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Egg washing: Improving efficacy and safety to optimise profitability

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Sub-Project No. 3.2.1

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Executive Summary

The annual production of eggs in Australia totalled 345 million dozen in 2009/10, of which 63.5% were cage eggs, 7.6% were barn laid, 26.6% were free range and 2.2% were organic (Australian Egg Corporation Limited, 2010). The vast majority of eggs are washed prior to packing to remove dirt and faecal material and to reduce the microbial contamination of the egg shell. Some eggs, known as 'black eggs', are so visually contaminated that industry currently do not attempt to recover them and it is estimated that these may constitute up to 2% of annual non-cage production (estimate based on discussions with egg producers). In addition, Food Standards Australia New Zealand estimates there are about 12,800 cases of egg-related salmonellosis per year in Australia, costing $44 million, and that the number of cases is rising (Anon, 2009).

The aims of this project were to improve the recovery of dirty and black eggs and to reduce microbial contamination on the egg shells, as measured by Total Viable Counts (TVC, used as hygiene indicator), *Enterobacteriaceae* (used as a faecal indicator and include *Escherichia coli* and *Salmonella*) and *Salmonella*. These aims were achieved through a series of laboratory and in-plant trials.

In the first of three laboratory trials three commercial chemical detergent and sanitiser combinations were assessed for their ability to clean artificially dirtied eggs from two hen ages. The detergent was applied either at 30 or 40°C and the sanitizer at 2°C higher or 8°C lower than the detergent. The two most effective combinations were

- Circhlor, a liquid alkaline chlorine based product, at 40°C used with Virogard, a liquid quaternary ammonium compound (200 ppm) based sanitiser, at 42°C, and
- Asepto LF, a liquid sodium hypochlorite based product, at 40°C used with Prochlor, a liquid sodium hypochlorite (200 ppm) based sanitiser.

These combinations were subsequently used in two further laboratory trials, which involved washing of black eggs and washing of eggs inoculated with *Salmonella*. Under the conditions used in this study, both combinations were effective at recovering black eggs after two repeat washes. TVC were reduced by over 3 $\log_{10}$ cfu/egg and the prevalence and concentration of *Enterobacteriaceae* were also reduced considerably. *Salmonella* was removed from inoculated eggs (10^6 cfu/egg) by washing with water alone, though greater reductions in *Salmonella* prevalence and concentration were observed when one of the two chemical combinations was used. While the differences between the two chemical combinations were not significant, Asepto LF and Prochlor resulted in a 13% (2/15) prevalence, while Circhlor with Virogard resulted in 33% (5/15) prevalence. However, three of five *Salmonella* detections after washing with Circhlor and Virogard were at 10^4 cfu/egg, though it is unknown why. The wash chemicals also reduced the cuticle coverage which could result in easier penetration of micro-organisms of the egg shell and this is being investigated in a companion Poultry CRC Sub-Project 3.2.2.

The efficacy of the two chemical combinations – Circhlor/Virogard and Asepto LF/Prochlor – to recover black eggs was also assessed during in-plant trials at three commercial egg washing businesses. Under the conditions used in this study, multiple washing with either of these chemical combinations resulted in an average of 29, 57 and 85% recovery of black eggs after two, three and four washes, respectively. However, the efficacy of the chemicals varied between plants and was affected by plant specific issues such as ability to accurately dose chemicals, blocked spray jets, brushes interfering with sprays, ability to measure and maintain the water temperature at the egg surface and recycling of water.

It was clear from the in-plant visits that egg handling and grading equipment has not been designed with ease and effectiveness of cleaning in mind. For example, much of the equipment has exposed electronic equipment which cannot withstand normal cleaning

processes. As a result, egg processing plants are faced with a difficult and expensive job to keep machinery clean which is not always effective, as evidenced by the presence of *Enterobacteriaceae* on grading equipment. Such contamination provides the potential for microbial cross-contamination of eggs following washing, increasing the food safety risk. Consequently, improvements in machinery design along with industry tailored cleaning chemicals and application methods would assist industry to minimise this risk.

While the benefits of washing eggs continue to be debated, this work clearly demonstrates that washing can remove faecal and microbiological contamination from the egg surface. Based on the 2009 production volumes of 125.9 million dozen non-cage eggs (AECL, 2010), the estimated 2% black eggs result in a total of 2.52 million dozen black eggs. If up to 85% of these can be recovered, then the potential retail value could be $9.48M and these figures are expected to increase as barn-laid, free range and organic egg production gain market share. In addition, reducing microbial contamination of egg shells through washing and preventing re-contamination during grading will help make eggs safer for the consumer and reduce the potential for foodborne outbreaks.

Contents

Introduction

The annual production of eggs in Australia totalled 345 million dozen in 2009/10, of which 63.5% were cage eggs, 7.6% were barn laid, 26.6% were free range and 2.2% were organic (Australian Egg Corporation Limited, 2010). The vast majority of eggs are washed prior to packing to remove dirt and faecal material and to reduce the microbial contamination of the egg shell. Eggs which are not visually clean after washing are frequently diverted for pasteurisation, along with cracked eggs, resulting in substantially lower returns to the processor.

The washing process consists of several stages – pre-washing, washing with the aid of a surfactant/cleaner, sanitising and blow-drying – and can take less than 30 seconds. Hence, the effectiveness of the surfactant's ability to penetrate and remove dirt and faecal matter is critical for the recovery of table eggs. An effective cleaner can also assist in the removal of bacteria while a suitable sanitiser, together with a clean post-wash processing environment, will assist in maintaining the hygienic status of the eggs.

It is estimated by the authors that dirty eggs constitute between 5 and 20% of total production, depending on the production system and management practices. Of these, up to 50% may not be recovered, depending on the operation and degree of contamination. In addition, up to 2% of non-cage eggs are black eggs (estimate based on discussions with egg producers) – these are deemed so visually contaminated that they cannot be recovered using current washing methods and hence are discarded.

Production of visually clean eggs, free from dirt and faecal contamination, is the primary concern in the supply of table eggs. However, clean eggs do not guarantee food safety, with *Salmonella* spp. being the main pathogen of interest internationally. In Europe and the USA *Salmonella* Enteritidis Phage Type 4 (PT4) is of greatest concern because of the ability of this organism to use the trans-ovarian transmission route into the egg. Even so, the majority of the published literature is on the effectiveness of different egg washing and processing methods to reduce or eliminate *Salmonella* Enteritidis from the outside of egg shells.

Salmonella Enteritidis PT4 has not been detected in the Australian egg industry, however, other *Salmonella* strains are still of interest. Previous work undertaken for the Australian Egg Corporation Limited (Daughtry *et al.,* 2005) indicated that *Salmonella* contamination of unwashed eggs in Australia was very low. Daughtry *et al*. (2005) found that these figures compare favourably to other countries with similar production systems.

Despite the low prevalence of *Salmonella* contamination of egg shells in Australia, outbreaks of *Salmonella* associated with eggs are still very common (Anon, 2009; OzFoodNet Working Group, 2010). Additional reductions in *Salmonella* contamination due to effective egg washing would further reduce the risk to consumers. Effective egg washing is also expected to improve the general microbiological status of eggs, as determined by Total Viable Counts (general hygiene indicator) and *Enterobacteriaceae* (indicator of faecal contamination).

The primary objective of this project was to improve the effectiveness of current washing practices to reduce the proportion of eggs downgraded or disposed. The secondary objective was to reduce potential food safety risks through the reduction of enteric microorganisms on the egg shell surface.

Objectives

1. Identify and trial effective egg washing chemicals to improve the visual appearance of eggs.

   Laboratory based trials will be undertaken to assess three surfactant and sanitiser combinations. These trials will be performed on eggs which are naturally and artificially contaminated with dirt and faeces. The most effective chemicals will then be assessed in several trials undertaken in commercial settings. The outcome will be improved recovery of table eggs which will provide economic benefits to egg processors.

2. Assess the effectiveness of surfactant and sanitiser combinations at improving the hygiene and safety of eggs.

   Laboratory trials to assess the effectiveness of reducing visual contamination will also be utilised to assess the effectiveness in reducing hygiene and faecal indicators on the surface of egg shells. Eggs will also be artificially inoculated with *Salmonella* and the reduction in levels after washing and sanitising will be measured.

Laboratory-Based Trials

Methods

To achieve the objectives of this project, a series of laboratory-based experiments were undertaken on artificially contaminated eggs to investigate the efficacy of three chemical/sanitiser combinations at different temperatures. The findings from this work then informed in-plant trials which were undertaken at three commercial egg washing plants.

Method for Making Artificially Dirty Eggs

A number of different approaches to making representative 'dirty' eggs were trialled using chicken faeces collected at an egg farm. It was originally envisaged that eggs would be coated with chicken faeces at five separate points using a template so that the total area cleaned could be calculated. After trialling different methods, however, this was found to be not feasible. It was then decided to cover the whole egg with faecal slurry. This method (detailed below) gave the most consistent coverage of eggs akin to that of naturally dirty eggs:

- Sterile water (2.5 mL) was added to chicken faecal matter (10 g) that had all feathers removed.
- This was mixed with a sterile wooden spatula until a consistency similar to fresh chicken faeces was achieved.
- The faecal slurry was then smeared onto clean eggs and massaged onto the eggs by hand to give an even coating.
- The dirty eggs were then carefully placed on three nails embedded in a wooden plank (to minimise surface contact with the egg) and dried overnight in a fume cupboard.

This method resulted in eggs that appeared similar to naturally dirty eggs collected from an egg farm (Figure 1) and hence was used for the production of dirty eggs used in Trial 1.

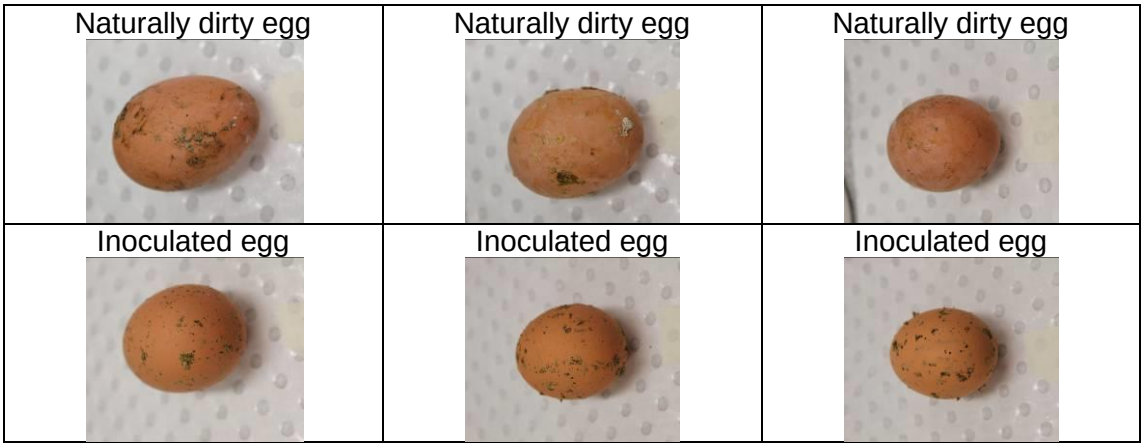


Figure 1: Naturally dirty and inoculated eggs. Eggs inoculated with a faecal slurry are visually similar to naturally dirty eggs.

Two different hen ages were used during these laboratory-based experiments: eggs from hens 20-40 weeks old and eggs from hens >50 weeks old. These were included to allow for differences in egg size and cuticle deposition between young and older hens.

Design of Laboratory Based Egg Washer

A laboratory based egg washer was built and installed at PIRSA Animal Health, Glenside, South Australia (Figure 2). The pump, fittings and spray system were built with materials

resistant to all chemicals used. Spray nozzles and rollers were sourced from and/or selected based on commercial washers currently used by egg processors (Solar Eggs, South Australia). Whilst brushes are often used in the commercial washing of eggs, they were not included in the laboratory based egg washer due to the potential for cross-contamination. The system was able to wash 15 eggs at a time. To wash the eggs, two water baths were filled with tap water and heated to the required temperature. The cleaner was dosed in one water bath and the sanitiser in the other. Prior to use, the spray system was flushed with hot water to warm the pipes and nozzles and hence prevent temperature loss between the water baths containing the cleaner and sanitiser, the hoses and the eggs. Eggs were placed in the washer in three rows of five on commercial rollers which were turned using a drill on a slow, fixed setting (10 rpm).

Figure 2: The laboratory based egg washer

Trial 1 – Washing Artificial Dirty Eggs

The cleaner/sanitiser combinations used to wash the eggs are outlined in Table 1. These were selected as they are the most widely used by industry (G. Bourne [Chemetall] and C. Kidd [Ecolab]). The chemicals and concentrations used were as follows:

- Circhlor (Chemetall): A liquid alkaline (pH 12) chlorine based product used at 1% solution (v/v). This was used with a liquid quaternary ammonium compound (QAC) (200 ppm) based sanitiser called Virogard.
- Asepto LF (Ecolab): A liquid sodium hypochlorite based product used at 0.45% solution (v/v) with a liquid sodium hypochlorite (200 ppm) based sanitiser called Prochlor.
- Automate (Ecolab): A solid alkaline (pH 12) chlorine based product used at 1% (w/v) also used with Prochlor.

The order of these treatments was selected at random. All products were used at concentrations recommended by the manufacturer.

Table 1: Cleaner/sanitiser combinations to be trialled for washing of soiled eggs

Cleaner	Sanitiser
Circhlor	Virogard
Automate	Prochlor
Asepto LF	Prochlor
Water	Water

The two detergent temperatures were chosen for the following reasons as per Srikaeo and Hourigan (2002):

- 30°C was chosen because often in winter time, eggs exposed to cold environmental temperatures just prior to washing may have a shell temperature between 5 and 10°C and if they are exposed to the normal wash temperatures of 40°C then thermal cracking is likely to occur because of the greater than 27°C temperature differential (Srikaeo & Hourigan, 2002).
- 40°C was chosen because it is frequently used by industry, as it is considered that this is a higher temperature than the internal temperature of the egg soon after it is laid and also the wash chemicals are more effective at higher temperatures.

