

# An Assessment of the Microbiological Risks Involved with Egg Washing under Commercial Conditions

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## ABSTRACT

The potential benefits of washing eggs is offset by a historical perception in the European Union that wetted eggs are prone to spoilage and water loss. This study describes the effects of spray jet washing under various processing conditions to shell surface counts of *Salmonella* and the presence of bacteria in egg contents. Experiments used eggs that were contaminated with *Salmonella* Enteritidis PT4 or *Salmonella* Typhimurium DT104 before cuticle hardening. Washing of contaminated eggs under optimum conditions resulted in a more than 5-log reduction of *Salmonella* counts from the shell surface. *Salmonella* was not isolated from the yolk or albumen of any egg washed by the optimal protocol, suggesting that when properly controlled, egg washing did not cause *Salmonella* to enter the contents. However, contamination did arise if strict control was not maintained over the wash and rinse water temperatures. Both *Salmonella* Enteritidis and *Salmonella* Typhimurium were shown to enter the egg contents when water temperatures were lowered, indicating that strict temperature control must be maintained in order to prevent the ingress of *Salmonella* into egg contents. Other washing machine parameters that were investigated did not significantly affect *Salmonella* entry into the egg contents but influenced shell surface kill levels to varying degrees.

The microbial quality of table eggs has been of concern to British consumers since eggborne *Salmonella* Enteritidis emerged as a major cause of food poisoning (10). Currently, the washing of class A table eggs to remove fecal material and glaze (yolk and albumen) is not allowed in the European Union. The reasons underlying this ban are largely historical and are linked to reports of increased rates of spoilage for eggs that were washed under less than optimum conditions (2). More recently, a number of studies have shown that some washing chemicals and sanitizers can cause physical damage to the egg surface by etching the shell cuticle (3, 16, 24). Although there has been consensus in reports that washing with inappropriate chemicals can allow potentially pathogenic bacteria to gain entry into the egg (16, 24), the effects of low-temperature washing are less clear. One study has reported that the practice did not appear to increase internal bacterial counts (19), whereas a number of historical publications indicated the opposite (1, 5, 18).

Despite these potential pitfalls, a number of countries, such as the United States, Australia, and Japan, have embraced egg washing. In these countries, it has become a routine and established practice, is regarded as safe, and is perceived by consumers as an essential part of the hygienic production of eggs. Although in the European Union egg washing is legally permitted for class B eggs intended for processing, no guidance has been issued by the European Commission to describe how it can be undertaken safely.

The purpose of this study was to practically assess the microbial implications of using a set of best practice washing guidelines conforming to the criteria previously listed as important (11). These best practice washing instructions took account of machine manufacturers' recommended optimum conditions, the results of previously published peer-reviewed egg washing studies, and egg washing guidance issued by the U.S. Department of Agriculture. Although a number of workers have previously reported reductions in shell bacterial levels caused by egg washing, this study expands these earlier findings. Specifically, we have determined the microbial implications of washing eggs that had been surface contaminated after laying and before oxidation of the shell cuticle proteins had occurred. Thus, the eggs were contaminated in a manner that mimicked the natural contamination process. In addition to measuring reductions in shell levels of bacteria, presence-absence testing for *Salmonella* was undertaken in the egg contents (i.e., the albumen and yolk) under a range of washing parameters. Deviations from ideal conditions were selected as those most likely to occur as a result of washing equipment failure. Furthermore, because the age of the laying bird has an influence on shell physiology and thickness (11), studies were undertaken over a complete laying cycle of 9 months. The results of this study allow an understanding of the microbiological implications for food safety when eggs contaminated externally with *Salmonella* are washed under the best practice discussed previously by us (11). These results provide the basis for European enforcement authorities to issue scientifically backed guidance as to how best to undertake egg washing.

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## MATERIALS AND METHODS

**Strains.** *Salmonella* Enteritidis PT4 strain 2C was supplied by the Public Health Laboratories Service, Exeter, UK, and was isolated originally from the contents of an uncooked chicken egg. The strain had been characterized as heat tolerant to 52°C and acid tolerant to pH 2.8 and also showed resistance to drying by air at 20°C (15). *Salmonella* Typhimurium DT104 strain 21008057 was a chicken strain isolated from routine analyses undertaken by the DLS laboratory.

**Experimental laying hens.** Experimental birds (110) used for the study were ISA Browns. They were delivered from the breeder (Hubbard ISA, Peterborough, UK) at 18 weeks of age and housed in standard battery laying cages (Patchett Engineering, Bradford, UK). The standard unit consisted of four back-to-back cages stacked three high. Cages were populated with either four or five birds. Birds were dual-vaccinated during the rearing period against *Salmonella* Typhimurium and *Salmonella* Enteritidis by injection with Salenvac (Intervet, Milton Keynes, UK) according to the manufacturer's instructions. After housing, artificial day length and light-on time were adjusted over several weeks by a maximum of 5 min each day to synchronize the egg laying time of all birds to within 2 to 3 h. In order to match as closely as possible standard UK production practices, after lay, eggs were held at between 8 and 20°C.

