Egg washing using a small-scale bucket washer

M.L. HUTCHISON[1]*, N. SPARKS[2], J. GITTINS[3], L. DRYSDALE[4] and T. MOORE[1]

[1]Direct Laboratories Research Division, Woodthorne, Wergs Road, Wolverhampton, UK
[2]Avian Science Research Centre, Animal Health Group, SAC, West Mains Road, Edinburgh, Scotland, UK
[3]ADAS Poultry Team, Ceres House, 2 Searby Road, Lincoln LN2 4DW
[4]Hannah Research Institute, Hannah Research Park, Ayr, Scotland KA6 5HL, UK
***mike.hutchison@directlaboratories.net**

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Summary

Preliminary results on the microbiological implications of using a small-scale bucket-style washer are reported for chicken eggs. The bucket washer reduced the total bacterial numbers on the surface of cage-produced eggs under manufacturer-recommended conditions on average by 1.5 log CFU egg^{-1}. When washing free range eggs which were visibly soiled, there was a tendency for the wash water to become dirty after only a few batches of eggs had been washed. Washing in dirty water could increase the levels of bacteria on the surfaces of the shells of visibly clean eggs. However, the additional bacteria did not manage to penetrate into the egg contents. A single batch of washed eggs (1 positive from 10 total) contained detectable numbers of bacteria in a pooled sample of contents after 2 weeks storage at 15ºC. None (0 positive from 10 total) of the batches of unwashed eggs cultured bacteria from their contents.

Eggs warmed to 37°C, 30°C, 25°C or 20°C were submerged in aqueous protein stain at 15°C for up to 7 minutes and hard boiled to determine if visible volumes of stain were taken into the egg contents. We did not observe take up of the stain solution unless it was 15°C cooler than the egg contents and eggs were immersed for at least 3 minutes.

Introduction

Avian eggs have evolved to protect the embryo and allow its development to the point at which it is able to hatch. Non-domesticated egg incubation environments are frequently contaminated not only with microbes, but also physical hazards such as mud and water. In spite of these challenges, the successful hatch rates for eggs incubated in the wild is remarkably high (Sparks, 1985). This success is due in part to the complex chemical and physical defence systems that the egg has developed that either prevents or hinders the movement of bacteria from the shell into the contents of the egg (Hutchison *et al.*, 2003). However it has been repeatedly demonstrated (Board *et al.*, 1986; Hutchison *et al.*, 2004) that water on the shell surface can undermine an egg's physical defences. Furthermore, if water contaminated with significant amounts of iron or organic matter enters the egg, the chemical defences can be compromised (Garibaldi, 1970). It is not unsurprising therefore that the washing of eggs can be associated with an increased incidence of eggs rotting during storage as a result of microbial action (Moats, 1978).

It is recognised that a main line of defence of the eggshell is the presence of the cuticle layer that plugs open pores and prevents bacterial penetration taking place. Washing eggs can erode the cuticle (Sparks, 1994), but the incidence of internal contamination resulting from washing may be reduced significantly provided certain basic rules are followed. In particular eggs should not be washed in water which is cooler than the egg contents because this can draw wash water into the egg as the egg contents cool and contract (Bartlett *et al.*, 1993; Leclair *et al.*, 1994). Additionally, if the wash water is too hot, thermal cracking of the shell can occur. While modern continuous washing machines do contain a number of failsafe mechanisms to help ensure that egg washing takes place under optimum conditions, the same often does not apply to simpler batch-type bucket washers. This style of washers allow the eggs to become completely submerged in water. Furthermore since they are simple to operate and inexpensive, they are frequently used by small- and medium-scale producers as a cost-effective way of removing organic material from the shell of table eggs. In most EU member states, washed eggs should only be sold as a class B product and be used only for processed food. Washing of grade A eggs in the EU is currently limited those establishments which, on June 1 2003, were

approved by their national governments. The derogation applies only until 31 December 2006 and does not cover bucket washing, however the low cost and simplicity of this style of washer, combined with a lack of understanding of legal and microbiological issues may mean that compromised food safety results from inappropriate use of these machines.

