MICROBIOLOGY OF SANITIZED BROILER HATCHING EGGS THROUGH THE EGG PRODUCTION PERIOD

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Primary Audience: Breeder Managers, Hatchery Managers, Researchers

SUMMARY

Nest-clean and dirty eggs were sampled monthly across the productive period of a commercial broiler breeder flock. Eggshells and membranes were examined for total aerobic bacterial and Enterobacteriaceae counts per egg. Paired nest-clean and dirty eggs were spray sanitized in a two-stage commercial egg sanitizing machine (a chlorine detergent wash followed by a quaternary ammonia sanitizing spray) and tested for bacterial contamination. As the flock aged, numbers of bacteria per nest-clean egg fluctuated without a noticeable trend (from $\log_{10}$ 4.1 to 5.3 aerobic bacteria). Bacterial populations were significantly lower on sanitized eggs ($\log_{10}$ 0.8 to 3.2 cells total aerobic bacteria and 2 to 5 cells Enterobacteriaceae per egg) regardless of hen age. Those eggs classified as dirty had visible fecal contamination and higher bacterial numbers than nest-clean eggs ($\log_{10}$ 5.9 to 7.6 cells total aerobic bacteria per egg). After sanitization, previously dirty eggs had bacterial populations comparable to those of sanitized nest-clean eggs. When eggs were examined in the hatchery at transfer, sanitized dirty eggs were still microbiologically indistinguishable from sanitized nest-clean eggs, though both groups had higher bacterial contamination levels than had been observed in samples taken immediately following sanitization.

Key words: Breeder flock, egg sanitization, hatching eggs

1997 J. Appl. Poultry Res. 6:298–305

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Description of Problem

Broiler hatching eggs that are classified by the producer as dirty due to adhering fecal or litter material are not sent to the hatchery. The producer and the integrated poultry company lose the revenue associated with potential chicks from these eggs. Although wetting or washing hatching eggs has been thought to lower the percentage hatch, research over the years has shown that proper wet egg sanitization does not adversely affect hatchability [1, 2, 3]. Washing dirty eggs with a spray sanitizing machine and sending the cleaned eggs to the hatchery can provide an appreciable increase in economic gain from a breeder flock, providing the eggs do not harbor undetected microbial contamination [4]. The economic benefit of sanitizing dirty eggs is especially noticeable if the flock is laying a high number of eggs on the floor.

Salmonellae and other bacteria can rapidly penetrate the shell of a freshly laid egg [5, 6]. An egg contaminated at the breeder farm can then cause contamination at the hatchery, creating a reservoir of salmonellae [7]. Researchers have tracked such hatchery contamination with salmonellae to the broiler grow-out farm [8] and then to the processing plant, where final product may be positive for the same serotype of *Salmonella* originally detected in the hatchery [9]. Any integrated poultry sanitation program should take into account the microbial load entering the hatchery on and within eggs. Automated spray sanitizing machines can be used to significantly reduce the microbial populations on nest-clean hatching eggs [10, 11]. The objectives of this study included examining the microbial populations on hatching eggs classified as dirty and clean through the productive life of a commercial broiler breeder flock. We also examined the microbiological consequences of on-farm sanitization of nest-clean and dirty eggs, both at the farm and in the hatchery.

Materials and Methods

All egg samples were taken from a commercial broiler breeder house in northeast Georgia stocked with Hubbard hens and Peterson roosters. The house was outfitted with automatic nests on 2/3 slatted raised floor. With previous flocks the producer had noted a high percentage of eggs being laid on the floor. Floor eggs in this house were estimated to represent about 10% of the total eggs produced.

In the study covering the entire productive life of the hens, eggs gathered at the midday collection were examined rather than those from initial or final collections. By using eggs from just the midday collection (11 a.m.) we eliminate the first or last eggs in a sequence (normally laid early in the morning or late in the afternoon respectively), thus focusing on the more uniform eggs found in the middle of a hen's egg sequence [12]. Further study included a separate group of experiments to test for differences in the microbiological profile of eggs from each collection time (morning, midday, and afternoon).

