

Application of a micro-aerosolized disinfectant to clear *Mycoplasma gallisepticum* from contaminated facilities¹

J. D. Evans,^{*,2} S. L. Branton,^{*} S. D. Collier,^{*} J. P. Brooks,[†] and J. L. Purswell^{*}

^{*}USDA-Agricultural Research Service, South Central Poultry Research Unit, Mississippi State, MS; and [†]USDA-Agricultural Research Service, Genetics and Sustainable Agriculture Research, Mississippi State, MS

Primary Audience: Poultry Health-Related Personnel/Veterinarians, Researchers, Hatchery Personnel, Flock Supervisors.

SUMMARY

Infectious agents and their associated diseases can be significant barriers in the production of poultry and zoonotic agents associated with poultry flocks can ultimately endanger consumers. To this end, poultry producers employ a variety of strategies to minimize associated risks. Disinfectants are widely utilized in the poultry industry to limit encounters with avian pathogens and zoonotic agents. These disinfectants are readily applied by a variety of means to both equipment and facilities to reduce pathogenic populations and minimize their associated risk. While a variety of disinfectants and application means are currently available, the search for more efficacious products and technologies continues. Recently, technology has been developed that may be applicable to the poultry industry for pathogen reduction. The NebuPure[™] disinfecting system was developed as a means to decontaminate facilities harboring human pathogens. The system utilizes a novel dispersal unit to suspend an electrochemically activated solution in enclosed facilities and allows for largely automated decontamination. To test the NebuPure[™] disinfecting system for poultry-related applications, a research facility was seeded with plate cultures of the avian respiratory pathogen *Mycoplasma gallisepticum* (MG). The facility was then treated with the NebuPure[™] disinfecting system with exposure times of 1 or 4 h. Following incubation, no growth was observed among exposed plates, while control plates were 100% positive for MG. The research demonstrates the efficacy of the NebuPure[™] disinfecting system for disinfecting MG-contaminated facilities and indicates that the system may be used against other poultry-associated pathogens.

Key words: Disinfectant, *Mycoplasma gallisepticum*, Poultry Sanitation, Poultry Disease

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DESCRIPTION OF PROBLEM

Diseases can have detrimental impacts on animal productivity and ultimately affect the availability of affordable sources of protein for the consumer. Furthermore, the association of certain microbial populations (e.g., *Salmonella*

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²Corresponding author: jeff.evans@ars.usda.gov

spp.) with production flocks and herds can lead to zoonotic disease among the consuming public. To minimize the occurrence of these incidences, producers have implemented various practices to reduce the populations of microbes linked to disease. In addition, the recent trend towards reduced reliance on antibiotics will further emphasize these practices.

Disinfectants and disinfecting regimens have been routinely utilized in various sectors of the poultry industry for many years. Producers have relied on the antimicrobial activity of these agents to reduce pathogen populations and limit pathogen encounters, thereby optimizing bird performance and reducing the occurrence of infected flocks [1]. Recently, a micro-aerosol disinfecting system was developed for gaseous decontamination of enclosed facilities. The system consists of a dispersal unit that produces a semi-dry fog from an electrochemically activated (ECA) solution. The system was originally developed through a collaboration of the U.S. Department of Energy, the Institute of Highly Pure Bio-Preparation, and the World Health Organization (WHO) Center “Institute of Influenza” in Russia, and has exhibited disinfecting efficacies of > 99.99% against numerous bacterial and viral agents [2]. While the technology is currently being assessed to reduce microbial loads in a wide range of industries, it has not been tested for poultry-associated applications.

Mycoplasma gallisepticum (MG) is an economically important poultry pathogen that affects both meat- and egg-type poultry. *Mycoplasma gallisepticum* infections are common among commercial egg-layers and occur more sporadically among poultry meat-type flocks. This bacterial pathogen is normally associated with respiratory disease, and the clinical presentation of the disease may be severely complicated in the presence of a number of secondary viral and bacterial agents and specific environmental factors [3].