This paper reports preliminary results from an ongoing series of experiments designed to assess the risks of bacterial contamination of egg contents if bucket washers are operated under inappropriate washing conditions.

Materials and methods

MICROBIOLOGICAL ANALYSES

Serial tenfold dilutions of egg contents or shell-sonicated washings were carried out in MRD for total viable aerobic bacterial counts (TVC) and coliform enumerations. Samples were plated out in duplicate onto Plate Count Agar (Oxoid) and incubated at 30°C for 3 days to determine TVC. For coliform numbers, samples were plated out in duplicate onto Violet Red Bile Agar (Oxoid) and incubated at 37°C for 24 hours.

BUCKET WASHING STUDIES

Studies were conducted using a Rotomaid 100 bucket-style egg washing machine. Unless stated otherwise, standard wash water conditions (3 minutes immersion into a wash water temperature of 38°C), as recommended by the manufacturer, were used. The wash water temperature was measured using a calibrated thermometer. The washing agent used was Antec egg wash powder (Antec, Suffolk, UK) at a concentration of 0.6% (w/v) according to the manufacturer's instructions. Eggs were obtained from a free-range production system and a commercial cage unit and were washed within 24h of lay. Eggs were candled pre- and post washing and those with cracked shells were excluded from any analysis. The purpose of including eggs from two systems in each experiment was to test the ability of visibly dirty eggs (obtained from the free-range unit) to contaminate nest clean eggs (taken from the cage unit).

EFFECT OF WASHING ON SHELL SURFACE BACTERIAL NUMBERS

Eggs (n=100) were collected from the belts of a standard commercial laying flock within 2h of lay. Eggs were randomly sorted into two groups (n=50 each group) and one group was washed using standard conditions. The other control group was unwashed. TVC were determined from the shell surface only.

REUSE OF WASH-WATER AS A VECTOR FOR CROSS CONTAMINATION OF SUBSEQUENTLY-WASHED EGGS

On two occasions, ten batches of eggs (n=100) were washed over a five day interval without replacing the wash water (i.e. two batches per day). Wash water temperature was allowed to cool to ambient temperature (22-25°C) between daily runs. Each batch of eggs consisted of 80 soiled eggs from a free-range unit and 20 visibly clean eggs.

After each batch of eggs was washed, the clean eggs and a random selection of the soiled eggs (n=20) were removed for determination of TVC and coliform numbers. Control samples of unwashed clean and unwashed soiled eggs were also tested on each day. Samples of wash water (one at the beginning and one at the end of each wash) were assessed for TVC and numbers of coliforms.

WASH-WATER WITH SANITISER ADDED AT REDUCED CONCENTRATION

Five batches of eggs (n=100) were washed in one volume of potable water without added sanitiser, or with sanitiser added at 50% or 100% the manufacturer's recommended concentration. Surface and content samples from unwashed visibly clean and soiled eggs, washed clean and soiled eggs and wash water samples were tested to determine TVC and coliform numbers as described above.

STORAGE OF INAPPROPRIATELY-WASHED EGGS

Ten batches of soiled eggs (n=100) were washed over a five day interval (i.e. two batches per day) in the same wash water. Washing chemicals were added at a concentration of 0.6% (w/v) at the beginning of the experiment and no subsequent additions or replacements were made. Wash water temperature was allowed to cool to ambient temperature (22-25^{o}C) between daily runs. After each batch of eggs had been washed, 20 eggs were selected at random and removed for bacteriological testing of contents. The remainder of the batch was stored (15^{o}C) for 14 days after which a further 20 eggs were randomly selected for bacteriological testing of contents. TVC and numbers of coliforms in unwashed eggs from the same laying flock (n=20) on day 0 and day 14 were assessed as comparative controls.

