Efficacy of Some Commercial Chemical Disinfectants on Salmonella enterica Serovar Typhimurium

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Abstract: Problem statement: Poultry industry is intensive and consistently applies an all-in, all-out system with the aim of minimizing infection pressure and targeting specific pathogens like Salmonella which remains one of the leading causes of food-borne illness, many questions regarding the introduction and persistence in animal production still remain. Therefore disinfection during production break is a routine part of the biosecurity programs of poultry houses. The correct usage of disinfectants is an important key of a successful biosecurity program in poultry farms and in-turn the role of the scientist was to evaluate the efficacy of these disinfection programs. Approach: In this study five commercial disinfectants [Green work (green non anionic surfactant), Sanidate RTU (hydrogen peroxide compound), Hi-yield® consan 20® (phenolic compound), Tektrol® (quaternary ammonium compound) and Kreso® D (phenolic compound)] were evaluated against Salmonella typhimurium in two different experimental conditions. In Experiment I, S. typhimurium was inoculated into fresh poultry litter (aluminum trays L: 30 cm × W: 25 cm × D: 6 cm filled with wood shavings) by inoculum size of ~10^7 CFU mL^-1 and then mixed with 100 g of fresh poultry droppings. Sample sizes of 3 g were obtained daily for the bacterial counts. Green work achieved 100% killing of S. typhimurium by day 7 (p≤0.0001); Sanidate RTU achieved 100% killing by day 6 (p≤0.001); Hi-yield® Consan®, Tektrol® and Kreso® D achieved 100% killing by day 5 (p≤0.001). Disinfectants were also compared to each other in their efficacy each day. At day 1, Green work was inferior to all other disinfectants at (p≤0.05). On day 2, Kreso® D was significantly superior to Tektrol®, Hi-yield® Consan®, Sanidate RTU and Green work at p≤0.01, p≤0.01, p≤0.01, p≤0.005; respectively. At day 4 Kreso® D was significantly superior to Hi-yield® Consan® at p≤0.01, Tektrol® was also significantly superior to Green work at p≤0.01. In experiment II; MIC use-dilution test was used to evaluate the five disinfectants against S. typhimurium (~10^7 CFU mL^-1) in the absence of organic matter. Results: Hourly samples were collected for the bacterial counts. Maximum efficacy (100% killing efficacy against S. typhimurium) was achieved for Green Work after 16 h (p≤0.0001), with Sanidate RTU after 8 h (p≤0.0001), with Hi-yield® Consan® and Kreso® D after 2 h (p≤0.0001) and with Tektrol® after 4 h (p≤0.0001). In presence of organic matter Green work and Sanidate RTU achieved 100% killing efficacy against S. typhimurium after 16 h (p≤0.0001), Hi-yield® Consan® and Kreso® D after 2 h at (p≤0.0001); Tektrol® after 8 h (p≤0.0001). When disinfectants were compared to each other in relation to time; we found that there was no kind of significance between their efficacies. When compared to other tested disinfectants, Kreso® D which is a phenolic compound revealed superior activity against Salmonella typhimurium in the two experiments. Conclusion: The study showed that many disinfectants regardless to their constituents continues to give a very powerful efficacy against the most virulent bacterial strains, but the question remain can they be used in the presence of live birds. Further studies are required to explore the safety and the efficacy of these compounds when applied in poultry farms in the presence of live birds.

Key words: Disinfectants, Salmonella typhimurium, poultry, organic matter

INTRODUCTION

The objective of disinfection is to reduce microbial population. Disinfectants act on microorganisms at several target sites resulting in membrane disruption, metabolic inhibition and lysis of the cell[4,14]. Removal of old litter followed by cleaning and disinfection of facilities helps reduce pathogen numbers and break

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disease cycles or at the minimum, keep pathogen numbers from reaching a level that can cause disease outbreaks. In addition, as live production becomes the target area of programs for the reduction of human pathogens such as Salmonella on poultry carcasses, it will become necessary to document that sanitation procedures are effective. Unfortunately, poor sanitation procedures and/or increased soil moisture levels have been linked to increased or sustained bacteria levels\textsuperscript{[18,24,26].}

