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Room Decontamination Using Ionized Hydrogen Peroxide Fog and Mist Reduces Hatching Rates of *Syphacia obvelata* Ova

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This study evaluated the efficacy of ionized hydrogen peroxide (iHP) fog and mist for environmental and surface decontamination of *Syphacia obvelata* ova in rodent rooms. Ova were collected by perianal tape impression from *S. obvelata* infected mice. In experiment 1, ova were exposed to iHP using a whole-room fogging decontamination system with a 15 min initial fog application cycle in unoccupied rodent rooms. Ova were removed from the fogged environment after a 15 min, 30 min, 90 min, or 240 min iHP exposure time. In experiment 2, a second cohort of ova were exposed to iHP using the whole-room fogging decontamination system. Ova were removed after 3, 4 or 6 continuous fog application cycles with 45 min dwelling time between each cycle and 15 h dwelling time for the last time point. In experiment 3, a third set of ova was exposed to an iHP surface misting unit with 1, 2, or 3 iHP mist applications. A 7 min contact time followed each application. After exposure, ova were incubated in a hatching medium for 6 h. Control ova were maintained at room temperature without iHP exposure before incubation in the hatching medium. After incubation, the number of ova hatched was assessed by microscopic examination. For experiment 1, results ranged from 46% to 57% of exposed ova hatched. For experiment 2, results ranged from 43% to 49% of ova hatched. For experiment 3, 37% to 46% of exposed ova hatched. Conversely, for the control groups above 80% of ova hatched for all 3 experiments. These data suggest that exposure to iHP fog and mist has variable effectiveness in reducing viability of *S. obvelata* ova at the time points tracked. Further studies are needed to identify iHP exposures that will further reduce or eliminate the hatching of rodent pinworm ova.

Abbreviations: iHP, ionized hydrogen peroxide; H_2O_2, hydrogen peroxide

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Although usually nonpathogenic in immunocompetent rodents, pinworms of the genus *Syphacia* are common contaminants of laboratory animal facilities.[1,9,14,20] Pinworms inhabit the cecum and colon of rodents, and in the research setting, infestation of rodent colonies with these parasites can have significant adverse effects on behavior, growth, intestinal physiology, and immunology of the affected animals.[17,19,25,28] Therefore, alteration and distortion of research data and conclusions may occur if study animals are infected with *S. obvelata*. These adverse effects of *S. obvelata* infestation can complicate collaboration between institutions and reduce confidence in the validity of research data. Due to these concerns, laboratory animal facilities need effective pinworm surveillance and eradication programs.

Traditional methods of eradication include treating rodents with a parasiticide, like fenbendazole. This agent has been successfully employed to eradicate *S. obvelata* from many affected colonies, but *S. obvelata* ova can remain viable in the environment for long periods of time.[2,10] Various methods and agents have been tested for their ability to destroy *S. obvelata* ova in the environment, including chlorine dioxide, heat, exposure to UV light, ethylene oxide, formaldehyde, and chlorhexidine.[6,7] H_2O_2-based environmental disinfection systems have gained popularity in the last few years in both the health care industry and the laboratory animal research setting.[3,15,16,26] To our knowledge, the effectiveness of using ionized hydrogen peroxide (iHP) to decontaminate rodent housing environments has not been evaluated for the specific destruction of *S. obvelata* ova. The aim of this study was to evaluate the efficacy of iHP fogging and misting systems in reducing the viability of *S. obvelata* ova.

Materials and Methods

Animals. A total of 40 mice were purchased from a local pet store. A local pet store was chosen as a source due to the unavailability of pinworm infected research mice from commercial vendors. Mice had an unknown health status. The animals were group-housed at 3 to 5 mice per cage in individually ventilated cages (Innovive, San Diego, CA) prefilled with corncob bedding and nesting enrichment (Innorichment, Innovive, San Diego, CA). Mice were provided with ad libitum rodent chow (Teklad 2014, Harlan Teklad) and prefilled water bottles (Aquavive, Innovive, San Diego, CA). Mice were housed in an AAALAC-accredited animal facility following standard husbandry practices. Environmental conditions were consistent with the *Guide for the Care and Use of Laboratory Animals*.[12] All procedures and protocols were reviewed and approved by the Iowa State University IACUC.