All eggs collected on the farm were separated by farm workers into groups classified as nest-clean, dirty, or cull (cracked, misshapen, etc.). Eggs with visible fecal, litter, or egg contamination on the surface were placed in the dirty group. All non-cull eggs collected on the farm were placed in plastic flats and subjected to automated sanitization. The sanitizing machine was a two-stage pressure spray machine (Surepip Model A-94, Agro Environmental, Dallas, GA 30132). The first tank contained a filtered, recycled chlorine-based (530 ppm) wash solution at 48°C (HI-KLOR @ 300 g/15 gal; BioSentry, Stone Mountain, GA 30083-9986). The second tank included a quaternary ammonia sanitizer (200 ppm) at room temperature (BIO-Quat 20 @ 18.5 mL/5 gal; BioSentry, Stone Mountain, GA 30083-9986). Full egg flats were placed on the conveyor line moving through the spray sanitizing machine. Nest-clean eggs were washed at a relatively high conveyor speed of 7 cm/sec (each egg passes through the machine in about 9 sec). Dirty eggs were first sent through the machine with only the first tank (chlorine-based wash solution) spraying at a slower conveyor speed of 3 cm/sec. The eggs were then examined and any remaining filth was manually rubbed, which allowed it to be cleaned off during the second pass though the egg sanitizer. The third and final passage through the machine for dirty eggs included both wash and sanitizer solutions and was done at the faster conveyor speed. At week 48 the chlorine level used in the wash tank was lowered to approximately

88 ppm (DBC-A @ 142 g/15 gal; BioSentry, Stone Mountain, GA 30083-9986) in order to test the performance of a more economical level of wash solution in an on-farm spray egg washer.

Eggs for microbiological monitoring were collected throughout the productive life of the flock. Samples were collected on weeks 29, 34, 35, 39, 42, 48, 52, 56, 58, and 59 of hen age. On each sample day, 10 nest-clean eggs and 10 dirty eggs were removed before washing. Two other sets of 10 eggs each were retrieved after washing, for a total of 40 eggs. When testing the effect of egg collection time, eggs were sampled at about 8 a.m., 11 a.m., and 3 p.m. for a total of 120 eggs per sample day. The collection time experiment was replicated four times, on weeks 52, 56, 58, and 59 of the production period. In all experiments, sample eggs were placed in separate new cardboard egg flats and transported to the laboratory within 1 hr.

HATCHERY SAMPLING

On some sampling days, nest-clean non-sanitized, nest-clean sanitized, and dirty sanitized eggs were marked and tracked through the commercial transportation and hatchery process. These eggs were then sampled after incubation (14 to 18 days) before transfer to the hatching cabinet. For these experiments 10 eggs from each of the three groups were removed from the setter and brought to the laboratory. The microbial populations on eggs removed from the setter were then compared to those of corresponding eggs on the farm. This procedure allowed a comparison of non-sanitized eggs on the farm, sanitized eggs on the farm, and eggs that had been in the hatchery for 14 to 18 days of incubation. This tracking was performed four times using eggs collected at weeks 48, 56, 58, and 59 of the flock life.

LABORATORY METHODS AND MICROBIAL ANALYSIS

Eggs were sampled by the method of Berrang *et al.* [13]. Briefly, eggshells were aseptically broken, the contents discarded, and the shell and membrane complex placed in 50 mL of Universal Preenrichment Broth (UP) [14] (Difco, Detroit, MI 48232). The shell and membranes were then subjected to a hand crush and thorough shaking in UP prior to removal of sample for serial dilution. Diluted samples were plated in duplicate on Plate Count Agar (PCA) (Difco, Detroit, MI 48232) for total aerobic bacterial counts and on Violet Red Bile Agar (VRBG) (Difco, Detroit, MI 48232) with the addition of 1% glucose (Sigma, St. Louis, MO 63178) to enumerate total Enterobacteriaceae. Following drying, VRBG plates were overlaid with a small amount of VRBG. All plates were incubated at 35°C for 24 hr prior to counting the resultant colonies. On PCA all colonies were counted; on VRBG only red or pink colonies were counted as Enterobacteriaceae.

STATISTICAL ANALYSIS

Individual means were compared using the t-test. The three means compared for collection time experiments (8 a.m., 11 a.m., and 3 p.m.) were examined together by one-way analysis of variance. Groups of eggs examined at the farm and at the hatchery were compared with one-way analysis of variance and Tukey's test analysis. All statistical analyses were conducted using Statmost statistical analysis package [15].