**Inoculation of belts with *Salmonella*.** To mimic as closely as possible a naturally inoculated egg, it was necessary to devise a method to infect the external shell surface before oxidation of the protein cuticle. Because oxidation and hardening occurs 2 to 3 min after laying, the chosen inoculation method was to paint the polypropylene belts (Edstrom Industries, Waterford, Wis.) that collected the freshly laid eggs with a mixture of fresh chicken manure and cultured *Salmonella*. *Salmonella* was cultured in a mixture of 25% fresh autoclaved chicken manure and 75% half-strength buffered peptone water (Oxoid CM509) supplemented with 1% (wt/vol) ammonium chloride and 0.9% (vol/vol) Tween 80. Cultures were grown into stationary phase at 37°C and painted onto the belts at the volume of 0.1 ml/cm<sup>2</sup>. With this inoculation procedure, levels of *Salmonella* did not fall significantly over the 2- to 3-h laying period, even though surface drying of the material was observed.

**Wash chemicals.** Wash chemicals added to the waters used were Chlorwash (a chlorine-based detergent washing agent [MS-Technologies, Kettering]) or the rinse sanitizer Quat 800 (a mixture of quaternary ammonium and nonionic surfactants [MS-Technologies, Kettering]). Chlorwash was added to the prewash and wash water tanks and Quat 800 to the rinse tanks. The two chemicals are not compatible for use together; thus, their effects on bacterial numbers were determined separately.

**Egg production and analyses.** The total number of eggs generated daily by the experimental flock varied between 80 and 100. Experiments ran for either 4 or 5 days each week. For each day that an experiment was undertaken, a minimum of 12 eggs were removed for control purposes. On the remainder of the days, batches were washed and analyzed for *Salmonella* as detailed below. Different sampling methods were used for shell surfaces and contents. Because methods were destructive, it was not possible to analyze a single egg for both surface and contents. Thus, post-wash eggs were divided evenly and randomly for analysis of either contents or surfaces.

**Culture of *Salmonella* from egg contents.** *Salmonella* was detected from egg contents with media combinations previously

found optimal (6). To release egg contents, a modification of the method described by Himathongkham et al. (9) was used. The blunt end of the eggs were dipped into 100% ethanol. The alcohol was then burned off. The end of the egg was broken by firm tapping onto a bench covered with an alcohol-soaked wipe. A pair of sterile forceps were used to gently remove loose pieces of shell from the internal membranes. Membranes were sprayed lightly with 70% ethanol before being completely torn off. Egg contents (25 ml) were pipetted into a sterile stomacher bag and homogenized, and 25 ml of homogenate was mixed with 225 ml of buffered peptone water and cysteine. Primary incubation was 37°C for 20 to 24 h, after which 0.5 ml of the buffered peptone water and cysteine culture was transferred into 10 ml tetrathionate broth and incubated further at 42°C for 20 ± 2 h. Sterile 10- $\mu$ l loops were used to inoculate brilliant green agar and xylose lysine deoxycholate agar plates. Presumptive salmonellae were confirmed by API 20E biochemical testing and randomly amplified polymorphic DNA fingerprinting.

**Enumeration of *Salmonella* from egg surfaces.** For shell surface enumeration, eggs were placed in stomacher bags containing 50 ml of buffered peptone water and cysteine. The entire shell surface was moistened with media before sonication of the egg in an ultrasonic bath (Grant Instruments Ltd., Royston, UK, model XB22) for 30 s. Aliquots (1 ml) were removed, and decimal dilutions were made in buffered peptone water and cysteine (*Salmonella*). Total aerobic counts were undertaken as described by ISO 4833:1991 (12). For enumeration of *Salmonella*, dilution aliquots (1 ml) were plated onto brilliant green agar plates and incubated for 48 h at 37°C. Conversion of diluted counts into bacterial numbers was as described by ISO 6887-1:1999 (13). Up to five presumptive *Salmonella* colonies were confirmed by API 20E biochemical testing and randomly amplified polymorphic DNA fingerprinting.

**Total aerobic viable counts from egg surfaces and contents.** Total aerobic viable counts were undertaken as described by ISO 4833:1991 (12). Briefly, aliquots of peptone salt water (10 g protease peptone, 5 g NaCl, 9 g Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O, 1.5 g KH<sub>2</sub>PO<sub>4</sub> to 1,000 ml) was added to samples that were sonicated (shell surfaces) or homogenized (contents) as described above. Samples were then diluted decimally, and 1-ml aliquots were added to appropriately labeled petri dishes. An aliquot (15 ml) of tempered (46°C) plate count agar (Oxoid CM325) was added to each petri dish, mixed, and allowed to harden. Plates were incubated at 30°C for 72 h before colonies were counted. Bacterial numbers on decimally diluted plates were converted into CFU/cm according to the criteria described by ISO 6887-1:1999 (13).

