Review Article

Dry Hydrogen Peroxide: one molecule for a One Health approach—a narrative review

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Background and Objective: While the most notable impacts of the coronavirus disease 2019 (COVID-19) pandemic have arguably been felt in the healthcare industry, there have been repercussions across virtually every vertical, including animal agriculture. Disruptions caused by the pandemic, including those related to regulatory responses, led to significant shortages in animal protein. Industry professionals are exploring environmental disinfection technologies that can reduce the presence of pathogens in food production plants. A fundamental point of differentiation among the options is their ability to be operated continuously in occupied settings, such as livestock/poultry production facilities. This review examines Dry Hydrogen Peroxide (DHP), a continuously operating technology, and its efficacy against pathogens in the context of suitability for optimizing food safety and processing in animal agriculture.

Methods: This review examines peer-reviewed research pertaining to DHP published between January 2019 and September 2022. These findings were supplemented by pertinent lab reports and observational findings describing DHP's efficacy against key foodborne pathogens and potential impact on chicken egg hatch rate and mortality.

Key Content and Findings: The materials reviewed indicate DHP's efficacy against a wide range of pathogens commonly present in clinical and animal agriculture settings. Additional observational evidence suggests a potential enhancement of poultry production outcomes, providing direction for future investigation.

Conclusions: The identification of effective environmental disinfection technologies that can be integrated into food production facilities requires evaluation of all aspects of system operation. DHP is a technology that appears to be well-suited to this industry, offering an automated, continuous strategy for reduction of pathogens that impact humans and livestock/poultry alike.

Keywords: Dry Hydrogen Peroxide (DHP); animal agriculture; food safety; environmental disinfection

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Introduction

Background

Described by the World Health Organization (WHO) as "one of the biggest global crises in generations", the coronavirus disease 2019 (COVID-19) pandemic has exacted an unprecedented toll on the world's healthcare systems, economies, and societies (1). The scale and scope of that toll continue to expand as research parses through the myriad ways in which lives were upended, economies disrupted, and resources depleted.

The most devastating consequence of the pandemic indisputably lies in the more than 6.63 million deaths from the virus worldwide (2). Even among survivors, the medical

repercussions of the disease have been significant, with one in five Americans who were infected reporting "long COVID" symptoms according to the United States (U.S.) Centers for Disease Control and Prevention (CDC) (3). And the impact of acute infection, particularly in the early stages of vaccine deployment and during the surge of the Delta variant, stressed global healthcare systems to a degree unparalleled in this era of modern healthcare. Healthcare revenues and employment plummeted and, in the U.S. alone, nearly a decade of progress against healthcare-associated infections was undermined as national standardized infection ratios (SIRs) for four key reportable infections spiked in late 2020 and again in 2021 (4,5).

Healthcare-associated infections were not the only diseases impacted by the pandemic. Mental health suffered across the globe, especially among healthcare workers. In the U.S., a survey of healthcare workers during the pandemic found 93%, 86%, and 77% reported experiencing stress, anxiety, and frustration, respectively (6). Worldwide, the WHO reports a 25 percent increase in anxiety and depression associated with the pandemic (7). Additionally, routine screenings for illnesses like heart disease and cancer, not to mention standard vaccinations for growing children, were missed in staggering numbers. The National Cancer Institute reports that over 9.4 million cancer screening tests failed to take place in the U.S. during 2020 while research estimates that diagnostic cardiovascular testing decreased by 64 percent in the early stages of the pandemic (8,9). The cumulative costs of those lapses, in terms of morbidity, mortality, and healthcare expenditures, remain to be seen.

The pandemic's economic injury extended well beyond the healthcare sector, with mandatory lockdowns, along with voluntary social distancing, negatively impacting a host of industries, most notably hospitality, travel, and entertainment (10). In fact, the International Monetary Fund estimates that the 3.9% drop in global median gross domestic product (GDP) during the first year of the pandemic was the worst economic downturn since the Great Depression (11). While some economic indicators such as unemployment are finally returning to pre-pandemic levels roughly two and half years later, others such as the global manufacturing purchasing managers index (PMI) continue to wax and wane (12,13).

Another industry dramatically affected by the pandemic is animal agriculture, specifically pork and poultry production. These effects manifested in multiple ways, most of which relate to human illness and the interruption or displacement of supply chains. Modern livestock production is highly efficient and often vertically integrated, where a single company will own all parts of the production system from birth/hatch to processing. This allows the industry to produce a standard and consistent product in the most cost-effective way possible, providing cheap and accessible protein to the world. Within this highly efficient system, a disruption at any point will lead to significant economic and health impacts. It has been widely shown that COVID-19 does not infect livestock directly, but the conditions necessary to produce and process these food animals necessitate close quarter working environments which are highly conducive for COVID-19 spread. Overall COVID numbers are hard to quantify in many of these facilities, but a 2020 survey of processing plants in 23 states reported that 9.1% of workers tested positive for COVID-19 over a 3-month period (14). The majority of these workers are from demographic minorities, which increased exposure to populations that seemed to be more susceptible to infection with COVID-19 (14).

