibody, CY3/C3-C3 provided by M. J. Arrowood (CDC) for 90 minutes. Cell layers were rinsed three times with 1.5 mL of PBS to remove unreacted antibodies. The cell layers were observed under an ultraviolet fluorescent microscopy.

Time-related survival

Washed pellets containing 10^7 oocysts were resuspended in 20 μL of 70% ethanol and transferred to sterile, stainless steel surgical blades and allowed to air dry (approximately 7 minutes). After the oocyst suspension was dried, the inoculated blades were transferred to a sterile petri dish and left under a laminar flow hood at room temperature and ambient humidity (45% relative humidity) for varying time periods. Immediately after incubation, oocysts were recovered from carriers as previously described under "Ethylene Oxide and Sterrad 100." Recovery controls were included. The oocyst titer after

Table 1.
Susceptibility of *Cryptosporidium parvum* to liquid chemical disinfectants/sterilants

Product	Chemical class	Concentration	Temperature (°C)	Time (min)	Log_{10} infectivity reduction
TBQ	Quaternary ammonium	1:128	20	10	No change
Clorox	Hypochlorite	5.25%	20	10	< 1.0
Vesphene IIse	Phenolic	1:128	20	10	0.6
Betadine	Iodophor	Undiluted (1% I_2)	20	20	No change
Ethyl alcohol	Alcohol	70%	20	10	No change
Hydrogen peroxide	Hydrogen peroxide	6%	20	20	> 3.0
		3%	20	20	2.0
		6%	20	10	2.7
		3%	20	10	1.8
Sporox	Hydrogen peroxide	7.5%	20	20	> 3.0
CIDEX	Glutaraldehyde	2.4%	25	45	0.3
Peracetic acid (Alrich)		0.35%	20	20	No change
		0.2%	50	12	1.8
Peracetic acid (Steris)		0.2%	23-25	12	No change
		0.2%	48-50	12	1.8
CIDEX-OPA	Ortho-phthalaldehyde	Undiluted	20	20	No change

Data represent mean values of triplicate samples using both 10^5 and 10^6 oocysts per experimental treatment, except Betadine, ethyl alcohol, and Cidex which represent a mean of six samples at 10^6 oocysts per experimental treatment.

recovery was determined by correcting initial concentration of test oocysts by recovery of controls. Recovered oocysts were diluted in Ultraculture medium to 10^5 and 10^6 oocysts/100 μL before addition to cell cultures. The oocysts used in these experiments were approximately 3 months old.

Data analysis

Microscopic examination of MDCK infected cell was performed to quantitate the extent of infectivity based on the frequency of presence of live parasitic stages in 100 random fields. Each experiment included an infectivity assay of serial 10-fold dilutions of a control oocyst suspension that was scored for the percent positive fields at each dilution. Oocyst titer was calculated using the dilutions at which only some of the 100 fields were infected. The percent positivity was converted to probit value and plotted against the log_{10} concentration of oocysts. A best fit linear regression was calculated as a dose-response. For treated samples, 100 microscopic fields were scored for infectivity, then percent positivity converted to probit value. The observed degree of infectivity of the treated samples was compared with those of the titer control providing an estimate of the infectivity of the treated oocysts exposed to a disinfection or sterilization process.

RESULTS

Die-off of *C parvum* with time

When *Cryptosporidium* oocyst air-dried onto surgical blades were exposed to room temperature (i.e., 23° to 25° C) and a relative humidity of 45%, the following log_{10} decreases in infective *C parvum* oocysts was observed: 30 minutes, 2.9; 60 minutes, 3.8; and 90 minutes, greater than 4.0.