**Best practice washing conditions.** A standardized set of optimal wash parameters was used for washing the eggs. These conditions were recommended by the equipment manufacturer and fell within the ranges discussed previously as best practice (11). The conditions used were: prewash water temperature 44°C, wash water temperature 44°C, rinse water temperature 48°C, Chlorwash wash agent concentration at 3 g/liter (or alternatively, Quat 800 rinse water agent at 2.5 ml/liter), prewash head pressure 138 kPa, wash head pressure 262 kPa, rinse head pressure 262 kPa, belt speed 111 cm/min. Eggs were warm air dried (42°C) for 2 min after washing. The water used was potable and soft and had a low iron content of 1.4 ppb.

**Deviation from best practice parameters.** To assess food safety implications of washing eggs under less than ideal conditions, several of the wash condition parameters were varied. A range of wash and rinse water additive concentrations and tem-

peratures were investigated for both *Salmonella* serovars. The efficiency of the drying process, the length of time the eggs were washed, and water jet pressure also were investigated. The parameter values that were used were those most likely to occur as a result of washing equipment failure.

**Randomly amplified polymorphic DNA fingerprinting of *Salmonella*.** PCR-based fingerprinting of *Salmonella* was performed using the method described originally by Hilton et al. (8) with minor modifications. Briefly, presumptive *Salmonella* isolates were cultured in 5 ml Luria Bertani broth at 37°C for 18 to 24 h.

DNA was crudely isolated by pelleting 1 ml of overnight culture (5 min, 13,000 × *g*), resuspending in 1 ml 100% ethanol, and incubating at ambient temperature for 10 min before final centrifugation as before. The pellet was resuspended in 1 ml of sterile deionized water. A 1:10 dilution of each sample of crudely extracted DNA was used for each PCR reaction. Each PCR tube contained 2 µl sample DNA, 1 µl Sal 1254 primer (7) (100 pmol/ml), 5 µl Mg-free PCR buffer (Promega), 7 µl 25 mM MgCl<sub>2</sub>, 2 µl *Taq* polymerase (Promega), 1.5 µl dNTP (Promega, 200 mM), 1 µl dimethyl sulfoxide, and 32 µl H<sub>2</sub>O. PCR was performed by 1 cycle at 94°C for 4.5 min; 4 cycles at 94°C for 30 s, 20°C for 2 min, 72°C for 2 min; 35 cycles at 94°C for 30 s, 32°C for 1 min, and 72°C for 2 min. A final extension for 5 min at 72°C was allowed before storage of reaction products at 4°C until required. Visualization of PCR products was by agarose gel electrophoresis with a 2% (wt/vol) agarose gel in 0.5× Tris–edetic acid–boric acid containing 0.5 µg/ml ethidium bromide. Gels were run typically for 70 min at 60 V in 0.5× Tris–edetic acid–boric acid buffer.

**Statistical analysis.** Student's *t* tests were carried out by SigmaStat 2.0 (SPSS, Erkarth, Germany).

**Determination of energy released by pressurized water on egg surfaces.** To quantify the force of water on the eggs, an electronic transducer was used to record the force generated by aqueous liquid fired through a hydraulic nozzle (MST 15-F65 2CM). The transducer was mounted on an artificial egg with a minor diameter of 46.5 mm and major diameter of 60 mm. The force read by the transducer over a 10-s interval was automatically logged and stored on a personal computer.

## RESULTS AND DISCUSSION

Initial studies were undertaken to determine the effect of the best practice washing protocol on the total aerobic counts of bacteria on egg shells and in egg contents. The results of these studies are summarized as Figure 1. For egg that had not been contaminated with *Salmonella*, the average total surface count was 9,500 CFU per egg (*n* = 50), which was reduced to 15 CFU per egg (*n* = 50) by the washing process. Although the reduction was significant (*t* test, *P* < 0.0001), there was also a significant increase from 2 CFU per egg (*n* = 50) to 15 CFU per egg (*n* = 50) in total aerobic bacterial numbers isolated from the egg contents (*t* test, *P* < 0.05). Thus, there was a small increase in the levels of bacteria in the egg contents caused by spray washing under ideal conditions. To determine the implications of this result for bacterial pathogens, a system was devised to undertake similar studies with the use of eggs contaminated with high levels of *Salmonella*.

Preliminary growth curve studies (data not shown) re-

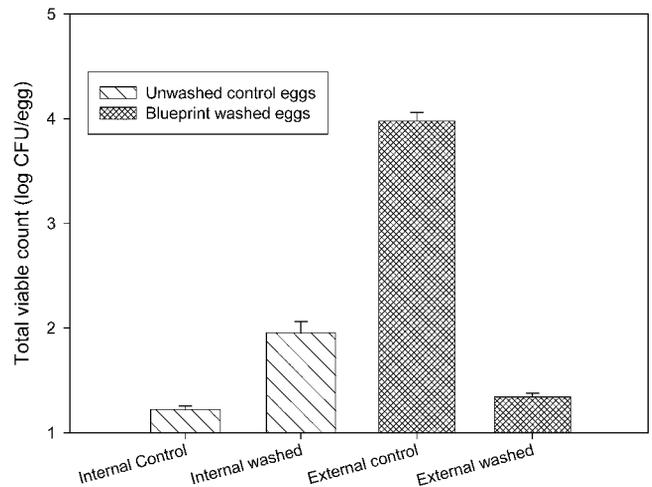


FIGURE 1. Effect of washing on the total aerobic bacterial levels measured on the egg shell and contents. Control eggs were unwashed; washed eggs were cleaned under standardized (blueprint) conditions as described in "Materials and Methods." Error bars for controls and treatments are the standard error of the mean of 50 eggs.