Secondary to the human health impact from COVID-19 were the impacts on supply chain. Food animal processing and packaging is divided into two separate supply chains where one supplies products for retail (e.g., grocery stores) and the other supplies products for the food service industry (e.g., restaurants). These two supply chains are governed by different regulations on processing, packaging, and delivery, so movement of product between the two chains is very difficult. During the height of lockdown, demand for food service industry product disappeared while the demand for retail product soared, creating a dramatic imbalance in the overall animal protein supply chain. Empty grocery store shelves were often publicized, but there was not a product shortage *per se*; there was an inability to divert food service product to retail stores to meet the increased demand. This imbalance was exacerbated by disease outbreaks among plant workers, which ultimately lead to slower production times or total plant closings.

Many aspects of both the outbreak of COVID-19, and international governmental responses to it, impacted the ability of companies to provide safe, economical, and consistent animal protein to the world. These impacts ranged from port and border restrictions limiting trade to reduced animal health and welfare from mass culling of overstock animals or product dumping from decimated markets to supply chain disruptions leading to more drastic food insecurities (15). And while these impacts were measured during the height of the pandemic, the long-reaching impacts are still unknown.

In an effort to mitigate the effects of these myriad negative pandemic-associated impacts, there was a surge in the development and use of a broad range of environmental disinfection technologies (16,17). As evidence demonstrating the potential for both airborne and surface-mediated transmission of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) virus accumulated, facilities across multiple industries looked to these adjunctive solutions as a means of mitigating the risk of viral spread (16,17). These technologies vary widely, however, in their application, operation, mechanism of action, and efficacy parameters, all of which are important considerations when choosing among commercially available options (16,17).

Ultraviolet-C (UV-C) irradiation

Ultraviolet germicidal irradiation (UVGI) is the most prevalent of these technologies, having been in use for over 70 years (18). UVGI technologies deliver UV-C energy, a form of short-wave (~200–280 nm) ultraviolet light that has been shown to effectively inactivate a host of viral, bacterial, and fungal organisms (18). UVGI can be used to treat microorganisms in the air and on surfaces, though its efficacy is dependent on a number of use parameters (17,19). Distance from the UV-C source, duration of exposure, presence of soils and other debris, and unobstructed exposure are all factors that impact efficacy. Farther distances between source and target, shorter exposure times, greater soiling, and shadowing or recessed surfaces all negatively influence the ability of UV-C to eradicate pathogens (17,19). UV-C energy can be delivered directly to a targeted space, via mobile robotic devices, to eliminate SARS-CoV-2 in the air and on surfaces; however, this must be performed in unoccupied spaces owing to the safety risks posed by direct human exposure to UV-C (18,19). UV-C can also be delivered in upper air systems that treat air in a room as it passes through the device, rendering airborne SARS-CoV-2 noninfective. The devices themselves shield occupants in the room below from exposure to the UVGI. While this addresses airborne infectious SARS-CoV-2 that passes through the device, it does not address infectious SARS-CoV-2 found on surfaces (18).

Pulsed xenon ultraviolet (PX-UV) irradiation

A more recent iteration of ultraviolet technology is PX-UV irradiation (20,21). In contrast to UV-C systems, which use low-pressure mercury bulbs to emit UV-C irradiation, PX-UV utilizes pulsed xenon flash bulbs that emit broad spectrum ultraviolet (UV-A, UV-B, and UV-C) and visible spectrum light (20). Like UV-C, PX-UV must be delivered in unoccupied spaces given the exposure risks associated with the UV irradiation (20). The purported advantage to PX-UV is that the broad spectra UV irradiation delivered in short, high-intensity pulses allows for shorter treatment times; however, some research has found it to be less effective than UV-C in reducing pathogens even when deployed for the same duration and at the same location within a hospital room (20,21). PX-UV's efficacy, like that of UV-C, is also dependent on distance from the device, though, at least one study found that, unlike UV-C, organic load and shading did not negatively influence PX-UV efficacy (20). Research demonstrates that PX-UV can significantly reduce SARS-CoV-2 on surfaces, though the dose deployed and the surface material can impact efficacy (22,23).