Collection of ova. Double-sided cellophane tape was used to collect ova by using anal impressions, performed in the afternoon, from all 40 mice.[9,23,27] Once the anal impression was

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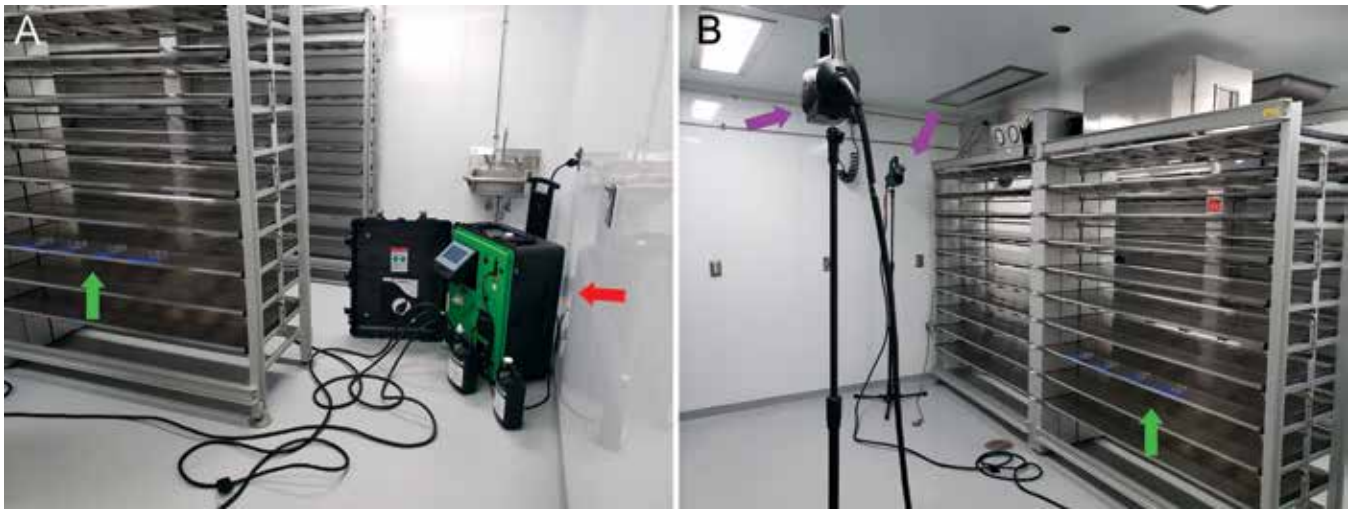

Figure 1. Whole-room fogging decontamination equipment using SteraMist (TOMI, Environmental Solutions, Frederick, MD) (A) Detail of the SteraMist fogging unit (red arrow) with slides placed individually inside culture dishes without lids on the third shelf of an empty rodent rack (green arrows). (B) SteraMist applicators securely mounted on Triad Orbital T3 tripod stands at a standard 6-foot height (purple arrows). The 3 applicators were positioned to maximize an even distribution of the fog through-out the area.

performed, the tape was affixed to a slide, with the anal impression side up.[6,7] Confirmation of *Syphacia* spp. ova presence on slides was performed by scanning the slides with a compound microscope under low power. Confirmation of genus and species was performed via submission of a representative sample of ova to an external lab for real time PCR (IDEXX BioAnalytics, Columbia, MO). Results confirmed the presence of *S. obvelata*. Other parasites screened for by PCR were *Aspiculuris tetraptera* and *Syphacia muris*. Neither of these parasites were detected in the sample submitted. Ova were identified according to distinguishing morphologic characteristics and size. The number of ova per tape was 20 or greater. Each slide had 3 tapes affixed to its surface. Slides were assigned randomly as either controls or experimental time points. Duplicate runs were performed for all time points and for the control groups.