vealed that 2.5 ml of an OD<sub>600</sub> = 0.6 (Eppendorf biophotometer) exponentially growing culture of either *Salmonella* Enteritidis or *Salmonella* Typhimurium was able to proliferate in the media-manure mixtures. Both strains reliably entered stationary phase by 18 h postinoculation, and cell numbers for both salmonellae were typically between 1 × 10<sup>8</sup> and 5 × 10<sup>8</sup> CFU/ml. Salmonellae that were painted onto belts were recovered at 5 × 10<sup>7</sup> CFU/cm belt, and populations were stable over the 2-h laying period despite visible drying of the material. It was not possible to control the distance that the eggs rolled along the collection belt and through the *Salmonella*/manure mixture. Thus, the levels of bacteria transferred to the egg were variable, and it was necessary to use a proportion (~8%) of the eggs produced from each days' lay to determine typical *Salmonella* levels deposited onto the egg surfaces. Over all experiments, the average level of *Salmonella* Enteritidis applied to each egg was 1.69 × 10<sup>9</sup> CFU per egg (*n* = 374; SEM = 8.38 × 10<sup>7</sup> CFU per egg). The average level of *Salmonella* Typhimurium applied to each egg was 4.7 × 10<sup>8</sup> CFU per egg (*n* = 60; mean SEM = 7.9 × 10<sup>7</sup> CFU per egg).

These artificially contaminated eggs were used to assess the effects of washing under best practice conditions and under less than ideal conditions to model those effects that could occur if washing equipment or monitoring systems failed during the course of the process. Over the entire course of the study, washing eggs under best practice conditions caused a reduction to the shell surface levels of *Salmonella* Enteritidis to 9.5 × 10<sup>3</sup> CFU per egg (*n* = 873; SEM = 5.24 × 10<sup>3</sup> CFU per egg; Fig. 2). Thus on average, washing under standardized conditions resulted in a 10<sup>5</sup> to 10<sup>6</sup> CFU per egg decline in *Salmonella* Enteritidis levels. The lowest reduction that was observed was 4 × 10<sup>4</sup> CFU per egg when eggs were contaminated with 4 × 10<sup>9</sup> CFU per *Salmonella* egg. Egg washing by the best practice treat-

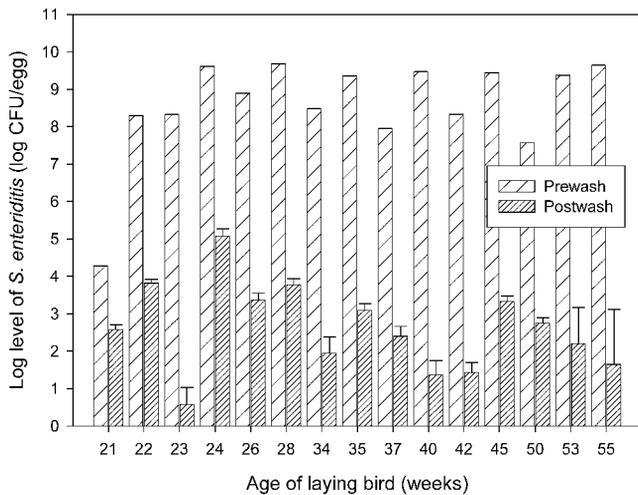


FIGURE 2. Effect of standardized (blueprint) conditions on the shell surface levels of *Salmonella Enteritidis* over an age range typical of that used in commercial laying hens. Eggs were inoculated, washed, and dried under standardized conditions as described in "Materials and Methods." Error bars are the standard error of the mean. Data are the average of 24 eggs (unwashed) or between 42 and 130 eggs (washed).

ment did not completely remove all *Salmonella* if high levels ( $>10^6$  CFU per egg) were present on the surface of the shell. Similar results were found for shell surface *Salmonella* Typhimurium, which were also significantly reduced when washed using the best practice protocol (Fig. 7).

In addition to enumeration of surface levels, egg contents were also examined for the presence of *Salmonella*. Throughout the course of the study, we never isolated either *Salmonella* Typhimurium or *Salmonella* Enteritidis from the contents of any egg washed by the best practice method (results not shown). Thus, it seems likely that washing, when performed under best practice conditions, does not cause recent external contamination with *Salmonella* to penetrate the egg and contaminate the yolk or albumen.

Almost all large-scale commercial washing machines of the type routinely used in the United States and Japan recycle water with a portion of the washing and rinsing water, being drained and replaced with fresh water on a continual basis. Although such a system is efficient in terms of water use, it means that a continual stream of washing additive has to be supplied to the machine or the levels of detergent or sanitizer will decrease. High-throughput commercial washers are not routinely equipped with monitoring equipment (4, 17, 22, 23) designed to detect whether there is an interruption to the flow of washing chemicals. To assess the likely effects of interruption of wash additive, either by failure of the pump used to supply the wash additive or by the container of additive running dry, the effect to shell levels of *Salmonella* was determined over a range of concentrations of Chlorwash and Quat 800.