Visible light technology

Visible light technologies achieve microbial reduction by utilizing light within the 405–410 nm wavelength—also referred to as "violet-blue" or "indigo" light (24). The light is generated from a matrix of light-emitting diodes and combined with white light to provide illumination and to produce light ("white" light) that conforms to safety guidelines for use in occupied rooms (24). Systems have occupancy sensors which allow the fixtures to toggle between the white mode and an indigo-only mode that delivers roughly 4-times the dose of 405 nm light when the room is vacant (24). As the majority of antimicrobial effect is achieved when the room is unoccupied and the indigo light is generated, these systems have been most often deployed in settings that are not continuously occupied such as operating rooms (ORs) or office spaces. Research has demonstrated these systems can reduce microbial bioburden and improving surgical outcomes when deployed in ORs (24). More recently, laboratory studies demonstrated susceptibility of SARS-CoV-2 to visible light technology when deployed at irradiance levels safe for occupied settings (25).

Bipolar ionization (BPI)

Another category of environmental disinfection technologies includes those that either disperse or generate chemical products such as bipolar ions or hydrogen peroxide. The chemical products are released into the treated space where they are intended to interact with and subsequently inactivate viral targets. BPI technology utilizes ambient humidity and oxygen to generate a plasma consisting of positive ions, negative ions, and free radicals (26). Needlepoint ionization is the most commonly deployed

form of BPI, owing to the manufacturer's claim that the oppositely charged electrodes used to generate the ions produce an electric field below 12 electron volts (eV), thereby preventing the possibility of ozone generation (26). A challenge with BPI is that the product generated is an unstable plasma, and the concentration of ions diminishes as distance from the device increases because the oppositely charged ions rapidly recombine, effectively neutralizing one another (26). The Environmental Protection Agency additionally cautions users that much of the existing efficacy data is limited to laboratory testing (27). Further, they report from their own efficacy testing of BPI against SARS-CoV-2, that there was less than a 1-$\log_{10}$ difference in the reduction of aerosolized virus between the control and intervention with no surface inactivation of the virus (17).

Vaporized hydrogen peroxide (VHP)

Hydrogen peroxide, a chemical with a long history of use as a liquid antiseptic or disinfectant, is the active agent of several other chemical disinfection technologies. VHP systems utilize an aqueous solution of hydrogen peroxide with a concentration typically ranging from 30–35% (21,26). The solution, as the name suggests, is vaporized into highly concentrated aqueous droplets that are then dispersed throughout a treated space (21). Following an appropriate dwell time during which the hydrogen peroxide droplets decontaminate the room, an active aeration process, in which fresh air is introduced into the room, is performed to facilitate the breakdown of the vapor into oxygen and water (21). The concentration of VHP allows for effective point-in-time sterilization of a room, but it exceeds safety thresholds for human exposure (26). Accordingly, the room must be vacated and all portals of air entry [e.g., doors, windows, heating, ventilation, and air conditioning (HVAC) units, etc.] must be sealed to avoid unintended dissemination of the aerosols into nearby spaces (26). Aqueous forms of hydrogen peroxide also form a weak acid which can have a degradative effect on some equipment, furnishings, and materials (26). While VHP has demonstrated efficacy in reducing the concentration of SARS-CoV-2 on N95 respirators (28-30), its necessarily intermittent use because of exposure risks limits its ability to mitigate transmission risk in active, occupied settings.

Aerosolized hydrogen peroxide (aHP)

aHP systems, sometimes referred to as hydrogen peroxide "mist" systems, also utilize an aqueous form of hydrogen peroxide for environmental disinfection. These systems use a solution of 5–7% hydrogen peroxide, combined with <50 part per million (ppm) Ag^+ cations or peracetic acid, which is dispersed into a room as an aerosol to decontaminate air and surfaces (21,28,31). Once the aHP is deployed into the room, it can be left to passively decompose into oxygen and water or actively removed via scrubbing if the solution contains peracetic acid (21,28). Research comparing the two categories of aqueous hydrogen peroxide decontamination systems has often found VHP systems to achieve more robust pathogen elimination or reduction than aHP systems, likely owing in part to the lower concentration of hydrogen peroxide utilized in the latter (21,31). Yet, the concentration of hydrogen peroxide achieved by aHP still exceeds U.S. Occupational Safety and Health Administrations (OSHA)'s permissible exposure limit (PEL) for workers, and therefore any treated space must be vacated and sealed. Accordingly, like VHP, aHP technologies are only utilized for intermittent, point-in-time disinfection.

Dry Hydrogen Peroxide (DHP)

DHP technology generates hydrogen peroxide as a true gas composed of molecules exhibiting near ideal gas behavior. The technology utilizes ambient humidity and oxygen, in a process involving photocatalysis and plasma separation, to create stable molecules of gas phase hydrogen peroxide at concentrations well below the 1 ppm safety threshold established by OSHA (26,32). The molecules then disperse throughout the treated space oxidizing bacteria, fungi, and viruses in the air and on surfaces, effectively traveling throughout the entire treated space. The lower concentration of hydrogen peroxide achieved allows for continuous use in occupied spaces and avoids the material compatibility issues seen with VHP.