Ionized hydrogen peroxide (iHP) fog and mist exposure. Slides holding tapes with attached ova were either exposed to iHP fog using a whole-room fogging decontamination system for experiments 1 and 2, or were exposed to a surface misting unit for experiment 3. In all 3 experiments, the SteraMist (TOMI, Environmental Solutions, Frederick, MD; Figure 1 A), a proprietary system for delivery of iHP for decontamination of environments and surfaces was used. The TOMI iHP system is different than traditional vaporized H_2O_2 systems that simply apply a high concentration of H_2O_2, often containing additives such as peracetic acid or silver, and then move it around the space using a fan. SteraMist uses Binary Ionization Technology (BIT), a patented 2-step process that activates and ionizes a 7.8% H_2O_2 sole active ingredient-based solution into a fine mist or fog known as iHP. This is achieved by aerosolizing, via an ultra-fine nozzle, a low concentration (7.8%, ready to use, non-hazardous to ship and store) solution of H_2O_2 and then passing it through an atmospheric cold plasma arc generated between 2 pin electrodes.[15] The mist moves like gas without the need for preconditioning humidity,temperature, or use of an external fan. The ionizing process allows a quicker contact time than VHP and a quicker aeration time, as the same high concentration of H_2O_2 is not required, reducing materials compatibility issues.

The whole-room fogging decontamination system (experiments 1 and 2) used unoccupied rodent housing rooms. Slides were placed individually inside culture dishes without lids at a height of 40 inches from the floor on the third shelf of an empty rodent rack located in the center of the room. Dispersion of the fog in the room was achieved through the use of 3 SteraMist applicators securely mounted on Triad Orbital T3 tripod stands at a standard 6-foot height. The 3 applicators were positioned to maximize an even distribution of the fog through-out the area (Figure 1 B). The air handling system was disabled at the room level. Air intake, exhaust ducts, and doors were sealed with tape and plastic sheeting. Iodine test papers (LaMotte, Chestertown, MD) were placed throughout the entire room and next to each slide to confirm fog penetration via color change. Iodine test papers are an accurate indicator of the presence of H_2O_2 .[5,8,21,22] The dosing instructions outlined on the EPA registered label were followed. The equipment used was put through rigid and documented quality control process to ensure it worked properly and it was calibrated on-site to confirm that the required dose was delivered. All iodine test papers changed color to purple confirming that the effective product concentration was achieved. In addition, the concentration of product used during this study of 7.8% H_2O_2 initial solution, applied in the room to be fogged at the rate of 0.5 mL/ft^3 of iHP for a 15 min application cycle, is the same that is routinely used to achieve a 6 log kill or greater for bacteria and viruses. At the time of sample collection, per the product label instructions, the technician retrieving the samples wore PPE including gloves, long pants/sleeves, and a PAPR with organic vapor filter and briefly accessed the room at the established time points to collect the slides. After the collection occurred the room door was resealed as described above.

For experiment 1, each room received an initial fogging application cycle of 15 min at 0.5 mL/ft^3 of iHP. After the 15 min application cycle, ova were continually exposed to fog and removed after 15 min, 30 min, 90 min or 240 min of exposure time.

For experiment 2, slides were continually exposed to fog being applied in the room. Slides were removed after 3, 4 or 6 whole room fog application cycles of 15 min each at 0.5 mL/ft^3 of iHP with 45 min of dwelling time between each cycle and 15 h of dwelling time for the last time point.

Experiment 3 used a surface misting unit to expose ova to iHP mist. The applicator is identical in use and design to the applicators used for the whole-room fogging decontamination system used in experiments 1 and 2. The main difference is that the applicator is handheld by a trained operator and the