WASH WATER TEMPERATURES AND CONTAMINATION OF EGG CONTENTS

Batches of eggs were incubated for 3-4h at either 37^{o}C, 30^{o}C, 25^{o}C or 20^{o}C. The eggs were removed from the incubator and completely immersed in a dilute filtered solution of Coomassie Blue protein stain [0.4% (w/v) Coomassie blue, 0.5% (v/v) glacial acetic acid, 2.5% (v/v) methanol, 96.5%(v/v) distilled water] which had been chilled to 15^{o}C. Immersion was for 3 min (n=20), 5 min (n=20) or 7 min (n=20). Excess stain was allowed to drain from the eggs at ambient temperature (24^{o}C). Eggs were stored air sac pointing upwards for up to 1h, before being hard boiled for immersing them in ambient temperature water and gradually raising its temperature over 12 minutes to 90^{o}C. Shells were carefully removed and individual blue spots on the shell-side surface of the shell membranes were counted.

Results

EFFECT OF WASHING ON SHELL SURFACE BACTERIAL NUMBERS

Log mean total bacterial numbers on the surfaces of unwashed eggs taken from the belts of a standard commercial battery flock were 4.75 log CFU egg^{-1}. After washing under manufacturer-recommended conditions, the total bacterial numbers were reduced to 3.32 log CFU egg^{-1}. A Mann Whitney comparison determined that the reduction was significant ($P<0.01$).

WASH-WATER WITH SANITISER ADDED AT REDUCED CONCENTRATION

The total numbers of bacteria recovered from the shells of originally clean and soiled eggs were analysed *en masse*. Comparisons were made between eggs washed without any sanitiser and with sanitiser at 100% or 50% manufacturer-recommended concentration and it was found that washing with sanitiser reduced significantly ($P<0.05$; t-test) shell surface total bacterial numbers (Figure 1) when compared with water that did not contain sanitiser. Coliform reductions were not significant when sanitiser was used in the wash water. A likely reason for this finding relates mainly to the fact that only small reductions were observed to the already low numbers of coliforms that were present initially on the visibly clean eggs. Bacteria were not recovered from any of the egg contents during this experiment.

REUSE OF WASH-WATER AS A VECTOR FOR CROSS-CONTAMINATION OF SUBSEQUENTLY-WASHED EGGS

A summary of our findings are shown as Figure 2. Analysed *en masse*, there were no statistically significant differences (ANOVA; $P>0.05$) between the log numbers of bacteria on the shell surfaces of the washed eggs and the unwashed controls. There was however a highly significant ($P<0.001$) positive correlation between the total number of bacteria recovered from the shells of the visibly clean eggs and the number of bacteria recovered from the wash water which suggests that clean eggs can be contaminated by bacteria in the wash water. The association was only for clean eggs because there was no correlation between the total number of bacteria recovered from the shells of soiled eggs and the number of bacteria recovered from the wash water. The bacterial numbers in the wash water steadily increased over the 5 day period (Figures 2E and 2F). A likely explanation for this observation relates to the active agent in the sanitiser. Since the antimicrobial activity was chlorine-based, we expect that it would have been rapidly neutralised by the accumulation of organic material from the

soiled eggs. As before, bacteria were not recovered from any of the egg contents during this experiment.

STORAGE OF INAPPROPRIATELY-WASHED EGGS

Although the previous experiment showed that washing 1000 moderately dirty eggs in the same wash water over 5 days did not cause any increase in the number of bacteria detected in the egg contents, there have been historical reports of increased spoilage of washed eggs (Moats, 1978). One of the two batches of eggs washed on day three of the study contained detectable TVC (1.0 Log CFU egg $^{-1}$) and numbers of coliforms (1.0 Log CFU egg^{-1}) in a single pooled (n=10) sample of egg contents. Aside from this single exception, storing eggs (washed or unwashed) for 14 days did not cause any increase to the detection of bacteria in the egg contents. These are preliminary results and there has not been enough replication of this experiment to determine by Chi-Squared or the Fisher's Exact Tests whether these findings are significant.

WASH WATER TEMPERATURES AND CONTAMINATION OF EGG CONTENTS

A summary of liquid uptake by eggs which were warmed to a temperature higher than a dye solution into which they were immersed is shown as Table 1. The majority of dye spots were observed at and around the air sac end of the egg. Although the eggs were treated identically, there was a range of susceptibilities to the dye; some eggs had far more dye spots than others. This observation is the reason why there are large standard deviations associated with some the means shown in Table 1. Although 20 eggs were used for each experiment, an average of 2 or 3 eggs from each batch cracked during boiling and had to be discarded.

