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Efficacy of Two Peroxygen-Based Disinfectants for Inactivation of *Cryptosporidium parvum* Oocysts

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Two commercial peroxygen-based disinfectants containing hydrogen peroxide plus either peracetic acid (Ox-Virin) or silver nitrate (Ox-Agua) were tested for their ability to inactivate *Cryptosporidium parvum* oocysts. Oocysts were obtained from naturally infected goat kids and exposed to concentrations of 2, 5, and 10% Ox-Virin or 1, 3, and 5% Ox-Agua for 30, 60, and 120 min. In vitro excystation, vital dyes (4′,6′-diamidino-2-phenylindole and propidium iodide), and infectivity in neonatal BALB/c mice were used to assess the viability and infectivity of control and disinfectant-treated oocysts. Both disinfectants had a deleterious effect on the survival of *C. parvum* oocysts, since disinfection significantly reduced and in some cases eliminated their viability and infectivity. When in vitro assays were compared with an infectivity assay as indicators of oocyst inactivation, the excystation assay showed 98.6% inactivation after treatment with 10% Ox-Virin for 60 min, while the vital-dye assay showed 95.2% inactivation and the infectivity assay revealed 100% inactivation. Treatment with 3% Ox-Agua for 30 min completely eliminated oocyst infectivity for mice, although we were able to observe only 74.7% inactivation as measured by excystation assays and 24.3% with vital dyes (which proved to be the least reliable method for predicting *C. parvum* oocyst viability). These findings indicate the potential efficacy of both disinfectants for *C. parvum* oocysts in agricultural settings where soil, housing, or tools might be contaminated and support the argument that in comparison to the animal infectivity assay, vital-dye and excystation methods overestimate the viability of oocysts following chemical disinfection.

Cryptosporidium parvum is a ubiquitous zoonotic protozoan parasite which can infect a wide range of host species, including humans and animals. In addition to being a cause of life-threatening disease in immunodeficient people, particularly AIDS patients, *C. parvum* has long been recognized as a common serious primary cause of diarrheic outbreaks in farm animals (especially newborn ruminants), resulting in significant economic losses (11). Infective thick-walled oocysts are passed with the feces of infected animals in very large numbers. These oocysts are highly resistant to most physical stress and environmental conditions, so infection can rapidly spread by means of the fecal-oral route when animals are communally housed or overcrowded. Additionally, oocysts shed from infected humans and animals contaminate food and drinking water, leading to human waterborne disease (10).

At present, there are no immunization or specific therapeutic regimens for the control of cryptosporidiosis, so measures to prevent or limit the spread of infection on farms must be targeted to eliminate or reduce infectious oocysts in the environment, for example, by hygienic measures such as removal of manure followed by disinfection of animal housings. In recent decades numerous studies focused on the viability, survival, and resistance of *Cryptosporidium* oocysts have shown that they are resistant to commonly used disinfectants. For most chemicals, effective concentrations are generally not practical for disinfection outside the laboratory, and highly concentrated chemicals that greatly reduce oocyst infectivity are either very expensive or quite toxic (14). *C. parvum* oocysts are resistant to chlorine-based disinfectants in any concentration that can be used to treat drinking water (12). Ozone, another popular water disinfectant, has proven to be much more efficient in killing oocysts, although its instability makes it difficult to maintain high levels in water for periods of time sufficient for treatment under practical conditions (20). The oocysts are also unaffected by commonly used laboratory disinfectants such as 6% sodium hypochlorite, 70% ethanol, and a variety of commercial preparations used domestically or in animal husbandry (28), although exposure to 10% Formol, aqueous or gaseous ammonia, or hydrogen peroxide has been reported to greatly reduce or eliminate oocyst infectivity (1, 9, 23). Low concentrations of ammonia (0.007 M) significantly decrease the viability of oocysts after 24 h of exposure, as determined with in vitro assays (19), and a 4-min exposure to 6% hydrogen peroxide or a 13-min exposure to ammonium hydroxide-amended windshield washer fluid reduces infectivity of *C. parvum* oocysts in cell culture 1,000-fold (28).

The objective of this research was to investigate the effects of two peroxygen-based disinfectants (commercialized for their use on farms) on the viability and infectivity of *C. parvum* oocysts in order to estimate the disinfectant concentrations and exposure times needed for oocyst inactivation. Two viability assays (an in vitro excystation assay and a 4′,6′-diamidino-2-phenylindole [DAPI]–propidium iodide [PI] stain procedure) and a mouse infectivity assay were used to evaluate the efficacies of the disinfectants.

