Chlorine Dioxide Gas Renders Syphacia Ova Non-Viable with a Four Hour Exposure Period

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Abstract

We evaluated the efficacy of chlorine dioxide gas for environmental decontamination of *Syphacia* spp. ova. *Syphacia* spp. ova were collected by perianal cellophane tape impression of pinworm infected mice. Tapes with attached ova were exposed to chlorine dioxide gas for periods of 1, 2, 3, and 4 hours (h). Following gas exposure, ova were incubated in a phosphate buffer medium for 6 hours to promote hatching. Tapes with attached ova maintained at room temperature for 1, 2, 3, and 4 hours and similarly incubated in a phosphate buffer medium for 6 hours, were designated as controls. Ova viability was assessed by microscopic examination following incubation.

The 4h chlorine dioxide gas exposure rendered 100% of *Syphacia* spp. ova non-viable. Conversely, only 17% of ova on the 4h control slide were rendered non-viable. Other chlorine dioxide gas exposure times resulted in variable effectiveness. These data suggest that chlorine dioxide gas at a minimal exposure time of 4h is effective for surface decontamination of *Syphacia* spp. ova.

Introduction

Pinworms of the genus *Syphacia* inhabit the cecum and colon of rodents and are common contaminants of contemporary laboratory animal facilities (Hill 2009). Although usually nonpathogenic in immunocompetent rodents, pinworm infections may have adverse effects on behavior, growth, intestinal physiology and immunology (Lubcke 1992, Mohn 1981, Pearson 1975, Wagner 1988). These effects, coupled with the demand for defined experimental rodents and possible hindrances to interinstitutional collaborations make effective pinworm surveillance and eradication important for many laboratory animal facilities. Eradication of *Syphacia* spp. infections is complicated by the ability of ova to aerosolize and remain viable in the environment for lengthy, but unknown, periods (Huerkamp 2000). Various agents and methods, including aqueous chlorine dioxide products, have been evaluated for destruction of *Syphacia* spp. ova. (Dix 2004). Chlorine dioxide gas has demonstrated efficacy as an environmental and surface biocide (Li 2012, Bhagat 2010, Kuroyama 2010, Newsome 2009, Han 2003), yet the agent's effectiveness in killing *Syphacia* spp. ova has not been evaluated. Here we report on the efficacy of chlorine dioxide gas for environmental decontamination of *Syphacia* spp. ova.

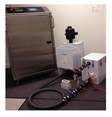
Materials and Methods (M&M): Animals

Thirty two mice of unknown health status were procured from two local vendors. The animals were group housed 3-4 mice per cage in micro- isolator cages (Lab Products, Inc., Seaford, DE) on corncob bedding (Teklad 7087 Soft Cobs Enriched Bedding, Harlan Teklad, Madison, WI.). Animals were housed in an AAALAC, International accredited dedicated animal facility under environmental conditions consistent with the Guide for the Care and Use of Laboratory Animals (ILAR). Mice were provided rodent chow (Teklad 8640, Harlan Teklad, Madison, WI) and tap water ad libitum. All manipulations were reviewed and approved by the University of Tennessee's Institutional Animal Care and Use Committee.

M&M: Collection of Ova

Ova were collected on the day of the experiment by anal cellophane tape impression. Double sided tape was used to affix the tape to a slide allowing the anal impression side up. Slides with affixed tape were scanned using a *compound microscope under low power to confirm the presence of Syphacia spp. ova.* Syphacia spp. ova were identified based upon distinguishing characteristics and size. Slides were randomly designated as either control or experimental for time points of 1, 2, 3 or 4 hours. Duplicate runs were performed for all time points except 1 hour. Collection of ova and exposure to the chlorine dioxide gas or room temperature took place on Day 0. The control and experimental slides were concurrently incubated in the phosphate buffer hatching medium on Day 1.