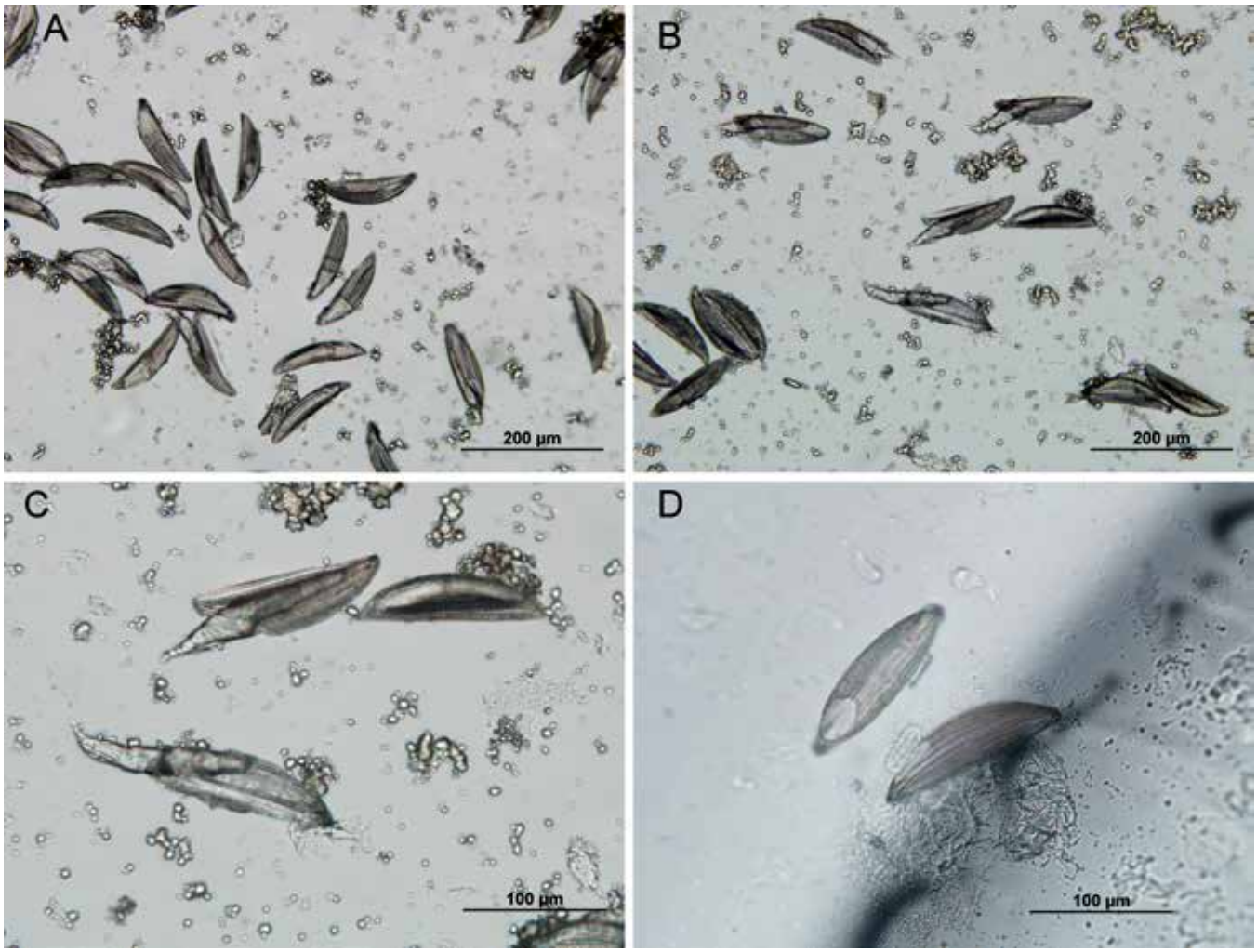

Figure 2. (A) Unhatched *S. obvelata* ova. (B) Hatched *S. obvelata* ova with emerging larvae. (C) Two hatched *S. obvelata* ova with emerging larvae next to an unhatched *S. obvelata* ovum. (D) Two hatched *S. obvelata* ova with characteristic open opercula.

iHP mist was sprayed on the slides from a distance of 20 to 24 in. for the duration of 5 s per square foot. In this experiment, slides were placed individually inside culture dishes without lids on the floor of an unoccupied rodent housing room and exposed to 1, 2, or 3 iHP mist applications, with each receiving 7 min of contact time. To reduce variability in the execution of the treatments, the same trained operator applied the iHP mist using the SteraMist surface misting unit. As in experiments 1 and 2, iodine test paper strips were placed next to each slide to confirm fog penetration via color change. All iodine test papers changed color to purple confirming that the effective product concentration was achieved. During the application of the product and subsequent retrieval of samples, per the product label instructions, the technician operating the device wore the same required PPE as described for experiments 1 and 2.

