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Bactericidal Treatment of Hatching Eggs V: Efficiency of Repetitive Immersions in Hydrogen Peroxide or Phenol to Eliminate *Salmonella* from Hatching Eggs

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Primary Audience: Production Managers, Hatchery Managers, Filed Service Personnel, Flock Supervisors, Quality Control Personnel

SUMMARY

In previous studies, immersion in hydrogen peroxide and phenol reduced *Salmonella*-positive eggs without reducing hatchability. This study was carried out to determine if three consecutive dips in a chemical was more effective than a single immersion to eliminate *Salmonella* from hatching eggs. Eggs were inoculated with a marker strain of *Salmonella typhimurium* by submerging the egg in a solution containing low numbers (10^3) or high numbers (10^5) of the organism. After allowing the eggs to dry, they were immersed for 1, 2, and 3 min in water, a 1.4% solution of hydrogen peroxide, or a 0.39% solution of phenol. With the low inoculation, *Salmonella* were eliminated from an additional 22% of the eggs when three dips were used as compared to a single dip.

Key words: disinfectant, hatching egg, multiple immersion, *Salmonella*

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DESCRIPTION OF PROBLEM

No chemical has been shown to eliminate 100% of *Salmonella* that has penetrated the fertile hatching egg without adversely affecting hatchability. The antibacterial agents that are used to disinfect hatching eggs are direct-contact chemicals. In order to kill *Salmonella*, or any other bacterium, direct physical contact of the chemical with the bacterial cell is required. *Salmonella* can rapidly penetrate the shell and shell membranes; therefore, elimination of these bacteria is very difficult. In previous studies, immersion in the chemicals used in this study for 1 min significantly reduced the number of *Salmonella*-positive eggs without depressing hatchability [1]. This study was carried out to determine if repeated application of chemicals could result in significantly fewer *Salmonella*-contaminated hatching eggs than one application.

MATERIALS AND METHODS

Broiler hatching eggs were obtained from a commercial hatchery, transported to the laboratory, and inoculated with a nalidixic acid resistant marker strain of *S. typhimurium* by submerging the egg in a solution containing the marker organism. Eggs were warmed to 42°C, and the

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inoculating solution was at 21°C. This was done to simulate a warm egg dropping onto room-temperature feces. For each of the repetitions, 90 eggs were used (30 per treatment: water, hydrogen peroxide, or phenol). The inoculum level was either 10^3 (low) or 10^5 (high)/mL of dip. After allowing the eggs to dry (4 h), they were then immersed for 1 min in water (control), 1.4% hydrogen peroxide [2], or 0.39% phenol [2] once, twice, or three times. For samples treated with multiple immersions, 5 min were allowed between successive dips. Recovery procedures for the marker *Salmonella* from all treated and control eggs were previously described by Berrang et al. [3]. This method consists of breaking the eggshell and discarding the contents and then gently hand crushing the shell and adhering membranes in 50 mL of buffered peptone (BP) in a sterile plastic bag. The bag and contents were then incubated for 24 h at 37°C. After this period of incubation, a sterile cotton swab was immersed in the BP broth and used to apply the solution onto the surface of BG sulfa agar [4] plates with an addition of 200 ppm nalidixic acid (to prevent growth of nonresistant salmonellae that might have been present naturally). Characteristic *Salmonella* colony-forming units appearing on the plate were confirmed to be our maker *S. typhimurium*, and the data were recorded as number of *Salmonella*-positive eggs per number of eggs treated. The entire experiment was replicated. Data were analyzed using chi-squared procedures [5].

RESULTS AND DISCUSSION

The data obtained when fertile hatching eggs were artificially inoculated with *S. typhimurium* (approximately 10^3), then immersed one, two, or three times (1 min each) in water, hydrogen peroxide, or phenol are shown in Table 1. After three consecutive 1-min immersions, 14/20 (70%) of the eggs treated with water (control) remained contaminated with *Salmonella*, whereas significantly ($P < 0.05$) fewer of the eggs treated with hydrogen peroxide (7/20, 35%) or phenol (7/20, 35%) remained *Salmonella* positive. Compared to a single 1-min immersion treatment, three consecutive immersions resulted in significantly ($P < 0.05$) fewer eggs being positive for *Salmonella*. When considering results from eggs treated with hydrogen peroxide or phenol, multiple applications were significantly ($P < 0.05$) more effective than a single application. A second 1-min chemical immersion eliminated *Salmonella* from an additional 10% of the eggs and a third 1-min dip eliminated *Salmonella* from an additional 22% of the eggs.