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MATERIALS AND METHODS

Source and purification of oocysts. Oocysts of *C. parvum* were obtained from naturally infected kids on a goat farm with an outbreak of the disease. The parasite was maintained, isolated, and purified using procedures described previously (24). Briefly, kid feces were mixed with 0.04 M phosphate-buffered saline (PBS; pH 7.2) and filtered through sieves (mesh size, 45 μm). After centrifugation (5 min at 1,000 × *g*), the resulting sediment was suspended in PBS (pH 7.2) and diethyl ether (2:1), shaken vigorously, and centrifuged at 1,000 × *g* for 5 min at 4°C. This step was repeated until the sediment was free of lipids. The pelleted oocysts were washed three times in PBS (pH 7.2) (5 min at 1,000 × *g*). Oocyst purification was performed with a discontinuous Percoll gradient consisting of four 2.5-ml layers with densities of 1.13, 1.09, 1.05, and 1.01 g ml^{-1} and centrifuged at 650 × *g* for 15 min at 4°C. The band containing oocysts was washed three times in PBS (pH 7.2), and the oocysts were counted in a modified Neubauer hemacytometer using a 0.16% malachite green solution. The oocysts were resuspended in 2.5% (wt/vol) potassium dichromate solution and stored at 4°C throughout the experimentation period. All assays were performed with oocysts less than 3 months old.

Disinfection procedure. The active ingredients of the disinfectants tested were as follows: 25% hydrogen peroxide plus 5% peracetic acid (Ox-Virin) and 48% hydrogen peroxide plus 0.05% silver nitrate (Ox-Agua) (OX-CTA, Huesca, Spain). These liquid disinfectants were diluted in distilled water at concentrations of 2, 5, and 10% (Ox-Virin) and 1, 3, and 5% (Ox-Agua) and incubated with oocyst suspensions for 30, 60, and 120 min. For each disinfectant concentration and exposure time assayed, aliquots of 2×10^6 oocysts were placed in 1.5-ml microcentrifuge tubes and washed three times at 4°C with Hanks ' balanced salt solution (HBSS) (pH 7.2) at 2,500 × *g* for 5 min in order to remove the potassium dichromate solution. Oocysts were suspended in 500 μl of freshly prepared chemical disinfectant solution, shaken and incubated at room temperature (22 to 24°C) for the required time, and then washed three times with HBSS (pH 7.2) (2,500 × *g* for 5 min) in order to remove the disinfectants. Neutralizing agents could not be applied consistently to both disinfectants tested; therefore, they were not used. Exposure time was considered to be the time oocysts were in suspension, excluding subsequent washing steps. Oocyst doses for mouse infectivity assays were washed with distilled water instead of HBSS and then resuspended in distilled water to yield 200,000 oocysts in 75 μl. A control assay with untreated oocysts was performed in conjunction with each series of disinfectant treatments. The control oocysts were subjected to all of the same experimental procedures as the oocysts from the experimental groups, except for the disinfection step.

Assessment of oocyst viability by excystation. The in vitro excystation procedure was a modification of a previously reported method for excystation of *Eimeria tenella* (22). Aliquots (200 μl) of control and disinfectant-treated oocysts ($\sim 2 \times 10^6$) were suspended in 500 μl HBSS, pH 7.2, containing 0.25% (wt/vol) bovine trypsin and 0.8% (wt/vol) sodium taurodeoxycholate and incubated for 90 min at 37°C. This was the optimal length of time for maximizing excystation, as determined in suspensions initially sampled at regular time intervals. Fresh excysting fluid was prepared for each experiment. The numbers of oocyst shells (ghosts) and intact oocysts were counted in 10-μl aliquots of excystation suspension (removed before and after incubation) under ×400 magnification by using a hemacytometer and phase-contrast microscopy. A minimum of 100 shells plus intact oocysts were counted for each trial. The percentage of excysted oocysts was determined by the formula [number of shells/(number of intact oocysts + shells)] × 100, where the number of shells equaled the number of shells preexcystation subtracted from the number of shells postexcystation (7). Excystation levels were corrected according to a formula adapted from one used for computing excystation of *Giardia* spp. (16). The percent excystation of control oocysts was corrected to 100%, and the percent excystation of disinfectant-exposed oocysts was then corrected by the same factor as follows: corrected percent excystation (exposed oocysts) = [100/percent observed excystation (control oocysts)] × percent observed excystation (exposed oocysts). The percent inactivation of the exposed oocysts was then calculated as follows: percent inactivation = 100 − corrected percent excystation (exposed oocysts). Trials for each disinfectant concentration and exposure time were conducted in triplicate.