RESULTS AND DISCUSSION

Before a breeder flock is placed, the house is cleaned and sanitized, and fresh litter is put down. As a breeder flock ages, the house becomes noticeably dirtier; some authors feel this should lead to higher levels of bacteria on eggs [16]. To develop this hypothesis, reference is made to experiments in which eggs laid in an area with high bacterial populations (such as the house floor) are more likely to become contaminated than eggs laid elsewhere [17, 18]. However, we found no increase in bacterial contamination of eggs during the production period. Figures 1 and 2 show microbiological data collected throughout the life of the flock. No significant increase or noticeable trend in the total aerobic bacterial or Enterobacteriaceae populations on eggs was detected as the flock aged. The use of automated nests that limit the surfaces that an egg contacts following oviposition may help to maintain consistent bacterial levels on eggs throughout the productive life of a flock. Also, maintaining shell quality may be important to the prevention of increased contamination with flock age.

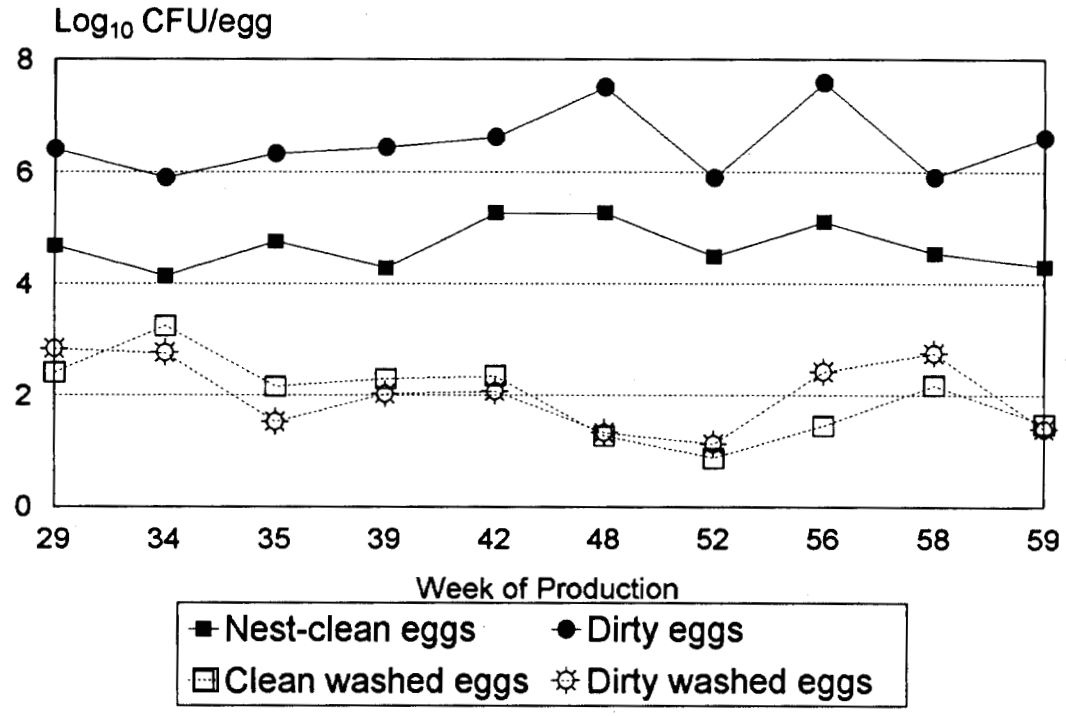

FIGURE 1. Total aerobic bacterial populations on hatching eggs through the production period