Data obtained when fertile hatching eggs were inoculated with a higher level of *Salmonella* (approximately 10^5), then immersed one, two, or three times (1 min each) in water, hydrogen peroxide, or phenol are shown in Table 2. After 3 consecutive 1-min immersions, 100% of the eggs treated with water (control) remained contaminated with *Salmonella*, whereas 60% of the eggs treated with hydrogen peroxide remained *Salmonella* positive, and 85% of the phenol-treated eggs were still contaminated with *Salmonella*. With a higher concentration in the inoculum, a second 1-min dip using hydrogen peroxide or phenol did not eliminate *Salmonella* from any more eggs than the first 1-min dip. When both sanitizers are considered together, the third consecutive immersion eliminated *Salmonella* from an additional 17.5% of the fertile eggs, a significant ($P < 0.05$) improvement over one or two dips.

Research has shown that salmonellae can rapidly penetrate the freshly laid fertile hatching egg [6]. Once microorganisms such as salmonellae get past the membranes of hatching eggs, there is no effective way to remove them, kill them, or prevent their further invasion of the egg contents and developing embryos, without endangering the developing embryo [7]. After incubation, the embryo can ingest salmonellae trapped in the membranes as it emerges from the egg [8]. In addition, during the process of hatching, one contaminated chick can spread salmonellae to most of the other chicks in the hatching cabinet due to the fan-driven air movement [8]. Low levels of *Salmonella* from breeder flocks and hatcheries can enter a young chick through an assortment of body openings and colonize the ceca, a process that then allows the salmonellae contamination to spread through the grow-out house via these seeder birds [9].

The abundance of salmonellae in commercial hatcheries is evidence that the presently used chemicals and methods of applying them are not killing all of the salmonellae or preventing the horizontal transmission of salmonellae through

TABLE 1. Number of *Salmonella*-positive hatching eggs following one, two, or three 1-min immersions in water, hydrogen peroxide, or phenol when eggs were artificially inoculated with a low number of cells

Immersions (n)	Immersion fluid						Total
	Replication 1[A]			Replication 2[B]			
	Water	H_2O_2	Phenol	Water	H_2O_2	Phenol	
1	9/10[C]	8/10	8/10	9/10	6/10	1/10	41/60[a]
2	8/10	5/10	6/10	6/10	3/10	5/10	33/60[ab]
3	9/10	5/10	5/10	5/10	2/10	2/10	28/60[b]
Total	26/30[a]	18/30[b]	19/30[b]	20/30[a]	11/30[b]	8/30[b]	

[a,b]Within sets of totals, values without like superscripts are significantly different ($P < 0.05$) by chi-squared test for independence.
[A]Inoculum for Replication 1 was 1.6×10^3/egg.
[B]Inoculum for Replication 2 was 4.0×10^3/egg.
[C]Number of *Salmonella*-positive eggs per number of eggs tested.

the shell and membranes of the egg. Also, in the laboratory, no chemical has been shown to consistently eliminate 100% of *Salmonella* that have penetrated the fertile hatching egg. One of the main reasons is that *Salmonella* can penetrate rapidly and deeply into the egg, and once this occurs, it is difficult to bring a chemical into direct contact with the *Salmonella* organism. These chemicals are only effective if they directly contact the bacterial cell.

In some previous studies, application of chemicals by immersion dips was used [10, 11, 12, 13, 14, 15]. Immersion application of chemicals was shown to be more effective than sprays or foams [11]. Hydrogen peroxide and phenol have been shown to be effective chemical treatments for eliminating or reducing *Salmonella* contamination of fertile hatching eggs [10, 11, 12, 13, 14, 15, 16].

The implications of salmonellae in the hatchery can be far reaching. Data from several field trials have been used to track *Salmonella* serotypes originating from the hatchery to the final processed broiler carcass [17, 18, 19]. Contamination and penetration of the shell of fresh and incubating eggs constitute the single most important link in the transmission of salmonellae from the breeder hen to the newly hatched chick. Salmonellae contamination is a critical control point and as such should be a primary target for any intervention procedure. This study was carried out to optimize treatments previously shown to be effective in an attempt to remove or destroy as many bacteria (such as salmonellae) as possible on the surface of a fertile hatching egg.

Although multiple-immersion application of sanitizers is effective at lowering *Salmonella*

TABLE 2. Number of *Salmonella*-positive hatching eggs following one, two, or three 1-min immersions in water, hydrogen peroxide, or phenol when eggs were artificially inoculated with a high number of cells

Immersions (n)	Immersion fluid						Total
	Replication 1[A]			Replication 2[B]			
	Water	H_2O_2	Phenol	Water	H_2O_2	Phenol	
1	10/10[C]	10/10	10/10	10/10	7/10	9/10	56/60[a]
2	10/10	10/10	10/10	10/10	7/10	9/10	56/60[a]
3	10/10	10/10	8/10	10/10	2/10	9/10	49/60[b]
Total	30/30[a]	30/30[a]	28/30[a]	30/30[a]	16/30[b]	27/30[a]	

[a,b]Within sets of totals, values without like superscripts are significantly different ($P < 0.05$) by chi-squared test for independence.
[A]Inoculum for Replication 1 was 2.6×10^5/egg.
[B]Inoculum for Replication 2 was 2.2×10^5/egg.
[C]Number of *Salmonella*-positive eggs per number of eggs tested.

levels on eggs, this approach may be impractical on the farm. Any successful immersion treatment requires careful quality control to maintain the dip at a temperature warmer than the egg and to minimize organic material [20]. The multiple immersion would be best used in a centralized location such a hatchery with careful company supervision, which, however, would move the chemical treatment away from the point of inoculation. Therefore, at this time an on-farm spray followed by spraying in the hatching cabinet may be our best solution to the problem of *Salmonella* contamination of eggs.