Discussion

The degree of soiling of the eggs used in this preliminary series of experiments was typical to that found in cage or free-range commercial production units. The cage-produced eggs that were used were visibly clean. For the free-range production studies, a mixture of visibly clean and eggs soiled with faeces and or other organic material were used. Eggs grossly contaminated with organic material were not included in the study since these would normally be discarded immediately after collection. Thus these experiments were a model for bucket-washing under typical commercial conditions.

When eggs from a commercial laying cage were washed under manufacturer-recommended conditions, there was a significant 1.5 log reduction in the total bacterial load associated with the shell surface. The majority of eggs from the free-range laying facility all had visible soiling. When these eggs were washed, the results were less clear-cut. In one experiment, when the bacterial numbers from clean and dirty eggs were analysed together and compared with 100%-concentration sanitiser, it was found that TVC were significantly lower than when no sanitiser was used. Although there was a general trend of fewer coliforms on the surfaces of sanitiser-washed eggs, coliform numbers were not significantly lower. Thus, although bucket-style washing does not sterilise the shell surfaces, egg washing under ideal conditions offers benefits in terms of reducing shell surface contamination and possibly cross-contamination between eggs.

When bacterial numbers from visibly clean and soiled eggs from a free-range production unit were analysed individually under conditions of worst practice, there was evidence that the washing process could increase shell bacterial numbers on the visibly clean eggs. Since there was a clear and significant correlation between the bacterial load in the wash water and the subsequent load on the shells of nest clean eggs, it seems plausible that under conditions of low sanitiser, bacteria could transfer from the surfaces of dirty eggs to the shells of clean eggs via the wash water. One very surprising finding of these studies was that it was very difficult to contaminate the egg contents using a bucket washer. Eggs which were washed in 5 day-old water which lacked active sanitiser and which contained enough bacteria to increase the numbers of total bacteria on the surface of the visibly clean shells, still did not have gross contamination of their contents. Previously we, and other groups, have reported the importance of maintaining wash and rinse water temperatures at least 5-15^{o}C above that of the egg contents (Bartlett *et al.*, 1993; Hutchison *et al.*, 2004; Leclair *et al.*, 1994; Lucore *et al.*, 1997;). All of the bacterial-based experiments in this study used wash water which was 12-16^{o}C higher than the egg contents. The role of temperature in prevention of wash water and bacterial ingress into the contents was investigated by immersing eggs into solutions of Coomassie blue at different temperatures. Although the sensitivity of dye-based experiments is probably not be equal to bacterial-

based studies, such studies are useful for the determination of gross contamination of egg contents. Dye solution that was 15°C lower than the egg contents and immersion of 3 minutes was required for visible staining of the egg contents. Thus the maintenance of an increased temperature difference for the wash water over the egg contents was probably a contributory factor in protecting the egg contents from bacterial contamination. However, the efficiency of innate egg defences, such as the shell membranes, and the iron chelation and lysozyme activities of the albumen in preventing the movement and growth of organisms into the egg contents should also be considered.

Innate egg defences are important for the long term storage of washed eggs (Moats, 1978). The storage of washed eggs has been previously reported to increase the number of eggs spoiling stored (Moats, 1978). With one exception, our findings were that there was no gross contamination of the contents of eggs stored for 14 days at 15°C after washing. Humphrey (1994) has noted that during ambient storage the vitelline membrane can break down, releasing iron into the egg white and allowing organisms passage into the yolk contents. Storage temperature is a key factor in this process and currently we are investigating the role of storage temperature on the bacterial contamination of washed egg contents.

