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BACTERIOLOGY OF WASHED AND UNWASHED EGGS

II. PENETRATION OF SALMONELLA BACTERIA THROUGH THE EGGSHELL*

By

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It is known since the investigations by *Haines* in 1938 that the contents of most eggs are sterile after laying and that thus most cases of egg spoilage depend on penetration of microorganisms through the eggshell. In evaluating the influence of washing on the hygienic and bacteriological properties of eggs it is consequently of great value to know if the shell's resistance to the penetration of microorganisms has changed after the washing procedure. There are several data available in the literature concerning the microbial penetration of the eggshell under various conditions, but the effect of egg washing upon the detailed penetration seems to be but little investigated.

Haines & Moran (1940) showed that microorganisms may be drawn through the shell by simple suction if an egg is immersed in a fluid containing bacteria and the temperature of the egg is higher than that of the fluid. The authors also found that a fluid more easily passes from the exterior to the interior of the egg than vice versa. *Rievel* (1939) found that "any form of humidity greatly favours the penetration" of fluorescent bacteria through the eggshell, an observation which is in accordance with experiences of egg storage under practical conditions (*McNally* 1953).The

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significance of an undamaged cuticle for the storage properties of the egg was shown by *Vadehra et al.* (1970), who demonstrated that removal of the cuticle reduced the storage properties of the egg.

As shown by *Östlund* (1971), no significant increases were seen in bacterial counts of the contents of washed eggs compared to unwashed eggs, suggesting that washing under strictly controlled conditions caused no substantial damage to the cuticle. The investigation was performed under conditions preventing bacterial recontamination of the eggshell after the washing procedure.

The aim of the present investigation was to compare the resistance of the shells of unwashed, machine washed and hand washed eggs to microbial penetration when the shell surface was heavily contaminated after the washing procedure. An attempt was also made to elucidate whether the most essential protection mechanisms of the egg may be damaged by washing, or, if the shell membranes after washing the egg still constitute an effective barrier against infection.

MATERIALS AND METHODS

General performance of the experiment

Trial a. Cultures of Salmonella bacteria were applied on a defined area of the shell surface of 60 machine washed and 60 unwashed eggs. Thirty eggs of each kind were then stored at 4°C for eight weeks and the rest of the eggs were stored at 30°C for 12 days. At the end of the storage period the egg contents were examined for the presence of viable Salmonella organisms.

Trial b. In 200 eggs of each kind of unwashed, machine washed and hand washed eggs the contents were removed through an opening in the shell and replaced by Salmonella enrichment broth. In every second egg of each kind the shell membranes were removed. Salmonella cultures were deposited on a defined area of the outer shell surface. After incubation at 37°C for four days, the presence of Salmonella bacteria in the broth was examined.

Eggs and washing of eggs. All eggs for the investigation were from a single strain of single-comb White Leghorns from one farm. They were in no case older than four days when the washing was performed. The eggs were selected at random on delivery to the grading station. Unwashed eggs and eggs washed in two

different ways, machine washed and hand washed, were used. The machine washing of eggs was performed as described by *Östlund* (1971), i.e. by sprinkling with detergent-water solution (43°C), rinsing in tap water (47°C), and drying in a stream of hot air (60—65°C). The hand washing of eggs was performed after storage of the eggs overnight at 4°C. The cold eggs were soaked for a few minutes in clean tap water (ab. 20°C) without additives and were then scrubbed in running water, with a nailbrush. They were left to dry at room temperature.

Preparation of eggs and bacteriological methods

Trial a. One hundred-and-twenty eggs, 60 machine washed and 60 unwashed eggs, were used in the experiment. On the side of each egg, 25 µl of a broth culture of Salmonella typhi murium (incubated 24 hrs. at 37°C) was spread over a previously marked area of 2.5 cm^2 using a micropipette (Microcap®, Drummond Scientific Co., Broomall, Pa., USA). Thirty unwashed and 30 machine washed eggs were then incubated at 4°C for 56 days and the same number of eggs of each kind were incubated at 30°C for 12 days.

At the end of the incubation period the eggs were opened under sterile conditions (*Östlund* 1971), and the contents were transferred to a flask, containing 100 ml of tetrathionate enrichment broth according to Kauffmann. After gentle stirring of the inoculated broth, the flasks were incubated at 37°C for two days. Cultivation from the enrichment broth for identification of Salmonella was performed using brilliant green agar ("Modified", Oxoid Ltd., London S. E. 1, England, incubation 24 hrs. at 37°C) and desoxycholate citrate agar (DC agar, incubation 48 hrs. at 37°C). Suspect colonies were subcultivated using brilliant green agar and lactose-sacharose-urea agar, "LSU-agar" (*Juhlin & Ericson* 1961) and tested biochemically and serologically.