Low concentrations of chemicals (Chlorwash at  $\leq 3$  g/liter) had no effect on the efficiency of washing (Fig. 3). An unexpected result was that completely omitting all Chlorwash from the wash water also gave on average a  $10^5$  reduction in *Salmonella* Enteritidis levels. Chlorine levels

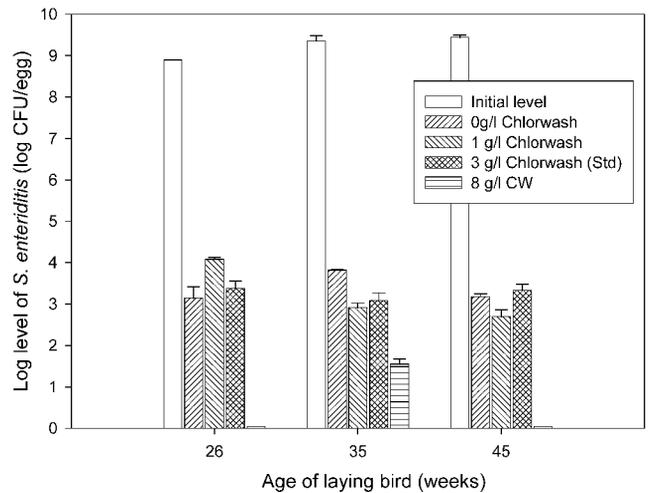


FIGURE 3. Effect of the concentration of the wash water additive Chlorwash on shell surface levels of *Salmonella Enteritidis*. Eggs were inoculated, washed, and dried under standardized conditions (Chlorwash at 3 g/liter) or with Chlorwash at 0, 1, or 8 g/liter as described in "Materials and Methods." Error bars are the standard error of the mean. Data are the average of between 24 and 118 eggs.

in the source water supply are routinely assayed by the Direct Laboratories water analyses section on a 6-month basis and are typically in the range of 50 to 80 ppb, making it unlikely that the water itself was a source of antimicrobial activity. At 5 and 8 g/liter, we saw a more pronounced bactericidal effect, although the equipment manufacturer and additive vendor recommends 3 g/liter. A minimum of  $10^5$  reduction in *Salmonella* numbers was observed for all concentrations of Chlorwash. This result is strongly indicative that, at recommended levels, Chlorwash might not be the main reason for the observed decline in levels of *Salmonella* when washing eggs.

Similar results were observed when using the Quat 800 rinse water sanitizing agent (Fig. 4). The equipment manufacturer and additive vendor recommends using Quat800 at a rinse water concentration of 2.5 g/liter (wt/vol). At this level, no statistically significant differences (*t* test,  $P > 0.05$ ) were found between the Quat800 and the water control. However, a significantly enhanced (*t* test,  $P < 0.001$ ) and almost complete kill was observed when Quat800 was used at either 5 or 10 g/liter.

One possible explanation for these results could be that the wash and rinse water temperatures were high enough to produce a bactericidal effect, and a temperature-dependent kill was significant enough to mask reductions in the Chlorwash or Quat800 concentrations. An alternative explanation centers on the design of the washer used and the fact that the water is partly recycled. A continuous input of fresh water into the machine mixes with the rinse wash tanks and causes an equal volume of water to be displaced through a drain into the wash tank. This has advantages in terms of controlling the temperature of the water tanks. Although the machine was operated under manufacturer's recommended conditions, a buildup of fecal materials was observed in the water, which might have been able to neu-

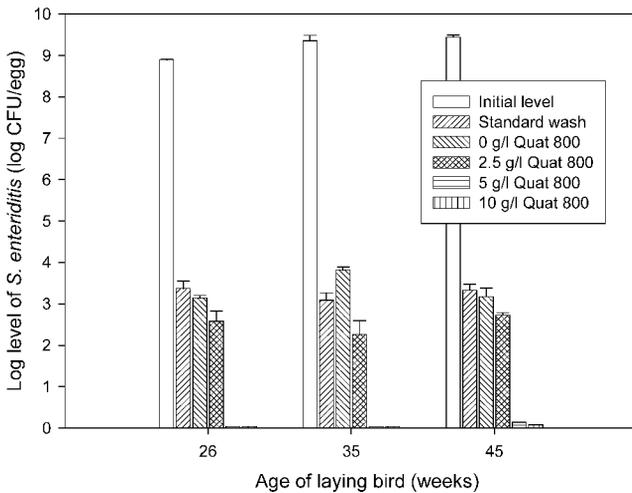


FIGURE 4. Effect of the concentration of the rinse water additive Quat 800 on shell surface levels of *Salmonella Enteritidis*. Eggs were inoculated, washed, and dried under standardized conditions with Chlorwash at 3 g/liter or with Quat 800 at 0, 2.5 (manufacturer's recommended dose), 5, or 10 ml/liter as described in "Materials and Methods." Error bars are the standard error of the mean. Data are the average of between 24 and 120 eggs.

tralize the wash additives in the water at manufacturer-recommended concentrations.