Photocatalytic oxidation (PCO)

Although DHP technology utilizes photocatalysis during part of its generation of hydrogen peroxide gas, it is distinctly different from the category of technologies referred to as PCO (26,33). PCO technology generates hydrogen peroxide in plasma; however, unlike the stable hydrogen peroxide generated by DHP, the PCO-generated hydrogen peroxide is rapidly consumed because it has a highly positive reduction potential (0.71 eV) and is immediately reduced to water by subatomic particles within the plasma (26,34). PCO devices contain a dense internal plasma zone which affects the airborne microbes that pass through the device, but like upper-room UVGI systems, only the air that travels through the device is treated and there is

Table 1 Search strategy summary

Items	Specification
Date of search	October 2022
Databases and other sources searched	ScienceDirect
Search terms used	"Dry Hydrogen Peroxide" or "DHP"
Timeframe	January 2019–September 2022
Inclusion and exclusion criteria	Include: journal publications, book chapters
	Exclude: "aqueous", "mist", "vaporized", "aerosolized"
Selection process	Study team conducted selection. Unanimous agreement on selection criteria
Any additional considerations, if applicable	Supplemented review with third-party laboratory reports provided by unit manufacturer and observational data from study team member

no antimicrobial effect outside of the device itself (26). Accordingly, this design inherently results in a very brief contact time between the targeted microbes and the photocatalyst surface, particularly at higher air velocities, which is often cited as a limitation to its application (35).

Objectives

The technologies introduced here differ in several important characteristics which affect the lens through which they are evaluated. The primary basis of categorization is the ability of a technology to operate continuously within occupied spaces. Technologies that employ UV-C radiation or aqueous hydrogen peroxide achieve the highest levels of disinfection within short periods of time, but the magnitude of these results are mitigated by their intermittent usage, especially within arenas that are occupied for extended periods of time, such as long-term healthcare facilities or livestock/poultry production facilities (21,26,31). Technologies that can continuously operate within occupied spaces achieve lower levels of disinfection than their terminal disinfection counterparts, but the dramatically extended time of exposure sets the stage for a potential reduction in a room's steady-state level of bioburden. This allows for greater versatility in the deployment of these technologies as adjuncts to standard environmental disinfection protocols.

One of these continuous technologies, DHP, has demonstrated effective inactivation of surface and airborne bioburden in laboratory and field settings, including clinical (26,36-41). This efficacy, combined with its automated deployment, makes DHP an attractive adjunctive solution for professionals in all verticals included within the One Health doctrine, including animal health. The study team reviewed existing published and previously unpublished evidence of DHP's impact on several pathogens related to foodborne illness. Additionally, the study team reviewed preliminary evidence of a potential connection between DHP exposure and changes in chicken egg hatch rate and early chick mortality. We present this article in accordance with the Narrative Review reporting checklist (available at https://jphe.amegroups.com/article/view/10.21037/jphe-22-105/rc).

Methods

The review process was conducted during the month of October 2022, the study team decided to limit the search for peer-reviewed literature concerning DHP to the dates of January 2019 to September 2022 (see *Table 1*). Published investigations of DHP have been concentrated within the last few years due to its relative novelty as an environmental disinfection technology. The study team used the ScienceDirect full-text database for the selection process, limiting the initial search to journal articles and book chapters published within the defined timeframe that specifically mentioned "Dry Hydrogen Peroxide" or "DHP". The initial search yielded 75 results, but once all references to "aerosolized", "vaporized", "aqueous", or "mist" were excluded, the final results yielded six peer-reviewed journal articles and one book chapter. Due to the limited publicly available research on DHP, the study team approached the unit manufacturer to obtain additional third-party laboratory reports detailing DHP's efficacy against several foodborne pathogens in the air and on surfaces. Additionally, a member of the study team provided

the results of an observational study detailing a potential impact of DHP on poultry production as a basis for future investigation.

Discussion

Narrative

Review of available published research

The database search for publications concerning DHP yielded six journal publications and a single book chapter. Of the journal publications, three pertained to DHP's efficacy against bacterial microbial load in a clinical setting, one described an observed reduction of hospital-acquired infections (HAIs) related to patient exposure to DHP, one detailed DHP's impact on eggshell contamination within a laboratory setting, and one described DHP's efficacy against SARS-CoV-2 on surfaces (36-41). The book chapter cataloged third-party laboratory reports of DHP's efficacy against several viruses, both on surfaces and in the air (26).