Trial b. At the middle of the long side of each egg the shell was peeled off over a circular area of about 4 cm^2, and the shell membranes were removed from the opening. The egg contents were poured out and discarded. The inside of the shell was rinsed twice with distilled water. In every second egg of each kind the shell membranes on the opposite side of the opening were scraped off using a specially designed knife and the inside was rinsed once more with distilled water. On the outside of the shell opposite the opening a circular area of 2.5 cm^2 was marked. On this

area 25 µl of a broth culture of Salmonella typhi murium (incubated 24 hrs. at 37°) was applied as described above. After drying the culture at room temperature the eggs were placed on egg trays and filled to about 4/5 with selenite broth (Difco Laboratories, Detroit 1, Michigan, USA). The openings were covered with Parafilm® (Gallencamp Inc., London E.C. 2, England) and the eggs incubated for four days at 37°C. Cultivation from the enrichment broth and the identification of Salmonella was performed as described above for Trial a.

RESULTS AND DISCUSSION

In this study a Salmonella species was chosen as test organism, partly because the occurrence of Salmonella infections in poultry — and thus the occurrence of Salmonella bacteria on the surfaces of eggs — constitutes a practical problem in egg production (*Marthedal & Velling* 1964), and partly because Salmonella differs from the normal microflora of eggs and can thus be detected and distinguished from other contaminants.

In Trial a, no Salmonella microorganisms could be detected in the egg contents after storage at 4°C or at 30°C. This was the case both for washed and unwashed eggs. The negative findings in unwashed eggs are in agreement with the results obtained by *Vadehra et al.* (1969). These authors found the egg contents free from Salmonella even after dipping warm eggs in a cold water suspension of Salmonella bacteria and storing them. The mode of application of the inoculum in the present investigation ought to result in still smaller probability of shell penetration since no temperature gradient was accomplished and only a small part of the surface of the egg was exposed to the infective agent.

The negative results in all the eggs indicate that potential damage to the protection mechanisms by washing was too small to be measured by the method used. The number of eggs in this study was fairly small (120 eggs), but even if a considerably larger number of test eggs may have given a few positive results, the conclusion must be that the method used was too insensitive for the measurement of a potential difference between washed and unwashed eggs. In order to facilitate the bacterial penetration and thus make a potentially occurring difference observable, the experiments of Trial b were performed.

The frequency of eggs in which the Salmonella bacteria had penetrated the shell in the experiments of Trial b, is given in

Table 1. Frequency of Salmonella penetration through the shell of unwashed, machine washed and hand washed eggs with intact and removed shell membranes. In each group n = 100, i.e. a total of 600 eggs were investigated.

Treatment of the eggs		Frequency of Salmonella penetration
shell membranes	washing	
intact	unwashed	0.12
	machine washed	0.10
	hand washed	0.22
removed	unwashed	0.27
	machine washed	0.42
	hand washed	0.60

Table 1. It will immediately be seen that the frequency in all cases was higher in eggs with removed shell membranes than in those with the membranes undamaged. It will also be seen that hand washed eggs had a higher frequency of Salmonella than had machine washed or unwashed eggs. In eggs with intact shell membranes, the difference between unwashed and machine washed eggs favours machine washed eggs (0.12 and 0.10, respectively). This difference, however, has no statistical significance ($0.8 < P < 0.9$, Table 2) and is thus of coincidental nature.

Table 2. Statistical comparison of frequency of Salmonella penetration through the shell between groups of eggs, subjected to different treatments.

Treatment F = frequency	χ^2-test	Treatment F = frequency	χ^2-test	Treatment F = frequency
shell membranes intact, unwashed. F = 0.12	$\chi^2 = 0.050$ $0.8 < P < 0.9$	shell membranes intact, machine washed. F = 0.10	$\chi^2 = 4.501$ $0.025 < P < 0.050$	shell membranes intact, hand washed. F = 0.22
shell membranes intact, hand washed. F = 0.22	$\chi^2 = 1.639$ $0.2 < P < 0.3$	shell membranes intact, unwashed. F = 0.12	$\chi^2 = 6.243$ $0.010 < P < 0.025$	shell membranes removed, unwashed. F = 0.27
shell membranes intact, hand washed. F = 0.22	$\chi^2 = 28.297$ $P < 0.0005$	shell membranes removed, hand washed. F = 0.60	$\chi^2 = 21.887$ $P < 0.0005$	shell membranes removed, unwashed. F = 0.27
shell membranes removed, unwashed. F = 0.27	$\chi^2 = 4.337$ $0.025 < P < 0.050$	shell membranes removed, machine washed. F = 0.42		