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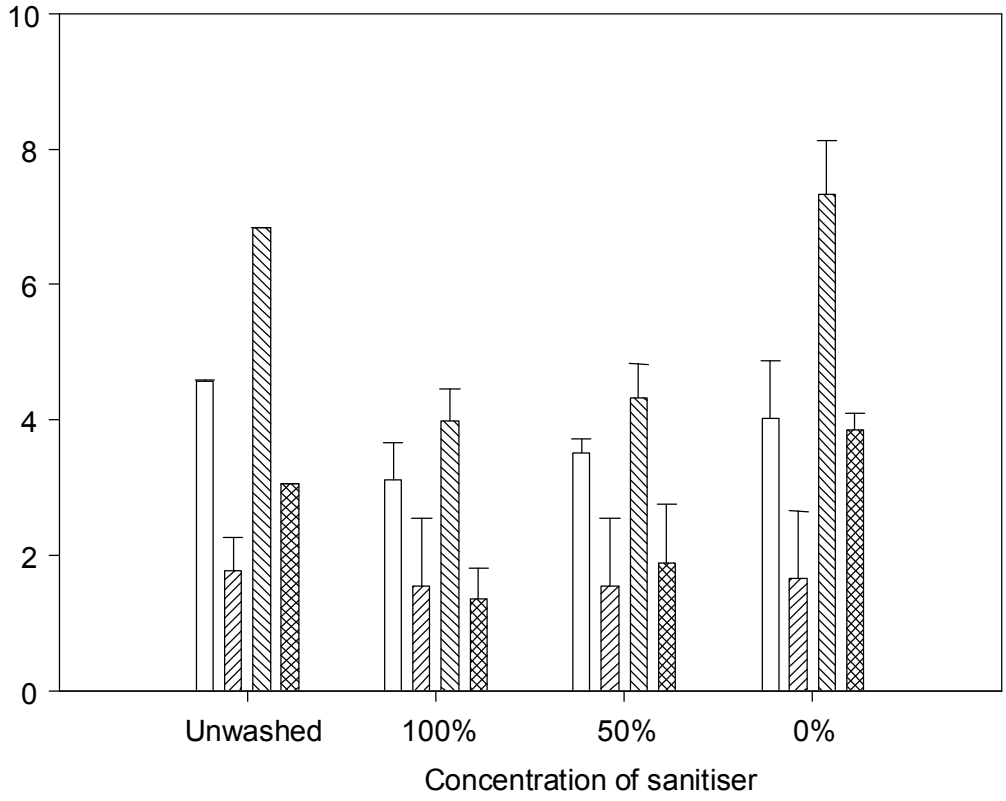

Figure 1 Bacterial counts associated with the shell surface of clean and soiled eggs washed in 100%, 50% and 0% of the manufacturer recommended sanitising agent. The TVC on clean eggs are depicted as □, numbers of coliforms on clean eggs as ▨, TVC on soiled eggs as ▧, and the numbers of coliforms on soiled eggs as ▩. Data for each bar are derived from five individual experiments and 50 eggs in total. Error bars are the standard deviation of the mean. Where no detections were made, half of the theoretical limit of detection (3.5×10^{1} CFU egg^{-1}) was substituted.