Assessment of oocyst viability using the vital-dye assay. The viability of oocysts was assessed using the vital-dye assay of Campbell et al., which relies on inclusion/exclusion of two vital dyes, PI and DAPI (7). A 1-h preincubation in acidified HBSS (pH 2.75) was performed prior to the incubation with the vital dyes in order to maximize vital-dye uptake (8). Aliquots of 10 μl of DAPI working solution (2 mg ml^{-1} in absolute methanol) and 10 μl of PI working solution (1 mg ml^{-1} in 0.1 M PBS, pH 7.2) were added to 100 μl of HBSS (pH 7.2) containing 2×10^6 oocysts and incubated at 37°C for 2 h. Oocysts were finally washed twice in HBSS (pH 4.0) in order to prevent DAPI crystallization (5). Ten-microliter aliquots of oocyst suspension were viewed under epifluorescence optics with appropriate filter blocks for visualization of the dyes. Proportions of ruptured (ghosts); PI-positive (permeable and dead); DAPI-positive, PI-negative (semipermeable and viable); and DAPI-negative, PI-negative (impermeable and viable after further trigger) oocysts were quantified by enumerating at least 100 oocysts in each trial. The sum of impermeable (DAPI-negative, PI-negative) and semipermeable (DAPI-positive, PI-negative) oocysts was considered the number of total viable oocysts (18). The percentage of viable oocysts was corrected according to the formula described above in order to determine the percent inactivation. Trials for each disinfectant concentration and exposure time were conducted in triplicate.

Assessment of oocyst infectivity using neonatal mouse assays. Litters (8 to 10 mice/litter) of 4-day-old neonatal BALB/c mice (Harlam Ibérica, S.L., Barcelona, Spain), individually housed with their dams in plastic boxes and given food and water ad libitum, were used for infectivity assays. Eighteen litters were infected per os with disinfectant-exposed oocysts at the different disinfectant concentrations and exposure times tested; four litters were infected with non-disinfectant-exposed oocysts and used as control groups. Pups were separated from their dams 1 h before inoculation and returned immediately afterward. Mice were each inoculated intragastrically with 2×10^5 *C. parvum* oocysts suspended in 75 μl of distilled water and killed 7 days postinoculation by cervical dislocation. The entire small and large intestine from each mouse was recovered in 5 ml of 0.04 M PBS (pH 7.2) and homogenized with an Ultra-Turrax (IKA Werke, Staufen, Germany) until a homogeneous mixture was obtained (three times for 10 s each time). The total number of oocysts present in each of the 5-ml homogenates was counted in a modified Neubauer hemacytometer.

Statistical analysis. Chi-square and Fisher's exact tests were used to analyze differences in the percentages of viable oocysts as determined by the in vitro excystation and dye permeability assays. The nonparametric Mann-Whitney U test was used to compare mean oocyst counts in mice. Significance was determined at a P of <0.05.

RESULTS

Ox-Virin. The results show that Ox-Virin has a deleterious effect on the survival of *C. parvum* oocysts, which could be enhanced by increasing both the time of exposure and the concentration of disinfectant. Viability results from control oocyst suspensions demonstrated considerable interassay variability. The lowest viabilities were recorded with the vital-dye-based assay (47.1%), while in vitro excystation assays revealed mean viabilities of 64.2%. Treatment of oocysts with Ox-Virin greatly reduced, and in some cases eliminated, the ability of oocysts to excyst; even the lowest concentration tested (2%) significantly decreased the excystation rate at the shortest exposure time of 30 min, in comparison with control oocysts ($P < 0.0001$) (Table 1). Excystation rates were significantly reduced by increasing disinfectant concentrations from 2% to 5% ($P < 0.01$), and addition of 10% Ox-Virin completely prevented excystation after 120 min of contact. Based on these data, exposures to 5% Ox-Virin for at least 120 min inactivated more than 99% of the oocysts. The dye permeability assay indicated a slightly higher survival rate than the in vitro excystation assay did, although the discrepancies between the two assays declined as both the exposure time and concentration of the disinfectant increased (Table 1). Measurements of oocyst viability determined by the vital-dye procedure indicated that treatment with 5% or 10% Ox-Virin for at least 30 min significantly reduced the percentage of viable oocysts, as did treatment with 2% Ox-Virin for 120 min ($P < 0.01$). As shown in the excystation assays, viability was significantly reduced by increasing the disinfectant concentration from 2% to 5% ($P < 0.05$), although a small proportion of oocysts remained viable following exposure to a 10% concentration for 120 min. Based on dye permeability assays, treatment with 5% Ox-Virin for

