Bactericidal Treatment of Hatching Eggs IV. Hydrogen Peroxide Applied with Vacuum and a Surfactant to Eliminate Salmonella from Hatching Eggs

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Primary Audience: Hatchery Managers, Hatchery Egg Producers, Poultry Researchers

Summary

Bacteria (including salmonellae) can be pulled into and through the shell and membrane of broiler hatching eggs as they cool. When this occurs, salmonellae are out of reach of a surface-applied chemical treatment, because direct contact is usually required to achieve a kill. Over many years of research, a large number of disinfectant chemicals have been tested on hatching eggs. We have found H$_2$O$_2$ (1.4%) to be a fairly effective disinfectant to reduce Salmonella that is artificially inoculated onto hatching eggs. The objective of the present study was to determine whether the efficacy of H$_2$O$_2$ could be enhanced by utilizing vacuum and a surfactant to remove air and reduce surface tension within the eggshell, enabling the bactericide to penetrate deeper into the egg to kill Salmonella. Eggs were inoculated with nalidixic acid-resistant Salmonella typhimurium. Inoculated eggs were treated with H$_2$O$_2$ with or without a surfactant and with or without the application of vacuum to facilitate shell penetration. Thirty percent of eggs exposed to H$_2$O$_2$ with surfactant and vacuum were still positive for the marker Salmonella. Although this represents a decrease in number compared with a water control, 30% remained contaminated. Results demonstrate the difficulty involved in reaching and killing Salmonella that has penetrated the hatching egg, even with an effective bactericide.

Key words: Disinfectant, hatching eggs, Salmonella, surfactant, vacuum

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Description of Problem

For over 100 yr, it has been known that salmonellae can penetrate the shells and membranes of hatching eggs. We now know that this can critically effect the salmonellae contamination on the final product (the processed broiler carcass). As the freshly laid egg cools, the contents contract, producing a negative pressure that in turn can draw bacteria such as salmonellae into and through the shell and adhering membranes. The salmonellae may then be ingested by the embryo as it emerges from the egg [1], or the salmonellae may proliferate as the embryo develops and be

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spread in the hatching cabinet [2]. Very few Salmonella cells are required to colonize the gut of the young animal [3], producing a seeder bird that will then spread contamination to the intestinal tract, skin, and feathers [4] of other birds in the flock. Several studies around the world have shown that the same Salmonella serotypes originating from hatcheries and breeder flocks can be found on fully processed broiler carcass [5, 6, 7]. Therefore, early contamination of the freshly laid fertile egg is a very important critical control point for preventing the entry of salmonellae and other human foodborne pathogens into poultry production and subsequent processing.

Research has shown that when Salmonella are able to penetrate the shell and membranes, they can avoid the killing effect of a chemical by avoiding direct contact with the chemical. Hydrogen peroxide has been shown to be an effective sanitizer for eggs by several researchers [8, 9, 10]. However, H₂O₂, like other chemicals, cannot be effective against Salmonella cells that have penetrated the shell, thereby avoiding direct contact with the chemical [11]. In an attempt to reach these microorganisms with a chemical treatment, researchers have explored the possibility of pushing or pulling chemicals deeper into the egg. Padron [9] found that H₂O₂ was a more effective bactericide when positive pressure was applied to push the chemical deeper in the egg during immersion treatment. Others have used a negative pressure approach. By placing an egg in a solution and evacuating the air within the egg with vacuum, a negative pressure is formed within the egg. When the vacuum is released, the liquid surrounding the egg is drawn deeper into the egg. This has been shown to be effective with chemical (H₂O₂) disinfection of a Salmonella-contaminated fertile egg [11]. Also, surfactants have been shown to assist chemicals to penetrate deeper into fertile eggs [12].

The objective of this study was 1) to evaluate the effectiveness of eliminating Salmonella from hatching eggs with H₂O₂ using vacuum, surfactant, and the combination of both and 2) to determine the effect of the most effective treatment on the hatchability and early chick mortality.

**Materials and Methods**

**EGGS**

All eggs were obtained from a commercial broiler hatchery and had been stored for less than 4 d at 18 °C. Eggs were transported to the laboratory within one-half hour and were warmed to 42 °C by placement in an incubator for 18 to 24 h. Following warming, eggs were inoculated by immersion for 1 min in a room temperature (approximately 25 °C) cell suspension of S. typhimurium in 1% peptone water and allowed to dry for 24 h prior to treatment. This was done to simulate a freshly laid warm egg contacting room temperature feces containing salmonellae. For each of the treatment groups, four replications were done with 10 eggs per replication.

**Salmonella Culture**

A nalidixic acid resistant strain of S. typhimurium was used. The marker organism was grown overnight on brilliant green (BG) sulfa agar [13] plate containing 200 ppm sodium salt of nalidixic acid (BGS + NAL). Cells were removed from the plate with a loop and suspended in sterile 0.85% NaCl. By measurement of optical density at 540 nm and comparison with a calibration curve, the Salmonella population was estimated at 10⁸ cells/mL. This primary cell suspension was diluted in 1% peptone water until an inoculum of 10⁴ cells/mL was reached. Inoculum levels were confirmed by plate count on BGS + NAL.