The combination temperatures were chosen for the following reasons:

- The combination with the sanitiser temperature being 2°C higher than the wash water was chosen because this is standard industry practice where it is considered that it is safer to have water temperatures increasing so bacteria are not drawn into the shell structure.
- The combination with the sanitiser temperature being lower than the wash water (by 8°C) goes against standard practice and was selected to confirm work carried out by Jones *et al* (2006). Those researchers showed benefits of drawing the clean sanitiser solution into the shell structure to increase its effect against microorganisms lodged there. In addition, chlorine is frequently used as the sanitiser and it is less effective at high temperatures.

The temperature of the cleaner and sanitiser was checked prior to use using a thermometer within the spray at egg level. Quaternary ammonium compound concentration was determined as appropriate using pHydrion Papers QT-40. Chlorine concentration was determined using either Precision Chlorine Test Paper or by titration using a Chlorine Test Kit (Ecolab).

A single cleaner/temperature combination was used per day. These are outlined in Tables 2 and 3. Eggs were at room temperature at time of use.

Eggs were selected from two different age groups of birds from commercial farms where production was standard for their age. The two age groups were 20-40 weeks of age and over 50 weeks of age. This was done to determine if age of hens had an effect on egg washing efficacy and this was further assessed in the collaborative Poultry CRC Sub-Project 3.2.2 entitled “Eggshell quality and risks of food borne pathogens.”

The treatment order was randomised within each day and between days. Each cleaner/temperature/hen age combination was used once. The washed eggs were then removed aseptically from the washer, photographed and assessed for total viable count (TVC) and *Enterobacteriaceae*.

Microbiological Assessment of Washed and Unwashed Eggs

To determine TVC and *Enterobacteriaceae* counts, washed and control eggs were placed aseptically into individual sterile stomacher bags. Sterile peptone saline solution (PSS) (10 mL) was added and the bag gently shaken by hand for two minutes. Serial decimal dilutions (1 mL) were plated onto 3M™ Petrifilm™ Aerobic Plate Count Petrifilm and incubated at 35°C for 48 hours to determine the TVC per mL of rinse. To determine the total *Enterobacteriaceae* count per mL of rinse, serial decimal dilutions of the rinse were plated onto 3M™ Petrifilm™ *Enterobacteriaceae* Count Plates and incubated at 35°C for 24 hours.

Table 2: Treatments for eggs from hens 20-40 weeks old

Cleaner	Temp	Unwashed	No Sanitiser	Sanitiser Low	Sanitiser High
Circhlor	30°C	8	13	13	13
Circhlor	40°C	8	13	13	13
Automate	30°C	8	13	13	13
Automate	40°C	8	13	13	13
Asepto LF	30°C	8	13	13	13
Asepto LF	40°C	8	13	13	13
Water*	30°C	8	13	13	13

* Denotes that only one temperature was used for washing eggs from young hens due to limited availability of eggs

Table 3: Treatments for eggs from hens more than 50 weeks old

Cleaner	Temp	Unwashed	No Sanitiser	Sanitiser Low	Sanitiser High
Circhlor	30°C	8	13	13	13
Circhlor	40°C	8	13	13	13
Automate	30°C	8	13	13	13
Automate	40°C	8	13	13	13
Asepto LF	30°C	8	13	13	13
Asepto LF	40°C	8	13	13	13
Water*	40°C	8	13	13	13

* Denotes that only one temperature was used for washing eggs from older hens due to limited availability of eggs

Cuticle Assessment

From each experimental treatment combination, two eggs were stained for one minute with MST Cuticle Blue (MS Technologies Limited, UK).

A light box was set up with 2 16 W Daylight Fluorescent tubes. A 10% grey card was used to determine the white balance which was set in-camera. Stained eggs were then placed inside the light box and all external light was blocked using a light-proof curtain. Each egg was then photographed using a Canon 400D digital SLR camera using an ISO of 100, aperture f/32 and ½ sec exposure time. The camera saved the resulting image as a JPG file using the Natural picture style, which does not modify brightness, colour or contrast.

The resulting JPG files were processed using the open-source Gnu Image Manipulation Program (GIMP: http://www.gimp.org/). For each image, the egg was selected using the scissor selection tool and the average RGB colour calculated across the selection using the average colour plug-in (http://registry.gimp.org/node/16678).

The RGB values were entered in the R software and converted to the L*a*b* (CIELAB) colour space using the ReadImages library (Loecher, 2012). The three coordinates of CIELAB represent different aspects of the colour:

- L* indicates the lightness with 0 = black and 100 = diffuse white
- a* indicates the level of green (negative values) and red/magenta (positive values)
- b* indicates the level of blue (negative values) and yellow (positive values)

Statistical Analysis

The variables used in the analysis of the microbiological data were:

- ***TVC:*** Total Viable Count (TVC) concentration (cfu/mL). The Limit of Detection (LOD) was 1 cfu/mL.
- ***Entero:*** *Enterobacteriaceae* concentration (cfu/mL). The LOD was 1 cfu/mL.
- ***Hen.Age:*** Age of the hen that laid the egg – 2 levels ("young" and "old"). Hen ages of 23, 24 and 25 weeks were coded as "young" and hen ages of 50, 54 and 69 were coded as "old."
- ***Detergent:*** Type of cleaner used to wash the egg – 4 levels ("Chemetall" (Circhlor), "Ecolab Asepto" (Asepto LF), "Ecolab Automate" (Automate) and "Water").
- ***Sanitiser:*** The sanitiser used – 4 levels ("unwashed", "no sanitiser", "sanitiser low", "sanitiser high"). "Sanitiser low" corresponded to a sanitiser temperature 8°C below the cleaner temperature and "sanitiser high" corresponded to a sanitiser temperature 2°C above the cleaner temperature. Sanitiser is nested within Detergent. The "unwashed" eggs were controls – they were not treated by the cleaner or a sanitiser, but were from the same batches of eggs as those that were washed. These were used to determine initial TVC and *Enterobacteriaceae* levels of inoculated eggs (for a specific Hen.Age and Temp).
- ***Temp:*** Temperature at which the eggs were washed – 2 levels ("30 deg" and "40 deg")
- ***Batch:*** Factor representing a batch of eggs – 12 levels. Each batch corresponds to a specific combination of Hen.Age, Detergent and Temp.

From visual inspection of the data it was clear that for both $\log_{10}$ TVC and $\log_{10}$ *Enterobacteriaceae* the variability of the results differed for each Sanitiser treatment within each batch of eggs.

The $\log_{10}$ TVC and $\log_{10}$ *Enterobacteriaceae*) data were analysed separately, but treated in the same way. A linear mixed effects model was fitted to determine differences in $\log_{10}$ TVC and $\log_{10}$ *Enterobacteriaceae* between the levels of Hen.Age, Detergent, Sanitiser and Temp and their interactions (Model 1). The data for Detergent="Water" were not included in this first model as these data existed for only two of the four treatment combinations of Hen.Age and Temp. Batch was fitted as a random effect. Since the variability was different within each Sanitiser treatment and Batch, the model allowed for different variances for each combination of Batch and Sanitiser. An ANOVA was used to test for significant differences in $\log_{10}$ TVC and $\log_{10}$ *Enterobacteriaceae* between the levels of Hen.Age, Detergent, Sanitiser and Temp and their interactions. Assumptions were checked using standard diagnostic plots. The function 'lme' in the 'nlme' package (Pinheiro *et al*, 2011) was used to fit the mixed effects models in R, version 2.11.1 (R Development Core Team, 2010).

A second model (Model 2) included the water only treatment, but excluded Hen.Age. This was due to the confounding of Hen.Age and Temp in the water only analysis and because Hen.Age is a variable not under the control of egg producers, unlike Temp, Detergent and Sanitiser.

For the cuticle assessment, the values of L*, a* and b* were compared only between Control eggs and eggs washed with either of the two most effective washing regimes using an Analysis of Variance.

Trial 2 – Washing Black Eggs

The most effective two cleaner/sanitiser/temperature combinations from Trial 1 were investigated for their ability to clean and sanitise black eggs. These combinations were

chosen based primarily on their ability make visually clean eggs, but their ability to reduce TVC and *Enterobacteriaceae* counts on artificially dirty eggs was also considered. An option sometimes utilised by industry is to wash black eggs multiple times to improve recovery. Therefore three treatment groups per trial were included in this trial (Table 4). These were:

- Unwashed control eggs
- Eggs washed once and sanitised once (SWS)
- Eggs washed and sanitised and then washed and sanitised a second time (DWS).

Eggs were room temperature at time of use. Each treatment was repeated twice (15 unwashed control eggs were assessed on each trial day). Photos were taken of all eggs before and after treatment. TVC and *Enterobacteriaceae* were determined as above.

Table 4: Treatment groups and number of eggs used for Trial 2

	Control	**SWS[1]**	**DWS[2]**	**Total**
Treatment 1*	15	15	15	45
Treatment 1	15	15	15	45
Treatment 2	15	15	15	45
Treatment 2	15	15	15	45
Total				**180**

* Denotes that treatment order was randomised.
[1] Eggs washed once and sanitised once
[2] Eggs washed and sanitised and then washed and sanitised a second time

There was a 15 minute interval between the first and second wash/sanitiser treatment for eggs in the DWS groups to allow the water baths to be refilled and reheated to the required temperature.

Statistical Analysis

The variables used in the analysis of the microbiological data were:

- ***TVC:*** Total Viable Count (TVC) concentration (cfu/mL). The Limit of Detection (LoD) was 1 cfu/mL.
- ***Entero:*** *Enterobacteriaceae* concentration (cfu/mL). The LoD was 1 cfu/mL.
- ***Enterobacteriaceae pos:*** Indicator variable for *Enterobacteriaceae*. Two levels – 0 (not detected) and 1 (detected).
- ***Detergent:*** Type of cleaner used to wash the egg – 3 levels ("Chemetall", "Ecolab Asepto" and “Water”).
- ***Wash:*** The number of washes used – 3 levels (“unwashed”, “single wash” and “double wash”). The “unwashed” eggs are controls for each detergent – they were not treated by the cleaner, but they were from the same batch of eggs as those that were.
- ***Rep:*** Factor representing the batch of eggs within each Detergent – 2 levels (1, 2).

From visual inspection of the data it was clear that the variability of the $\log_{10}$ TVC results differed for each wash method within each batch of eggs. A linear mixed effects model was fitted to determine differences in $\log_{10}$ TVC between the levels of Detergent and Wash and their interaction. Batch was fitted as a random effect. Since the variability was different within each Wash treatment and Batch, the model allowed for different variances for each combination of Wash and Batch. An ANOVA was used to test for significant differences in $\log_{10}$ TVC between the levels of Detergent and Wash and their interaction. Assumptions were checked using standard diagnostic plots. The function ‘lme’ in the ‘nlme’ package (Pinheiro *et al*, 2011) was used to fit the mixed effects model in R, version 2.11.1 (R Development Core Team, 2010).

Because there were too few detections on which to fit a model for the *Enterobacteriaceae* concentrations, a logistic regression model was fitted to investigate whether there was a difference in *Enterobacteriaceae* prevalence between the levels of Wash and Detergent and their interactions. Rep within Detergent was treated as a blocking variable. An ANOVA was used to test for significant differences in *Enterobacteriaceae* prevalence between the levels of Detergent and Wash and their interaction.

Trial 3 – Washing Eggs Artificially Contaminated with *Salmonella*

Salmonella Hoffitt was grown overnight at 37°C in 3 L of nutrient broth. This strain was used as it is the current laboratory reference culture. Visually clean eggs were subsequently contaminated with *Salmonella* Hoffitt by immersion for five minutes with gentle shaking (36 rpm) as per Hierro *et al*. (2009), except that eggs were dried in a biological safety cabinet for 30 minutes before use as per Keklik *et al*. (2010) instead of under cool flowing air for five minutes as described. Eggs were room temperature at time of use.

The two most effective methods for washing artificially dirty eggs (chemical treatment and temperature combinations) from Trial 1 were chosen based on their ability to reduce both TVC and *Enterobacteriaceae* counts on artificial dirty eggs. These were used to wash eggs as described above. Control and washed eggs were treated as per Trial 1 to determine total *Salmonella* per mL of rinse, except serial decimal dilutions (100 µL) were spread plated onto *Salmonella* Chromogenic Medium Agar (Oxoid).

Statistical Analysis

There were too few detections on which to fit a linear model for the *Salmonella* concentrations and too few detections for a logistic regression model for the *Salmonella* prevalence to produce reliable estimates. Fisher's Exact Test was used to investigate differences in prevalence between the treatments ("Unwashed", "Water", "Ecolab Asepto", "Chemetall"). All analyses were conducted in R, version 2.11.1 (R Development Core Team, 2010).

Results

Trial 1 – Washing Artificial Dirty Eggs

The following sections provide results obtained from washing artificially contaminated eggs.