Each of the 3 experiments had one slide and a duplicate as the control. Control slides were maintained at room temperature without fog and mist exposure before incubation in the hatching medium.

Hatchability. The hatching medium used was adapted from a previously published paper.[7] Briefly, sodium phosphate dibasic bio reagent (Na_2HPO_4, Sigma-Aldrich, St Louis, MO) in the amount of 1.6 g was added to 95 mL sterile water, and then heated, and stirred to dissolve. Then, 0.07 g of potassium dihydrogen phosphate (KH_2PO_4, Sigma-Aldrich, St Louis, MO) was dissolved in 5 mL sterile water. These 2 solutions were combined to produce the phosphate buffer at a final molar concentration of 0.118 M. In addition, 1.0 g trypsin (1000 to 2000 BAEE U/mg solid, Sigma–Aldrich, St Louis, MO), 0.26 g ox bile (dehydrated, purified for microbiology, Sigma–Aldrich, St Louis, MO) dissolved in 3 mL sterile water, and 0.2 g cysteine (Sigma-Aldrich, St Louis, MO) dissolved in 2.5 mL of 1 normal solution of hydrochloric acid (1 N HCl, Sigma-Aldrich, St Louis, MO) were added to the phosphate buffer. Slides were all placed individually in single culture dishes. Hatching medium was added to the dish in an amount sufficient to cover the slide and the affixed tape. After being covered with lids, culture dishes were placed in an ambient air incubator at 37 °C for 6 h. After incubation, slides were rinsed with sterile water, allowed to air dry and scanned at 40× magnification under a compound microscope to quantify percentage of hatched *S. obvelata* ova. Quantification of hatching was accomplished by counting how many ova hatched out of 20 on each tape. Results were reported as an overall average percentage of ova hatched for each exposure group in each experiment. Ova were considered as not hatched if they contained a larva (Figure 2 A). Ova without larva and with an open operculum, or with a larva emerging were considered as a hatched (Figure 2 B through D). To reduce variability in scoring, the same person quantified all slides for hatching. This person was not blind to the exposure groups.

Statistical Analysis. All statistical analyses were performed by using Statistical Analysis System Software (SAS version 9.4, SAS Institute, Cary, NC). Briefly, a generalized linear model was fit to explain the effect of a given treatment on the probability of an ovum hatching for each experiment. Significance was set at $P < 0.05$.

Results

Experiment 1 showed that the 15-min, 30-min, 90-min, and 240 min dwelling time points had 57, 55, 52, and 46% hatching respectively, with controls showing 87% hatching (Figure 3 A).

Experiment 2 showed 49, 42, and 47% hatching for the 3, 4 and 6 whole room fog application cycles respectively, with controls showing 81% hatching (Figure 3 B).

Experiment 3 showed 44, 37, and 46% hatching for the 1, 2, or 3 iHP mist applications with 7 min contact time each respectively, with controls showing 91% hatching (Figure 3 C).

Differences in ova hatching percentages were statistically significant for exposed groups compared with control groups in all 3 experiments. Differences in the percentage of ova hatched were not statistically significant between any exposed groups within each experiment (Table 1, Table2 and Table 3).