Other explanations that should be considered is the diluting effect of the water tanks themselves. The combined volume of the water tanks that were used for washing and rinsing was 300 liters. Much of the *Salmonella*-containing fecal material was removed from the egg surface by strong water jets during the washing process, and this material was returned to the wash and prewash tanks. The large volumes of slowly recycled water present in these tanks would effectively dilute even high levels of *Salmonella* present in the fecal materials; thus, dilution would be expected to contribute to the observed reduction in shell surface bacterial levels. The antimicrobial effects of the shear associated with high pressure release of wash water through the spray nozzles was not investigated as part of this study.

*Salmonella* was not isolated from either the wash water or the contents of any egg washed during this phase of the study leading to the conclusion that wash water additive concentration does not directly influence the survival of *Salmonella* or its transfer into egg contents during the commercial washing process. However, content contamination can arise if the washing process is not undertaken carefully and with strict adherence to best practice conditions.

The effects of lowering the wash and rinse temperatures on *Salmonella* isolation from egg contents are shown in Figure 5. Lowering the wash and rinse temperatures to 25 and 27°C, respectively, caused the egg contents to become contaminated with either *Salmonella Enteritidis* or *Salmonella Typhimurium*, even in the presence of 200 ppm chlorine. Other authors have described the difficulties in reliably isolating *Salmonella* from egg contents (9) without contamination from the shell surface. We adhered strictly to the recommendations given by Himathongkham et al. (9), which state that laboratories testing egg contents should

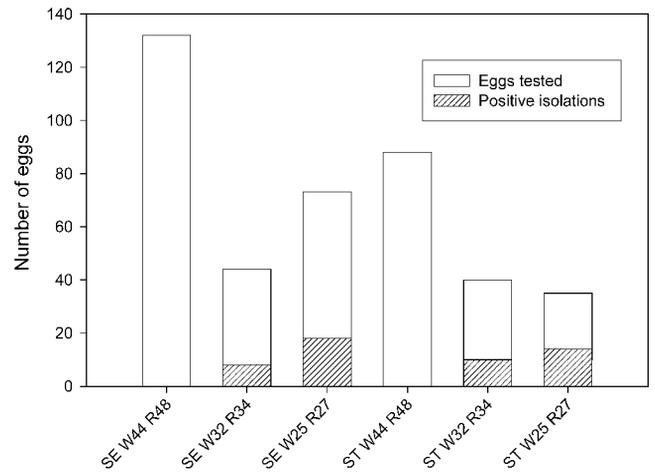


FIGURE 5. Effect of lowering wash and rinse water temperatures on contamination of albumen and yolk by externally applied *Salmonella Enteritidis* (SE) and *Salmonella Typhimurium* (ST). Figures after W and R denote the temperature of the wash and rinse waters, respectively. Plain bars are the number of washed eggs that were examined; shaded bars show the numbers of postwashed eggs that contained salmonella in the yolk and albumen.

surface-sterilize eggs to avoid contamination of contents from the shell. In addition, egg contents were pipetted from the egg, further reducing the chances of cross-contamination (6). Egg contents were tested for all of the treatments used in this study, but *Salmonella* was only isolated from eggs in a small number of specific treatments. This gives confidence that both *Salmonella Enteritidis* and *Salmonella Typhimurium* entered the egg contents when water temperatures were lower than those recommended. To ensure that the salmonellae isolated were the experimental strains used and to protect against the outside possibility that the birds had acquired other strains (e.g., through consumption of contaminated feed), basic PCR-based fingerprinting was used to confirm that the *Salmonella* strains isolated from the egg contents were the same as those used to contaminate the shell surfaces (gel photographs not shown).

These results are in contrast to those reported previously (19). This previous study used a spray wash system to compare the effects of three wash water temperatures (15.5, 32.2, and 48.9°C) on internal and external shell surface bacterial counts. The treatments used were shorter in duration than would normally be applied in commercial practice—10 s for washing and 3 s for rinsing. Under these conditions, they concluded that spray washing of eggs at the lowest temperature did not increase internal shell bacterial counts.

Temperature of wash and rinse water did not appear to have a significant effect on the surface populations of *Salmonella Enteritidis*. *Salmonella Enteritidis* showed a 5- to 6-log reduction irrespective of which temperatures were used for washing and rinsing (Fig. 6). This result is puzzling because it does not support the hypothesis discussed previously that temperature is responsible for the death of *Salmonella Enteritidis* during egg washing. It seems likely that the combination of dilution, temperature, and chlorine concentration all contribute to *Salmonella Enteritidis* de-