As the aforementioned disinfecting technology may afford the various sectors of our poultry industries with an immediate and readily applicable means to reduce pathogen encounters and pathogen-induced disease, the objective of this study was to evaluate the ability of the NebuPure™ disinfecting system against an environmentally-deposited avian pathogen.

MATERIAL AND METHODS

Preparation of Mycoplasma Gallisepticum

Fresh cultures of MG were derived from a live, attenuated MG vaccine [4] obtained from a commercial source. Vials of the Poulvac Myco F were resuspended and seeding solutions were prepared as outlined in [5]. The seeding solutions were inoculated (20 μ L) onto Frey’s plate medium [6] in 35-mm Petri plates [7]. Following seeding, plates were incubated at 37°C for 12 h (trial I) or 20 h (trials II & III) prior to placement in the research chambers for treatment application.

Experimental Setup & Facilities

Pursuant to this research, 3 separate trials were conducted. For each trial, 2 isolated steel research chambers were utilized [8]. Each enclosed research chamber measured 2.4384 m \times 2.4384 m \times 2.4384 m, and the chambers were designated as either control or experimental units. The experimental chamber was modified with a port through which the components of the MAG 50 dispersal unit [2] were linked. The dispersal unit was placed in the center of the floor in the interior of the chamber while the remainder of the system was maintained on a cart exterior to the chamber. No modifications were made to the control chamber.

In the experimental chamber, lids were removed from the inoculated Petri plates, which were affixed vertically on 3 walls of the chamber. In addition, plates were inverted and attached to the ceiling of the experimental chamber. All plates were affixed immediately prior to treatment application in the experimental chamber utilizing a double-sided adhesive. The plates were spatially aligned on each surface according to an equidistant grid. In trial I, plates were arranged on a 3 \times 3 grid (9 plates/surface). In trials II and III, plates were arranged on a 2 \times 2 grid, but at each position, 2 plates were affixed (8 plates/surface). In all trials, control plates without lids ($n > 8$) were maintained in a separate control chamber throughout the treatment application.

Treatment Application and Assessment

Immediately prior to treatment application, the NebuPure™ ECA solution [2] was prepared and added to the dispersal unit by the manufacturer. The experimental and control chambers were sealed and the solution was dispersed via the dispersal system, which converts an aqueous solution to a semi-dry micro-aerosol containing 0.5- to 10- μ M particles [2]. Doors remained sealed and exposure times of 1 or 4 h were assessed in all trials. Following exposure, lids were immediately replaced and all plates were inverted and incubated at 37°C. Qualitative assessments were performed through 21 d of incubation [9], during which time the plates were assessed daily for the presence or absence of MG colonies. For simplicity, the results of each trial were combined and overall average responses reported.

RESULTS AND DISCUSSION

Disinfectants have been vital components of sanitation regimes utilized across the poultry industry for many years. Their primary function is to negate or reduce the microbial load associated with the various components making up poultry environments and equipment utilized throughout poultry production. They may be applied following a disease outbreak or in between flocks or hatches [1, 10, 11]. They have been shown to control important poultry pathogens, including *Salmonella* spp., *Pseudomonas* spp., *Proteus* spp., *E. coli*, *Staphylococcus* spp., and *Aspergillus* spp. [12]. With the current trend toward reduced reliance on antibiotics in animal agriculture, the disinfectant agents may be increasingly relied upon to reduce pathogen encounters.