Several studies were carried out on disinfectants and many of these disinfectants are not considered to be environmentally safe e.g., gluteraldehyde, formaldehyde to show their effectiveness against Salmonella\textsuperscript{[7,8,21].} Further, poultry houses have inaccessible equipment and considerable amounts of organic matter and high contents of protective compounds (fats, carbohydrates and proteins) from which Salmonella are difficult to remove\textsuperscript{[8].} On the other hand, water hardness, low temperature and biofilm development also decrease efficacy of disinfectants\textsuperscript{[8,12,25].}

Disinfectant efficacy is often tested against laboratory bacterial suspensions\textsuperscript{[1,16].} However, this approach may not always prove to simulate commercial production conditions, thus, making it difficult to determine the true effectiveness of the disinfectant. Disinfectants that are effective against bacterial suspensions may have a reduced effect against bacteria that adhere to surfaces\textsuperscript{[15].}

The main objective of this study was to compare the efficacy of some new commercial disinfectants against S. typhimurium, in the presence or absence of organic matter as an extra-challenge for the disinfectants.

MATERIALS AND METHODS

Propagation of Salmonella typhimurium:  
S. typhimurium (ATCC 1331) genomic DNA strain NCTC74 was propagated and counted using drop plate technique, Zelver et al\textsuperscript{[27]} and Herigstad et al\textsuperscript{[10].} The procedures were carried out by pipetting 1 mL of bacterial suspension into a dilution tube containing 9 mL of tetrathionate broth; making dilution 10\textsuperscript{1}. Tenfold serial dilutions were made to obtain dilutions of 10\textsuperscript{2}, 10\textsuperscript{3}, 10\textsuperscript{4}, 10\textsuperscript{5}, 10\textsuperscript{6} and 10\textsuperscript{7} mL\textsuperscript{-1}. Bacterial count in each dilution was obtained by inoculating on CHROMagar Salmonella plates (Becton-Dickinson, VMR Int.) The plates were incubated overnight for 17-20 h at 35-37°C. Viable cell counts were expressed as CFU/surface area. The calculation was carried out using the following formula:

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\text{Log (average CFU/drop vol.) (dilution factor) (Vol. scrapped into/surface area)}
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Biochemical identification: The biochemical identification of Salmonella was carried out using automated method (MICROSCAN auto SCAN4, Dade Behring), which confirmed that the suspension was positive for Glucose, Lysine, Citrate utilization, Raffinose, Hydrogen Sulphid, Sorbitol, Arabinose, Meltonin and Ornithin.

Experiment I: Efficacy of chemical disinfectants against Salmonella typhimurium under conditions simulating naturally ventilated poultry houses.

Inoculation of the litter with Salmonella typhimurium:  
Six trays of aluminum foil (L: 30 cm × W: 25 cm × D: 6 cm) were filled with litter (wood shavings). All the trays were sterilized by autoclaving at 121°C for 1 h. Sterilization was confirmed by placing 25 g of autoclaved litter into 225 mL of buffered peptone water (BPW; Oxoid, Fisher Scientific Int.) and incubated in rotatory incubator for 3 h; followed by spread plating on CHROMagar Salmonella plates (BD, VMR Int.). All trays were incubated at 37°C for 20-24 h and then the colonies were enumerated.

Autoclaved fresh poultry droppings were added to the trays as a challenge to the chemical disinfectants at rate of 100 g tray\textsuperscript{-1}. The trays were labeled, one for each disinfectant, then the six trays were inoculated with S. typhimurium suspension (4.8\times10\textsuperscript{7} CFU mL\textsuperscript{-1}), five trays were used for treating with the disinfectants and the last one was used as control.

Application of the chemical disinfectants:  
The following disinfectants were chosen to be tested against S. typhimurium: Hi-Yield\textsuperscript{®} Consan20\textsuperscript{®} (Parkway Research Corp., Houston, TX) a mixture of quaternary ammonium compounds as 20 with 80% inert ingredients. The recommended dilution rate was 1 floz per 30 gallon water (1.85 mL of the disinfectant was added to 483 mL of distilled water, pH 9.22).