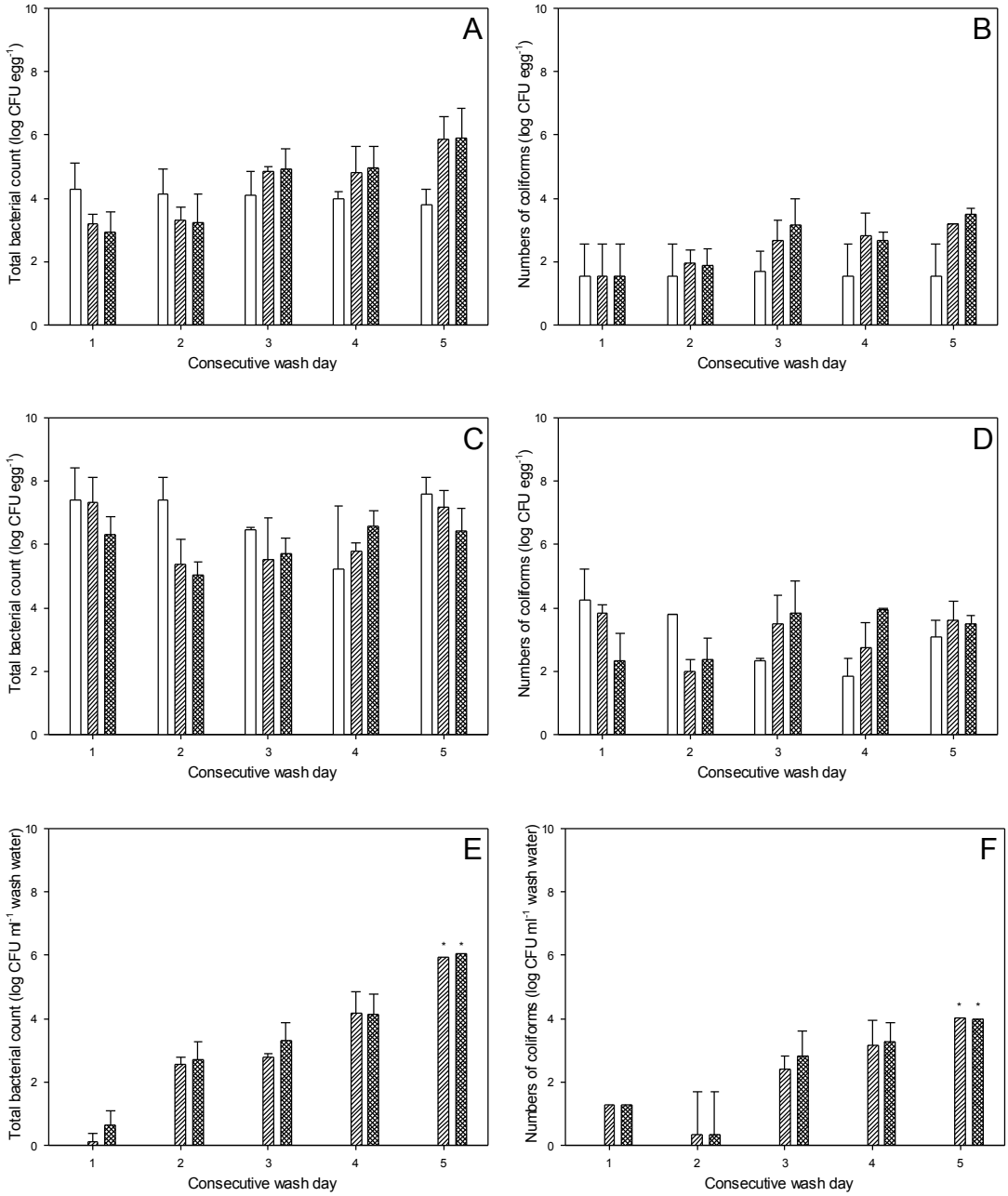

Figure 2 Bacterial counts associated with the shell surfaces of clean (A and B) and soiled eggs (C and D) washed on consecutive days without changing the wash water (E and F). Unwashed controls are depicted as □, bacterial numbers derived from the first batch of eggs washed on each day as ▨, and bacterial numbers from the second batch of eggs washed as ▩. The mean log numbers of total bacteria (A and C) and coliforms (B and D) on shell surfaces are shown. Data for each bar (A-D) are derived from two experiments and 40 eggs in total. Error bars are the standard deviation of the mean. Wash water determinations were undertaken on one experiment only for bars marked *. Where no detections were made, half of the theoretical limit of detection (3.5×10^1 CFU egg^{-1}) was substituted.

Table 1 The mean numbers of Coomassie blue dye spots counted on the shell membranes of eggs warmed to the temperatures shown and immersed in dye solution at 15°C for the lengths of time shown. Numbers shown in brackets are the standard deviation of the mean of up to 20 replicates.

Immersion time (minutes)	Egg contents temperature (°C)			
	20	25	30	37
	Mean number of dye spots counted (standard deviation)			
3	0 (0)	0 (0)	0.047 (0.22)	2.381 (1.56)
5	0 (0)	0 (0)	0.381 (0.74)	3.095 (1.48)
7	0 (0)	0 (0)	1.238 (1.37)	12.381 (9.87)