

Bacterial Infection of Washed and Unwashed Eggs with Reference to Salmonellae

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A survey of eggs in the Fraser Valley area of British Columbia was conducted to determine the level of bacterial contamination on washed and unwashed shell eggs and the incidence of *Salmonella* infection in shell eggs. Samples of eggs were taken from 15 grading stations. Determinations on eggs from 87 shipments showed that a higher proportion of eggs was heavily contaminated (> 5-million organisms per egg) after washing than as received at the grading stations. This finding suggests the need for the development of more satisfactory washing procedures. No salmonellae were detected in the 180 samples (3,995 eggs) which were examined. It was concluded that *Salmonella* contamination of intact shell eggs does not constitute a serious health hazard in eggs from this area.

Salmonellosis is regarded as a public health problem of serious magnitude. Because of the ubiquity of the salmonellae in man and animals, there are numerous vectors for transmission of the organisms in the environment and cycles leading to continuous reinfection. Poultry are considered to be a significant reservoir of salmonellae and one from which transmission to man can readily occur. In recent years there have been outbreaks of *Salmonella* food poisoning in which dried or frozen egg products have been implicated. It is, accordingly, of concern to the poultry industry to ascertain the ways by which bacterial infection of eggs or egg products is most likely to occur in order to devise measures to minimize infection in general and to eradicate salmonellae in particular.

The following survey of eggs in the Fraser Valley area of British Columbia was conducted to determine (i) the level of bacterial contamination on unwashed and washed shell eggs, and (ii) whether the incidence of *Salmonella* infection in shell eggs is sufficiently high to constitute a serious health hazard.

MATERIALS AND METHODS

Bacterial contamination of unwashed and washed eggs. Samples of eggs were drawn from 87 shipments of eggs to 15 grading stations.

The sampling procedure was as follows for each shipment. The second and fourth trays were taken from one side of each of two cases as received at the station. Eggs of rows 1, 3, and 5 from each tray (30 eggs in all) were placed in plastic egg trays which had been soaked in a 10% solution of Formalin and dried. Eggs of rows 2, 4, and 6 from each tray

were marked and placed on the conveyor leading to a washing machine. The eggs under test were washed with the eggs undergoing washing in the normal operation of the station. The washed eggs were picked up from the conveyor at the point of packaging and were placed in plastic bags sterilized as above. The trays of unwashed and washed eggs were put into plastic bags and kept overnight at room temperature.

To avoid cross-infection among the samples, the eggs were handled throughout with disposable plastic gloves. The gloves were changed after each sampling and again when each sample of eggs was picked up after washing.

Plate counts were made as follows. Eggs of each sample were broken as aseptically as possible. The shells were placed in a sterile 1,000-ml flask and crushed with a large, sterile glass rod. A 300-ml amount of phosphate buffer (14.2 g of anhydrous Na_2HPO_4 in distilled water, plus 20 ml of 1 N HCl, diluted to 1,000 ml; final pH 7.4) was added. This amount completely covered the crushed shells. The flask was then shaken for 30 min.

A 10-ml portion was added to 90 ml of sterile phosphate buffer in a dilution bottle. Serial dilutions in buffer were made to give final dilutions of 1 egg per 1,000, 1 egg per 10,000, and 1 egg per 100,000; 1-ml samples were used for plating with nutrient agar. Triplicate plates were made at each dilution. Incubation was at 37 C for 48 hr.

The effect of washing on the bacterial count of sterile eggs was determined after the following procedure. Eggs were individually wrapped in aluminum foil and autoclaved at 121 C for 1 hr. The autoclaved eggs were taken to a grading station for washing under normal commercial conditions. For each lot of eggs put through the washer, 15 eggs were unwrapped carefully, by use of plastic gloves. Any eggs which showed cracks were discarded. The eggs were marked and put through the washer with the eggs being washed

in the normal operation of the plant. They were removed from the conveyor at the point of packaging and were placed on a sterile plastic egg tray. Samples of eggs were washed in this way at 30- to 60-min intervals during a 4-hr shift at the station. The trays of washed eggs were placed in plastic bags for holding overnight. The following day, plate counts were made on all samples as well as on a control sample which had been autoclaved but not washed.

Effects of the washing procedures were determined in two grading stations. The effect of washing was studied separately on eggs with smooth- and rough-textured shells in trials 3 and 4 (see Table 1). In the latter two trials, samples from the commercial eggs (after washing) were also taken for counting.

Salmonella contamination of shell eggs. In test 1, the samples of eggs which were used in the determinations of total aerobes were also examined for the presence of *Salmonella*.