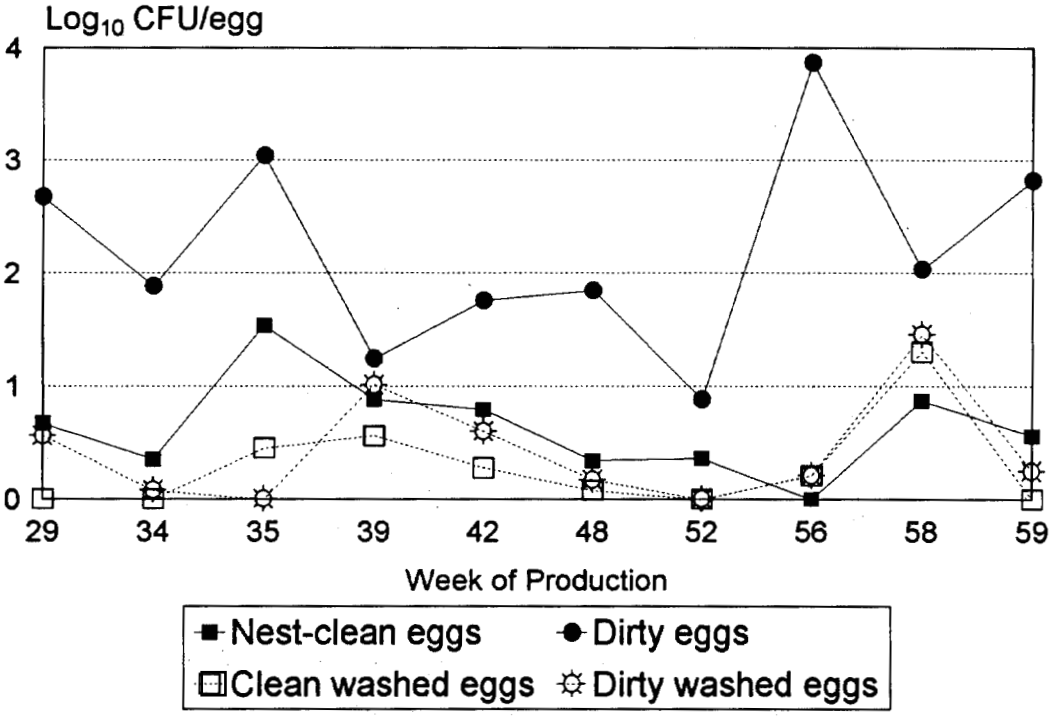

FIGURE 2. Enterobacteriaceae populations on hatching eggs through the production period

Research to address this point is now being performed.

Eggs classified as dirty by the producer had significantly higher populations of total aerobic bacteria and Enterobacteriaceae than eggs classified as clean ($P \leq .05$). However, after sanitizing there was no significant difference in levels of bacteria between sanitized dirty and sanitized clean eggs (Figures 1 and 2). Using, the measures taken in this study sanitized dirty eggs were microbiologically indistinguishable from sanitized clean eggs. Furthermore, we found that lowering the level of chlorine (from 530 to 88 ppm) in the first stage wash solution did not lead to higher microbial populations recovered from eggs. Apparently the first wash primaraly removes debris, so the detergent component is more important than the chlorine level. When followed by a quaternary ammonia sanitizer the level of chlorine in the egg wash solution can be varied widely without affecting the microbial reduction.

Generally, eggs are collected in a breeder house three times daily. Eggs collected in the morning include those that were laid late in the afternoon the previous day and have consequently been in the house all night. Eggs collected at midday or afternoon would have been in the house for a maximum of 3 to 4 hr. The longer time that a morning egg spends in the nest or on the floor could lead to higher levels of bacterial contamination. However, we found no significant difference in total aerobic microbial populations on eggs collected in the morning compared to the other collection times (Figure 3). Furthermore, although Enterobacteriaceae tend to be highest on dirty eggs collected in the morning (Figure 4), this difference is not significant at the $P \leq .05$ level. Apparently in a house with automated nests, the time of day eggs are collected does not affect the number of aerobic microorganisms present on the egg.

Sanitized eggs have lower numbers of bacteria than non-sanitized eggs when tested at the farm (Figures 1–4). However, after 14 to 18 days of commercial incubation, sanitized eggs had undergone a significant ($P \leq .05$) increase in numbers of bacteria from that noted in on-farm sampling (Figure 5). In fact, after incubation, the bacterial populations of