Effect of liquid chemical disinfectants on *C parvum* infectivity

The infectivity of *C parvum* oocysts was not decreased compared with controls when exposed to several disinfectants, including TBQ, Betadine, ethanol, Alrich peracetic acid (0.35%, 20° C, 20 min), Steris peracetic acid (0.2%, 23° to 25° C, 12 min), and CIDEX-OPA (Table 1). A detectable infectivity decrease of less than 1 log was observed with exposure to Clorox, Vesphene IIse, and CIDEX. A greater than 1 log_{10} decrease in infectivity was observed for Alrich peracetic acid (0.2%, 50° C, 12 min), Steris peracetic acid (0.2%, 48° to 50° C, 12 min), and 3% hydrogen peroxide. Only 6% hydrogen peroxide (20° C, 20 min) reduced oocyst infectivity greater than 3 log_{10}, which was the detection limit of the assay. Inactivation by hydrogen peroxide was both concentration and time dependent. Hydrogen peroxide at a 6% concentration was more effective than at a 3% concentration for both 20- and 10-minute exposures. Hydrogen peroxide exposure at both 3% and 6% was more effective at 20 minutes than after 10 minutes.

Effect of sterilization processes on *C parvum* infectivity

Complete inactivation (> 3 log_{10}) of *C parvum* was observed after sterilization using a standard gravity displacement steam sterilizer, ethylene oxide, or the Sterrad 100 (Table 2). The latter two methods were effective even if the carrier inoculated with *C parvum* was placed in the center of a 40 cm tube of diameter of 1 or 3 mm.

Table 2.
Susceptibility of *Cryptosporidium parvum* to sterilization processes

Sterilization process	Level or concentration	Carriers	Log_{10} reduction/ infectivity*
Moist heat (under pressure)	250°C (121°C) for 18 min	Microtubes	> 3
Ethylene oxide	450-500 mg/L at 55-60°C	Stainless steel blades in 1 mm lumen test unit	> 3
		Stainless steel blades in 3 mm lumen test unit	> 3
		Stainless steel blades in petri dish	> 3
Sterrad 100	Hydrogen peroxide plasma	Stainless steel blades in 1 mm lumen test unit	> 3
		Stainless steel blades in 3 mm lumen test unit	> 3
		Stainless steel blades in petri dish	> 3

*Data represent mean of triplicate samples at both 10^5 and 10^6 oocysts per experimental treatment.

DISCUSSION

Cryptosporidium species are small coccidian pathogens of the intestinal tract, and the species *C parvum* is the important human pathogen that is also found in many other mammals. Infection is initiated by the ingestion of the environmentally stable thick-walled oocysts. The most common symptom of infection is watery diarrhea but other symptoms are also common including abdominal pain, low-grade fever, and vomiting. Infection is generally confined to the GI tract although in immunocompromised persons there may rarely be involvement of the respiratory or biliary tracts.

A review by Guerrant of 78 reports of more than 131,000 patients with diarrhea and more than 6000 control subjects without diarrhea found *Cryptosporidium* infection in 2.1% to 6.1% of immunocompetent persons in industrialized and developing countries, respectively, versus 0.2% to 1.5% in asymptomatic control subjects.[35] Guerrant also noted that 22 reports of nearly 2000 HIV-infected persons showed *Cryptosporidium* infection in 14% to 24% of HIV-infected persons with diarrhea versus 0% to 5% infection of asymptomatic HIV-infected persons. Seroepidemiologic surveys suggest approximately 25% of young adults in the United States have had infection with *Cryptosporidium*.

Endoscopy is widely used for both diagnosis and therapy of GI disorders. Failure to appropriately clean and disinfect endoscopes has been associated with person-to-person transmission of infectious agents via a contaminated endoscope.[42-45] Data regarding the susceptibility of GI pathogens to disinfectants used for surface decontamination as well as disinfectants used for high-level disinfection of endoscopes are important to minimize the risk of disease transmission via contaminated surfaces in endoscopy suites and/or via endoscopes.

We have used an in vitro cell culture infectivity assay to evaluate chemical disinfection efficacy against *C parvum*. This assay provides for a quantitative estimate of inactivation and has a high degree of correlation with animal infectivity assays (M. J. Arrowood, personal communication). Previous investigations of the activity of disinfectants against *C parvum* have the important flaws that either the concentration used or the duration of exposure[46,47] were not consistent with current use practices or the assay method did not measure infectivity.[46,48]