Each of the studies performed in clinical settings described statistically significant impacts of DHP on bacterial microbial load on surfaces. A study from a tertiary care hospital in the Western U.S. reported a 99.47% reduction in surface microbial load on privacy curtains within patient rooms after 24 hours of exposure to DHP (37). Another article from the same tertiary care facility reported significant reductions linked to DHP exposure on other key clinical surfaces within patient rooms, such as bedrails, bedside tables, and counters (36). A study from a pediatric oncology hospital located in Central U.S. also reported statistically significant reductions in surface microbial load in a patient room exposed to DHP, while no reductions were observed in an identical unexposed room (38). Subsequently, this same facility observed a reduction in HAI that was significantly linked to patient exposure to DHP while controlling for several covariates in a multivariate regression model (39). Two of the studies that reported significant surface microbial load reductions observed reductions in airborne microbial load that did not achieve statistical significance, citing low baseline levels of airborne contamination and a limited sample size as contributing factors (36,38).

The laboratory study detailing reductions of SARS-CoV-2 in the presence of DHP was performed by a research institute at a university in the Midwest U.S. (40). Accelerated decay of SARS-CoV-2 surface concentrations were observed in the DHP-treated samples, with a 98.7% reduction compared to the untreated control within 120 minutes (40). The laboratory study detailing DHP's impact on microbial load on chicken eggshells performed by a study team at a university in the Southeast U.S. (41). The study reported statistically significant reductions in microbial load, as well as increases in hatch rate (41).

The book chapter yielded by the search cataloged several third-party laboratory reports detailing reductions in surface and airborne viral titers in the presence of DHP, compared to the control (26). On surfaces, sizable reductions were observed in titers of Influenza A (H1N1) and Feline Calicivirus, a non-enveloped virus often used as a surrogate for human norovirus (26). The chapter also detailed reductions in airborne MS2 bacteriophage, another common surrogate for human norovirus (26).

Methicillin-resistant *Staphylococcus aureus* (MRSA) on surfaces

Staphylococcus aureus is a species of gram-positive bacteria commonly found on human skin and in nasal passages that can cause a wide range of infections, both within the community and in clinical settings (42). Antibiotic-resistant strains, such as MRSA, can be particularly problematic, causing serious skin and tissue infections, as well as bacteremia if it enters the bloodstream (43). MRSA is also classified as a foodborne pathogen that typically contaminates livestock-associated food, with certain strains possessing the ability to generate enterotoxins that may cause food poisoning if consumed (44). MRSA has been documented to survive on surfaces for days to months at a time in residential, healthcare, and livestock settings (43,44). *Staphylococcus aureus*, including MRSA, intracellularly produces the enzyme catalase which breaks down hydrogen peroxide into water and oxygen gas, thereby mitigating the decontamination efficacy of aqueous forms of the chemical such as VHP and aHP (45,46).

A DHP device was tested against cultures of MRSA, with a starting concentration of 6.76 $\log_{10}$ colony-forming units (CFUs), in both a laboratory biosafety hood and a room approximately 30 m^3 in size. The test sought to determine if DHP effectively inactivated MRSA in comparison to the control condition over the course of 48 hours of exposure.

Glass slides 1" × 3" in size were inoculated with the MRSA test culture prepared with 5.0% fetal bovine serum. Twenty carriers, in total, were prepared for this experiment, with duplicate slides for baseline and each combination of post-intervention timepoint and condition (Control, Room, Hood): Time Zero, T =6 hours, T =24 hours, T =48 hours.

Once inoculated, the carriers were allowed to dry at room conditions [21–25 ℃, 30–50% relative humidity (RH)] until no visible liquid remained. The dried carriers were placed in their respective experimental conditions, with the room having a DHP unit that had been operating for 48 hours, and the hood having a DHP unit that had been operating for 12 hours. All samples remained at room temperature and humidity for the duration of their respective exposures. The Time Zero samples were immediately retrieved and harvested in 10.0 mL of Dey Engley (D/E) broth, vortex mixed, then diluted with phosphate buffered saline (PBS) and plated on tryptic soy broth (TSB). The plates were then incubated for 24 hours at 36 ℃.

After incubation was complete, the plates were removed and enumerated, and the average count from each pair of duplicate samples was calculated. In comparison to the control, the DHP-treated samples from the room yielded an 83.053% (0.77 $\log_{10}$) reduction, and the DHP-treated samples from the safety hood yielded a 95.150% (1.31 $\log_{10}$) reduction after 6 hours of exposure. At the 24- and 48-hour timepoints, there was minimal recovery of MRSA in the DHP-treated samples (limit of detection =5 CFU), and significant die-off was observed in the control (47).

Salmonella enterica on surfaces

Salmonella is a genus of gram-negative bacteria that is extremely prevalent in verticals related to animal health, specifically in the poultry production sector (48,49). Animals that are infected with *Salmonella* typically carry the pathogen in their gastrointestinal tracts, which can then be transmitted to other animals via fecal contact (50). Consequently, food products derived from these animals (e.g., eggs, poultry products), can be contaminated with certain species of *Salmonella*, causing salmonellosis in humans if these products are consumed raw or are improperly cooked (51). *Salmonella* can also be transmitted between humans via fecal contact, most commonly in scenarios when food service workers fail to follow proper hygiene guidelines in their workplace (52).