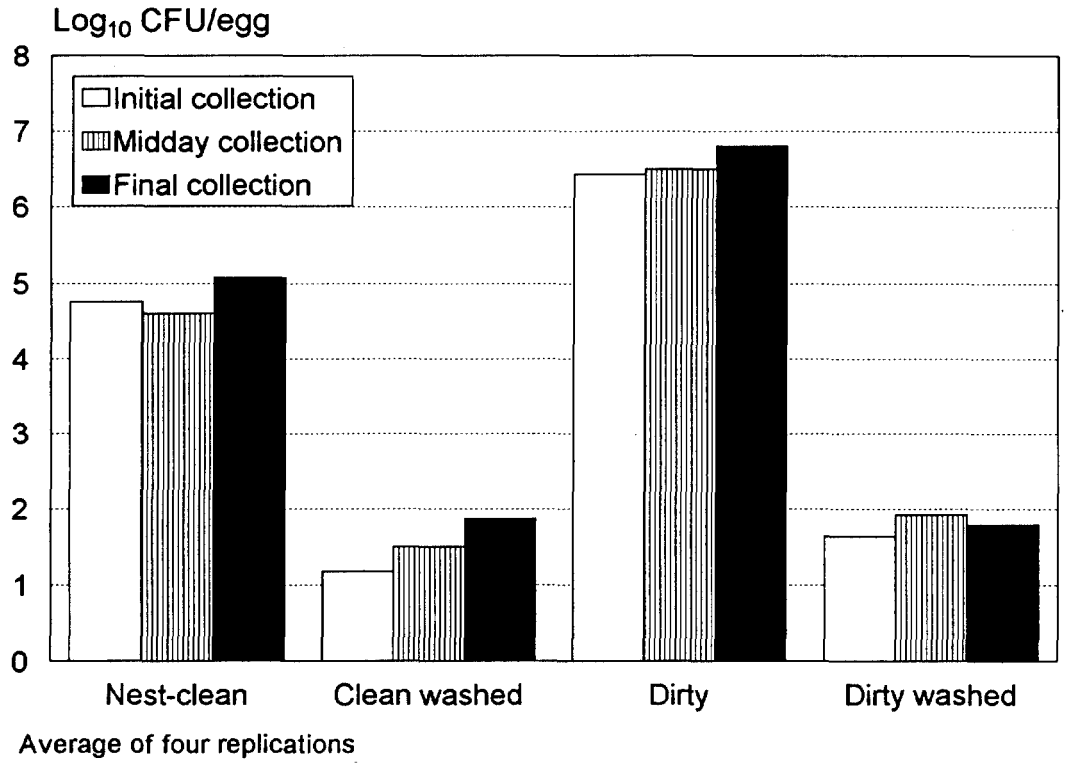

FIGURE 3. Effect of collection time on total aerobic bacterial populations on hatching eggs

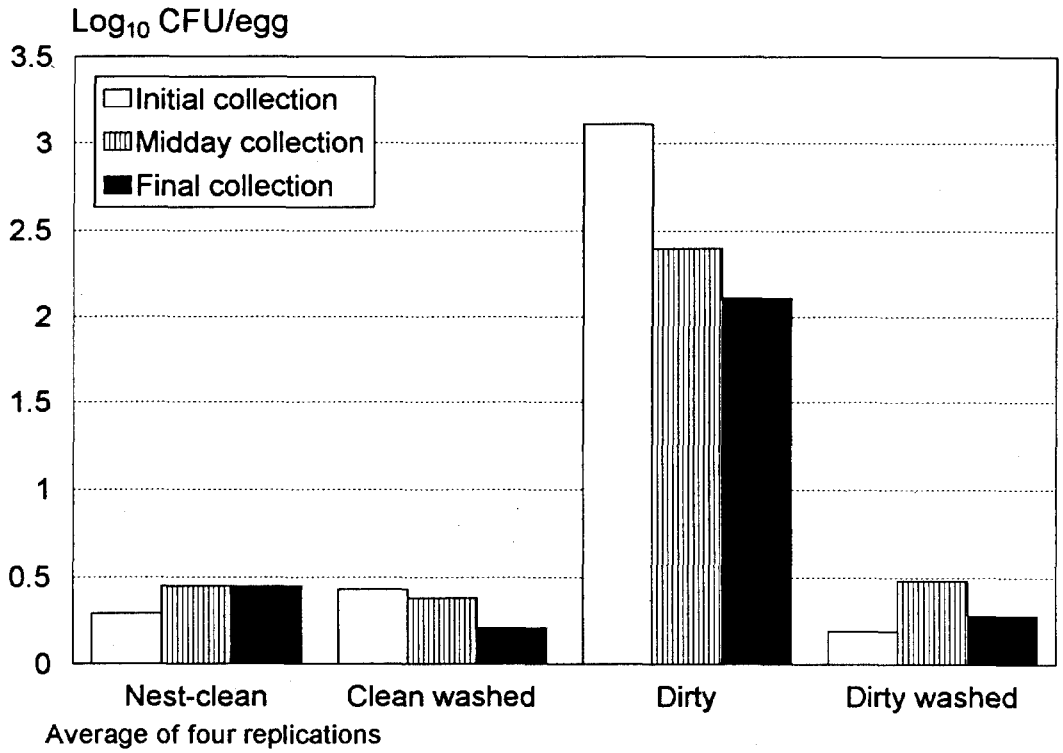

FIGURE 4. Effect of collection time on Enterobacteriaceae populations on hatching eggs