Visual Assessment of Washed Eggs

Typical visual results from each of the washing trials are as follows:

Unwashed eggs:



Circhlor 30°C, hen age 24 wks:


No Sanitiser


Sanitiser 22°C

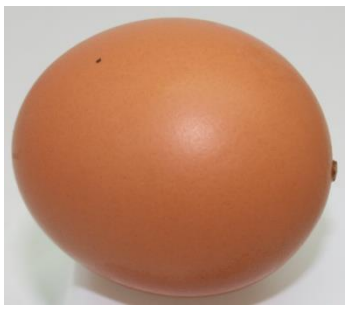

Sanitiser 32°C

Circhlor 30°C, hen age 50 wks



Circhlor 40°C, hen age 25 wks


No sanitiser


Sanitiser 32°C


Sanitiser 42°C

Circhlor 40°C, hen age 69 wks:

No sanitiser

Sanitiser 32°C

Sanitiser 42°C

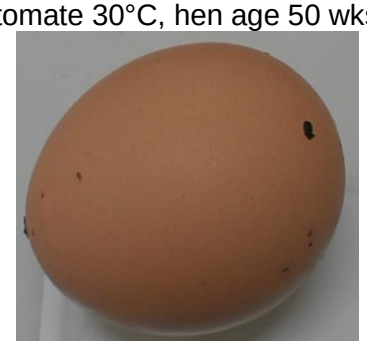
Automate 30°C, hen age 24 wks:

No sanitiser

Sanitiser 22°C

Sanitiser 32°C

Automate 30°C, hen age 50 wks



No sanitiser

Sanitiser 22°C

Sanitiser 32°

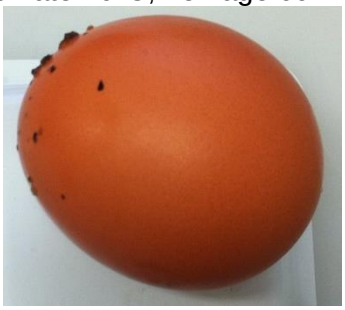
Automate 40°C, hen age 25 wks

No sanitiser

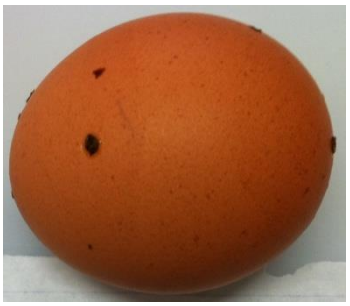
Sanitiser 32°C

Sanitiser 42

Automate 40°C, hen age 69 wks:



No sanitiser



Sanitiser 32°C

Sanitiser 42°C

Asepto LF 30°C, hen age 25 wks

No sanitiser

Sanitiser 22°C

Sanitiser 32°C

Asepto LF 30°C, hen age 69 wks:

No sanitiser

anitiser 22°C

Sanitiser 32°C

Asepto LF 40°C, hen age 24 wks:

No sanitiser

Sanitiser 32°C

Sanitiser 42°C

Asepto LF 40°C, hen age 50 wks

No sanitiser

Sanitiser 32°C

Sanitiser 42°C

Key observations

- Eggs cleaned under the conditions used in this study with Circhlor at 40°C and sanitised with Virogard at either 32°C or 42°C produced the visually cleanest eggs. This was closely followed by eggs cleaned with Asepto LF at 40°C and sanitised with Prochlor at either 32°C or 42°C.
- Eggs cleaned with Automate were often left with patches of faecal matter.

TVC

Box plots of the $\log_{10}$ TVC (cfu/ml) data for eggs from young and old hens are given in Figures 6 and 7, respectively.

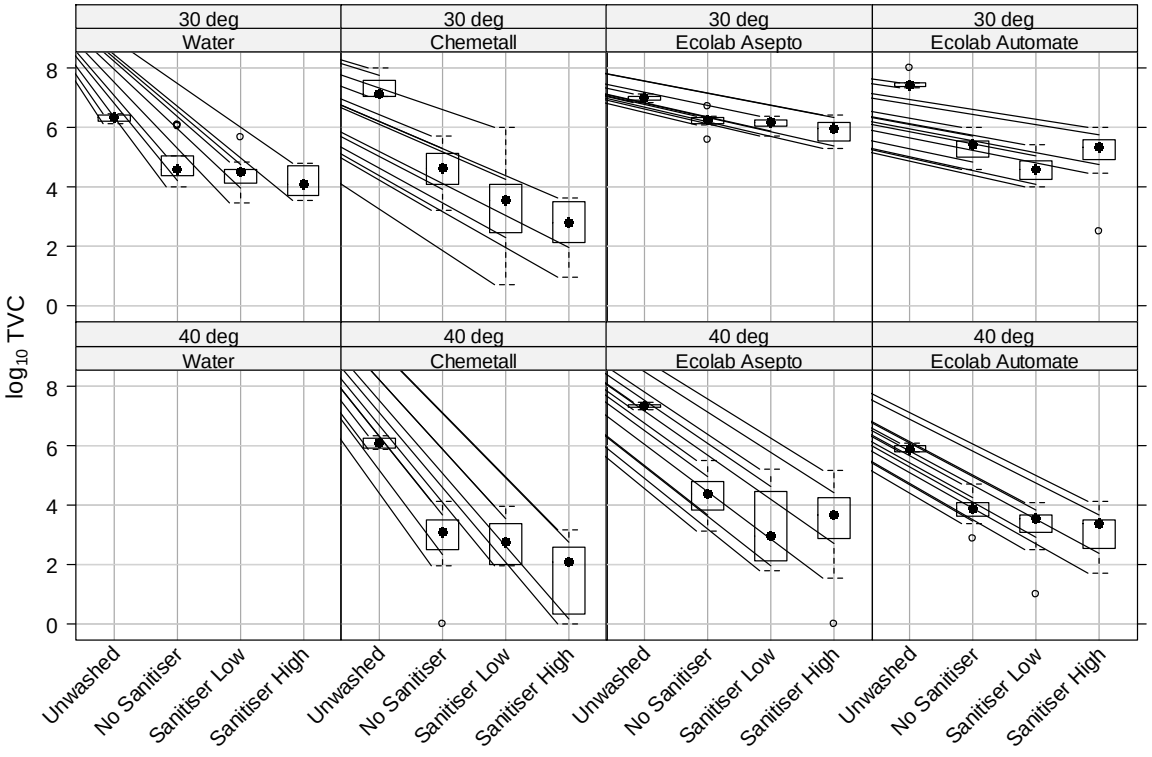


Figure 6: Box plots of $\log_{10}$ TVC (cfu/ml) for eggs from young hens

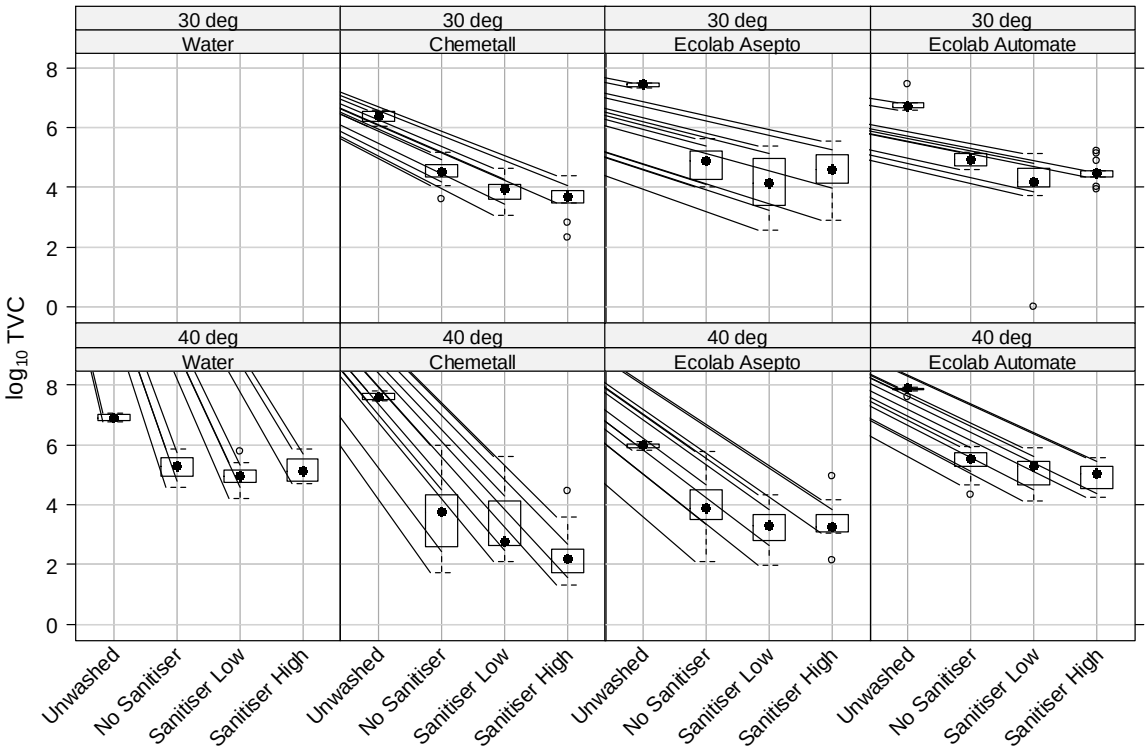


Figure 7: Box plots of log_{10} TVC (cfu/ml) for eggs from old hens

Key observations

- The biggest reduction in TVC was observed when Circhlor at 40°C and its associated sanitiser at 42°C (sanitiser high) were used to wash eggs laid by old hens.
- Asepto LF was not very effective at reducing the TVC on eggs laid by young hens when used at 30°C without its sanitiser.
- Washing with water (no detergent or sanitiser) was in some cases similarly effective to washing with a detergent.
- The variability in the results differs between the treatment combinations, indicating the need for a statistical model that can allow for these differences (i.e. a simple ANOVA was not appropriate).

There was a significant difference in log_{10} TVC between Hen Age, Detergent, Sanitiser and Temperature and their interactions. When the data for Water (control) were included in the model and Hen Age removed, the interaction between Detergent, Sanitiser and Temperature was significant. Within each batch of eggs (one combination of Hen Age, Detergent and Temperature) the difference between the estimate for each Sanitiser and the estimate for the unwashed eggs was calculated for each model. These differences are ranked from largest to smallest for Model 1 and Model 2 in Tables 5 and 6 respectively.

Table 5: Estimated microbial reduction between treatment combination and relevant control for log_{10} TVC (using estimates from Model 1) from largest to smallest

Detergent	Detergent Temp (°C)	Hen Age	Sanitiser Temp (°C)	Estimated Reduction (log_{10})
Circhlor	40	Old	42	5.29
Circhlor	30	Young	32	4.68
Circhlor	40	Young	42	4.47
Circhlor	40	Old	32	4.28
Asepto LF	40	Young	32	4.13
Circhlor	40	Old	None	4.01
Circhlor	30	Young	22	4.00
Asepto LF	40	Young	42	3.91
Circhlor	40	Young	32	3.38
Asepto LF	30	Old	22	3.36
Circhlor	40	Young	None	3.27
Asepto LF	40	Young	None	2.96
Asepto LF	30	Old	32	2.92
Automate	40	Old	42	2.90
Automate	30	Young	22	2.89
Automate	40	Young	42	2.82
Automate	30	Old	22	2.79
Circhlor	30	Old	32	2.76
Circhlor	30	Young	None	2.74
Automate	40	Old	32	2.72
Asepto LF	40	Old	32	2.69
Automate	40	Young	32	2.63
Asepto LF	30	Old	None	2.57
Asepto LF	40	Old	42	2.56
Automate	40	Old	None	2.43
Circhlor	30	Old	22	2.41
Automate	30	Young	32	2.38
Automate	30	Old	32	2.29
Automate	30	Young	None	2.22
Automate	40	Young	None	2.05
Asepto LF	40	Old	None	2.05
Automate	30	Old	None	1.90
Circhlor	30	Old	None	1.87
Asepto LF	30	Young	32	1.10
Asepto LF	30	Young	22	0.87
Asepto LF	30	Young	None	0.77

Table 6: Estimated differences between treatment combination and relevant control for log_{10} TVC (using estimates from Model 2 with Hen Age excluded) from largest to smallest.

Detergent	Detergent Temp (°C)	Sanitiser Temp (°C)	Estimated Reduction (log_{10})
Circhlor	40	42	5.03
Circhlor	40	32	3.64
Circhlor	40	None	3.58
Asepto LF	40	32	3.00
Circhlor	30	32	2.97
Asepto LF	30	32	2.93
Automate	40	42	2.87
Automate	30	22	2.84
Asepto LF	40	42	2.77
Asepto LF	30	22	2.72
Automate	40	32	2.68
Asepto LF	40	None	2.61
Asepto LF	30	None	2.59
Circhlor	30	22	2.44
Automate	30	32	2.38
Automate	40	None	2.23
Water	30	32	2.16
Automate	30	None	2.03
Water	40	32	1.93
Water	30	22	1.87
Circhlor	30	None	1.81
Water	40	42	1.73
Water	40	None	1.66
Water	30	None	1.49

Enterobacteriaceae

Box plots of the $\log_{10}$ *Enterobacteriaceae* (cfu/ml) data for eggs from young and old hens are given in Figures 8 and 9, respectively.

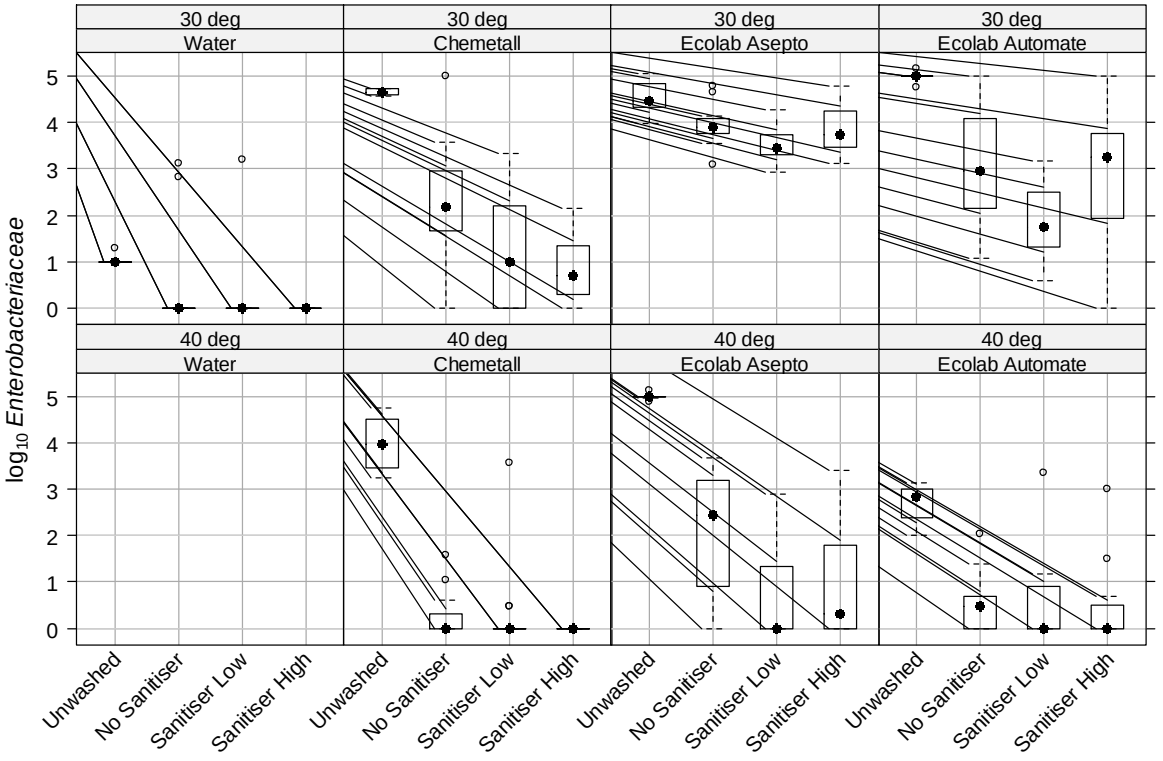


Figure 8: Box plots of $\log_{10}$ *Enterobacteriaceae* (cfu/ml) for eggs from young hens