The sanitizing chemical tested in this study was 1.4% H₂O₂. A non-treated control and distilled water treatment were used for comparison. Chemicals were applied as an immersion dip or as an immersion dip with the addition of a vacuum step and/or the addition of a surfactant [0.05% Triton X 100 (TX)]. Eggs were submerged in each test chemical within a stainless steel pan. For application of the vacuum, the pan was placed in a vacuum chamber with an attached vacuum pump. A vacuum of 12 to 13 inches mercury (0.4 bar) was applied for 4 min. The vacuum was released, and the eggs remained submerged in the test solution for an additional 6 min at atmospheric pressure (1.0 bar). Eggs that were treated by immersion without vacuum were left submerged in the chemical for a total of 10 min concurrent with those undergoing the vacuum treatment. Eggs were inoculated and treated in replicate groups of 10. Four replications were done for each treatment with and without vacuum applied.
CULTURE OF EGGS

Following sanitization treatment, eggs were removed from the chemical and allowed to dry at room temperature. Eggs were sampled by the method of Berrang et al. (14). Briefly, eggs were aseptically opened, the contents discarded, and the shell and membranes were placed into a sterile plastic bag with 50 mL buffered peptone (BP). The shell and membranes were crushed by hand and thoroughly submerged in the BP. The bags of BP were incubated for 24 h at 35 °C. Following incubation, a sterile cotton-tipped applicator was moistened in the BP and was used to streak the surface of a BGS + NAL agar plate, which was incubated 24 h at 35 °C. Plates with characteristic Salmonella colonies were counted as positives. Positive samples were confirmed as Salmonella by test with Poly O and Poly H antisera [13].

HATCHABILITY STUDY

Application of H₂O₂ and water with vacuum and/or TX were examined for the effect on hatchability compared with untreated controls. Broiler hatching eggs were obtained from a local commercial hatchery 1 d prior to treatment. Following treatments, eggs remained at room temperature for 4 h prior to setting. For each of two replicate settings, 180 eggs were placed into 88 egg plastic racks, set in a NatureForm NMC-2000 [15] incubator operating at 37.8 °C and 55% relative humidity, and were turned every hour. On Days 7 and 18 of incubation, eggs were candled, and candled-out eggs were opened to macroscopically distinguish between infertile and embryo mortality. On Day 18 of incubation, eggs containing viable embryos were transferred into mass hatching baskets by treatment and placed into a single NatureForm hatcher. The hatcher operated at 36.9 °C, and humidity was increased to 70% relative humidity on Day 19.5 of incubation. On the day of hatch (Day 21), all unhatched eggs were broken-out, and embryos were staged according to Hamburger and Hamilton [16] to determine embryo age. Percentage fertility of eggs set, percentage hatchability of eggs set, percentage hatchability of fertile eggs, and percentage hatchability of viable embryos transferred on Day 18 of incubation was determined. Hatchability of viable embryos at transfer was used as an indicator of late embryonic mortality immediately prior to hatching.

RESULTS AND DISCUSSION

The effect of applying a chemical along with a vacuum and a surfactant to eliminate Salmonella on fertile broiler eggs is shown in Table 1. When eggs inoculated with 10⁴ Salmonella each were immersed in water with a surfactant (TX) or with a vacuum and TX, 95% of the eggs remained positive for the marker Salmonella. When inoculated eggs were similarly immersed in H₂O₂ plus a surfactant (TX) but no vacuum, 62% of the eggs remained positive. When eggs were immersed in H₂O₂ plus a vacuum, but no surfactant, 40% of the eggs remained positive. Finally, when the inoculated eggs were immersed in H₂O₂ plus vacuum plus surfactant, only 30% of the treated eggs remained positive for Salmonella. When one considers the method used to inoculate the eggs (temperature differential immersion), which allowed

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¹ Triton X 100 (TX) at 0.0005% (3.5 mL per 7 L water).
² Vacuum specifications: 4 min at vacuum of 13 inches Hg, brought down over a period of 1.5 min, remained at atmospheric pressure for an additional 6 min.
³ Not Done.
the *Salmonella* to be drawn deeply into the egg, making the microorganism more difficult to reach by the chemical and considers that the method used to recover *Salmonella* from the treated eggs, the hand crush method of Berrang et al. [14], is a very sensitive, reliable method to recover low populations of *Salmonella* on and inside of the egg; then the elimination of $10^4$ *Salmonella typhimurium* from 70% of the inoculated eggs is impressive.

Hatchability and livability were unaffected by the most effective of the tested treatments in this study (Table 2). To date, no chemical treatment has been shown to work perfectly for eliminating salmonellae from fertile hatching eggs. However, the combination of using an effective chemical (H$_2$O$_2$) along with a vacuum and a surfactant in an immersion treatment maximizes the elimination of salmonellae from fertile hatching eggs. However, in a commercial setting, a continuous spray wash treatment may be more practical than an immersion treatment, which will probably have to be applied as a batch operation. Nevertheless, breeder flocks and commercial hatcheries represent the earliest and probably the most critical control points for salmonellae entry into the integrated poultry industry and as such, perhaps a more aggressive approach (such as immersion treatment of eggs) may be required to prevent extensive reservoirs of salmonellae from being established in commercial broiler and broiler breeder hatcheries.

### CONCLUSIONS AND APPLICATIONS

1. Chemical (H$_2$O$_2$) immersion sanitization of broiler hatching eggs with a vacuum step and a surfactant (Triton X) maximized elimination of the *Salmonella* on and in the eggs.
2. Hatchability and early chick mortality were not adversely effected by the above treatment.
3. Because of the batch nature of the process, it would probably require large, expensive equipment to work in a commercial setting.
4. Because of the critical nature of this early salmonellae contamination, all possibilities, regardless how drastic, must be researched and considered if we are to attain zero salmonellae contamination.

### REFERENCES AND NOTES


13. Difco Laboratories, Division of Becton Dickinson and Company, Sparks, MD 21152.


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