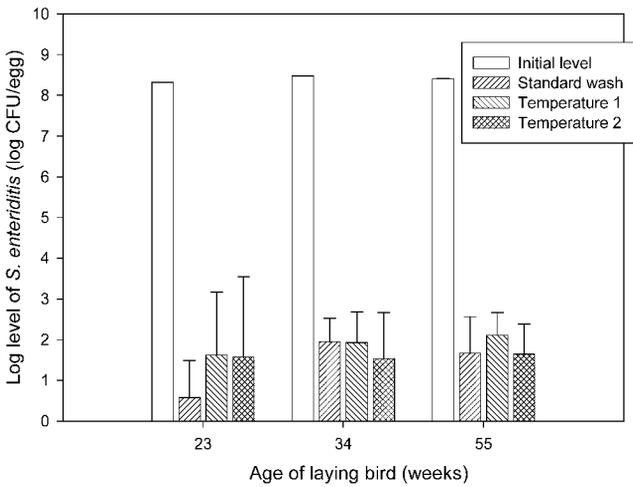


FIGURE 6. Effect of wash and rinse water temperatures on shell surface levels of *Salmonella Enteritidis*. Eggs were inoculated, washed, and dried under standardized conditions (44°C wash water, 48°C rinse water) as described in “Materials and Methods.” Temperature treatment 1 used wash and rinse water temperatures of 25 and 27°C, respectively. Temperature treatment 2 used temperatures of 32 and 34°C, respectively. Error bars are the standard error of the mean. Data are the average of between 38 and 130 eggs.

cline during egg washing and that reduction of any of these parameters can still result in efficient kill.

In contrast to *Salmonella Enteritidis*, *Salmonella Typhimurium* appeared to show a temperature-dependent decline in shell surface numbers (Fig. 7).

The effect of wash and rinse water pressures also were investigated as part of this study. Raising (slightly) or lowering the pressure of the wash and rinse water forced through the wash nozzles did not introduce *Salmonella* into the egg contents, although higher pressures did show a tendency to remove more *Salmonella* from the shell surface (Fig. 8).

For one set of experiments run during week 40 of the study, *Salmonella Enteritidis* was isolated from 12 of 50 (24%) eggs that were not dried after washing. There are good reasons why eggs that are not effectively dried after washing should be contaminated internally. Haines and Moran (5) first observed that when eggs are placed in a cooler bacterial suspension, a pressure gradient is set up that draws bacteria through the shell into the interior as the egg contents cool. If eggs are warmed to a temperature higher than ambient by the washing process, then postwash, as they cool, their contents will contract and produce a negative pressure to the inside of the egg. This negative pressure could assist bacterial entry into the egg as gas (and water if the surface of the egg is wet) are sucked into the egg (11, 14, 21). It is difficult not to warm eggs during the washing process. An increasing water temperature gradient as eggs move through the washing machine helps prevent bacterial ingress into the egg contents. Brant and Starr (1) have concluded that the temperature of the wash water should be at least 11°C higher than the egg content temperature to provide protection against ingress. However, it is not certain that *Salmonella* was able to colonize the egg

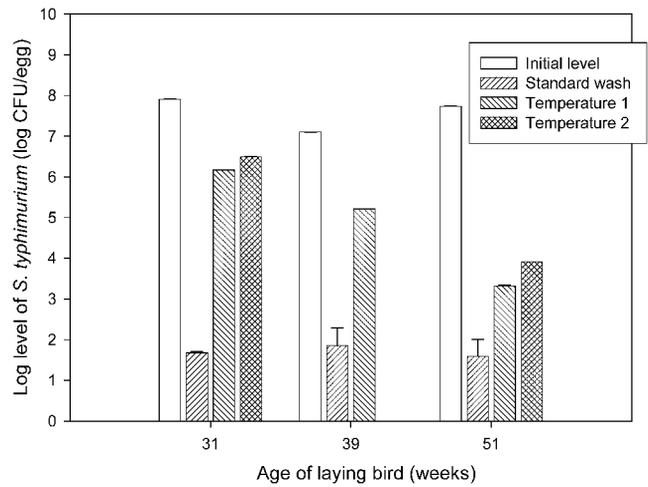


FIGURE 7. Effect of wash and rinse water temperatures on shell surface levels of *Salmonella Typhimurium*. Eggs were inoculated, washed, and dried under standardized conditions (44°C wash water, 48°C rinse water) as described in “Materials and Methods.” Temperature treatment 1 used wash and rinse water temperatures of 25 and 27°C, respectively. Temperature treatment 2 used temperatures of 32 and 34°C, respectively, and was not undertaken for week 39. A different number of eggs were used for each data point. Between 40 and 151 eggs were used to calculate each data point. Error bars are the standard error of the mean.