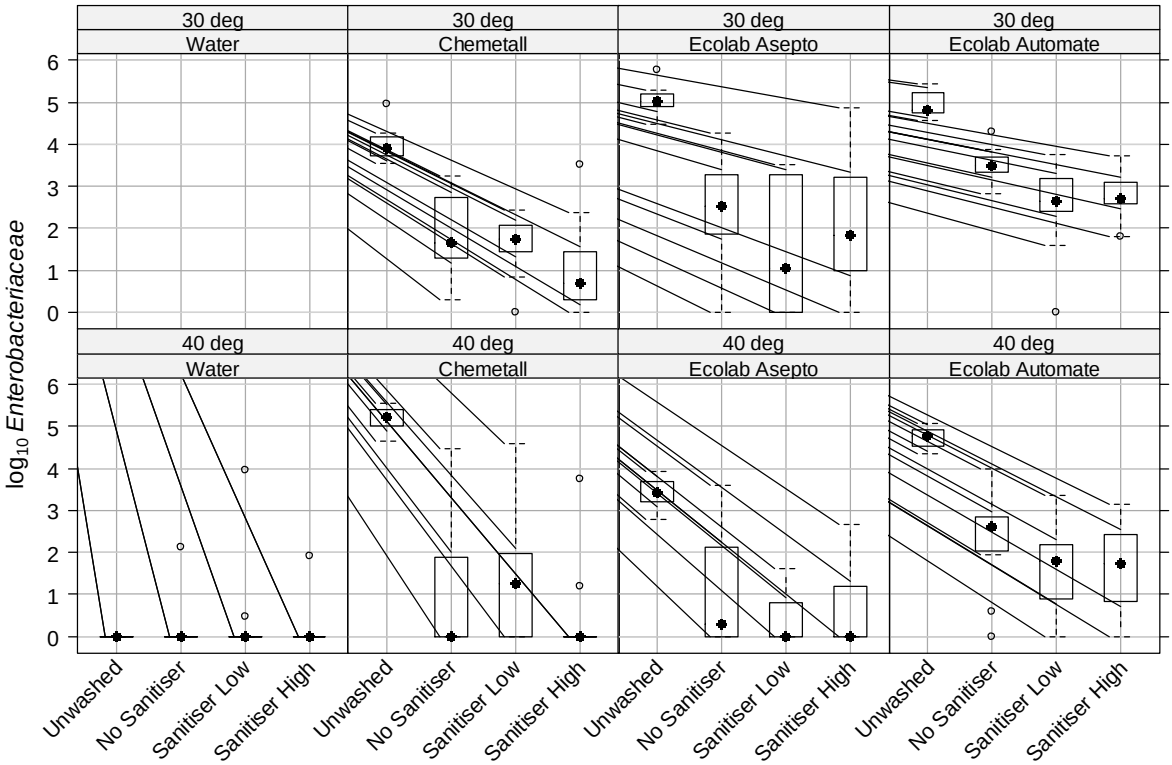


Figure 9: Box plots of $\log_{10}$ *Enterobacteriaceae* (cfu/ml) for eggs from old hens

Key observations

- The biggest reduction in *Enterobacteriaceae* counts was observed when Circhlor was used at 40°C with Virogard at 42°C (sanitiser high) to wash eggs laid by old hens.
- Asepto LF was not very effective at reducing the *Enterobacteriaceae* counts on eggs when used without its sanitiser at 30°C on eggs laid by young hens.
- The variability in the results differed between the treatment combinations, indicating the need for a statistical model that can allow for these differences (i.e. a simple ANOVA was not appropriate).
- Detection of *Enterobacteriaceae* was low for the batch of eggs that were washed with water only – both before and after washing. The reason for this lack of detection is unclear.

There were difficulties fitting the first model to the $\log_{10}$ *Enterobacteriaceae* data as the *Enterobacteriaceae* results for eggs from young hens when treated with Chemetal at 40°C and using the higher temperature sanitiser all below the limit of detection. This meant that the variance of this subset of the results was zero, and thus the variance for the model could not be estimated. To address this issue, a small sensitivity analysis was performed to determine the effect on the overall model if the results in this particular combination of factors were allowed to vary. This sensitivity analysis was executed as follows:

- Three different scenarios were assessed using standard deviations of small (0.00001 $\log_{10}$), medium (0.5 $\log_{10}$) and large (1 $\log_{10}$).
- A new dataset was created for each standard deviation. For a dataset with standard deviation σ this was done by randomly generating new $\log_{10}$ data and replacing all "<LoD" for this combination of factors. The new data was obtained from a normal distribution with a mean of $\log_{10}$(LOD)-3σ and a standard deviation of σ. The means for each dataset were:

Size of SD	Mean
small	0.000
medium	-1.595
large	-3.236

- Model 1 was fitted to each dataset and the resulting estimates and ANOVA saved.

It was found using the model that the interaction between Hen Age, Temperature, Detergent and Sanitiser was significant for each scenario. The effect and associated standard error for the "affected" treatment combination corresponded to that generated for each scenario. Neither scenario affected the remaining estimates, which was not surprising given the structure of the model. Since the estimate for the small standard deviation was already very low (0 $\log_{10}$ – the limit of detection) it seemed sensible to use a small standard deviation for the final analysis.

Further difficulties were encountered when fitting the second model to the $\log_{10}$ *Enterobacteriaceae* data because two of the treatment combinations involving Water only (control) had results only at the limit of detection (see Figures 8 and 9)[1]. Thus, for this dataset there were three separate treatment combinations for which the variance was zero. A similar approach was employed for this sensitivity analysis as above. However, rather than considering all 27 possible combinations of small, medium and large standard deviation with the three treatment combinations, the size of the standard deviation was fixed at one of small

[1] These treatment combinations related to (1) Detergent="Water", Temp="30 deg", Sanitiser="Sanitiser High" and (2) Detergent="Water", Temp="40 deg", Sanitiser="Unwashed."

(0.00001 $\log_{10}$), medium (0.5 $\log_{10}$) and large (1 $\log_{10}$) for each dataset and the associated means were:

Size of SD	Affected Treatment Combination	Mean
small	Circhlor 40°C, Sanitiser 42°C, Young hens	-0.00003
small	Water 30°C, Sanitiser 32°C	-0.00003
small	Water 40°C, Unwashed	-0.00003
medium	Circhlor 40°C, Sanitiser 42°C, Young hens	-1.5
medium	Water 30°C, Sanitiser 32°C	-1.5
medium	Water 40°C, Unwashed	-1.5
large	Circhlor 40°C, Sanitiser 42°C, Young hens	-3
large	Water 30°C, Sanitiser 32°C	-3
large	Water 40°C, Unwashed	-3

The interaction between Temp, Detergent and Sanitiser was significant for each scenario. As was the case above, the effect and associated standard errors for the "affected" treatment combinations corresponded to those generated for each scenario. Neither scenario affected the remaining estimates considerably[2] which was not surprising given the structure of the model. Since the estimate for the small standard deviation was already very low (0 $\log_{10}$ – the limit of detection) it appears sensible to use a small standard deviation for the final analysis.

There was a significant difference in $\log_{10}$ *Enterobacteriaceae* between Hen Age, Detergent, Sanitiser and Temperature and their interactions. When the data for Water only (control) were included in the model and Hen Age removed, the interaction between Detergent, Sanitiser and Temperature was significant. Within each batch of eggs (one combination of Hen Age, Detergent and Temperature) the difference between the estimate for each Sanitiser and the estimate for the unwashed eggs was calculated for each model. These differences are ranked from largest to smallest for Model 1 and Model 2 in Tables 7 and 8 respectively.

[2] Some estimates were slightly affected, however all differences were less than 0.2 $\log_{10}$

Table 7: Estimated differences between treatment combination and relevant control for log_{10} *Enterobacteriaceae* (using estimates from Model 1) from highest to lowest

Detergent	Detergent Temp (°C)	Hen Age	Sanitiser Temp (°C)	Estimated Difference (log_{10})
Circhlor	40	Old	42	4.79
Asepto LF	40	Young	32	4.27
Asepto LF	40	Young	42	4.15
Circhlor	40	Old	None	3.99
Circhlor	40	Young	42	3.98
Circhlor	40	Old	32	3.83
Circhlor	30	Young	32	3.79
Circhlor	40	Young	None	3.71
Circhlor	40	Young	32	3.64
Asepto LF	30	Old	22	3.51
Circhlor	30	Young	22	3.38
Automate	40	Old	32	3.15
Automate	30	Young	22	3.14
Automate	40	Old	42	3.13
Asepto LF	40	Old	32	3.03
Circhlor	30	Old	32	2.97
Asepto LF	40	Young	None	2.95
Asepto LF	30	Old	32	2.91
Asepto LF	40	Old	42	2.80
Asepto LF	30	Old	None	2.70
Circhlor	30	Old	22	2.43
Automate	40	Old	None	2.39
Automate	30	Old	22	2.38
Asepto LF	40	Old	None	2.33
Circhlor	30	Young	None	2.30
Automate	40	Young	42	2.22
Automate	30	Old	32	2.19
Circhlor	30	Old	None	2.17
Automate	40	Young	32	2.15
Automate	40	Young	None	2.14
Automate	30	Young	32	2.12
Automate	30	Young	None	1.93
Automate	30	Old	None	1.44
Asepto LF	30	Young	22	0.99
Asepto LF	30	Young	32	0.67
Asepto LF	30	Young	None	0.57

Table 8: Estimated differences between treatment combination and relevant control for log_{10} *Enterobacteriaceae* (using estimates from Model 2 with Hen.Age excluded) from highest to lowest

Detergent	Detergent Temp (°C)	Sanitiser Temp (°C)	Estimated Difference (log_{10})
Circhlor	40	42	4.22
Circhlor	40	None	3.95
Circhlor	40	32	3.85
Asepto LF	40	42	3.68
Asepto LF	40	32	3.65
Circhlor	30	32	3.60
Asepto LF	40	None	2.88
Automate	30	22	2.85
Circhlor	30	22	2.80
Automate	40	42	2.73
Automate	40	32	2.70
Automate	40	None	2.38
Circhlor	30	None	2.36
Automate	30	32	2.25
Automate	30	None	1.56
Asepto LF	30	22	1.08
Water	30	32	1.04
Water	30	22	0.77
Asepto LF	30	32	0.76
Asepto LF	30	None	0.65
Water	30	None	0.61
Water	40	42	-0.15
Water	40	None	-0.16
Water	40	32	-0.34

From the results obtained from Trial 1, two treatments were chosen for use in the subsequent remaining trials based on visual assessment of washed eggs and the decreases in microbial loads (TVC and *Enterobacteriaceae)* irrespective of hen age. These were Circhlor at 40°C with its sanitiser (Virogard) at 42°C and Asepto LF at 40°C and its sanitiser (Prochlor) at 32°C.

Trial 2 – Washing Black Eggs

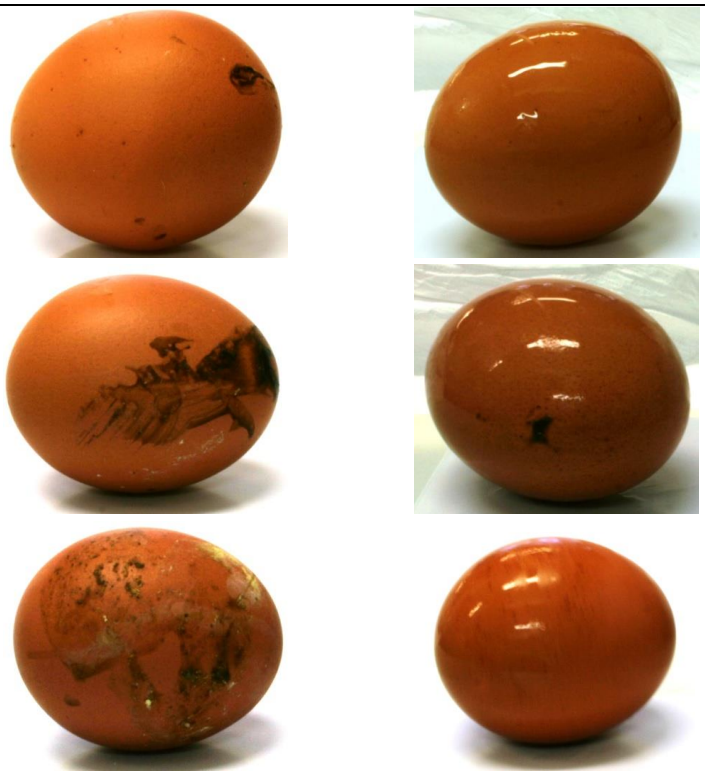
The following sections provide results obtained from washing naturally contaminated black eggs.

Visual Assessment of Washed Black Eggs

Typical results for the washing of black eggs, before and after treatment, are shown below.