However, hand washing seems to lower the shell's ability to resist Salmonella penetration (frequency 0.22, Table 1), in spite of the fact that the washing was performed in clean water and with a positive temperature gradient from the egg to the water. The difference in frequency between machine washed and hand washed eggs was almost significant ($\chi^2 = 4.501$; $0.025 < P < 0.050$). This difference supports the conclusion that the way of washing the eggs is of great significance for the result.

Within the group of eggs with removed shell membranes there is an almost significant difference in Salmonella frequency between unwashed and machine washed eggs (0.27 and 0.42 respectively; $\chi^2 = 4.337$; $0.025 < P < 0.050$). This difference must be explained by a certain injury, accomplished by the machine washing procedure, but it also seems as if this damage was of no significance as long as the shell membranes were intact. It is also obvious that the negative effect of hand washing of the eggs on the resistence to microorganisms is fairly moderate when the shell membranes are intact (frequency 0.22), but the hand washing has severe consequences if the shell membranes are simultaneously damaged (frequency 0.60). The difference between these groups is statistically highly significant ($\chi^2 = 28.297$; $P < 0.0005$).

When the shell membranes were intact, no significant difference was seen between unwashed and hand washed eggs ($\chi^2 = 1.639$; $0.2 < P < 0.3$), while the difference between the same groups was statistically highly significant ($\chi^2 = 21.887$; $P < 0.0005$), when the shell membranes were removed. These facts stress the importance of intact shell membranes in preventing washing injuries from influencing the bacterial resistance of the egg.

It is of interest to note that, while machine washing of untreated eggs had no measurable effect on the resistance to Salmonella (frequencies 0.10 and 0.12, respectively), removal of the shell membranes resulted in an increase in the frequency of positives to 0.27 (the difference to untreated eggs: $\chi^2 = 6.243$; $0.010 < P < 0.025$). It seems as if intact shell membranes are more important as concerns the protection mechanism of the egg toward microorganisms than are the structures that may be injured by a washing procedure.

From the present study it may be concluded that industrial large-scale washing of eggs under strictly controlled conditions

in no way favours the penetration of Salmonella organisms through the shell of uncracked eggs. Furthermore, the conclusion made in part I of this investigation (*Östlund* 1971) has been supported, that eggs which had been subjected to the machine washing procedure after storage had bacterial counts equal to those of unwashed eggs. It is consequently obvious that the washing of eggs according to the applied method in no way harms essential storage properties of uncracked eggs. It is also possible that the bacteriological quality of machine washed eggs would be still better than this study has indicated if the most dirty eggs are picked out at the arrival to the grading stations and used for other purposes than washing and marketing.

Starr et al. (1952) observed severe spoilage in eggs washed under practical conditions by methods essentially similar to those, which gave satisfactory results in the laboratory. Also *Forsythe et al.* (1953) stressed the necessity of studies in commercial scale experiments, before any recommendations could be made to the poultry industry.

In this study, however, the washing was done at a grading station together with the current large-scale washing of eggs for market, and therefore the results of the investigation may be assumed to be directly applicable to practical conditions. It is a well known fact that washing of eggs under badly controlled conditions results in increases in rotting during storage (e.g. *Rievel* 1939, *Haines & Moran* 1940, *Wright* 1948, *Miller et al.* 1950, *Lorenz & Starr* 1952, *Trussel* 1955, *Brown et al.* 1966). It is also known that such washing occurs to a considerable extent at Swedish farms prior to delivery of the eggs to the grading stations. It seems clear that this washing under uncontrolled conditions — probably done because reduced rates are received for dirty eggs — results in an uneven hygienic quality of eggs for market, and a deterioration of the average storage properties.

Considering the results of this investigation it thus seems probable that a higher average storage quality of the eggs for market would be the result if the practice of paying reduced rates for dirty eggs was discontinued and all eggs were subjected to industrial machine washing so that all hand washing could be eliminated.

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SUMMARY

The ability of the eggshell to resist penetration of Salmonella bacteria was studied in unwashed and in hand washed eggs, and in eggs, which had been subjected to industrial, large-scale machine washing under strictly controlled conditions.