A wide range of disinfecting agents and chemicals are currently utilized within the poultry industry, and generally, poultry-related disinfectants are applied by spraying, misting, fogging, or fumigation [10]. Selection criteria for disinfection and means of application to be considered include properties of both the disinfectant (e.g., active ingredient, residuals) and target material (e.g., composition, position, accessibility) as well as the presence of target-associated

organic matter (e.g., soil, dust, feathers, litter), which can impede the action of the disinfectants [1, 11]. Commonly utilized disinfecting agents include chlorine dioxide, hypochlorite, phenolic compounds, quaternary ammonium compounds, iodophors, glutaraldehyde, ozone, and hydrogen peroxide, with the choice of disinfectant varying with specific application [10, 13]. Recently, ECA solutions have been considered for their disinfecting properties towards poultry-related applications and their environmentally friendly status [2, 14, 15, 16]. The ECA solutions are prepared by passing water and substances (e.g., NaCl) dissolved in it through an electrochemical cell. This enables the synthesis of chemical reagents and a solution that ultimately can contain a mixture of chlorine, peroxide, and chlorine oxides. These ECA solutions have been shown to kill a wide variety of microorganisms, and protein and nucleic acid destruction has been implicated as the primary means of cell death [2, 14, 16, 17, 18].

To qualitatively evaluate the efficacy of a micro-aerosol disinfecting system [2] and its potential applicability for the control of poultry pathogens, an avian pathogen MG was grown on plate media at rates of 20 cfu/plate (Trial I) or 200 cfu/plate (Trial II & III) and affixed to the walls and ceiling of an enclosed facility. A disinfecting ECA solution was suspended as airborne particles and dispersed within the facility. As illustrated in Figure 1, no MG colony growth was observed on exposed plates for 1 or 4 h, while MG colonies were observed on all (100%) non-exposed control plates. Results also demonstrate that the inhibitory action of the disinfectant was independent of the position of the plates during their exposure. It should be emphasized that the plates on vertical walls were affixed in that plane and that plates on the ceiling were inverted. Therefore, the associated results are indicative of the production of particles that filled the experimental chamber and were small enough to remain suspended for the length of time necessary for contact with the MG on the plate media.

It is also of interest that the presentation of the avian pathogen in association with plate media did not appear to limit the disinfecting activity of the solution. Organic matter has previously been shown to impede the active components of disinfectants [1, 11], and culture media can

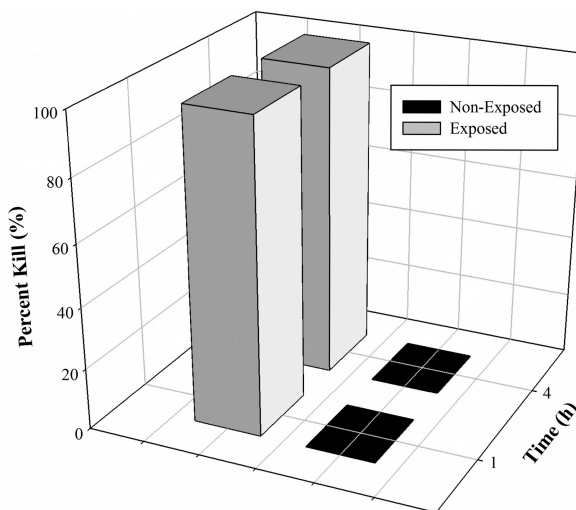


Figure 1. The effect of a micro-aerosolized disinfectant on the viability of environmentally deposited *Mycoplasma gallisepticum* exposed for 1 or 4 h.

be a rich source of organic matter (e.g., proteins, carbohydrates). Therefore, this presentation of the avian pathogen cells was selected over more commonly utilized suspension and carrier tests to more closely mimic the real-life conditions encountered in poultry production facilities [2, 19, 20, 21].

CONCLUSIONS AND APPLICATIONS

1. The disinfecting solution was effective against actively growing cells of the avian pathogen MG.
2. The disinfectant solution was effective independent of MG location (wall or ceiling) and position (vertical or inverted) in the chamber indicating the ability of the dispersal unit to saturate the environment inside the enclosed facility with small disinfecting particles resistant to gravitational effects.
3. The disinfecting system may be applied to clear poultry facilities following MG outbreaks and may be an effective means to clear facilities and equipment of other environmentally deposited avian pathogens.

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