Black eggs before and after being washed once with Circhlor at 40°C and Virogard at 42°C:

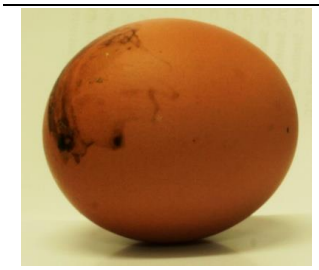
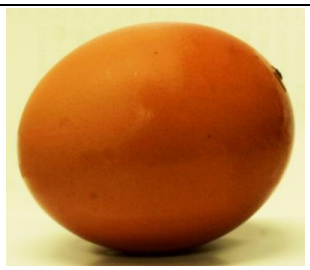
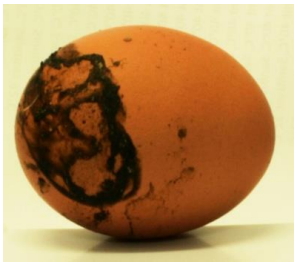
Before **After**



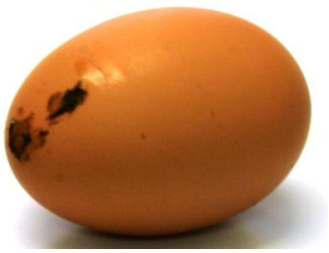
Black eggs before and after being washed twice with Circhlor at 40°C and Virogard at 42°C:

Before **After**

Black eggs before and after being washed once with Asepto LF at 40°C and Prochlor at 32°C:

Before	After
	
	
	

Black eggs before and after being washed twice with Asepto LF at 40°C and Prochlor at 32°C:

Before	After
	
	
	

Key observations

- Heavy faecal contamination of eggs was particularly difficult to remove with a single wash, regardless of cleaner/sanitiser used.
- A single wash/sanitiser treatment with Circhlor and Virogard was more effective than a single wash/sanitiser treatment with Asepto LF and Prochlor.
- A double wash/sanitiser treatment was better than a single wash/sanitiser treatment, regardless of cleaner used, Indeed, between 73% and 93% of eggs were visually clean after two cleaner/sanitiser treatments.
- A double wash/sanitiser treatment with Asepto LF and Prochlor was more effective than a double wash/sanitiser treatment with Circhlor and Virogard.

TVC

Box plots of the $\log_{10}$ TVC (cfu/ml) data are given in Figure 10.

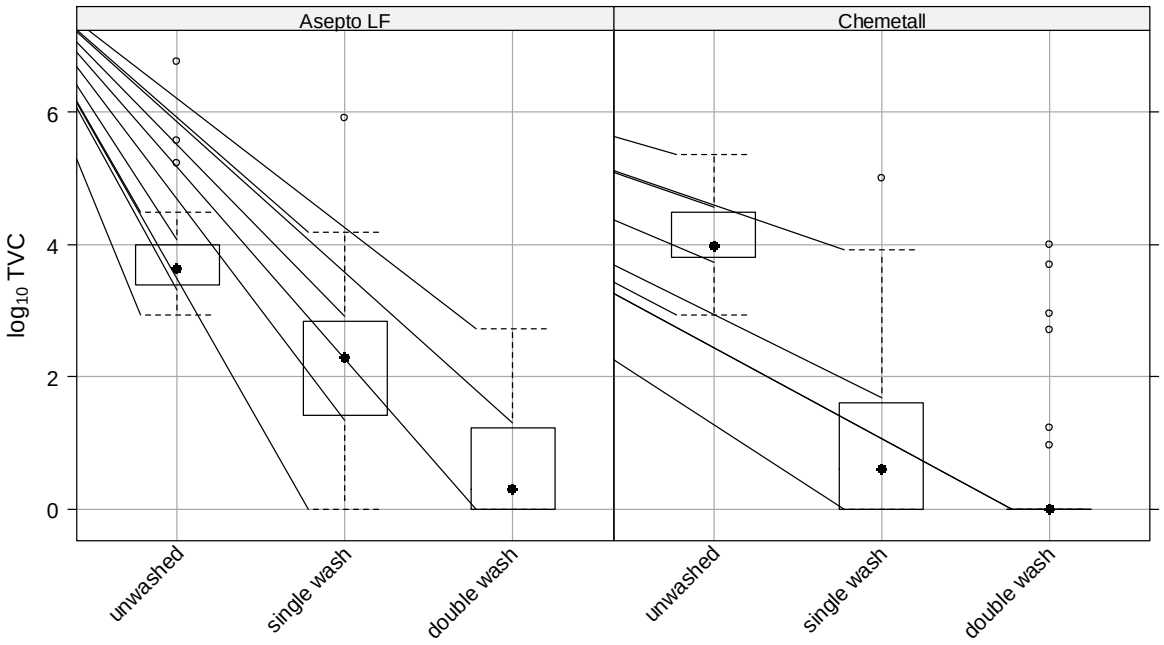


Figure 6: Box plots of the $\log_{10}$ TVC (cfu/ml) data

Key observations

- The biggest reduction in TVC was observed using a double wash of Chemetall at 40°C and Virogard at 42°C.
- Using a double wash resulted in greater reduction than using a single wash regardless of cleaner used.
- The variability in the results differed between the treatment combinations, indicating the need for a statistical model that can allow for these differences (i.e. a simple ANOVA is not appropriate).

There was a significant difference in $\log_{10}$ TVC between the chemical combinations used, the number of washes and their interaction. The difference between the estimate for each single or double wash and the estimate for the corresponding unwashed eggs was calculated within each batch of eggs washed. These differences are ranked from largest to smallest in Table 9.

Table 9: Differences in log_{10} TVC (cfu/ml) between treatment combinations and unwashed eggs

Detergent	Wash	Estimated Difference
Circhlor	double wash	3.72
Asepto LF	double wash	3.27
Circhlor	single wash	3.09
Asepto LF	single wash	1.68

Enterobacteriaceae

Box plots of the log_{10} *Enterobacteriaceae* data are given in Figure 11 and the proportion of times that *Enterobacteriaceae* were detected are presented in Table 10.

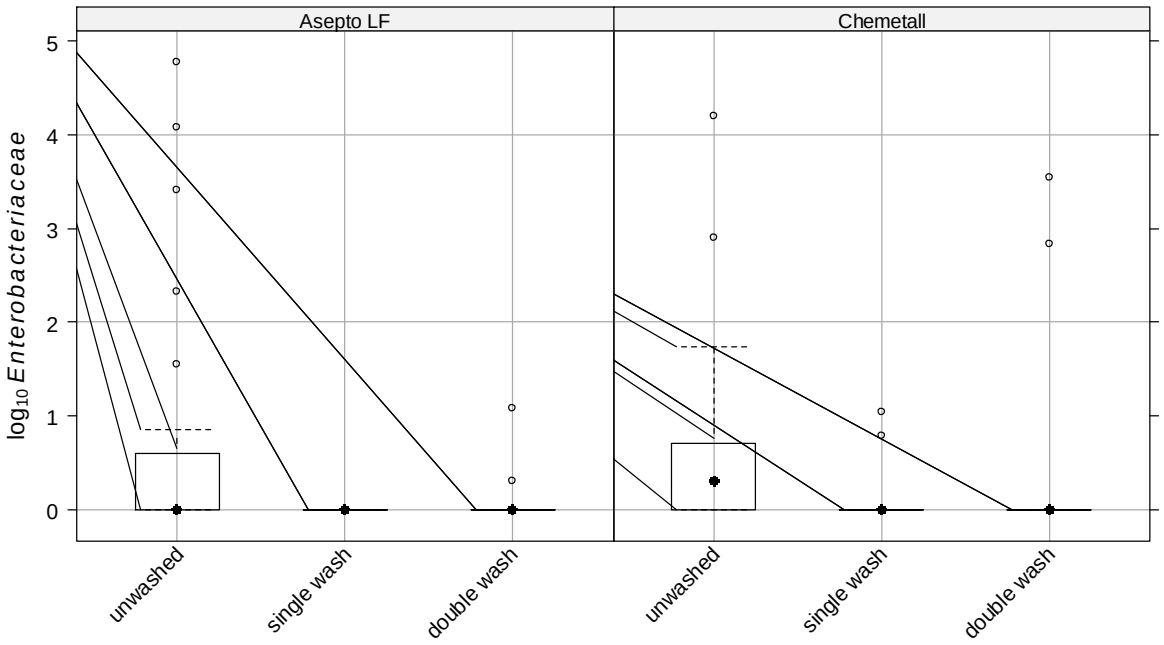


Figure 11: Box plots of the log_{10} *Enterobacteriaceae* (cfu/ml)

Key observations

- Concentration of log_{10} *Enterobacteriaceae* were much lower than log_{10} TVC, but had more unusually high counts.
- There were many non-detects (125 non-detects out of 165 eggs tested), including the unwashed eggs.
- Generally, the lowest prevalence was for single wash eggs, though this may be an artefact of the contamination on unwashed eggs, which varies greatly as indicated above.
- The eggs washed twice with Circhlor at 40°C and Virogard at 42°C had a higher prevalence of *Enterobacteriaceae* overall across the Wash methods.

The difference in *Enterobacteriaceae* prevalence between the number of washes used was highly significant ($P < 0.001$). There was no significant difference in prevalence between the interactions between the number of washes and the chemicals used ($P = 0.320$). The difference in prevalence between replicate batches (for each chemical used) was also significant ($P = 0.015$), indicating that there was variability in the results between repeated

trials (using different batches of eggs). When the model was refitted excluding replicate as an explanatory variable, the difference in prevalence between the number of washes was still highly significant ($P < 0.001$), but there was no significant difference in prevalence between the chemicals ($P = 0.477$) or the interactions ($P = 0.285$). These results are in agreement with the observations made from the plots above.

The estimated prevalence and associated 95% confidence interval for each combination of chemical, replicate and number of washes from the full model are given in Table 10. The estimated prevalence and associated 95% confidence interval for each number of wash from the model excluding replicates and chemical information are given in Table 11 – the observed difference between single and double washing is little practical importance.

Table 10: Estimated *Enterobacteriaceae* prevalence (%) for each Detergent, Rep and Wash

Detergent	Rep	Wash	Tests	Detections	Model estimate (%)	95% CI
Asepto LF	Rep 1	unwashed	15	9	54.68	(31.89, 75.66)
		single wash	14	0	1.77	(0.31, 9.62)
		double wash	14	0	3.92	(0.93, 15.02)
	Rep 2	unwashed	15	9	65.68	(42.06, 83.46)
		single wash	15	0	2.79	(0.51, 13.76)
		double wash	12	2	6.08	(1.54, 21.16)
Circhlor	Rep 1	unwashed	14	7	45.87	(23.97, 69.48)
		single wash	15	0	1.25	(0.21, 7.25)
		double wash	14	0	2.79	(0.62, 11.63)
	Rep 2	unwashed	11	9	86.58	(64.87, 95.75)
		single wash	13	2	8.81	(2.04, 30.94)
		double wash	13	2	17.93	(6.23, 41.80)

Table 11: Estimated *Enterobacteriaceae* prevalence (%) for each Wash

Wash	Tests	Detections	Model estimate (%)	95% CI
unwashed	55	34	61.82	(48.45,73.61)
single wash	57	2	3.51	(0.88,12.97)
double wash	53	4	7.55	(2.86,18.45)

Trial 3 – Washing Eggs Artificially Contaminated with *Salmonella*

Box plots of the $\log_{10}$ *Salmonella* data are given in Figure 12. Unfortunately, it was only possible to wash seven eggs with water due to losses from broken and cracked eggs throughout the trial.

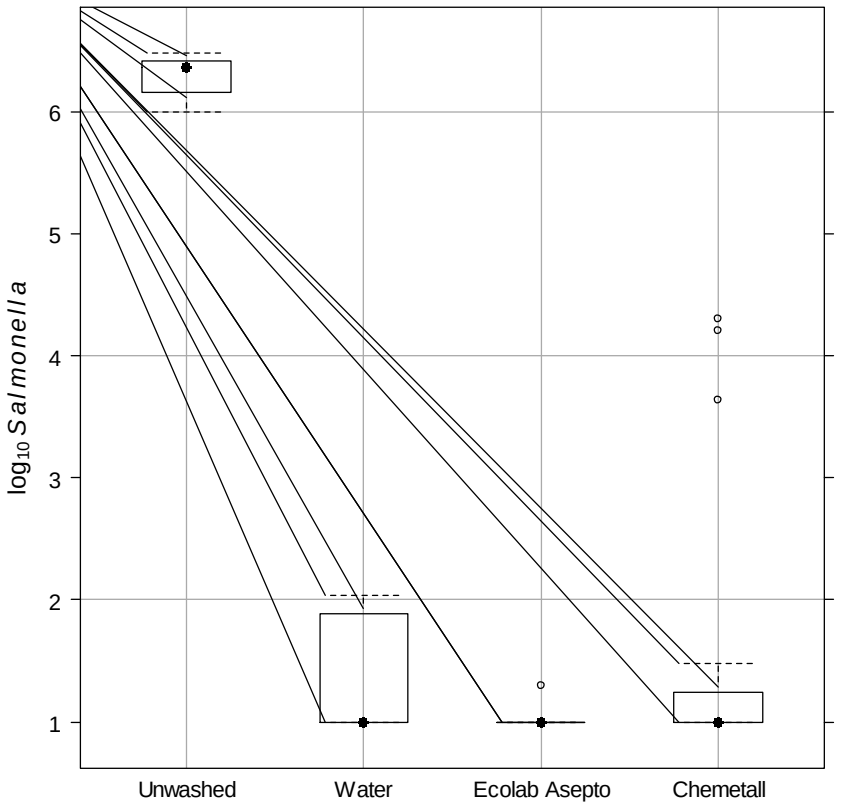


Figure 12: Box plots of the $\log_{10}$ *Salmonella* counts

Key observations

- Eggs treated with Asepto LF appeared to have the lowest detectable levels of *Salmonella* under the conditions used in this study.
- There were three *Salmonella* counts for eggs washed with Circhlor and its associated sanitiser that appeared to be unusually high.

There was a significant difference in the *Salmonella* prevalence between all four treatments ($P < 0.001$). There was also a significant difference in the *Salmonella* prevalence between Water, Asepto LF and Circhlor ($P = 0.028$), but not between Asepto LF and Circhlor ($P = 0.390$). The significant difference in the *Salmonella* prevalence for all four treatments appears to be driven by the difference between unwashed and washed eggs. Table 12 gives the estimated prevalence and associated confidence interval for each treatment. Table 13 gives the means and standard deviations for the detections in each treatment.