In test 2, samples of shipments of unwashed eggs from 93 farms were obtained at grading stations. Precautions to avoid cross-infection among samples were taken as in test 1. Each sample contained 15 eggs and was tested for the presence of salmonellae only.

In test 1 for isolation and identification of salmonellae, a 100-ml sample of the shaken buffer suspension described above was added to 100 ml of (double-strength) lactose broth. The mixture was incubated for 5 hr at 37 C. Samples (10 ml) were added to duplicate flasks containing 90 ml of selenite-cystine broth (1 g of L-cystine dissolved in 10 to 20 ml of 1 N NaOH, diluted to 100 ml with distilled water; 1 ml of this solution added to 1 liter of selenite F broth). After incubation at 37 C for 24 and 48 hr, respectively, the selenite-cystine broth cultures were streaked on Brilliant Green agar plates.

All pink colonies which appeared on the Brilliant Green agar were transferred to MacConkey's agar. Lactose-negative colonies were inoculated onto a triple-sugar-iron slope. All slopes showing an alkaline slant and H₂S butt were tested with *Salmonella* polyvalent O serum. Urea broth was used to eliminate *Proteus* colonies.

For test 2, the eggs were broken aseptically as possible. The shells were crushed and shaken with 150 ml of sterile phosphate buffer solution. Three 1-ml samples were taken. Two were added to tubes containing 9 ml of selenite broth and the third to a tube containing 9 ml of lactose broth. All tubes were incubated at 37 C and subcultured to Brilliant Green agar after 24 and 48 hr. The ensuing procedure for identification of salmonellae was as in test 1.

Recovery of salmonellae. A 24-hr culture of *Salmonella thompson* grown in lactose broth was diluted with lactose broth to give dilutions of 10³, 10⁶, 10⁷, and 10⁹. Plate counts were made of each dilution. Four flasks containing 15 crushed egg shells in 150 ml of phosphate buffer were inoculated with 1 ml of the respective dilutions of *Salmonella thompson* and shaken for 30 min. Samples (1 ml) were inoculated into three tubes containing 9 ml of selenite broth.

The tubes were incubated at 37 C and subcultured at 24 and 48 hr onto Brilliant Green agar plates. All

pink colonies which appeared were identified as above.

The recovery experiment was repeated with a 24-hr culture of *Salmonella derby*.

RESULTS AND DISCUSSION

Bacterial contamination of unwashed and washed eggs. The results of the bacterial counts made in the samples of unwashed and washed eggs taken from the grading stations are summarized in Fig. 1. The graph shows the distribution of the samples with respect to the average numbers of bacteria found per egg. The range in bacterial counts is in general agreement with the levels reported by other investigators (1, 3, 6). The proportion of the samples contaminated with bacteria beyond an average of 5-million organisms per egg was higher with the washed eggs (21.8%) than with the unwashed eggs (8.0%).

Although note was made of the type of washing machine and the use of sanitizing chemicals in the various grading stations, no consistent relationship appeared between egg-washing procedures and bacterial counts. The variations in counts are probably attributable mainly to variations in the cleanliness of eggs as received at the stations from different producers.

Increased bacterial infection leading to spoilage of shell eggs after washing has been observed during storage (4). Trussell (7), from a study of the bacteriology of spoiled eggs, concluded that wet-washing may be a more serious cause of infection than the heavy visible soiling of eggs. It has been shown experimentally (5) that eggs in which the shell moisture content has been increased have a higher percentage of bacterial infection than do eggs in which the shells have been somewhat dried.

The relatively high proportion of washed eggs, which was heavily contaminated, suggested the importance of determining the numbers of bacteria which may be picked up by sterilized eggs put through an egg-washing machine during the regular operation with the commercial eggs which are being washed. The results are given in Table 1, and lead to a number of conclusions. It is apparent that the egg shell was altered by the autoclaving procedure so that it was more subject to bacterial contamination. The counts obtained in trials 3 and 4 on the sterilized eggs after washing were far in excess of those found on the plant samples of eggs washed at the same time. In trial 2, consistently high counts were found, but two of the samples showed counts even above the generally high level of contamination of the eggs in this trial. In both these samples it was noted that two eggs were cracked. The cracked eggs had bacterial counts perhaps 1,000 times higher than