M&M: Chlorine Dioxide Exposure



Chlorine dioxide gas (ClO_2) was generated using the Minidox-M Decontamination System (ClorDiSys Solutions, Lebanon, NJ). To generate chlorine dioxide gas, 2% chlorine gas was passed through sodium chlorite catalyst cartridges. The resulting chemical reaction produced pure chlorine dioxide gas.

The Minidox system delivered chlorine dioxide gas via Kynar® tubing to an airtight, fabricated polypropylene chamber (ClorDiSys Solutions, Lebanon, NJ) measuring 0.028 m^3. The slides with attached pinworm ova were positioned nine centimeters above the floor of the experimental chamber. A manufactured fan (Minebea, Mita,Tokyo) was placed on the floor of the chamber to continuously circulate the air. Temperature and relative humidity were monitored. Between treatment groups, relative humidity ranged from 52-67% and temperatures ranged from 23-26^{0}C. Chlorine dioxide gas was delivered to the chamber until a concentration of 360 ppm (1 mg/L) was obtained. This level of chlorine dioxide gas was maintained until the parts per million hours (ppm-hours) of 360, 720, 1080, and 1440 (equivalent to 1hr, 2hr, 3hr, and 4hr respectively) exposure periods were achieved. At the end of the exposure period, the chamber was aerated using a charcoal scrubber (ClorDiSys Solutions, Lebanon, NJ), and the slides with treated ova were removed from the chamber.

M&M: Hatching Procedures

The hatching medium was adapted from and prepared according to a method provided by J. Dix et al. (2004). 1.6 g sodium phosphate dibasic bio reagent (Na_2HPO_4, Sigma-Aldrich, St. Louis, MO) were added to 95 ml of sterile water; heated and stirred to dissolve. 0.07 g potassium phosphate (KH_2PO_4) (Sigma-Aldrich, St. Louis, MO) was dissolved in 5 ml of sterile water. These two solutions were combined as the phosphate buffer. 1.0 g of trypsin (1000-2000 BAEE units/mg solid, Sigma-Aldrich, St. Louis, MO), 0.26g ox bile (dehydrated, purified for microbiology, Sigma-Aldrich, St. Louis, MO) dissolved in 3 ml of sterile water and a solution of 0.2 g cysteine (DL-$C_3H_7NO_2S$) (Sigma-Aldrich) dissolved in 2.5 ml 1N HCl were added to the phosphate buffer. Both control slides and slides exposed to chlorine dioxide gas were placed in covered petri dishes with a sufficient amount of hatching medium to cover the slide and the affixed tape. Incubation took place in an ambient air incubator at 37° C for 6 hours. Slides were rinsed with sterile water and observed microscopically.

M&M: Percent Hatchability

Slides were air-dried and scanned at 40X magnification under a compound microscope to quantify hatched vs. not hatched *Syphacia* ova. Ova were considered nonviable and scored as not hatched if it contained larva (Fig. 2). Ova without larva or those with an open operculum with larva emerging were considered viable and scored as a hatched (Figs. 3, 4, 5). To reduce variability in quantification of each condition, the same person quantified all slides for hatching percentage. The person quantifying was not blinded to treatment groups

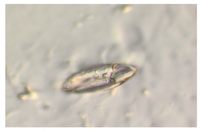


Figure 2. Non-viable *Syphacia* spp.ovum at 40X magnification.

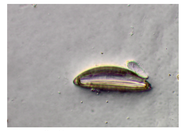


Figure 3. Syphacia spp. ovum with open operculum at 40X magnification

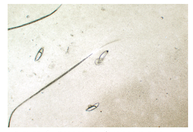
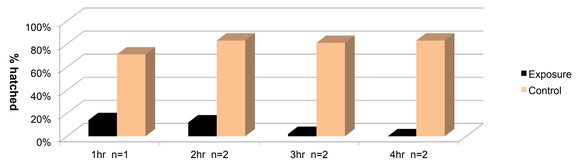


Figure 4. Emerging *Syphacia* spp. larva at 10X magnification

Hatched (%)				
Exposure time (h)	ClO_2	Control	t	df
1	14	71	6.96	1,129
2	12	82.5	19.99	1,134.26
3	2	80.5	32.69	1,487.44
4	0	83	17.18	1,590

Table 1. Hatch percentage comparison using unpaired *t* tests for *Syphacia* spp. Ova exposed to chlorine dioxide gas. $P < 0.001$. Except for 1-h exposure time, all hatch percentages are reported as means of duplicates.