Table 12: Estimated *Salmonella* prevalence for each treatment and associated confidence limits

	Tests	Detections	Prevalence (%)	95% CI*
Unwashed	15	15	100.00	(78.20, 100.00)
Water	7	5	71.43	(29.04, 96.33)
Asepto LF	15	2	13.33	(1.66, 40.46)
Circhlor	15	5	33.33	(11.82, 61.62)

*Clopper-Pearson confidence limits for the proportion of positive detections

Table 13: Mean *Salmonella* concentrations and associated standard deviations (in brackets) for each treatment (detections only)

Unwashed	Water	Asepto LF	Circhlor
6.28 (0.2)	1.56 (0.5)	1.15 (0.2)	2.92 (1.6)

Cuticle Assessment

A box plot of the a* values for unwashed control eggs (n=8) and eggs treated with Asepto (Asepto at 42°C/Prochlor at 32°C; n=6) or Circhlor (Circhlor at 42°C/Virchlor at 45°C; n=5) is shown in Figure 13. From this figure it can be seen that control eggs had generally a lower a* value than washed eggs (either treatment) and this was confirmed by the ANOVA (p-value = 0.025). Control eggs resulted in more negative a* values, indicating more green staining and hence better cuticle coverage. However, there was considerable variability in the cuticle disposition of control eggs, which is also clearly evident from the photos (Figure 14) – this makes practical interpretation more difficult. In addition, neither the L* or b* values were significantly different between the three groups of eggs, which indicates that the difference was due to the green staining.

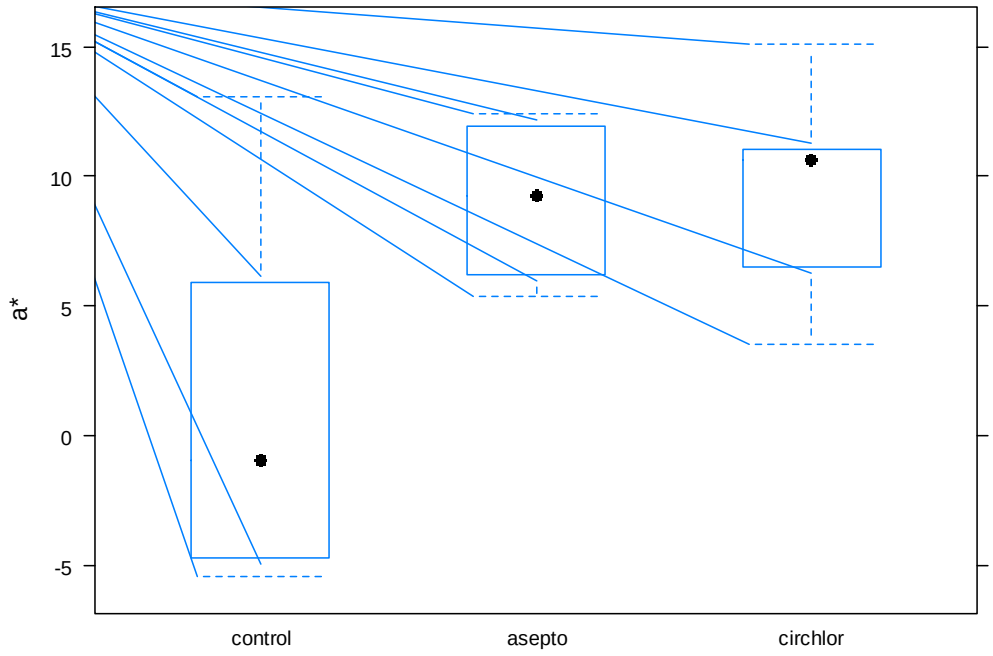


Figure 13: Box plots of a* values for Control eggs (n=8) and eggs treated with Asepto (Asepto at 42°C/Prochlor at 32°C; n=6) or Circhlor (Circhlor at 42°C/Virchlor at 45°C; n=5).

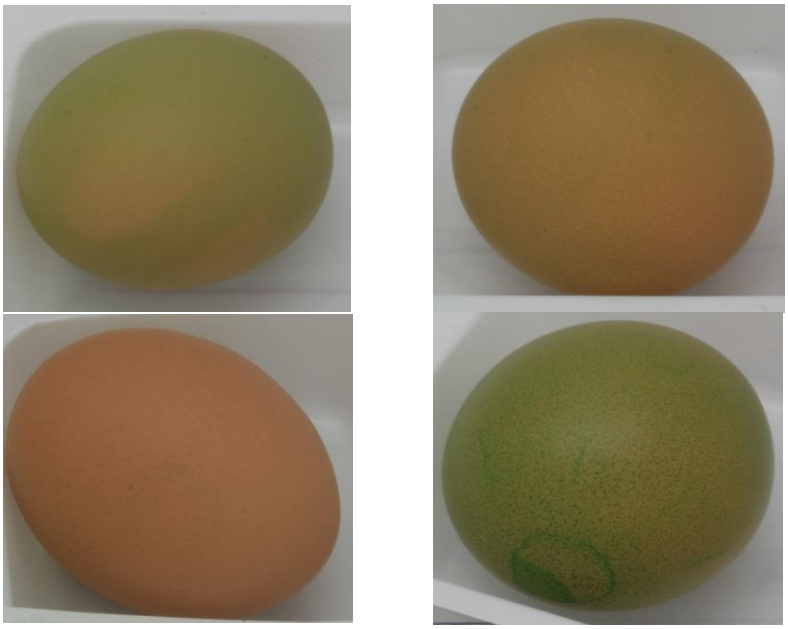


Figure 14: Unwashed control eggs stained with MST Cuticle Blue

Discussion

The ability of three commercial egg wash detergents and their associated sanitisers to clean artificially dirtied eggs was examined during laboratory-based trials. The three detergent-sanitiser combinations trialled were:

- Circhlor: a liquid alkaline (pH 12) chlorine based product used with a liquid quaternary ammonium compound (QAC) (200 ppm) based sanitiser called Virogard.
- Asepto LF: a liquid sodium hypochlorite based product used with a liquid sodium hypochlorite (200 ppm) based sanitiser called Prochlor.
- Automate: a solid alkaline (pH 12) chlorine based product also used with Prochlor.

The efficacy of these products to clean dirty eggs under the conditions used in this study was based on visual inspection of the washed eggs and statistical analysis of TVC and *Enterobacteriaceae* compared to unwashed eggs. *Enterobacteriaceae* (a family of bacteria in which *Salmonella* and *E*. coli are found) were used as an indicator of faecal contamination. Based on these analyses there were clear differences in the treatments used. When Circhlor and Virogard were used at 40°C and 42°C respectively, the estimated drop in TVC was approximately 5 $\log_{10}$ cfu/mL and *Enterobacteriaceae* counts dropped by approximately 4.2 $\log_{10}$ cfu/mL. Asepto LF was also effective when used at 42°C and Prochlor at 32°C with the estimated drop in TVC of approximately 3 $\log_{10}$ cfu/mL and *Enterobacteriaceae* by 3.7 $\log_{10}$ cfu/mL. In contrast, Circhlor or Automate were no more effective at 30°C, when used without the sanitiser, than using water only (TVC: 1.5-2.5 $\log_{10}$ cfu/mL; *Enterobacteriaceae*: 1.5-2.5 cfu/mL respectively). Prochlor was consistently more effective when used at either 22°C or 32°C than at 42°C. This was likely influenced by the chlorine content which would have volatilised more rapidly at the higher temperature. This is contrary to traditional thinking, where it has been advocated to increase water temperatures through the washing and sanitising process to prevent micro-organisms being sucked into the egg. However, recent work (Jones. *et al* 2006) indicates that lower sanitiser temperatures produce acceptable results possibly by drawing the sanitiser into the pores of the shell. Overall it appears that Virogard gave the best sanitising results.

These results have important consequences as foodborne illness associated with the consumption of eggs and egg products was estimated to cost the Australian economy $44 million per annum, mainly due to *Salmonella* (Anon. 2009). This is despite the lack of *Salmonella* found on eggs by Daughtry *et al*. (2005). The two most promising detergent-sanitiser combinations – Circhlor at 40°C with Virogard at 42°C and Asepto LF at 40°C and Prochlor at 32°C – were effective at substantially reducing TVC, *Enterobacteriaceae* and *Salmonella* on artificially contaminated eggs. Plain water was also able to reduce *Salmonella* levels significantly although a better reduction in prevalence was achieved by using a chemical. The efficacy of plain water may be related to a lack of adherence of *Salmonella* onto the egg shell. This could be addressed either by allowing the eggs to dry for longer after artificial inoculation, using different strains of *Salmonella* or inoculating eggs using *Salmonella* mixed with chicken faecal material. Regardless, these results show promise of these detergent-sanitiser combinations to reduce contamination of eggs by enteric bacteria, including *Salmonella*. In addition, both QAC and sodium hypochlorite treatments have previously been shown to also reduce bacterial penetration into eggs during storage up to 21 days (Wang & Slavik, 1998). This may also be an additional benefit of the wash regimes investigated in this study and may be worth further investigation for flow on effects, such as prolonged shelf life.

In-Plant Black Egg Wash Trials

Methods

Three egg processing plants were visited to assess the recovery of black eggs using the best two chemical/temperature combinations identified in the bench-top trials under commercial conditions.

Cleaner/Sanitiser Combinations

Two cleaner/sanitiser combinations, selected based on the best outcomes achieved during laboratory based trials, were used for in-plant trials. These were:

- Circhlor: A liquid alkaline (pH 12) chlorine based product used at 1% solution (v/v) and 40°C (or as near as practical). This was used with a quaternary ammonium compound (QAC) based sanitiser called Virogard (0.25% (v/v), final QAC concentration 400 ppm) at 42°C (or as near as practical)
- Asepto LF: A liquid sodium hydroxide based product to be used at 0.45% solution (v/v) at 40°C (or as near as practical) with a sodium hypochlorite based sanitiser called Prochlor (now called XY12) (0.16% (v/v), final hypochlorite concentration of 200 ppm) at 32°C (or as near as practical).

Final QAC concentration and pH were determined using Hydrion Papers QT-40 (Microessential Laboratories Inc) and pH Test Strips (Sigma), respectively. Final hypochlorite concentration was determined either using Precision Chlorine Test Paper (Precision Laboratories) or by titration.

Microbiological Assessment of Washed and Unwashed Eggs

Eggs were microbiologically assessed for TVC and *Enterobacteriaceae* as per the laboratory trials.

Assessment of Plant Hygiene

The hygiene of plants and equipment was visually assessed on initial entry. Swabs were taken from up to 20 different sites on each of the two trial days. This was done by adding Letheen diluent (10 mL) to the sterile sponge swab, which was removed from the package using a sterile glove. Surfaces were selected for swabbing because they were in direct contact with eggs after washing. The surfaces were vigorously rubbed with a swab which was subsequently placed into a Whirl-Pak bag, the bag was sealed and labelled with the location, date and time of sampling. The swabs were then stored at 4°C for transport to the laboratory and tested within 24 hours. Peptone saline solution (10 mL) was added and the swab stomached for 60 seconds. Serial decimal dilutions (1 mL) were plated onto 3M™ Petrifilm™ Aerobic Plate Count Petrifilm and incubated at 35°C for 48 hours to determine the TVC per mL of rinse. To determine the total *Enterobacteriaceae* count per mL of rinse, serial decimal dilutions of the rinse were plated onto 3M™ Petrifilm™ *Enterobacteriaceae* Count Plates and incubated at 35°C for 24 hours.

Presence/absence of *Salmonella* was also determined by the addition of Buffered Peptone Water (50 mL) to the swabs. Swabs were then incubated at 37°C for 18-24 hours. Aliquots (3 × 33 µL) were spot inoculated onto the surface of a Modified Semi-solid Rappaport-Vasilliades medium plate and incubated upright at 42°C for 18-24 hours. The plates were then examined for zones of growth surrounding the inoculation spots. Suspect growth was then streaked onto CLED agar for single colonies and incubated at 37°C for 18-24 hours. The plates were examined for typical non-lactose fermenting (blue) colonies and latex

agglutination performed using a *Salmonella* polyvalent latex test kit. Those colonies giving a positive reaction to the latex were reported as *Salmonella* species. Colonies that were of typical appearance but gave a negative reaction to the latex were tested biochemically using the Microbact 24E system. If this still gave a result that was not *Salmonella* the sample was reported as negative for *Salmonella* species. Isolates were serotyped at the Australian *Salmonella* Reference Centre (Adelaide, South Australia).

Plant 1

Plant 1 trials were run using a Kuhl 2-lane Model Dew 10-2 (Serial Number 6864). This machine had two lanes and eggs took 32 seconds to enter and exit the washer. The machine originally had brushes but these were removed for the purpose of the trials as they were not functioning correctly and were interfering with the spray jets. Spray pressure was 15 psi. The detergent was fed from a 47 L recycled reservoir and the sanitiser from a 25 L drum. However, following spraying onto the eggs, the sanitiser solution was collected in the wash water tank and mixed with the detergent at a rate of ~1.35 L per minute effectively diluting the detergent. Circhlor was used at 40°C and Virogard at 42°C (usual processing temperatures at this plant). Asepto LF was used at 42°C and Prochlor at 24-26°C due to difficulties maintaining a constant water temperature, as temperature adjustments had to be made by turning the hot water system on or off.

Figure 3: Egg washer used at Plant 1

Plant 2

Plant 2 trials were run using a MAK60-HR(FT) supplied by MOBA capable of washing 60,000 eggs per hour. This machine had six lanes and eggs took 30 seconds to enter and exit the washer. In this time they were exposed to 18 separate roller brushes with five rows of recycled wash water (three sprays per row), six rows of fresh detergent (three sprays per row) and two rows of sanitiser (three sprays per row). Nylon brush rollers were used which blocked the sprays from directly contacting the eggs. The detergent and sanitiser were mixed via a dosage pump directly into the appropriate water supply line. Circhlor was used at 41°C and Virogard at 45°C (usual processing temperatures at this plant). Asepto LF was used at 41°C and Prochlor at 29-30°C. Temperature was adjusted by opening or closing taps.