TABLE 1. Viability of *C. parvum* oocysts exposed at room temperature to Ox-Virin or Ox-Agua based on in vitro excystation and dye permeability assays[a]

Disinfectant and concn	Excysted oocysts (%)			Viable oocysts (%)		
	30 min	60 min	120 min	30 min	60 min	120 min
2% Ox-Virin	25.3*	14.2*	7.6*	43.4	33.8	23.1*
5% Ox-Virin	7.4*	2.9*	0.4*	30*	7.1*	2*
10% Ox-Virin	7.8*	0.8*	0*	9.6*	2.3*	1*
Control	64.6	65.2	62.7	46.7	47.4	47.2
1% Ox-Agua	35.2*	22*	12*	40.4	30.8*	16.4*
3% Ox-Agua	15.1*	11.8*	4.4*	34	16*	11.1*
5% Ox-Agua	17.9*	9*	0*	24*	16*	0*
Control	62.3	59.8	60.8	47.3	47.3	47.2

[a] Percentages of excysted oocysts and viable oocysts were determined by the in vitro excystation and dye permeability assays, respectively. Each number represents the mean of three replicates of at least 100 oocysts observed per replicate. *, statistically significant differences between disinfectant-exposed and control oocysts.

120 min inactivated more than 95% of the oocysts. Disinfection of oocysts with Ox-Virin also resulted in a dose-dependent reduction of infectivity for BALB/c mice. The inoculum with non-disinfectant-exposed oocysts (2×10^5 oocysts/mouse) allowed the establishment of infection in all of the mice inoculated. Mice infected with oocysts exposed to Ox-Virin at a concentration of 5% or greater for at least 30 min showed a statistically significant reduction in oocyst count compared to that of the controls ($P < 0.05$), and infection was completely prevented in mice infected with oocysts exposed to 5% Ox-Virin for 120 min or 10% Ox-Virin for 60 min (Table 2). These results indicate that both in vitro excystation and dye permeability assays slightly underestimated inactivation in comparison to mouse infectivity assays.

TABLE 2. *C. parvum* infection in neonatal BALB/c mice inoculated with oocysts exposed to Ox-Virin or Ox-Agua[a]

Disinfectant and concn	Exposure time (min)	Oocyst count (mean ± SE) (10^5)	Reduction of oocyst count (%)
None	0	14.2 ± 2.4	
2% Ox-Virin	30	10.5 ± 0.3	26.5
	60	9 ± 3.2	36.5
	120	6.5 ± 1.3	54.4
5% Ox-Virin	30	5.4 ± 0.5*	62.4
	60	1.5 ± 0.9*	89.5
	120	0*	100
10% Ox-Virin	30	1.5 ± 0.3*	89.6
	60	0*	100
	120	0*	100
1% Ox-Agua	30	9.2 ± 0.7	35.1
	60	6.6 ± 1.5*	53.3
	120	0.7 ± 0.2*	94.8
3% Ox-Agua	30	0*	100
	60	0*	100
	120	0*	100
5% Ox-Agua	30	0*	100
	60	0*	100
	120	0*	100

[a] Mice were inoculated with 2×10^5 oocysts exposed to the indicated concentrations of Ox-Virin or Ox-Agua. *, statistically significant differences between disinfectant-exposed and control oocysts.

