

Peer Review File

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Reviewer A

Comment 1: This appears not to be an unbiased scientific review of the technology - for example it at no point references the UK government 2019 review of the DHP technology to which this papers efficacy data relates: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/823215/2203-01_synexis_dry_biodefence_system_review.pdf

I found the paper poorly structured, confusing and not well linked to the title. The first two pages discuss the COVID-19 epidemic, but this has very little relation to animal agriculture and is not related to the data provided on DHP.

Reply 1: Rather than a full literature/scientific review, this was submitted under the guidelines of a narrative review within the context of the One Health initiative.

CHANGE MADE: The reference to the narrative review checklist that was added is in lines 146-148 now due to the addition of the additional author information.

Previously published research (primarily in the past three years) provides substantial evidence that DHP significantly reduces environmental bioburden within dynamic spaces (e.g., hospital rooms). Our team sought to evaluate this evidence through the lens of the One Health initiative with a focus on pathogens related to foodborne illness and animal health. As part of this endeavor, we acquired third-party lab data from the manufacturer related to DHP's impact on several of these pathogens.

The UK government's 2019 review of DHP was an evaluation of available published evidence at that time. Since then, several studies, which we include in this narrative review, have been published that directly address the gaps in the research previously identified by the UK government evaluation. Additionally, the database search guidelines listed in Table 1 did not yield this document from ScienceDirect.

Comment 2: Section 3.1.5 speaks to vaporised hydrogen peroxide as an aerosol (which it is not) and then Section 3.1.6 describes aerosol hydrogen peroxide systems - this is just one example of the confusing nature of the paper.

Reply 2: Thank you for this correction. We have adjusted the statement accordingly.

CHANGE MADE: The reference to aerosol in the Vaporized Hydrogen Peroxide paragraph (3.1.5) was removed. The addition of the author information after the creation of this document shifted the line number of the change to 229.

Comment 3: The literature review seems very limited based on DHP technology being around since circa 2008. The limited number of references identified and subsequent reliance on manufacturer data, results in a substantial part of the paper being a publication of 2016 laboratory testing (references 53, 59 and 65).

Reply 3: While DHP has been around since 2008, the majority of the peer-reviewed literature pertaining to DHP has been generated in recent years. The 2016 manufacturer data was included in this paper because it pertained to pathogens related to animal health and foodborne illness. Therefore, the study team believed it to be an important inclusion to a discussion about the One Health Initiative.

Comment 4: The data presented in Section 5.1.7 is interesting and related to the paper title. Authors should present figures in full - for example "Over the course of testing, hatch of fertile eggs set was increased on the DHP treated side by .65% compared to the non treated side". The .65% should be written as 0.65%, as it could be easily misinterpreted. Likewise, "lower mortality rate (.07%)" should be presented as 0.07%.

Reply 4: Thank you for this feedback. These requests were addressed in the manuscript and corrected.

CHANGES MADE: Lines 579, 580, 587, Page 13

Comment 5: The results data are not statistically significantly different between the treated and non-treated side and the conclusions reached are tenuous.

Reply 5: While the results were not statistically significant, they are very biologically significant for the commercial poultry industry and highlight the difference between laboratory and real-world datasets. The authors have added more context for these data, including a value metric for increased production. P-values were also added to the manuscript where appropriate.

CHANGES MADE: Lines 581-583, 588-593, 596-604, Pages 13-14

Comment 6: The statement "it is probable that this is a result of the reduction in bacterial and fungal loads measured in the hatchery during the study" is not supported by data. If bacterial and fungal loads have been measured during the study they should be presented as supporting data in this paper.

Reply 6: A statement was added with fungal measurements which were significant.

CHANGES MADE: Lines 591-592, Page 13

Comment 7: I would recommend to the authors that they rewrite the paper, removing the 2016 lab study data, the references to COVID-19, other technologies, etc and focus the paper on the poultry production study, producing a detailed paper on this study, with its methods, results, data and data supported conclusions.

Reply 7: Based on comments received from other reviewers and the guest editors, the study team believes that the revised manuscript appropriately addresses and discusses DHP's mechanism of action, identifies relevant industries it can potentially impact within the scope of the One Health Initiative, and presents previously unpublished data as evidence of this potential impact.

Reviewer B

Comment 1: This is a critical and important review, not only for the livestock industry but the greater infection control community as well. The review was constructed beautifully, with the data from the supporting manuscripts facilitating the reader to reach the same conclusion as the authors.

One aspect that I had hoped the authors might have addressed in the discussion was the emerging issue of the generation of viable but not culturable (VBNC) communities of microbes upon environmental exposure to strong oxidative insults such as they are advocating with dry hydrogen peroxide. Unfortunately, this is an emerging area of focus for the food and infectious diseases fields with little direct evidence in support of the significance that strong oxidants can do with respect to VBNC rates and clinical outcomes. For background see the following manuscripts (this reviewer has no involvement with the manuscripts)

Highmore CJ, Warner JC, Rothwell SD, Wilks SA, Keevil CW. 2018. Viable-but-Nonculturable *Listeria monocytogenes* and *Salmonella enterica* Serovar Thompson Induced by Chlorine Stress Remain Infectious. *mBio* 9. and

Wilks SA, Koerfer VV, Prieto JA, Fader M, Keevil CW. 2021. Biofilm Development on Urinary Catheters Promotes the Appearance of Viable but Nonculturable Bacteria. *mBio* 12.

Overall, well done.

Reply 1:

Thank you for addressing this issue in your review and for providing these very interesting manuscripts. When assessing the issue of VBNC microbe communities left behind by oxidative disinfection technologies, we must consider the intended use of the technology (i.e. terminal clean vs. continuous pressure). The continuous use of DHP is intended to supplement terminal cleaning procedures by targeting a reduction in the steady-state level of microbial load within the space, rather than a total elimination of bioburden. Results such as the hatchery data presented in this manuscript indicate a measurable impact on metrics associated with the reduction of bioburden that is linked to DHP. Further investigation is needed to determine if VBNC microbe communities are present after prolonged exposure to DHP, but the impacts of the steady-state reduction in bioburden are apparent.

As DHP is a relatively novel technology compared to other oxidants, we have minimal insight into how DHP's continuous oxidative pressure could potentially impact a microbe's ability to reach and maintain a VBNC state. Thank you again for providing us with a potential direction for future research.

Reviewer C

Comment 1: The article clearly articulates the case for using DHP as one technology that can be implemented in healthcare and food services to reduce pathogen impact.

Reply 1: Thank you for your feedback.