Discussion

Previous studies have evaluated the efficacy of other decontamination agents on *Syphacia* ova.[6,7] Many of those evaluated had several drawbacks to their use. For example, methods involving heat can be damaging to heat sensitive materials and equipment.[7] Methods involving the use of formaldehyde, ethylene oxide or chlorine dioxide, although effective, are strictly regulated by authorities due to their potential to be carcinogenic and toxic.[4,7] H_2O_2 vapor, delivered by fog or mist systems, is a widely used decontamination method in hospital settings and the food industry.[15,24] This trend has encouraged their use for the disinfection of laboratory animal rooms as an alternative to other more labor-intensive methods of disinfection.[3,16,26] H_2O_2 is a potent oxidizing agent, whose killing activity is mediated by the production of free hydroxyl radical (OH^-). OH^- kill bacteria and fungal spores and inactivate viral particles by destroying their proteins, carbohydrates, and lipids, leading to cellular disruption and/or dysfunction. The ionization process allows for iHP to contain a high concentration of Reactive Oxygen Species (ROS), consisting mostly of OH^-. A secondary effect conveyed by the ionization process is the addition of an electrostatic charge to the H_2O_2 fog or mist. This charge improves the dispersive characteristics of the product, allowing it to disinfect hard to reach surfaces and spaces. At the time of the study, the SteraMist system held EPA labels for use as a decontaminant for bacteria like *C. difficile* and viruses like norovirus and influenza H1N1 (EPA lists K, L, G and M, Registration Number 90150 to 2 | EPA Est. Number 72038-DE-001) and is registered with the FDA as a disinfectant, Medical Devices (Reg. no. 3012117386). This product is used to kill or inactivate viruses and bacteria in hospitals, vivaria, clean rooms, pharmaceutical manufacturing facilities and many other locations.[24] Despite this wide spectrum of use, the efficacy of H_2O_2 has a decontamination method for *S. obvelata* ova has not been documented. Furthermore, to our knowledge, our study is the first to test the efficacy of an iHP whole-room decontamination and surface decontamination systems on *S. obvelata* ova in the laboratory animal environment.

Our results showed a statistically significant reduction of ova hatching across all 3 experiments for iHP exposed groups as compared with nonexposed controls (Table 1, Table 2 and Table 3). The lowest percentage of hatching of *S. obvelata* ova was achieved during Experiment 3 using the surface misting unit, with 37% hatching for the slides in the 2 iHP mist applications group. Our statistical analysis did not show any significant difference in hatching between slides from different exposure or application groups within each experiment (Table 1, Table 2 and Table 3). All control groups for each experiment showed a hatching rate of 80.8% or more.

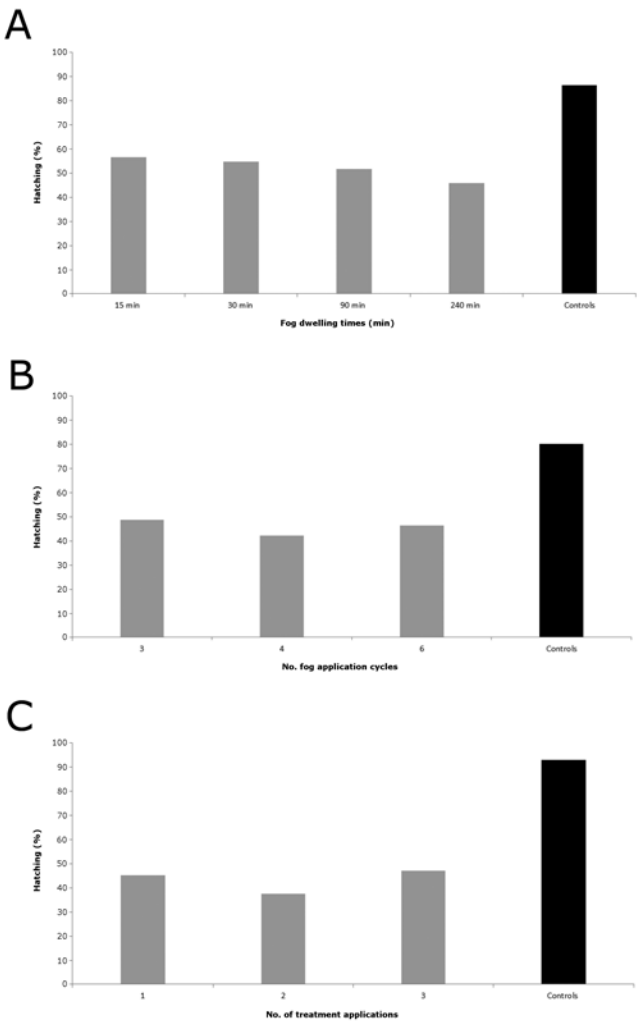

Figure 3. (A) Hatching percentage of ova exposed to different iHP fog dwelling times using the whole room decontamination system in Experiment 1 compared with unexposed (control) ova. (B) Hatching percentage of ova exposed to multiple iHP fog application cycles of 15 min each using the whole room decontamination system in Experiment 2 compared with unexposed (control) ova. (C) Hatching percentage of ova exposed to different iHP mist applications with 7 min contact time each using the surface misting unit in Experiment 3 compared with unexposed (control) ova.