KRESO\textsubscript{®}D (Elmwood Park Station, Omaha) a mixture of coal tar neutral oil, coal tar phenols, soap and water. The recommended dilution rate was 1 part of KRESO\textsubscript{®}D to 72 parts warm water (5 mL of the disinfectant was added to 360 mL distilled water, pH 9.57).

TekTro\textsubscript{®} (ABC Compounding Co, Int., Atlanta, GA) is a mixture of Paratertiary Amylphenol (40%), Orthobenzyl Para Cholorophenol (10%), Orthophenyl phenol (12%) and inert ingredients (74%). The recommended dilution rate was 15 cc per one gallon.
Evaluation of the efficacy of the chemical disinfectants in the absence of organic matter:

Tenfold serial dilution was carried out for each disinfectant in 15 mL tubes using PBS to obtain the dilutions of 10^1, 10^2, 10^3, 10^4 and 10^5 cfu mL^{-1}. 0.1 mL (100 µL) of the bacterial suspension (4.8×10^7 cfu mL^{-1}) was added to the tubes containing 10 mL of the disinfectant dilutions. The treated tubes were vortexed. One mL was transferred from the bacterial/organic matter-disinfectant tubes to 15 mL tubes containing 9 mL of each of the disinfectant dilutions. The treated tubes were vortexed. One mL was transferred from the bacterial/organic matter-disinfectant tubes to 15 mL tubes containing 9 mL of each of the disinfectant dilutions. The treated tubes were vortexed. One mL was transferred from the bacterial/organic matter-disinfectant tubes to 15 mL tubes containing 9 mL of each of the disinfectant dilutions. The treated tubes were vortexed.

Salmonella typhimurium was counted on chromagar Salmonella plates using plate drop techniques Zelver et al.[25] and Herigstad et al.[10] as described previously. Viable cell counts were expressed as CFU/surface area. The calculation was carried out using the following formula:

$$\text{Log (average CFU/drop vol.) (dilution factor) (Vol. \, scrapped into/surface area)}$$

Statistical analysis: The statistical analysis was carried out by performing Analysis Of Variance (ANOVA, GLM, MIXED) using SAS 9.2.0 software.

RESULTS

Efficacy of the tested chemical disinfectants on the survival of Salmonella Typhimurium in poultry litter:
The objective of this study was to evaluate the efficacy of some new commercial disinfectants some of which are considered environmentally safe and possibly could be used in poultry houses while the birds are still present.
In this experiment (experiment I), the object was to compare the efficacy of these disinfectants in poultry litter with or without organic matter. The results showed superiority in action that was faster time and higher significant when compared with the other tested chemical disinfectants.

DISCUSSION

Commercially available disinfectants are not classified as broad spectrum agents. Multiple factors should be considered when a disinfectant is chosen, such as organic matter on the surface to be treated.
presence of organic matter in the diluents, quality of water, corrosiveness or toxicity of the product, application method, temperature, porosity of the surface being treated, length of contact time, infectious organisms targeted, susceptibility of the infectious organisms and correct dilution.[17,19]

It is well known that elimination of *Salmonella* from poultry houses is a difficult task.[13] The main risk of *Salmonella* contamination of poultry flocks are the *Salmonella* status of the previous flock,[23] *Salmonella* status of day old chicks,[25] contaminated litter, feed and water,[11] presence of contaminated carriers,[3] rodents, flies and beetles and inadequate disinfection of abattoir trucks.[21]

Data showed that Green work was effective against *S. typhimurium* starting at day 5 (p≤0.001) with killing ratio of 98.48% and showed 100% killing efficacy on day 7 (p≤0.0001). Sanidate RTU was effective starting at day 4 (p≤0.05) with killing ratio of 99.51 and showed 100% killing efficacy starting at day 6 (p≤0.001) and day 7 (p≤0.0001). Hi-yield® Consan® was effective starting at day 4 (p≤0.05) with killing ratio of 99.66% and showed 100% killing efficacy at day 5 (p≤0.001), day 6 (p≤0.0005) and day 7 (p≤0.0001). Tektrol®, was effective starting at day 4 (p≤0.05) with killing ratio of 99.91% and showed 100% killing efficacy at day 5 (p≤0.001), day 6 (p≤0.0001) and day 7 (p≤0.0001). Kreso®, D was effective starting at day 2 (p≤0.05) with killing ratio of 99.76% and showed 100% killing efficacy at day 5 (p≤0.001), day 6 (p≤0.0005) and day 7 (p≤0.0001).