CONCLUSIONS AND APPLICATIONS

1. With a low inoculum, *Salmonella* was completely eliminated from an additional 10% of eggs with a second 1-min immersion and from an additional 22% with a third dip in the same chemical.
2. With a high inoculum *Salmonella*, a second dip showed no difference from one dip, whereas a third immersion in the same chemical eliminated *Salmonella* from an additional 17.5% of the eggs.
3. Performances of phenol and hydrogen peroxide were similar with the low inoculum, but neither was effective with the high inoculum.

REFERENCES AND NOTES

1. Cox, N. A., M. E. Berrang, and J. S. Bailey. 1998. Bactericidal treatment of hatching eggs I. Chemical immersion treatments and *Salmonella*. J. Appl. Poult. Res. 7:347–350.

2. Sigma, St. Louis, MO.

3. Berrang, M. E., N. A. Cox, J. S. Bailey, and L. C. Blankenship. 1991. Methods for recovery of *Salmonella* from chicken eggs. Poult. Sci. 70:2267–2270.

4. Difco Laboratories, Division of Becton-Dickinson and Company, Sparks, MD.

5. Incidences of *Salmonella* positive samples were compared by chi-squared test for independence. Significance was assigned at $P < 0.05$.

6. Williams, J. E., and L. H. Dillard. 1968. *Salmonella* penetration of fertile and infertile chicken eggs at progressive stages of inoculation. Avian Dis. 12:629–635.

7. Cason, J. A., N. A. Cox, and J. S. Bailey. 1994. Transmission of *Salmonella typhimurium* during hatching of broiler chicks. Avian Dis. 38:583–588.

8. Bailey, J. S., R. J. Buhr, N. A. Cox, and M. E. Berrang. 1996. Effect of hatching cabinet sanitation treatments on *Salmonella* cross contamination and hatchability of broiler eggs. Poult. Sci. 75:191–196.

9. Cox, N. A., J. S. Bailey, and M. E. Berrang. 1996. Alternative routes for *Salmonella* intestinal tract colonization of chicks. J. Appl. Poult. Res. 5:282–288.

10. Cox, N. A., and J. S. Bailey. 1991. Efficacy of various chemical treatments over time to eliminate *Salmonella* on hatching eggs. Poult. Sci. 70(Suppl. 1):31. (Abstr.)

11. Cox, N. A., and J. S. Bailey. 1991b. Effect of chemical treatments to eliminate *Salmonella* on hatching eggs. Poult. Sci. 70(Suppl. 1):154. (Abstr.)

12. Cox, N. A., and J. S. Bailey. 1992. Chemical treatment of fertile hatching eggs to control salmonellae at the breeder flock and hatchery level. Poult. Sci. 71(Suppl. 1):7. (Abstr.)

13. Cox, N. A., J. S. Bailey, M. E. Berrang, and R. J. Buhr. 1995. Use of vacuum with chemical disinfectants to eliminate inoculated Salmonella from hatching eggs. Poult. Sci. 74(Suppl. 1):49. (Abstr.)

14. Padron, M. 1995. Egg dipping in hydrogen peroxide solution to eliminate *Salmonella typhimurium* from eggshell membranes. Avian Dis. 39:627–630.

15. Shane, S. M., and A. Faust. 1996. Evaluations of sanitizers for hatching eggs. J. Appl. Poult. Res. 5:134–138.

16. Sheldon, B. W., and J. Brake. 1991. Hydrogen peroxide as an alternative hatching egg disinfectant. Poult. Sci. 70:1092–1098.

17. Bhatia, T. R. S., and G. D. McNabb. 1980. Dissemination of *Salmonella* in broiler-chicken operations. Avian Dis. 24:616–624.

18. Goren, E. W., A. DeJong, P. Doormenbal, J. P. Koopman, and H. M. Kennis. 1988. Reduction of *Salmonella* infection of broilers by spray application of intestinal microflora: A longitudinal study. Vet. Q. 10:249–255.

19. Blankenship, L. C., J. S. Bailey, N. A. Cox, N. J. Stern, R. Brewer, and O. Williams. 1993. Two step mucosal competitive exclusion flora treatment to diminish salmonellae in commercial broiler chickens. Poult. Sci. 72:1667–1672.

20. Bailey, J. S., N. A. Cox, and M. E. Berrang. 2000. Bactericidal treatment of hatching eggs III. Effect of organic contaminants on efficacy of egg sanitizers. J. Appl. Poult. Res. 10:117–120.

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