Ox-Agua. Ox-Agua also considerably reduced the viability and infectivity of *C. parvum* oocysts, at even lower concentrations than those of Ox-Virin. Viability results from control oocyst suspensions were similar to those observed with Ox-Virin-treated oocysts. As reported above, the highest level of inactivation was predicted by mouse infectivity assays, whereas excystation assays demonstrated considerably lower viability values than vital-dye assays. Addition of Ox-Agua at a concentration of 1% or greater significantly reduced the excystation rate of oocysts after 30 min of contact, in comparison with control oocysts ($P < 0.0001$) (Table 1). Increasing the concentration of Ox-Agua from 1% to 3% significantly decreased excystation rates ($P < 0.05$), and no excystation occurred after addition of 5% Ox-Agua for 120 min. Based on these data, exposure to 3% Ox-Agua for 120 min inactivated more than 92% of the oocysts. Vital-dye permeability assays revealed that the lowest concentration of Ox-Agua tested (1%) significantly decreased the viability of oocysts after 60 min of exposure ($P < 0.05$) (Table 1). Viability declined with increasing concentrations of the disinfectant, and no viable oocysts remained after exposure to 5% Ox-Agua for 120 min. Disinfection also reduced the infectivity of oocysts for BALB/c mice in a dose-dependent model. The mean oocyst count in mice infected with oocysts which had been exposed to 1% Ox-Agua for at least 60 min was significantly lower ($P < 0.01$) than that in mice infected with untreated oocysts, and addition of Ox-Agua at a concentration of 3% or greater completely eliminated infectivity of oocysts for all mice after 30 min of exposure (Table 2).

DISCUSSION

Cleaning and chemical disinfection currently play a major role in the control of cryptosporidiosis, due to the limited availability of drugs effective against *Cryptosporidium*. However, the ability of *C. parvum* oocysts to withstand chemical treatment has long been recognized (4). *Cryptosporidium* oocysts are resistant to many forms of environmental stress that would prove lethal to other species of infectious agents and are invariably among the microorganisms most resistant to chemical disinfectants (21, 25). The reasons for this are not well known, but it would be reasonable to assume that the robust and impervious wall of the oocyst makes disinfection by chemical methods extremely difficult (13).

In the current survey, the abilities of two commercial peroxygen-based disinfectants to inactivate *C. parvum* oocysts were evaluated. According to the manufacturer's instructions, Ox-Virin is a biocide recommended for disinfection of livestock and poultry buildings, utensils, and environment, whereas the manufacturer's label for Ox-Agua claims its ability to disinfect tanks, cisterns, pipes, and drinking troughs. The results of the above-described experiments indicate that both disinfectants constitute a means to inactivate *C. parvum* oocysts, since disinfection resulted in a significant dose-dependent reduction of viability and infectivity, as demonstrated by in vivo and in vitro assays. The chemical compositions of the two products suggest that their anticryptosporidial activities were related to the concentration of hydrogen peroxide, the

main active ingredient common to both disinfectants. Ox-Agua contains hydrogen peroxide (48%) in combination with silver nitrate (0.05%) and was effective at lower concentrations and over shorter periods of time than was Ox-Virin (25% hydrogen peroxide, 5% peracetic acid); infectivity was completely eliminated after exposure to 3% Ox-Agua (equivalent to 1.44% hydrogen peroxide) for 30 min, whereas exposure to 5% Ox-Virin (equivalent to 1.25% hydrogen peroxide) for 120 min was needed to obtain the same effect. These results agree with those of previous studies demonstrating the effect of hydrogen peroxide on *C. parvum* infectivity in cell cultures at concentrations and exposure times different than those reported in the current study. Weir et al. found that oocyst infectivity in HCT-8 cells was reduced 1,000-fold after 4 min of exposure to 6% hydrogen peroxide, and no foci of infection were observed after exposure to 6% hydrogen peroxide for 13 or 33 min (28). Barbee et al. reported a 500-fold reduction in infectivity in MDCK cells after a 10-min exposure to 6% hydrogen peroxide and a >1,000-fold reduction in infectivity after 20 min, although this treatment at lower concentrations or exposure times did not completely inactivate the parasite (2). Hydrogen peroxide also reduced excystation rates of *C. parvum* oocysts, and oocysts treated with hydrogen peroxide failed to infect neonatal mice at a dose of 10^5 treated oocysts per mouse (4). The use of hydrogen peroxide gas has also been reported useful for sterilization of endoscopic material containing *C. parvum* oocysts (27).

Besides hydrogen peroxide, additional minor active ingredients of both disinfectants may have contributed to oocyst inactivation. Peracetic acid, another peroxygen compound considered to be a more potent biocide than hydrogen peroxide (21), is a component of Ox-Virin and has also been found effective against *C. parvum* oocysts at low concentrations. Holton et al. reported that 0.35% peracetic acid completely abrogated the viability of *C. parvum* oocysts, as no excystation was reported after 5 min of exposure (17). However, peracetic acid did not completely eliminate infectivity of oocysts in MDCK cells (2). The anticryptosporidial activity of silver nitrate, a minor ingredient of Ox-Agua, has not previously been reported, although this disinfectant has long been used as an antimicrobial agent (21). Our results suggest that peracetic acid did not greatly contribute to oocyst inactivation, since infectivity was reduced but not eliminated after exposure to 10% Ox-Virin (equivalent to 2.5% hydrogen peroxide and 0.5% peracetic acid) for 30 min. In contrast, exposure for the same period to 3% Ox-Agua (equivalent to a lower concentration of hydrogen peroxide, 1.44%, but used in combination with 0.0015% silver nitrate) completely eliminated oocyst infectivity for mice, suggesting that silver nitrate has significant anticryptosporidial activity at low concentrations and, in combination with hydrogen peroxide, clearly contributes to oocyst inactivation.