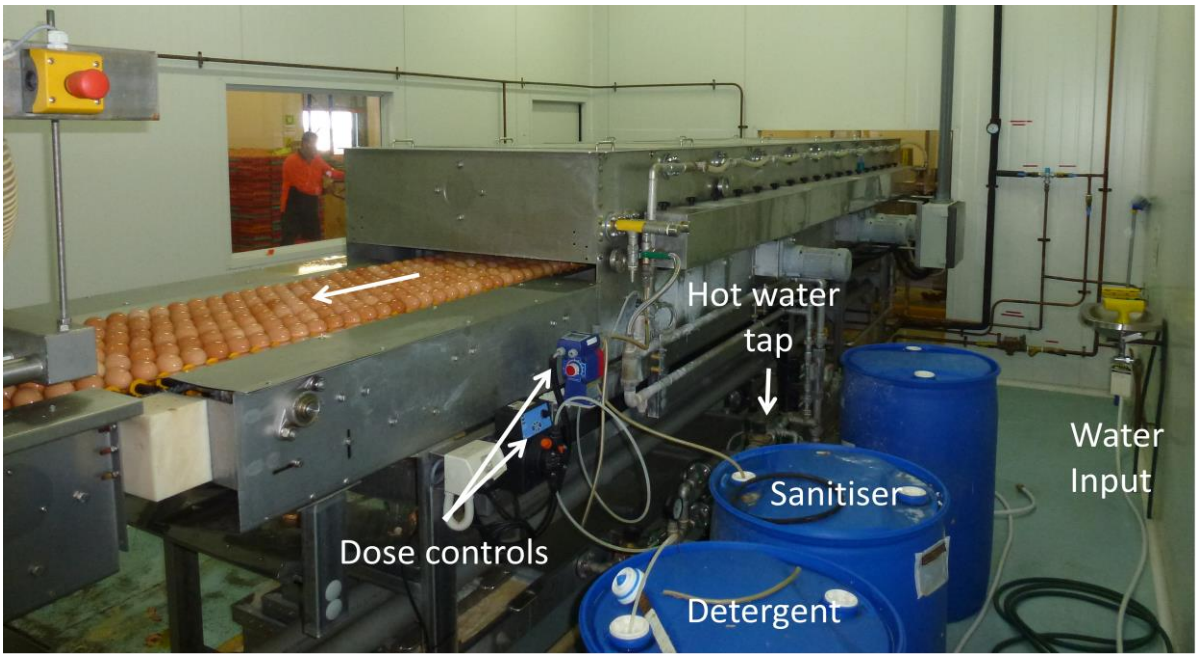


Figure 4: Egg washer used at Plant 2

Plant 3

Plant 3 trials were run using an Aoyama Egg Washer (Model MAK-180SR) supplied by MOBA. It used two rows of 18 nylon brush rollers with 11 detergent nozzle pipes above each row (total 110 nozzles per row) and two final rinse (sanitiser) nozzle pipes (total 20 nozzles per row). The brushes prevent direct contact of the sprays with the eggs as per Plant 2. The machine is capable of washing 180,000 eggs per hour with a wash time of 30 seconds. All water for the washer was Reverse Osmosis (RO) treated bore water. The detergent was dosed into a full recycle water tank which was monitored via a conductivity meter. The sanitiser was freshly pumped into fresh RO water. Again, the water temperature was difficult to maintain and hence Circhlor was used at 26-39°C and Virogard at 50-53°C while Asepto LF was used at 31-41°C and Prochlor at 25-30°C.

Figure 5: Egg washer used by Plant 3

Statistical Analysis

TVC and *Enterobacteriaceae* counts were $\log_{10}$ transformed for analysis. Results below the lower limit of detection (LoD) or above the upper LoD were set equal to the corresponding LoD.

Results were analysed separately for each company. A two-way analysis of variance model was used to assess differences in the mean microbial levels due to processing step, chemical and sanitiser combination used and their interaction. The chemical and sanitiser combination used was confounded with the day of application, as only one chemical pair could be used on one day. Statistical significance was assessed using a significance level of 0.05. The highest order non-significant terms were removed from the model using backward elimination.

All analyses were undertaken in the statistical software R, version 2.15.1, (R Development Core Team, 2012).

Results and Discussion

General Observations

Accurate dosage of chemicals at all plants proved difficult. In particular, maintaining the correct dose in recycled systems was especially difficult with there being a frequent need to measure and re-dose chemicals. However, measuring and adjusting dosage rates required a different approach in each plant. There was limited knowledge among plant operators on how to conduct such analysis with operators relying on test paper strips or conductivity meters which gave inaccurate readings due to particulate matter within recycled water. Often, these were only occasionally checked and operators were surprised to see how quickly the chemical dissipated under normal operating conditions. If the dosage was higher than the limit of the test strips then it was impossible to know the true concentration without the use of titration, which is beyond the expertise of most operators. Furthermore, in two of the plants the chemical in the recycled wash water was significantly diluted by the sanitiser

solution which flowed into the wash tank solution. Dosage pumps were in operation at all plants for the sanitiser and at one plant for the wash chemical. It was very difficult to adjust these so that the required concentration of chemical was achieved, with any adjustment in water flow requiring a re-adjustment of the pumping rate.

Accurate adjustment of the wash and sanitiser water temperature was particularly challenging – wash water temperature was difficult to adjust at two plants and sanitiser water temperature was difficult to adjust at all plants. Plant 1 had a thermostat on the heater element in the wash tank that could be set at the required temperature after measuring the temperature at the egg but all other spray systems required turning boilers on and off and adjusting flow rates through hot (and cold) water taps. This meant that maintaining the correct temperature was difficult and almost impossible in Plant 3. Furthermore, whilst the water temperature should be measured at the egg surface, normal practice in all plants was to measure the temperature of the incoming water (although Plant 2 had recognised this problem and placed temperature probes directly under the spray nozzles). This meant that the temperature at the egg surface was generally below that being monitored by the plant operators (especially where the sprays were only spraying above the rotating brushes).

Blocked jets were another issue which was observed, to varying degrees, at all plants visited. Jets were blocked by:

- chemical precipitation when the system was turned off,
- faecal matter in the recycled water and
- hard water scaling or calcium precipitation from egg shells (particularly where RO water was used).

Plant 1

The following are general observations made at this plant.

- A member of the research team visited the facility prior to the in-plant trials and observed that the insides of the washer and tank were covered with scale. The plant manager was provided with information and chemicals to de-scale the machinery periodically and subsequently the washer was in good condition for the trial.
- The brushes inside the washer were hanging loosely and moving back and forth only a little. However, this caused the brushes to interfere with at least four of the five sprays and hence sprays were deflected without reaching the eggs. In addition, the brushes were set too high and thus were ineffective at cleaning the eggs. For this reason the brushes were removed prior to commencing the trial which achieved improved cleaning of eggs by the high pressure sprays (15 psi). This resulted in an immediate improvement in egg recovery and was noted by the plant manager.
- Another observation was that the washer was leaving two ‘rings’ around washed eggs, which corresponded with the edges of the depressions that hold the eggs on the rollers. While these had been largely addressed through de-scaling the machine prior to the trials, some evidence of this problem was still apparent. However, running the machine with the correct chemical concentration in the wash water resulted in cleaning of the rubber rollers and subsequently the rings on eggs disappeared.
- The retrofitted sanitiser spray used fresh potable water; sanitiser was added at the correct dilution via a volumetric pump using 1.3 L of sanitiser per minute. However the spent sanitiser water ran into the wash water tank, causing the concentration of the wash chemical to be diluted very quickly as the total initial volume which was hand dosed with chemical at the start was only 47 litres In addition, the wetters in the sanitiser chemical caused the wash water to foam up and overflow.

- For the Circhlor/Virogard trial it was necessary to regularly add wash chemical to the reused water to counteract the diluting effect of the large volume of sanitiser water being added.
- The heating element, located in the wash water tank, was fitted with a thermostat and hence the water temperature was easy to adjust. Because the temperature of the sanitiser spray was several degrees higher than that of the wash water the temperature control worked well using Circhlor. However, for the Asepto LF trial the sanitiser needed to be at a lower temperature than the wash water and as a result, the used sanitiser water had to be diverted to reduce the cooling effect (on the wash water).
- As an immediate solution to this dilution problem for normal washing it was recommended to use Circhlor in both the wash water and the sanitiser, which would at least maintain adequate chemical concentrations in the wash water. Since Circhlor is a chlorinated alkali with at least 200 ppm chlorine it will work as a sanitiser and at the same time provide fresh chemical to maintain the wash solution.
- A longer term solution would be to capture the sanitiser water and reuse it for prewashing of eggs at the start of the washer (water is not further reused). Such a process would also assist in warming the egg shells prior to washing as this was a considerable problem during this trial. In particular, egg shell temperatures had quickly dropped to ambient temperatures (8-9°C) meaning that it was necessary to warm the shells before washing eggs to prevent cracking which can result when temperature differential between shell and water exceeds >27°C.
- The blower/drier air should be sourced from outside wash room to improve the efficiency of drying eggs, as damp or wet eggs can lead to increase in contamination, microbial growth and result in cross contamination of equipment.
- It should be ensured that chemical levels are regularly measured in wash water and re-dosed when required and that wash water is replaced when required chemical levels cannot be achieved.

After completion of the in-plant trials the plant manager replaced the brushes, lowering them and fixing them in position so that they did not interfere with the excellent jetting action of the sprays. These changes resulted in notable improvement in egg recovery, as noted by the plant manager.

Recovery of Black Eggs

The black eggs used at this plant were collected from the sheds in the morning just prior to the trials and so would be considered to be very freshly soiled which may be easier to clean compared with eggs used at other plant trials.

Table 14: Recovery of black eggs following repeated washing in Plant 1. Typical examples of washing and unwashed eggs are provided in Appendix 1.

Treatment	No. of washes	Eggs Recovered	Percent (%)
Circhlor & Virogard	2	24	53.33
	3	8	17.78
	4	11	24.44
	Rejected after 4	2	4.44
Asepto LF & Prochlor	2	11	24.44
	3	15	33.33
	4	11	24.44
	Rejected after 4	8	17.78

Plant 2

The following are general observations made at this plant.

- The egg washer in Plant 2 originally used recycled wash water (from the tank underneath) for the first section of the washer. As a result, the eggs were first sprayed by two rows of recycled water (three sprays each), then went into a section with three rows of wash sprays and four brushes which took approximately 12 seconds followed by fresh rinse water with eight rollers and six rows of jets (taking approximately 16 secs) and finally two rows of sanitiser sprays (two secs). Chemical was measured by a conductivity meter in the recycle tank which gave very inconsistent results depending on the amount of organic soils and calcium (from the egg shells) dissolved in the water. Overall, it took 30 secs for eggs to enter and leave the washer.
- The washer was subsequently re-plumbed so that the rinse section delivered fresh chemical. Dosage was measured by a volumetric pump. The water collected and recycled in the first section had a temperature between 30-35°C and this allowed slight warming of eggs, which typically were washed straight from the chiller at 15°C, and hence prevented thermally induced cracking. The continuous addition of fresh chemical to the re-used water caused constant overflow of the tank which kept the water reasonably clean. Because a large section of this machine uses fresh chemical and hot water it can operate quite effectively at cleaning eggs. However, this also means that significant amounts of chemical and hot water are used and so the process needs to be monitored very closely.
- To achieve the correct temperatures at the egg level the temperature of the in-feed water had to be around 60°C because of loss of heat in the sprays and rotating brushes. This could cause problems if the machine starts and stops because the high temperature in the pipes means that the water evaporates quickly and precipitated chemical blocks the nozzles. In contrast, the sanitiser water was just a direct spray onto the eggs and the inlet temperature required was approximately 45°C. Consequently, it appears as if the rinse/sanitiser spray was at a lower temperature than the wash water. This highlights the importance of determining the actual temperature at the egg surface for process control and auditing purposes. To facilitate this, a long temperature probe has now been placed directly under the sprays.
- Since both sanitiser and wash chemicals are delivered fresh onto the eggs it was easier to monitor active chemical on the egg shell. However, this process still required constant monitoring and adjusting of the flow rates of water and chemicals to obtain the right balance between temperature and chemical concentration. This proved very difficult in the trial with Prochlor and hence resulted in twice the desired chemical concentration (400 versus 200 ppm, which could only be identified by titrating the chemical in the laboratory and not from the test strips in plant).
- The installed brushes appeared ineffective for egg cleaning as they were very thin and soft. The suggestion was made to lower the brushes and to investigate the efficacy of a new stiffer set.

This machine has already been adjusted and modified considerably to improve its effectiveness at washing eggs. However, additional alterations may include:

- Redesigning the washing section so that fresh wash chemical is sprayed at high pressure (low volume) in only two rows of fan jets directly onto the eggs as the last part of the wash section prior to sanitising to cut the already softened soil from the eggs. This would reduce the amount of fresh, hot water needed but may then lower the temperature too much for effective recycled washing in the first section. This could be address by placing a heating element in the tank of recycled water.
- Redesigning wash chemicals with substances which can penetrate and solubilise soils, hold them in solution and enable free rinsing – all at a lower temperature thus removing the requirement above for a heating tank in the recycled wash water tank.

- Keeping sanitiser sprays but increase pressure, reduce water volume and increase water temperatures to assist quick drying.
- The blower/drier air should be sourced from outside wash room to improve the efficiency of drying eggs, as damp or wet eggs can lead to increase in contamination, microbial growth and result in cross contamination of equipment.

Recovery of Black Eggs

The black eggs at this plant had been collected and stored in the cool room for several days so the soils were well adhered and dried.

Table 15: Recovery of black eggs following repeated washing in Plant 2. Typical examples of washing and unwashed eggs are provided in Appendix 1.

Treatment	No. Of washes	Eggs Recovered	Percent (%)
Circhlor & Virogard	2	2	4.44
	3	11	24.44
	4	19	42.22
	Rejected after 4	13	28.89
Asepto LF & Prochlor	2	21	46.67
	3	14	31.11
	4	8	17.78
	Rejected after 4	2	4.44

Plant 3

The following are general observations made at this plant.