The disinfectants were compared to each other in their efficiency each day and the results showed that on day 1 there was a significant difference between Sanidate RTU and Green work (p≤0.05). On day 2 there was a significant difference between Kreso®, D and Tektrol®, Hi-yield®, Consan®, Sanidate RTU, Green work (p≤0.01), (p≤0.01), (p≤0.01), respectively. On day 4 there was a significant difference between Kreso®, D and Hi-yield®, Consan® (p≤0.01), Tektrol®, and Green work (p≤0.01). On day 3, 5, 6 and 7 there were no significant differences between the efficacy of the disinfectants and each others.

The intention of disinfectant programs in poultry facilities is to reduce the pathogenic micro-organisms. However, if disinfectants are used without properly cleaning the facility prior to application, then the effectiveness of the disinfectant may be compromised. Organic matter provides a physical barrier that protects microorganisms from contact with the disinfectants.[5] In this study both Green work and Sanidate RTU showed delayed action. Although they are environmentally safe their action seems to be affected by the presence of organic matter. It would be prudent to study their efficacy at higher concentrations.

The pH of the litter did not show any obvious influence on the activity of the disinfectants, although it is well known that each disinfectant has its own favorable pH range to act. *Salmonella* is known to survive in pH range of up to 5.3. In this study the pH values broadly were ranged from 8.90-6.13 which is considered to be within the working range pH for the optimum action of all the disinfectants as well as optimum range for the growth of *Salmonella*. It is to be mentioned that Sanidate RTU and green work when diluted to the recommended concentrations, pH was highly acidic 4.75 and 2.40, respectively but when mixed with the litter the pH was in same range of the litter treated with other disinfectants (Table 2).

These data showed that in the absence of organic matter Green work started to show high efficacy after 2 h (p≤0.0001) with killing ratio of 97.50% and showed 100% killing efficacy after 16 h (p≤0.0001). Sanidate RTU started to show high efficacy after 2 h (p≤0.0001) with killing ratio of 99.62% and showed 100% killing efficacy after 8 h (p≤0.0001).

Hi-yield® Consan® and Kreso® D showed 100% killing efficacy after 2 h (p≤0.0001). Tektrol® started to show high efficacy after 2 h (p≤0.0001) with killing ratio of 99.97% and showed 100% killing efficacy after 4 h (p≤0.0001) (Table 3).

In the presence of organic matter Green work and Sanidate RTU, both starting to show high efficacy after 2 h (p≤0.0001) with killing ratio of 93.75 and 94.58%, respectively and showed 100% killing efficacy after 16 h (p≤0.0001). Hi-yield® Consan® and Kreso® D showed 100% killing efficacy after 2 h (p≤0.0001). Tektrol® started to show high efficacy after 2 h (p≤0.0001) with killing ratio of 99.97% and showed 100% killing efficacy after 4 h (p≤0.0001) (Table 3).

Latasa et al.[13], reported that life in a biofilm state protects the bacteria against environment insults like chemical sanitizers which are generally unable to eliminate most biofilm-associated bacteria.

The disinfectants were compared to each other in their efficacy at each specific time by taking samples from the disinfectant/bacterial mixture. There were no significant differences between Green work, Sanidate RTU, Hi-yield®, Consan®, Tektrol® and Kreso® D.

Quinn and Markey,[20] suggested that phenolic compounds should be used for any application where excessive organic matter may be present, due to their efficacy even in the presence of organic matter.
CONCLUSION

In summary both of Kreso® D and Hi-yield® Consan 20® which are phenolic compounds have shown higher efficacy against S. typhimurium compared with the other compounds. In experiment I in the presence of organic matter in the litter, they achieved 100% lethal effect by day 5 (p≤0.0001) (Table 1) and in experiment II they achieved 100% lethal activity after 2 h (p≤0.0001) (Table 3 and 4) irrespective of the absence or the presence of organic matter.