In one part of the investigation, concerning unwashed and machine washed eggs, a defined amount of a broth culture of Sal-

monella typhi murium was applied on the shell surface, whereafter the eggs were incubated at 4°C for eight weeks or at 30°C for 12 days. At the end of the storage period, the egg contents were examined for the presence of Salmonella. No Salmonella bacteria were detected in the 120 eggs investigated.

In another part of the investigation, dealing with 200 unwashed, 200 hand washed and 200 machine washed eggs, a broth culture of Salmonella was applied on the shell surface of eggs, whose contents had been poured out through an opening. In every second egg of each kind, the shell membranes had been scraped off over an area, corresponding to the placement of the bacterial inoculum. The eggshells were filled with enrichment broth and the occurrence of Salmonella bacteria in this broth was examined after incubation.

In the eggs with intact shell membranes, the frequencies of Salmonella positive enrichment broths were for unwashed eggs 0.12, for machine washed eggs 0.10, and for hand washed eggs 0.22. The corresponding frequencies in eggs with removed shell membranes were 0.27 (unwashed), 0.42 (machine washed), and 0.60 (hand washed).

No statistical difference occurred between unwashed and machine washed eggs when the shell membranes were intact. When the shell membranes were removed this difference was statistically almost significant. The difference between machine washed and hand washed eggs with intact shell membranes was statistically almost significant, while the same difference was highly significant in eggs with removed shell membranes.

The author assumes, from the results of the present and a preceding investigation, that the applied washing procedure in no way harms any essential storage properties of uncracked eggs. Considering the known occurrence of hand washing of eggs in Swedish farms — a treatment with documentedly unfavourable influence — it would be desirable if all eggs could be subjected to machine washing under strictly controlled conditions at the grading stations. Such an arrangement ought to result in an increase in the average bacteriologic-hygienic quality of eggs for market.

SAMMANFATTNING

Bakteriologiska förhållanden hos tvättade och otvättade ägg. II. Salmonellabakteriers penetration av äggskalet.

Äggskalets förmåga att motstå penetration av salmonellabakterier jämfördes hos otvättade ägg, hos ägg som handtvättats samt hos ägg som under strängt kontrollerade betingelser tvättats maskinellt i industriell skala.

I ett delförsök, som omfattade otvättade och maskintvättade ägg, fick en bestämd mängd på skalet applicerad buljongkultur av Salmonella typhi murium torka in, varpå äggen förvarades vid 4°C i 8 veckor eller vid 30°C i 12 dygn. Vid lagringsperiodens slut undersöktes äggens innehåll med avseende på Salmonella. Icke i något av 120 undersökta ägg kunde viable salmonellabakterier påvisas.

I ett annat delförsök, som omfattade 200 otvättade, 200 maskintvättade och 200 handtvättade ägg, applicerades salmonellakulturen på utsidan av ägg som genom en öppning tömts på sitt innehåll. Hos halva antalet ägg av varje slag skrapades skalhinnorna bort över det område där bakteriekulturen anbringats. Äggskalen fylldes med anrikningsbuljong, i vilken förekomsten av salmonellabakterier undersöktes efter inkubering.

Hos ägg med intakta skalhinnor var frekvensen salmonellapositiva buljonger för otvättade ägg 0,12, för maskintvättade ägg 0,10 och för handtvättade ägg 0,22. Hos ägg med avlägsnade skalhinnor var motsvarande frekvenser 0,27 (otvättade), 0,42 (maskintvättade) och 0,60 (handtvättade).

Ingen statistisk skillnad förelåg mellan otvättade och maskintvättade ägg så länge skalhinnorna var intakta, men när dessa avlägsnats var skillnaden statistiskt nästan signifikant. Skillnaden mellan maskintvättade och handtvättade ägg med intakta skalhinnor var statistiskt nästan signifikant, medan samma skillnad var höggradigt signifikant för ägg med avlägsnade skalhinnor.

I diskussionen anför författaren med ledning av resultaten av detta och ett föregående arbete att det inte synes som om den tillämpade maskintvättningen skulle ha någon menlig inverkan på lagringsegenskaperna hos oknäckta ägg. Med kännedom om den på gårdarna förekommande handtvättningen av ägg — ett förfarande med dokumenterat ofördelaktig inverkan — skulle det därför vara önskvärt att alla ägg i stället kunde tvättas centralt och under strängt kontrollerade betingelser. Ett sådant arrangemang torde resultera i en höjning av konsumtionsäggens genomsnittliga bakteriologisk-hygieniska kvalitet.

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