A DHP device was tested against cultures of *Salmonella enterica*, with a starting concentration of 5.27 $\log_{10}$ CFU, in a room approximately 30 m^3 in size. The test sought to determine if DHP effectively inactivated *Salmonella* in comparison to the control condition over the course of 6 hours of exposure using American Society for Testing and Materials (ASTM) E1153 test method.

Glass slides 1" × 3" in size were inoculated with the *Salmonella enterica* test culture. Fifteen carriers, in total, were prepared for this experiment, with triplicate slides for baseline and each combination of post-intervention timepoint and condition (Control, Test): Time Zero, T =2 hours, T =6 hours. Once inoculated, the carriers were allowed to dry in an incubator (36 ℃) until no visible liquid remained. The dried carriers were placed in their respective experimental conditions, with the treatment room having a DHP unit that had been operating for 24 hours. All samples remained at room temperature and humidity for the duration of their respective exposures. The Time Zero samples were immediately retrieved and harvested in 20.0 mL of D/E broth, vortex mixed, then diluted with PBS and plated on TSB. The plates were then incubated for 24 hours at 36 ℃.

After incubation was complete, the plates were removed and enumerated, and the average count from each set of triplicate samples was calculated. The DHP-treated samples yielded similar results to the control at 2 hours, but a 91.62% (1.08 $\log_{10}$) reduction compared to the control after 6 hours of exposure (53).

Escherichia coli (*E. coli*) on surfaces

E. coli is a species of gram-negative bacteria that normally resides in the intestines of humans and animals. While some serotypes of *E. coli* are part of a healthy intestinal microbiome, others are pathogenic and can cause significant gastrointestinal disease via the production of Shiga-type endotoxins (54,55). Pathogenic strains are transmitted via the fecal-oral route which can occur by ingesting the bacteria directly from contaminated food, typically raw or improperly cooked meat, or through hand contact with a contaminated surface or substance and subsequent oral contact. Like *Salmonella*, *E. coli* is transmitted between animals via fecal contact, and the bacteria can survive and grow on the bodies of livestock animals. Accordingly, hand hygiene and surface cleaning and disinfection, particularly for those surfaces involved in food processing or preparation, are important prevention strategies (56).

A DHP device was tested against cultures of *E. coli*, with a starting concentration of 6.75 $\log_{10}$ CFU, in a room approximately 30 m^3 in size. The test sought to determine if DHP effectively inactivated *E. coli* in comparison to the control condition over the course of 2 hours of exposure using ASTM E1153 test method.

Glass slides 1" × 3" in size were inoculated with the *E. coli* test culture. Fifteen carriers, in total, were prepared for this experiment, with triplicate slides for baseline and each

combination of post-intervention time point and condition (Control, Test): Time Zero, T =1 hour, T =2 hours. Once inoculated, the carriers were allowed to dry in an incubator (36 ℃) until no visible liquid remained. The dried carriers were placed in their respective experimental conditions, with the treatment room having a DHP unit that had been operating for 24 hours. All samples remained at room temperature and humidity for the duration of their respective exposures. The Time Zero samples were immediately retrieved and harvested in 20.0 mL of D/E broth, vortex mixed, then diluted with PBS and plated on TSB. The plates were then incubated for 24 hours at 36 ℃.

After incubation was complete, the plates were removed and enumerated, and the average count from each set of triplicate samples was calculated. In comparison to the control, the DHP-treated samples from the room yielded a 64.42% (0.45 $\log_{10}$) reduction after 1 hour and an 86.31% (0.86 $\log_{10}$) reduction after 2 hours of exposure (53).

Airborne *E. coli*

While the primary mode of *E. coli* transmission within animal health is related to fecal contamination on surfaces and other animals, *E. coli* has also been documented to survive aerosolization long enough for airborne transmission to occur between livestock animals (57,58).

A DHP device was tested against airborne cultures of *E. coli*, with a starting concentration of 6.01 $\log_{10}$ CFU, in a room approximately 30 m^3 in size. The test sought to determine if DHP effectively inactivated *E. coli* in comparison to the control condition over the course of 4 hours of exposure.

The test inoculum containing the culture of *E. coli* was equally divided between two nebulizers within the test chamber, which were then activated for 60 minutes prior to the collection of the Time Zero samples. Samples were collected using an SKC Inc. (Eighty Four, PA, USA) Biosampler® (500 L collected at 12.5 L/min) equipped with PBS. After collection, the sample was serially diluted and plated onto Tryptic Soy Agar (TSA), then incubated for 48 hours. Subsequent samples were then collected each of the following 4 hours, with no DHP present. The chamber was subsequently decontaminated, and the process was repeated, with the DHP machine being activated after the collection of the Time Zero sample.