Results



The one hour exposure had a 14% viability rate compared to 71% control rate. The two hour exposure had a 12% viability rate compared to 83% control rate. The three hour exposure rate had a 2% viability rate compared to 81% control rate. The four hour exposure rate had a 0% viability rate compared to 83% control rate. Viability rates decreased as the exposure times increased.

Under 1-h exposure, 14% of ova were viable compared to 71% for the control group. The 2-h exposure group had a 12% viability rate compared to 82.5% for controls. The 3-h exposure group had a 2% viability rate compared to 80.5% for controls, and the 4-h exposure group had a 0% viability rate compared to 83% for the control group (Figure 6; Table 1). Thus, viability rates decreased as exposure times increased.

There was a statistically significant difference in the proportion of hatched to not hatched ova for each group ($P < 0.001$ for all).

Discussion

Chlorine dioxide destroys microorganisms by disrupting the transportation of nutrients across the cell wall.[20] In 1967, the Environmental Protection Agency first registered chlorine dioxide (in the form of a liquid) for use as a disinfectant and sanitizer at a variety of commercial sites, such as animal farms, and food processing, handling, and storage plants. Chlorine dioxide gas has been registered as a sterilizer since 1988,[20] and all microbial agents so far tested have been eradicated by the chlorine dioxide gas.[2] For example, chlorine dioxide gas has been shown to be effective at eradicating interior surfaces of *Bacillus anthracis* spores as well as effective against *Salmonella*.[15,19]

Our study is the first, to our knowledge, that tests chlorine dioxide gas effectiveness against *Syphacia* spp. ova. All chlorine dioxide treatment times resulted in significant differences in the hatching rates of the ova. The 1-h exposure decreased the hatching rate from 71% to 14%. Because some ova were still considered viable at 1 h, the exposure rate was increased. At a 2-h exposure time, hatching decreased slightly more to 12%, but some of the ova were still viable. Increasing the exposure rate to 3 h further reduced the hatching rate to 2%. Chlorine dioxide gas fumigation effectively rendered 100% of the *Syphacia* ova nonviable at the 4-h exposure time. Hatching rates were significantly different between the treated verses untreated eggs at each exposure time; however, the 4-h exposure time was required to make 100% of the ova nonviable. Because the 4-h exposure time consistently rendered the *Syphacia* ova nonviable, longer exposure times were not evaluated. We shortened the hatching media incubation time from overnight to 6 h because the overnight hatching time was digesting larvae and damaging eggs. A previous study reported altered egg walls after incubating the eggs overnight in the hatching media.[5]

Syphacia spp. ova are known to be resilient in the environment,[8] and eradication of these eggs from the environment can be challenging. Using a gas would be ideal in eradicating *Syphacia* spp. ova from a room because areas typically inaccessible to liquid decontaminants, such as light covers and vent covers, would be susceptible to the gas. Our study addresses only a chamber method. For smaller items contaminated with *Syphacia* spp. ova, the chamber method would be ideal. Items that could be damaged by high heat could also be disinfected with the chlorine dioxide gas chamber system.

The ability of the chlorine dioxide gas to permeate hard-to-reach areas makes it an attractive method for room-level eradication of *Syphacia* spp. Unlike liquid chlorine dioxide products, gaseous chlorine dioxide is not corrosive and is thus safe for many materials that could be damaged by other disinfectant methods or agents.[3] While the 4-h chlorine dioxide gas exposure rendered 100% of *Syphacia* spp. ova nonviable in an enclosed environmental chamber, results may differ under other environmental conditions such as those present in animal housing rooms. Additional large-scale studies should be performed to ensure the reproducibility of these data.

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