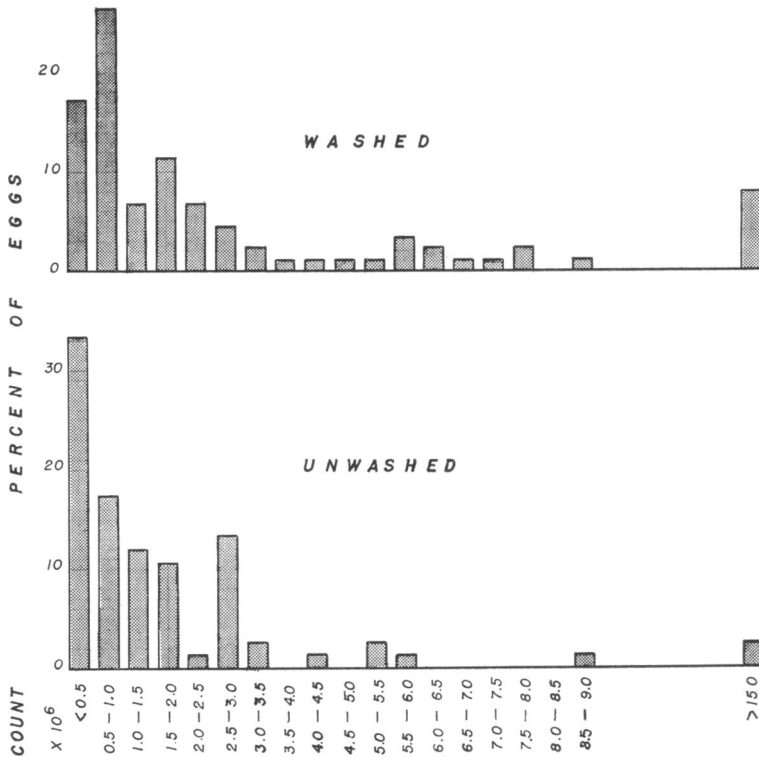


FIG. 1. Bacterial counts on shells from washed and unwashed eggs.

TABLE 1. Bacterial counts on sterilized eggs put through commercial egg washers

Trial 1 ^a		Trial 2		Trial 3 ^b			Trial 4 ^c		
Time (PM)	Counts/egg	Time (PM)	Counts/egg	Time (PM)	Counts per		Time (PM)	Counts per	
					Smooth egg ^d	Rough egg		Smooth egg	Rough egg
1:30	420,000	12:15	5,700,000	12:15	915,000	740,000	12:30	32,000	2,500,000
2:00	5,700,000	12:45	11,100,000	1:30	473,000	1,190,000	1:45	910,000	4,300,000
3:00	144,000	1:15	216,000,000 ^e	3:00	353,000	650,000	3:00	6,400,000	15,800,000
3:30	149,000	1:45	8,000,000	4:00	17,000	98,000	4:15	14,000,000	30,500,000
4:00	190,000	2:15	27,200,000						
4:30	159,000	3:00	142,800,000 ^e						
		3:30	23,900,000						

^a Trials 1 and 4 were conducted at one station and trials 2 and 3 at another.

^b At 4:00 PM, two plant samples (oiled) had bacterial counts of <math><10,000</math>.

^c At 1:45 PM, one plant sample had a bacterial count of <math><10,000</math>; at 4:15 PM, one plant sample had a bacterial count of 59,000.

^d Eggs in this sample were from a pullet flock and averaged only 47.3 g in weight.

^e Two cracked eggs were detected in this lot when the eggs were broken.

did the intact eggs in the sample. Brown (2) reported that the shell of cracked eggs is a barrier to bacteria only so long as the egg is dry. When a cracked egg is wet, bacteria may be swept into the shell crack and onto the shell membrane.

Although the differences in the physical characteristics of smooth and rough shells would

logically suggest that a rough shell would provide greater surface area and increase the likelihood of bacteria adhering to the shell during washing, the results do not give conclusive evidence that this is actually the case. In the four trials on washing sterilized eggs, the eggs were washed at different times during the shift; therefore, any buildup

of bacteria in the wash water and on the equipment would probably have been detected. Only in trial 4, however, was there any indication that this occurred. Even in this trial, although the sterilized eggs became heavily contaminated, the plant samples had low bacterial counts.

Salmonella contamination of shell eggs. *Salmonellae* were not isolated from any of the 180 samples of eggs tested. The experiment on the recovery of *Salmonella* demonstrated that at least 400 organisms per shell would have been detected with the technique employed. Assurance is accordingly provided that the incidence of *Salmonella* infection in shell eggs in the Fraser Valley area is low. It should be emphasized, nonetheless, that only intact shell eggs were studied in this test. McNally (5) considers that, with clean, unwashed eggs, cracked or checked eggs are probably the most important cause of bacterially infected eggs. The increase in bacterial numbers noted when the samples in trial 2 (Table 1) contained cracked eggs vividly illustrates the potential hazard of cracked eggs should pathogenic bacteria be present on the shell or in the environment.

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