In vitro excystation, live-dead vital dyes (DAPI and PI), and infectivity in neonatal BALB/c mice were used in this study as indicators of oocyst viability and infectivity. Viability assays by DAPI/PI vital staining have been reported to demonstrate a very good correlation with the maximized in vitro excystation assay (7, 26), although evidence exists to suggest that in vitro excystation assays may overestimate viability in comparison to animal infectivity assays (3, 15). Our findings show that the dye permeability assay indicated a slightly higher survival rate than the in vitro excystation assay did, as previously observed (18, 19), although both methods clearly underestimated the inactivation of *C. parvum* oocysts in comparison with the animal infectivity methods. When in vitro assays were compared with the infectivity assay as an indicator of oocyst inactivation after treatment with 10% Ox-Virin, the excystation assay showed 98.6% inactivation after 60 min of exposure, while vital dyes showed 95.2% and infectivity revealed 100% inactivation. Discrepancies in results were even greater for Ox-Agua, since addition of 3% Ox-Agua for 30 min completely eliminated infectivity of oocysts for mice, while we were able to observe only 74.7% inactivation by excystation assays and 24.3% with vital dyes (which proved to be the least reliable method for predicting *C. parvum* oocyst viability).

These results are in agreement with earlier studies reported for oocysts treated with ozone, UV light, or chlorine compounds, which showed neonatal mouse infectivity as the most sensitive indicator of oocyst inactivation (3, 6, 15, 20). The factors responsible for these differences are unclear, although they might be explained by what each procedure is measuring. According to Bukhari et al., in vivo assays provide an indication of viability by measuring changes in oocyst permeability or oocyst response to biochemical stimulus, whereas animal infectivity is a measure of the ability of oocysts to excyst, invade, and multiply within the enterocytes of the host (6). By comparing viability and infectivity assays following ozone treatment of *C. parvum* oocysts, the authors suggested that the damage caused by low disinfectant concentrations and short contact times was insufficient to change the permeability of the outer wall to vital dyes but not to cause changes in the sporozoite to prevent the attachment to or invasion of enterocytes. Other investigators have attributed differences between in vitro and in vivo surrogates to the higher sensitivity of the mouse infectivity assay. The low number of oocysts enumerated in the in vitro assays (usually limited to 100 oocysts) may reduce confidence when results are used for extrapolation to large populations of oocysts, whereas only a small dose of oocysts is required to produce a conspicuous infection in mice, since the production of sporulated thin-wall oocysts makes it possible for the parasite to persist inside the host (20). The age (storage time) of oocysts has also been reported to affect the sensitivity of the estimate, and oocysts stored in dichromate solution for longer than 6 months cannot reliably be used for disinfection studies because low levels of excystation are frequently observed (20). Our results with control oocyst suspensions demonstrated low percentages of viability in comparison to those reported in other studies. Maximum levels of excystation were only around 60%, and that percentage was not improved by increasing the time of incubation beyond 90 min. Viabilities were lowest with the vital-dye assay (47.1%). These low numbers of viable oocysts may also have affected the accuracy of the counting procedure, despite the fact that the oocysts used were stored in dichromate solution for no longer than 3 months.

In conclusion, the current findings demonstrate the efficacies of the disinfectants tested against *C. parvum* oocysts and their potential usefulness in disinfecting contaminated buildings, where poultry, livestock, or other animals might acquire cryptosporidiosis, as well as contaminated instruments, tools, or

utensils. Ox-Virin at a concentration of 5% can effectively disinfect *C. parvum* oocysts within 120 min of exposure, whereas the more potent disinfectant Ox-Agua at a concentration of 3% completely eliminates infectivity after 30 min of exposure. This study also supports the argument that vital-dye and excystation methods overestimate the viability of oocysts following chemical disinfection, in comparison to animal infectivity assays.

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