Pinworm ova can be extremely resilient in the environment and have a high infectious potential, even at low numbers.[9,10,18] Although not studied in depth, the outer membrane of pinworm ova is composed primarily of chitin, a strong and impermeable polymer that is also found in the exoskeleton of arthropods.[11,13,29] This particular structure and the toughness of the pinworm egg could provide an explanation of why any of the methods using iHP mist evaluated in this study did not achieve a complete inhibition of hatching.

In this study, we used the ability of ova to hatch as a surrogate measurement for viability. Ova were scored as hatched when observed to be without larva and with an open operculum, or

Table 1. Hatch means (mean [95% CI]) for *S. obvelata* ova exposed to iHP in Experiment 1.

Exposure	Mean	Interval
30 min	0.5667	0.4924–0.6380
60 min	0.5500	0.4758–0.6220
90 min	0.5167	0.4429–0.5897
240	0.4583	0.3860–0.5324
Control	0.8667	0.8079–0.9095

All differences between exposure groups and controls were significant. There were no differences between exposure groups.
Significance set at $P < 0.05$, all P values adjusted for multiple comparisons.

Table 2. Hatch means (mean [95% CI]) for *S. obvelata* ova exposed to iHP in Experiment 2.

Exposure	Mean	Interval
3 application cycles	0.4917	0.4246–0.5591
4 application cycles	0.4250	0.3599–0.4929
6 application cycles	0.4667	0.4002–0.5344
Control	0.8083	0.7494–0.8561

All differences between exposure groups and controls were significant. There were no differences between exposure groups.
Significance set at $P < 0.05$, all P values adjusted for multiple comparisons.

Table 3. Hatch means (mean [95% CI]) for *S. obvelata* ova exposed to iHP in Experiment 3.

Exposure	Mean	Interval
1 application	0.4417	0.3438–0.5443
2 applications	0.3667	0.2747–0.4695
3 applications	0.4583	0.3595–0.5606
Control	0.9100	0.8221–0.9568

All differences between exposure groups and controls were significant. There were no differences between exposure groups.
Significance set at $P < 0.05$, all P values adjusted for multiple comparisons.

with a larva emerging. In previous papers, the same observations and scoring system were used but the ova were deemed as either viable or nonviable, rather than hatched or nonhatched.[6] Whether nonhatched ova were alive or dead is not known, and no in vitro tests can readily make this determination. To test viability, infection studies using treated ova would be necessary. This information is extremely important in the research animal setting, where immunodeficient animals might be susceptible to infection even with exposure to low levels of *S. obvelata* ova.[14]

Ionized H_2O_2 fog and mist systems are attractive methods of decontamination of laboratory animal housing rooms due to their ability to reach inaccessible surfaces and spaces, their safety with delicate equipment that might be sensitive to heat or liquids, and their overall safety to personnel. Other methods of decontamination of *Syphacia* ova from the environment such as chlorine dioxide, formaldehyde, ethylene oxide, and heat have been validated previously, although most of these previous studies investigated efficacy of these products in small chambers, surfaces or spaces.[6,7] None of these studies attempted to decontaminate the entire room. Although complete inhibition of hatching was not achieved with the decontamination protocols used in this study, the statistically significant reduction in hatching percentages was promising and indicates that further studies are needed to determine whether different exposure parameters of iHP treatments can further reduce or eliminate hatching of rodent pinworm ova. The results of this study suggest that this decontamination method is not adequate for eradication of *S. obvelata* ova from the laboratory animal room environment.

Acknowledgments

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