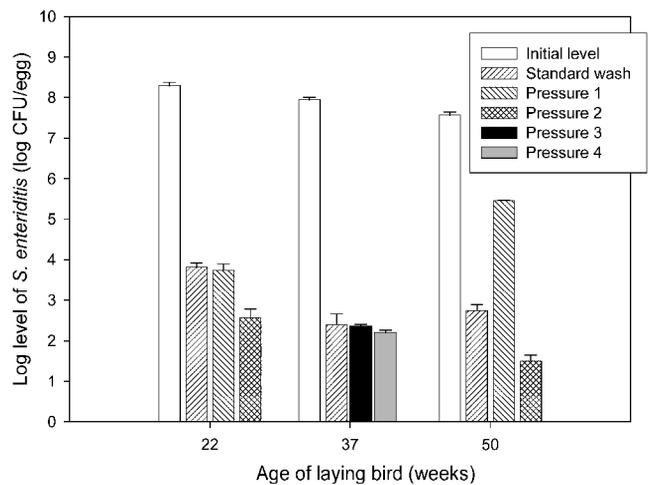


FIGURE 8. Effect of wash and rinse water pressures on shell surface levels of *Salmonella Enteritidis*. Eggs were inoculated, washed, and dried under standardized conditions (prewash head pressure 138 kPa, wash head pressure 262 kPa, rinse head pressure 262 kPa) as described in “Materials and Methods.” Pressure treatment 1 used 30 kPa prewash head pressure and 55 kPa wash and rinse head pressures. Pressure treatment 2 used 70 kPa prewash head pressure and 140 kPa wash and rinse head pressures. Pressure treatment 3 used 100 kPa prewash head pressure and 200 kPa wash and rinse head pressures. Pressure treatment 4 used 150 kPa prewash head pressure and 280 kPa wash and rinse head pressures. Treatments 3 and 4 were undertaken during week 37 only. A different number of eggs were used for each data point. Data are the average of between 18 and 82 eggs. Error bars are the standard error of the mean.



FIGURE 9. Effect of inefficient drying on the surface levels of *Salmonella Enteritidis*. Eggs were inoculated, washed, and dried under standardized conditions (2 min warm air at 42°C) as described in “Materials and Methods.” A different number of eggs were used for each data point. Data are the average of between 24 and 90 eggs. Error bars are the standard error of the mean.

contents as a result of improper drying because around this time and despite a number of precautions, the experimental flock became infected by the *Salmonella Enteritidis* strain being used for the experiment.

The birds were vaccinated for dual protection against *Salmonella Typhimurium* and *Salmonella Enteritidis*. The experiments commenced when the birds came into full lay at 21 weeks, and their eggs were used for 34 weeks until the birds were just over 1 year old. Despite these vaccinations, there were concerns that the use of live *Salmonella* cultures on the laying belts in close proximity to the bird feed could lead to infection. Therefore, fecal materials generated by the hens and their eggs were routinely analyzed to monitor the microbiological status of the flock. Over the course of the 34 weeks that the experiments ran, 37 pooled fecal samples, 696 external egg, and 401 internal egg controls were analyzed for *Salmonella* to monitor the infection status of the laying flock. The results of this monitoring revealed that 19 weeks after commencing the experiment, the birds became colonized with *Salmonella Enteritidis*. The infections were confirmed by cloacal swabbing. Because it is possible that *Salmonella*-infected birds can give rise to eggs with *Salmonella*-colonized contents by infection in the birds’ uterus before shell formation, we did not undertake examination of the contents after week 40 of the experiment, and we cannot report definitively that poor post-wash drying of eggs contaminated before cuticle hardening can cause colonization of contents by *Salmonella*. There were no significant differences between the surface counts of eggs dried at 42°C for 2 min and those that were not dried (Fig. 9).

One of the original objectives of this study was to determine whether the age of the bird that produced the egg had any effect on the infection rate of the egg contents. It was thought laying bird age might influence the infection of egg contents because, as birds age, the eggs become

larger and the thickness of the calcite layer of the shell decreases (20). However, we did not find any statistically significant correlations between bird age and penetration by salmonella from the limited internal data that we generated.

We also found no effect to surface levels of contamination when eggs inoculated with *Salmonella Enteritidis* were washed by the machine with different belt speeds (data not shown). Velocities of 27, 54, 80, and 160 cm/min were used, which resulted in 5.5, 2.8, 1.9, and 1 min as the respective amounts of time taken to pass through the machine. Belt speed experiments were undertaken during weeks 22, 37, 42, and 50 with a minimum of 38 eggs at each belt speed.

Although, for convenience, the pressure of water being supplied to the washing machine nozzles is used in the description of the best practice washing conditions, we determined the energy released by the wash and rinse water as it impacted the egg. At 262 kPa, the nozzle produced a 65° fan; the eggs are washed with their longest axis in line with the fan. Both the pressure supplied to the nozzle and the distance between nozzle and the surface of the egg were varied and the impact force recorded over a 10-s period. The nozzle to egg distance investigated ranged from 55 to 60 mm, the prewash nozzle supply pressure ranged from 35 to 150 kPa, and the wash nozzle range was 50 to a maximum of 280 kPa. Representative values for the energy released by water forced through the prewash nozzles using the above parameters was 0.22 to 0.81 N. For the wash nozzle, the range was 0.31 to 1.07 N.

The results of this study have demonstrated that, when undertaken according to a strictly controlled set of best practice conditions, washing eggs that have been contaminated with *Salmonella* before cuticle hardening does not lead to contamination of contents with these pathogens. Other washing parameters such as wash chemical concentration, the length of washing time, lowered jet pressure, and the age of the laying bird do not appear to influence the contamination of contents. However, if wash and rinse water temperatures are allowed to fall below 34°C, there is a detectable amount of content contamination.

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