Green work and Sanidate RTU are considered environmentally safe disinfectants. Although their efficacy was less compared to Kreso® D in this study, future experiments are necessary to see if they would be effective at higher concentrations. Future studies are also needed to study the efficacy of environmentally safe disinfectants while the birds are present in poultry houses.

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REFERENCES

1. Bloomfield, S.F., M. Arther, E. Looney, K. Begun and H. Patel, 1991. Comparative testing of disinfectant and antiseptic products using proposed European suspension testing methods. Lett. Applied Microbiol., 13: 233-237. DOI: 10.1111/j.1472-765X.1991.tb00617.x
2. Cardinale, E., F. Tall, E.F. Guèye, M. Cisse and G. Salvat, 2004. Risk factors for Salmonella enterica subsp Enterica infection in Senegalese broiler-chicken flocks. Prev. Vet. Med., 63: 151-161. http://cat.inist.fr/?aModele=afficheN&cpsidt=14716669
3. Davies, R.H. and M. Breslin, 2003. Observations on Salmonella contamination of commercial laying farms before and after cleaning and disinfection. Vet. Rec., 152: 283-287. http://veterinaryrecord.bvapublications.com/cgi/content/abstract/152/10/283
4. Denyer, S.P. and G.S.A.B. Stewart, 1998. Mechanisms of action of disinfectants. Int. Biodeterior. Biodegradat., 41: 261-268. DOI: 10.1016/S0964-8305(98)00023-7
5. Dvorak, G., 2005. Disinfection 101. Reviewed by J. Roth and S. Amass. The Center for Food Security and Public Health. Iowa State Univ. Ames, IA. http://www.cfsph.iastate.edu/BRM/resources/Disinfectants/Disinfection101.pdf
6. Eckman, M.K., 1994. Chemicals used by the poultry industry. Poult. Sci., 73: 1429-1432. http://www.ncbi.nlm.nih.gov/pubmed/7800644
7. Gradel, K.O., J. Chr Jorgensen, J.S. Anderson and J.E.L. Corry, 2003. Laboratory heating studies with Salmonella spp. and Escherichia coli in organic matter, with a view to decontamination of poultry houses. J. Applied Microbiol., 94: 919-928. http://cat.inist.fr/?aModele=afficheN&cpsidt=14716669
8. Gradel, K.O., J. Chr Jorgensen, J.S. Anderson and J.E.L. Corry, 2004. Monitoring the efficacy of steam and formaldehyde treatment of naturally Salmonella infected layer houses. J. Applied Microbiol., 96: 613-622. http://cat.inist.fr/?aModele=afficheN&cpsidt=15521217
9. Gradel, K.O., L. Randall, A. Sayers and R.H. Davies, 2005. Possible association between Salmonella persistence in poultry houses and resistance to commonly used disinfectants and a putative role of mar. Vet. Microbiol., 107: 127-138. http://cat.inist.fr/?aModele=afficheN&cpsidt=16654537
10. Herigstad, B., M. Hamilton and J. Heersink, 2001. How to optimize the drop plate method for enumerating bacteria. J. Microbiol. Methods, 44: 121-129, DOI: 10.1016/S0167-7012(00)00241-4
11. Heyndrickx, M., D. Vandekerchove, L. Herman, I. Rollier, K. Grijspeerdt and L.D. Zutter, 2002. Routes for Salmonella contamination of poultry meat: Epidemiological study from hatchery to slaughterhouse. Epidemiol. Infect., 129: 253-265. http://www.ncbi.nlm.nih.gov/pubmed/12403101
12. Lapidot, A., U. Romling and S. Yaron, 2006. Biofilm formation and the survival of Salmonella typhimurium on parsley. Int. J. Food. Microbiol., 109: 229-233. http://www.ncbi.nlm.nih.gov/pubmed/16616389