After incubation was complete, the plates were removed and enumerated. In comparison to the control, the DHP-treated sample yielded a >99.85% (>2.82 $\log_{10}$) reduction after 1 hour, with a count less than the limit of detection (17 CFU). The subsequent two-hour sample also yielded a count less than the limit of detection (59).

***Listeria monocytogenes* (*L. mono*) on surfaces**

L. mono is a species of gram-positive bacteria capable of causing the severe foodborne illness listeriosis as the result of ingesting contaminated food products, most commonly meat and dairy (60). While listeriosis is a mild infection for the general population, immunocompromised individuals are at extreme risk of severe symptoms that can be fatal. Additionally, listeriosis in pregnant women can lead to transmission to the fetus or newborn, commonly manifesting as meningitis or encephalitis (61,62). *L. mono* possesses the ability to survive and grow at refrigeration temperatures, and so contamination at any step of a food product's lifecycle is particularly dangerous. *L. mono* is commonly transmitted between livestock animals, most commonly cows, sheep, and goats, via fecal contamination. While animals can carry *L. mono* without any signs of illness, listeriosis can be fatal to livestock (63). Additionally, unprocessed fertilizer from contaminated livestock can infect crops used in human food products (64).

A DHP device was tested against cultures of *L. mono*, with a starting concentration of 5.68 $\log_{10}$ CFU, in a room approximately 30 m^3 in size. The test sought to determine if DHP effectively inactivated *Listeria* in comparison to the control condition over the course of 3 hours of exposure using ASTM E1153 test method.

Glass slides 1" × 3" in size were inoculated with the *L. mono* test culture. Nine carriers, in total, were prepared for this experiment, with triplicate slides for: Time Zero, T =3 hours (DHP), T =3 hours (Control). Once inoculated, the carriers were allowed to dry at room conditions (21–25 ℃, 30–50% relative humidity) until no visible liquid remained. The dried carriers were placed in their respective experimental conditions, with the treatment room having a DHP unit that had been operating for 24 hours. All samples remained at room temperature and humidity for the duration of their respective exposures. The Time Zero samples were immediately retrieved and harvested in 20.0 mL of D/E broth, vortex mixed, then diluted with PBS and plated on TSB. The plates were then incubated for 24 hours at 36 ℃.

After incubation was complete, the plates were removed and enumerated, and the average count from each set of triplicate samples was calculated. The DHP-treated samples yielded a 65.77% (0.47 $\log_{10}$) reduction compared to the control after 3 hours of exposure (65). See *Table 2*: summary

Table 2 Summary of bacteria and subsequent reductions in the lab efficacy studies

Bacteria	Strain	Culture medium	Peak reduction compared to control	Timepoint of peak reduction
Staphylococcus aureus (MRSA)[†]	ATCC 33592	5.0% FBS	95.15%	6 hours
Salmonella enterica[‡]	ATCC 10708	TSB	91.62%	6 hours
Escherichia coli[‡]	ATCC 8739 (surface); ATCC 10798 (airborne)	TSB (both)	86.31% (surface); >99.85% (airborne)	2 hours (surface); 1 hour (airborne)
L. mono[‡]	ATCC 15313	TSB	65.77%	3 hours

[†], testing performed at Antimicrobial Test Laboratories, Round Rock, Texas, USA; [‡], testing performed at Microchem Laboratory, Round Rock, TX, USA. MRSA, methicillin-resistant *Staphylococcus aureus*; ATCC, American Type Culture Collection; FBS, fetal bovine serum; TSB, tryptic soy broth; *L. mono*, *Listeria monocytogenes*.

of laboratory studies.

Preliminary evidence of DHP's impact on poultry production

As previously mentioned above, animal agriculture was dramatically affected by the COVID-19 outbreak, but it is vulnerable to animal-specific pathogens each year. These pathogens have potential impacts on animal protein production, and mitigation strategies for reduction of these pathogens is continually sought and used. It is well known that environmental contamination leads to negative health outcomes for commercial poultry, for instance. Bacterial infection of broiler chicks during the incubation and hatching phase can cause increased incidences of omphalitis and salmonellosis as well as increased mortality within 7 days of age (66-68). Infected chicks can spread disease within the hatcher tray and later within the flock at the broiler farm, causing economic losses for both the farmer and the integrator (69). For this reason, DHP was tested in poultry production facilities in several laboratory and field studies to evaluate the impact on poultry health and production.

Within poultry operations, the hatchery is the most likely site where DHP use would have the largest influence and a point where all poultry produced could be efficiently reached. Preliminary lab studies showed that DHP could significantly reduce microbes on the surface of eggshells, which would have chick and human health relevance (70). This led to a more comprehensive laboratory study evaluating the effect of those cleaner eggshells on hatchery performance metrics, namely total hatchability and hatch of fertile eggs set. This study resulted in a significant increase in chicks hatching from fertile eggs, as well as less bacterial contamination in eggs that didn't hatch (41).

