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 infected mice. Tapes were incubated at 37°C and 100% humidity for 1 to 4 hours. Following gas exposure, ova were incubated in a phosphate buffer medium and examined at 1 hour. Slides with attached pinworm ova were scored as contaminated, achieving a 360 ppm (1 mg/L) concentration of chlorine dioxide gas exposure for 4 hours, was designated as controls. Ova viability was assessed by microscopic examination following incubation. The 4th chlorine dioxide gas exposure rendered 100% of Syphacia spp. ova non-viable. Conversely, only 17% of ova on the 4th control 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 temporary laboratory animal facilities (Hill 2009). Although usually nonpathogenic to immunocompetent rodents, pinworm infections may have adverse effects on behavior, growth, intestinal physiology and immunology (Lubke 1992, Mohn 1981, Pearson 1975, Wagner 1988). These effects, coupled with the demand for defined experimental rodents and other potential 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 aerotolerate 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 disinfectant (Dix 2007, Bell 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)

Two thirty mice of unknown health status were procured from two local vendors. The animals were grouped housed in 3-4 mice per cage in micro-isolator cages (Lab Products, Inc., Seco/box, DB) on a corncob bedding (Teklad 7087 Soft Cobs Enriched Bedding, Harlan Teklad, Madison, WI.). Animals were housed in a AAALAC accredited dedicated animal facility under environmental conditions consistent with the Guide for the Care and Use of Laboratory Animals (LAMC). Mice were provided rodent chow (Teklad 8040, 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 attached tape were scanned using a compound microscope under low power to confirm the presence of Syphacia spp. ova. Ova were identified based upon distinguishing characteristics and observed microscopically. 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 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.

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 (NaH2PO4, Sigma-Aldrich, St. Louis, MO) were added to 95 ml of sterile water; heated and stirred to dissolve. 0.07 g potassium phosphate (KH2PO4) (Sigma-Aldrich, St. Louis, MO) added to 5 ml of sterile water. These two solutions were combined as the phosphate buffer. 1.0 g of trypsin (1000-2000 BAUE units/ml solid, Sigma-Aldrich, St. Louis, MO). 0.26g ox bile (dehydrated, purified for use in bile salts, St. Louis, MO) dissolved in 3 ml of sterile water and a solution of 0.2 g cysteine (DL-C3H7NO2S) (Sigma-Aldrich) dissolved in 2.5 ml 1N HCl 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 chamber at 33°C for 6 hours. Slides were rinsed with sterile water and observed microscopically.

M&M: Exposure Times
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 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.

M&M: Analysis
The ability of the chlorine dioxide gas to permeate hard-to-reach areas makes it an attractive method for decontamination of areas, such as inside toilet covers, vent covers, would be susceptible to the gas. Our study addresses only a chamber method. For environmental and surface decontamination, the chlorine dioxide gas exposure atmosphere 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.

Discussion
Chlorine dioxide destroys microorganisms by disrupting the transportation of nutrients across the cell wall. In 1967, the Environmental Protection Agency first registered chlorine dioxide in the form of a liquid for use in water treatment. Chlorine dioxide gas has been registered as a disinfectant since 1988 and all recent studies have been eradicated by the chlorine dioxide gas. 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. 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 rate of the ova. The 1h 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 2h time exposure, 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 4h exposure time. Hatching rates were significantly different between the treated versus untreated eggs at each exposure time; however, the 4h exposure time was required to make 100% of ova nonviable. Because the 4h exposure time consistently rendered the Syphacia ova nonviable, longer exposure times were evaluated. We shortened the hatching media incubation time from overnight to 6 h because an 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. Syphacia spp. ova are known to be resilient in the environment, 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.

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References