Our data demonstrated that no chemical agent currently used as a surface disinfectant or antiseptic (i.e., TBQ, Clorox, Vesphene IIse, betadine, ethyl alcohol) is capable of inactivating ≥ 3 $logs_{10}$ of *Cryptosporidium* after a contact time of 20 minutes or less. Thus, with the use of currently available agents, surface disinfection cannot be ensured. With the exception of 6% and 7.5% hydrogen peroxide at a contact time of 20 minutes, no chemical (i.e., CIDEX, peracetic acid, CIDEX-OPA) that may be used as a high-level disinfectant on endoscopes was capable of inactivating 3 $logs_{10}$ of *Cryptosporidium* when used at the recommended contact time. The glutaraldehyde product produced a 0.3 log_{10} decrease in *C parvum* oocyst infectivity. Peracetic acid when used at 48° to 50° C, the operating temperature of the Steris System 1, was able to inactivate almost 2 $logs_{10}$ of *C parvum*. However, our data demonstrated that the elevated temperature was the important factor in inactivation of *C parvum*.

Because *C parvum* is a common cause of gastroenteritis, endoscopes are likely to be contaminated with this agent. Our data demonstrate that steam sterilizers, ethylene oxide sterilizers, or the Sterrad 100 were effective in completely inactivating *C parvum* on endoscopes. However, most endoscopes either undergo high-level disinfection with 2% glutaraldehyde (or other high-level disinfectants) or are treated in a chemical sterilization process with peracetic acid (i.e., Steris System 1). Immersion in a 2% glutaraldehyde solutions for 20 minutes at room temperature has been recommended for the disinfection of endo-

scopes by several professional organizations.[42,45] Immersion at 25° C for 45 minutes has been required by the Food and Drug Administration as the label claim for one 2% glutaraldehyde.[49] Our data suggest that immersion in 2% glutaraldehyde is incapable of inactivating *C parvum*.

The environmental survival of *C parvum* has been investigated. Robertson et al.[50] used a vital dye test to evaluate *C parvum* oocyst survival on surfaces at room temperature and observed 97% loss of viability by 2 hours and 100% by 4 hours. Our data confirm this loss of infectivity with time. We found a greater than 2.9 log reduction in oocysts infectivity within 30 minutes. However, it should be noted that *Cryptosporidium* in diarrheal stool smeared on a wooden surface survived for up to 72 hours.[51]

We believe that endoscopes likely do not represent an important vehicle for the cross-transmission of *C parvum* because mechanical cleaning removes approximately 10^4 organisms,[49] and our data and that of other investigators has demonstrated that *C parvum* viability is reduced over time on dry environmental surfaces.[50] Meticulous cleaning and high-level disinfection or sterilization is crucial to preventing the possibility of person-to-person transmission of *C parvum* via endoscopes. Our data suggest that commonly used disinfectants would be incapable of inactivating infectious *C parvum* oocyst present on environmental surfaces. To prevent the possibility of healthcare workers acquiring *Cryptosporidia* or person-to-person transmission via surface contamination, endoscopy personnel should use standard precautions as defined by the Centers for Disease Control and Prevention which include avoiding eating, smoking, or apply lip balm in areas that may be contaminated with stool or blood and handwashing after contact with stool and after endoscopic procedures. Mechanical cleaning of potentially contaminated environmental surfaces may also be important.

Our data demonstrate that the most common sterilization processes (steam sterilization and ethylene oxide) effectively inactivated high numbers of *C parvum* oocysts with greater than 3 $\log_{10}$ reduction. The Sterrad 100 which uses a plasma phase of hydrogen peroxide was also highly effective. However, one should note that our control data demonstrated that *C parvum* viability decreases with time and with exposure to high temperature. Thus, time and/or temperature may play the critical role in the inactivation of *C parvum* by ethylene oxide sterilization and the Sterrad 100 system. The vacuum cycle included in these sterilization processes may also damage *C parvum* oocysts, but we did not assess the separate role of exposure to a vacuum.

Our data represent the most complete set of experiments to assess the infectivity of *C parvum* to disinfection and sterilization processes used in healthcare. One must use caution in comparing our data with those of other studies that used different chemicals, exposure times, concentrations and assay techniques, especially assays that did not assess oocyst viability. In conclusion, we believe that our data do not warrant any change in the current recommendations for the cleaning and disinfection of endoscopes.

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