These data were significant, but the trials were performed in a controlled laboratory setting. As can be the case for production animal research, laboratory derived data may not translate to the same impact when applied to true field situations. Accordingly, an observational study evaluating DHP for poultry production was conducted in a commercial hatchery that hatches 1.2 million chicks per week. This hatchery had two identical halves so that a true "treated with DHP" versus "non-treated" comparison could be made. On the treated side, DHP was applied in the egg storage cooler, the ambient air space in the room with incubators, and in the air plenum space around the hatchers. By treating in the air space around the incubators and hatchers, the DHP was drawn into the machines to continually treat the eggs for 21 days during the entire hatching process. Data was collected weekly for 26 weeks, during which time 31.2 million chicks were hatched. Over the course of testing, hatch of fertile eggs set was increased on the DHP treated side by 0.7% compared to the non-treated side. While not statistically significant (P=0.09), this resulted in 8,000 more chicks per week produced from this hatchery, on average, over the duration of our study.

Additionally, early mortality of chicks after placement on the poultry farm was evaluated, and chicks that had been treated with DHP in the egg during incubation had a lower mortality rate (0.07%) than those hatched from eggs that did not receive DHP treatment resulting in 840 additional chicks per week in production. Again, while not statistically significant (P=0.17), this is a biologically significant metric for poultry production. This reduction in early mortality is likely associated to the significant reduction in the percent of chicks with fungus (Aspergillus) in the lungs also found during this study (70.4% positive in non-treated chicks;

54.7% positive in treated chicks; P=0.02), though no difference was seen in percent of chicks positive for bacteria in the yolk sac.

In summary, DHP treatment in a commercial poultry hatchery was associated with higher chick hatch rate and survivability. This increase in production is biologically significant for the poultry industry and provides a mechanism to increase production without increasing inputs (eggs set). When the cost of production is factored in, this resulted in an increased value to the company of ~$34,000 per month. Reduced fungal loads in chicks were seen during this study, though no differences in bacterial loads were measured warranting further investigation to elucidate the mechanism for the DHP's positive impact on production parameters.

Limitations and recommended future research

The previously published evidence included in this review contains several peer-reviewed publications that describe statistically significant microbial reductions in the presence of DHP, observed in both laboratory and field settings. Due to the limited results yielded by the database search, this review was supplemented with previously unpublished laboratory reports provided by the unit manufacturers detailing laboratory studies that were performed by a third-party entity, as well as an observational study performed by university researchers within a chicken hatchery. While reductions were observed in all titers of foodborne pathogens during exposure to DHP in comparison to the control, it is unclear what the magnitude and impact of the microbial reductions due to DHP would be in animal production settings over long periods of time. The review team recommends that future research should focus on the potential impact of DHP on the environmental and direct transmissions of zoonotic pathogens within animals. Subsequent studies simulating human pathogen transmission using animal surrogates would also be warranted.

Conclusions

DHP's demonstrated ability to reduce common foodborne pathogens, along with the outcomes observed in a poultry production setting, indicate that the technology could be an asset in livestock production (41,47,53,59,65). By reducing the presence of microbes in the environment, DHP can not only facilitate conditions that beget healthier animals at hatch or birth, but also improve the efficiency of the production system itself.

The impact of an efficient, optimized animal production system cannot be overestimated, given the concurrent rise in the human population and decline in the number of companies producing animal protein. It is widely acknowledged that all agricultural production systems will have to enhance efficiency in order to meet the growing food demands of the global population. Technologies will undoubtedly play an important role in this undertaking, but they must be vetted for their efficacy, application, and operation. DHP is a strategy well suited to this task. With evidence-based efficacy and the ability to operate continuously in occupied settings, DHP may be an important resource to shepherd animal agriculture and food safety through the world's current challenges and those that lie ahead.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jphe.amegroups.com/article/view/10.21037/jphe-22-105/coif). The series "Global Environmental Health and COVID-19" was commissioned by the editorial office without any funding or sponsorship. CL reports that Synexis has contracted CL's consulting company to provide research-related services and to provide technical insights. CL is a Consulting Epidemiologist at EpiClear Consulting. BJJ reports that Synexis has provided their equipment to BJJ's laboratory at the University of Georgia for research testing, as well as outfitted commercial poultry hatcheries with their

technology for field testing. These projects were supported by external grants. Synexis has also paid BJJ consulting fees for working in hatcheries with/on their technology. DG reports that Synexis provides financial reimbursement for time spent on consulting services related to the infection prevention and control guidelines across the continuum of care. DG is IPC Consultant and President at DDG Associates, LLC. The authors have no other conflicts of interest to declare.

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