13. Latasa, C., R. Agnès, A. Toledo-Arana, J. M. Ghigo, C. Gamazo, J. R. Penadés and I. Lasa, 2005. Bap A, a large secreted protein required for biofilm formation and host colonization of Salmonella enteric serovar Enteritidis. Mol. Microbiol. 58:1322-1339.
14. Maillard, J.Y., 2002. Bacterial target sites for biocide action. J. Applied Microbiol., 92: 16S-27S. DOI: 10.1046/j.1365-2672.92.5s1.3.x
15. Mosteller, T.M. and J.R. Bishop, 1993. Sanitizer efficacy against attached bacteria in a milk biofilm. J. Food Prot., 56: 34-41. http://cat.inist.fr/?aModele=afficheN&cpsidt=4633699
16. Parkinson, E., 1981. Testing of Disinfectants for Veterinary and Agricultural Use. In: Disinfectants: Their Use and Evaluation of Effectiveness, Collins, C.H., M.C. Allwood, S.F. Bloomfield and A. Fox (Eds.). Acad. Press, London, ISBN: 0121813800, pp: 33-36.
17. Payne, J.B., E.C. Kroger and S.E. Watkins, 2005. Evaluation of disinfectant efficacy when applied to the floor of poultry grow-out facilities. J. Applied Poult. Res., 14: 322-329. http://japr.highwire.org/cgi/content/abstract/14/2/322
18. Pepper, I.L., K.L. Josephson, R.L. Bailey, M.D. Burr and C.P. Gerba, 1993. Survival of indicator organisms in Sonoran Desert soil amended with sewage sludge. J. Environ. Sci. Health, 28: 1287-1302.
19. Prince, H.L., D.L. Prince and R.N. Prince, 1991. Principles of viral control and transmission. In: Disinfection, Sterilization and Preservation, Block, S.S. (Ed.), 4th Edn., Lippincott, Williams and Wilkins, Philadelphia, PA., pp: 411-444. http://iodine4health.com/research/gottardi_1991_chapt8_iodine_compounds.pdf
20. Quinn, P.J. and B.K. Markey, 2001. Disinfection and Disease Prevention in Veterinary Medicine. In: Disinfection, Sterilization and Preservation, Block, S.S. (Ed.), 5th Edn., Lippincott, Williams and Wilkins, Philadelphia, PA., pp: 1069-1103.
21. Ramesh, N., S.W. Joseph, L.E. Carr, L.W. Douglass and F.W. Wheaton, 2002. Evaluation of chemical disinfectants for the elimination of Salmonella Biofilm from poultry transport containers. Poult. Sci., 81: 904-910. http://poultsci.highwire.org/cgi/content/abstract/81/6/904.
22. Robinson, R.A., H.L. Bodily, D.F. Robinson and R.P. Christensen, 1988. A suspension method to determine reuse life of chemical disinfectants during clinical use. Applied Environ. Microbiol. 54: 158-164.
23. Rose, N., J.P. Mariain, P. Drouin, J.Y. Toux, V. Rose and P. Colin, 2003. A decision-support system for Salmonella in broiler-chicken flocks. Prev. Vet. Med., 59: 27-42. DOI: 10.1016/S0167-5877(03)00056-4
24. Rudolfs, W., L.L. Falk and R.A. Rgotzki, 1950. Literature review of the occurrence and survival of enteric pathogenic and relative organisms in soil, water sewage and sludge and on vegetation. Sewage Ind. Wastes, 22: 1261-1281. http://www.jstor.org/stable/25031419.
25. Tylor, J.H. and J.T. Holah, 1996. A comparative evaluation with respect to the bacterial cleanability of a range of wall and floor surface materials used in the food industry. J. Applied Bacteriol., 81: 262-266.
26. William, J.E., Mallenson and G.H. Soeynebos, 1975. Isolation and identification of avian pathogens. AM. ASS. Avian Pathologist.
27. Zelver, N., M. Hamilton, D. Goeres, D. Walker and J. Heersink, 2001. Development of a Standardized Antibiobfilm Test: In Microbial growth in Biofilms, Doyle, R.J. (Ed.). Gulf Professional Publishing, ISBN: 0121822389, pp: 363-376.