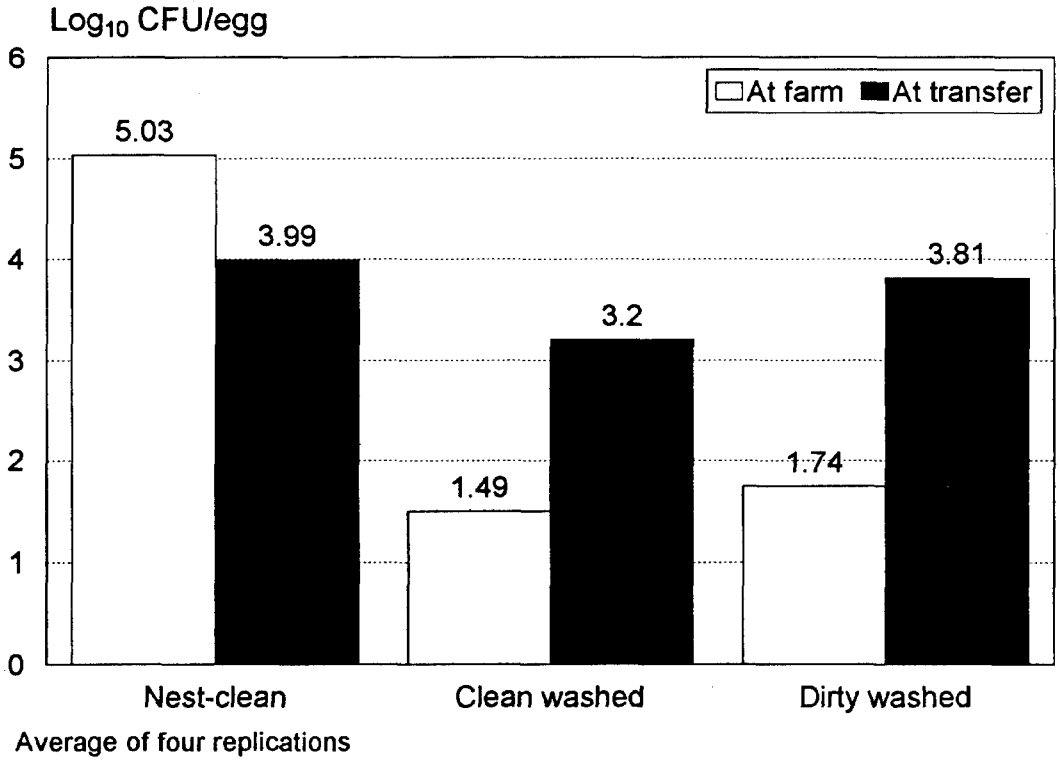

FIGURE 5. Total aerobic bacterial populations on hatching eggs examined at the farm and after incubation

sanitized eggs did not significantly differ from those of non-sanitized eggs. Since most of the eggs in the incubator were from five other farms and not washed, the circulating air may have caused cross-contamination of the washed eggs. On two occasions eggs were examined after transport to the hatchery (prior to set) and again prior to transfer. These results (data not shown) indicate that the transport process itself may lead to recontamination of the eggs before set. This recontamination may result from environmental conditions when eggs are removed from the farm cold room and placed in the hatchery truck. Any step that causes condensation on the egg surface ('sweating') is a potential point of recontamination [19]. Eggs are probably recontaminated at several steps in the process between sanitization and hatch. At any rate, recontamination or growth of surviving bacteria seems to have effectively negated the reduction in numbers that earlier egg sanitization achieved. This observation shows the importance of careful hatchery sanitation, including the possibility of misting antimicrobial agents in the incubator or hatching cabinet [20]. Future research efforts will include large-scale egg sanitizing experiments that will produce a commercial incubator full of cleaned eggs, thus reducing the likelihood of cross-contamination in the cabinet. Further examination of the transport and storage system – from farm cold room to hatchery egg room, set, and transfer – is planned to identify possible recontamination points.

Conclusions and Applications

1. Hatching eggs (nest-clean or dirty) do not become contaminated with higher numbers of aerobic bacteria as the flock ages.
2. Time of day that eggs are collected does not significantly affect the total aerobic bacterial or Enterobacteriaceae populations on or in eggs.
3. Eggs can be effectively sanitized on the farm. Through sanitizing, eggs classified as dirty can be made microbiologically indistinguishable from those classified as clean.
4. In a two-stage spray egg sanitizer, the first chemical primarily to cleans off visible filth. Thus the first tank should be filled with a detergent or surfactant, not necessarily a sanitizer.
5. Egg sanitization on the farm may be an important control point for hatchery sanitation.
6. Sanitized eggs must be handled carefully to prevent recontamination.

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