- The egg washer used at this plant has only been in operation for a short period of time and is still under warranty. Consequently, modifications would void the warranty and thus it suffers similar problems to those faced in Plant 2 prior to modifications being made there.
- All the washing sprays used recycled water from the tank which was filled with hot water from a boiler. The boiler also contains a heat exchanger that has been designed to keep the recycled water hot, though in the plant trials it was not possible to maintain the correct temperatures.
- A conductivity meter is used in this system to monitor the chemical level in solution and pump wash chemical into the tank when required. However these meters have been found to be unreliable in the presence of organic materials in the water and especially when high levels of calcium are present in the water (This system was also originally used in Plant 2 and removed due to its inconsistency). This plant uses Reverse Osmosis (RO) water which is highly reactive and was causing a very high level of calcium to be extracted from the egg shells. Therefore, frequent measuring of the active chemical in the water was recommended.
- The temperature in the recycled wash water tank was difficult to maintain during the trials as heat was lost from the sprays above the brushes. Spray temperatures fell as low as 26°C by the end of the first trial, which was well below the recommended effective usage temperature of the Circhlor. The reason for the temperature loss was the rapid air movement created by extractor fans that had been installed to remove the steam from the room in an attempt to achieve better blow drying of eggs after sanitising and the fact that the sprays are set well above the roller brushes (which create a lot of air movement).

- The brushes had been set well down on the eggs (lower than Plant 2) and were in good functional condition. They appeared to be operating as well as could be expected.
- The rinse water was collected for re-use in the wash tank and hence it was important to ensure that the sanitiser was compatible with the wash chemical. In addition it was important to consider the dilution effect of the sanitiser addition on the wash water solution (flow rates were not measured but could be done as the solution is collected separately). Consequently, it was necessary to regularly measure the chemical concentration during the trials and dose the wash water tank as required.
- It was not possible to measure the actual temperature of the wash water at the level of the egg due to the design of the machine (however knowledge from Plant 2 would indicate that it would be several degrees lower than that under the sprays).
- There was a large problem with calcium scale build-up inside the pipes and nozzles causing blockages of the sprays. Replacement sets were available but preventing or minimising the problem would save a lot of time and effort and ensure continual functioning. This could be achieved by preventing calcium leaching from the eggs or broken egg shells or keeping it in suspension using chelating agents. In addition, polymers which may be added to keep the calcium in suspension may be worthwhile investigating. Alternatively, larger nozzles or a pipe with slots may be just as effective as the sprays when they are only used for wetting the brushes and not for spraying eggs.
- The sanitiser nozzles were in a better condition but a more effective rinse could be achieved through an increase in pressure – to give a solid cutting fan jet over each egg. This would also be a good machine to trial a chemical that can penetrate and solubilise organic matter, hold it in solution and be free rinsing at lower temperatures.
- In addition, it may be feasible to collect the used sanitiser water into a small holding tank and pump the water over the eggs as they enter the washer. This would help warm the egg shells gradually before they enter the higher temperature of the washer, thus preventing the risk of thermal cracking of the eggs which is a problem suffered in winter when environmental temperatures are very low. This would be even more useful if the sanitiser contained chemicals which helped to wet the soils such as Virogard (containing QACs and surfactants)

Recovery of Black Eggs

These eggs had been collected within the last few days and were heavily contaminated with well caked on soils and were at room temperature when used for the trials.

Table 16: Recovery of black eggs following repeated washing in Plant 3

Treatment	No. of washes	Eggs Recovered	Percent (%)
Circhlor & Virogard	2	13	28.89
	3	18	40.00
	4	8	17.78
	Rejected after 4	6	13.33
Asepto LF & Prochlor	2	8	17.78
	3	8	17.78
	4	19	42.22
	Rejected after 4	10	22.22

Microbiological Analysis

Box plots of the $\log_{10}$ TVC (cfu/egg) are shown in Figure 15. From these plots the following key observations can be made.

- Dirty eggs ("before") have considerably higher levels of TVC than any washed eggs.
- There appears to be little difference in TVC between eggs that have been washed repeatedly (to achieve visual cleanliness). Graded eggs appear marginally higher in some cases.
- At Plant 1, Circhlor and Virogard appear to be much more effective (yielding approx. 6 $\log_{10}$ cfu/egg reduction) than at any other plant under the conditions used in this study. The reason for this is unknown, but may be related to the actual temperatures achieved, effective sprays, constant chemical levels measured and achieved and possibly the freshness of the dirty eggs. Asepto and Prochlor were fairly consistent in the final microbial load on eggs (achieving about a 4 $\log_{10}$ cfu/egg reduction).

For Plant 1 there was a significant interaction between chemical/day and process step ($P < 0.001$ – excluding dirty eggs from the analysis). This interaction was due to graded eggs being higher on Day 1 than on Day 2, which is expected to be the result of the plant being thoroughly cleaned overnight.

For Plants 2 and 3, excluding dirty eggs prior to washing, there were no significant differences between multiple washes ($P = 0.09$ and 0.15), chemical /day ($P = 0.75$ and 0.85), nor their interaction ($P = 0.43$ and 0.35).

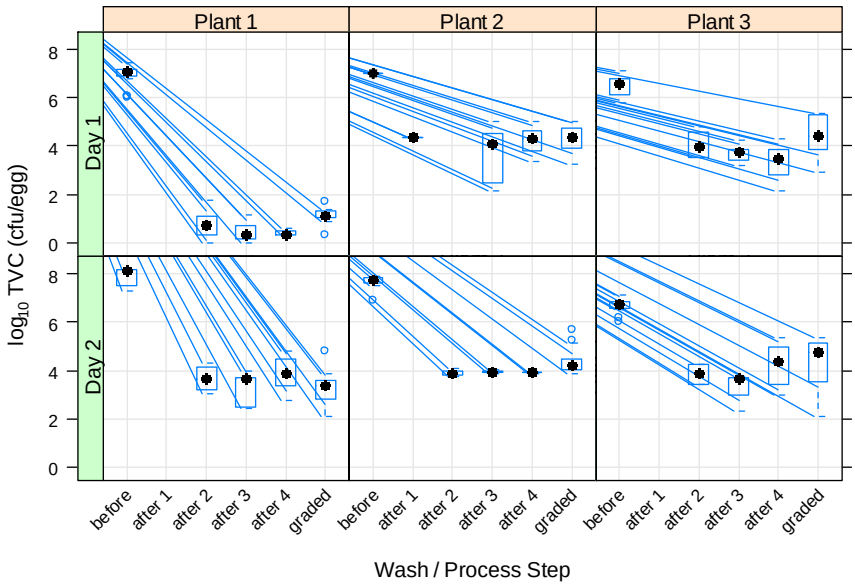


Figure 15: Box plots of the $\log_{10}$ TVC (cfu/egg) on eggs before washing, after washing one to four times, and after grading. Eggs were collected over two days from three plants after being washed using two detergent/sanitiser combinations.

Box plots for *Enterobacteriaceae* counts per egg are shown in Figure 16. The key observations from this figure are:

- Unwashed eggs can be substantially contaminated with *Enterobacteriaceae*.
- Washing under the conditions used in this study removes most *Enterobacteriaceae* from the egg – most washed eggs had less than detectable levels, i.e. < 1 cfu/egg.
- At Plant 1, only one graded egg had detectable levels of *Enterobacteriaceae* and these were too numerous to count. However, the other two plants resulted in multiple

detections of *Enterobacteriaceae*. This may be related to the hygiene status of the post-wash equipment at these plants.

Because of the limited post-washing *Enterobacteriaceae* detections no formal statistical analysis was undertaken.

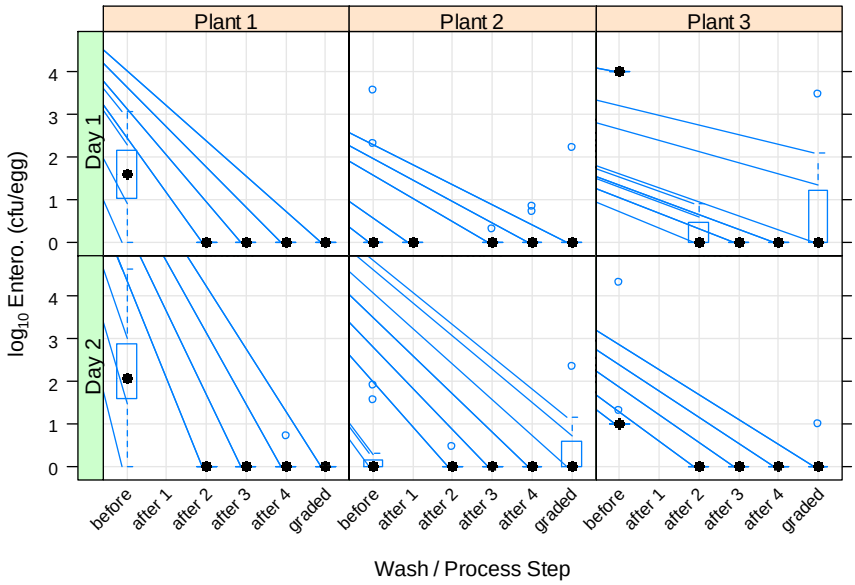


Figure 16: Box plots of the log_{10} *Enterobacteriaceae* (cfu/egg) on eggs before washing, after washing one to four times, and after grading. Eggs were collected over two days from three plants after being washed using two detergent/sanitiser combinations.

General Sanitation of Egg Processing Plants

Swabbed areas were of different sizes and depended on the equipment surface being swabbed and hence levels of TVC and *Enterobacteriaceae* are not directly comparable (Table 17). Nevertheless, *Enterobacteriaceae* were isolated from post-wash equipment at all plants. In particular, all swabs at Plant 2 resulted in isolations of *Enterobacteriaceae*, at high levels (per swab) compared to the other plants, indicating the presence of considerable faecal contamination. This poses a potential risk if foodborne bacteria, such as *Salmonella*, are present.

Table 17: Summary of levels of hygiene indicators obtained by swabbing various equipment surfaces at the three plants.

Plant	Before/after washing	Average log_{10} TVC (cfu/swab)	Percent *Enterobacteriaceae* detections (pos/n)	Average log_{10} *Enterobacteriaceae* (cfu/swab)
1	before	4.58	33 (2/6)	0.05
	after	4.77	37 (10/27)	0.40
2	before	6.93	100 (10/10)	4.25[1]
	after	5.97	100 (30/30)	2.78[2]
3	before	5.86	89 (8/9)	1.22
	after	4.67	35 (7/20)	0.62

[1] excludes two swabs which resulted in overgrown plates
[2] excludes five swabs which resulted in overgrown plates

Conclusions

Effective washing and sanitation of eggs to maximise food safety and profitability is difficult to achieve in commercial plants. Specific problems include:

- Large variation between (measured) input water temperatures and actual temperature of water at egg surface.
- Inability to accurately measure active chemical concentrations at the egg shell surface and make appropriate adjustments.
- Inadequate agitation of chemical solution on the egg due to blocked spray nozzles and brushes that are ineffective or interfere with sprays.
- The design of large scale egg washing machines makes it difficult to measure the temperature of water at the egg shell surface, especially where brushes have been introduced between sprays and eggs. Also the drive to recycle water has made it very difficult to control chemical application. These problems can result in ineffective washing and sanitising.

It was also clear from these site visits that cleaning of washing and grading equipment is often difficult and hence is not as thorough as it needs to be. However, clean equipment and contact surfaces are critical to maintain the microbial quality and safety of eggs after washing.

Costs associated with water use, heating of water and chemical use are placing increasing pressure on egg processors. This issue was raised during a project Steering Committee meeting (dated 09/02/2012) and subsequently confirmed by egg processors during plant visits. Any advancement in this area that does not negatively impact egg quality and safety and increases profit margins would be beneficial to the industry. Key components to effective egg washing are contact time, water temperature, water pressure, physical agitation, chemical composition and strength of cleaning and sanitation chemicals (pers. comm. G. Bourne, Chemetall and Dr S. Pahl, SARDI). As such, further research into chemicals and washing systems would likely be welcomed by the industry. Potential approaches include the use of wetting agents to loosen faecal matter from the surface of eggs, investigation of the chemistry that leads to calcium precipitation and the loss of active ingredients particularly evident in recycled water systems. Considerations would also need to include practicality, cost (including energy and water), water recycling/reduction, egg recovery and reduction in *Salmonella.* It would also be beneficial to investigate factors causing blocking of jets (e.g. chemical precipitation, faecal matter, calcium deposition) so that chemical application can be kept constant and prevent the constant need to remove and clean jets.

Increasing understanding of correct chemical use, machine effectiveness, temperature control, and plant hygiene is also likely to lead to better egg recovery and safety. Improving the performance of the wash system at two plants led to improved egg recovery noted by plant staff. Importantly, information regarding plant hygiene (as assessed by swabs of equipment) assisted in improving cleaning practices and reducing potential for post wash contamination. Such improvements industry wide can lead to better egg recovery and improved safety of eggs and egg products. This in turn will help make eggs safer for the consumer and reduce the potential for foodborne outbreaks.

Implications

The annual production of eggs in Australia totalled 345 million dozen in 2009/10, of which 63.5% were cage eggs, 7.6% were barn laid, 26.6% were free range and 2.2% were organic (Australian Egg Corporation Limited, 2010). Black eggs, which are so visually contaminated that they are currently discarded by industry without washing, can constitute up to 2% of non-cage eggs and the ability to recover these eggs could result in significant financial benefits. During in-plant trials an average of 29, 57 and 85% of black eggs were recovered after two, three and four washes respectively under the conditions used. Based on the 2009 production volumes of 125.9 million dozen non-cage eggs (AECL, 2010) and the estimate of 2% black eggs, this results in a total of 2.52 million dozen black eggs. Consequently, the recovery of 85% of these, or 2.14 million dozen, has a potential retail value of $9.48M.[3] These figures are expected to increase as barn-laid, free range and organic egg production gain market share. However, the financial benefit of washing eggs multiple times will depend on individual processors and their plant setup. In particular, the ability to return still dirty eggs for rewashing, chemical dosing set-up and washer design is critical. Therefore it is difficult to accurately measure the overall potential financial benefit to industry. However, improved wash systems and chemicals can make this process easier especially if satisfactory results can be achieved with single washing.

In addition, eggs and egg products continue to be associated with foodborne illness outbreaks (OzFoodNet Working Group, 2010). Consequently, improvements in removing visual and microbiological contamination on the egg surface and better hygiene of post-wash equipment are expected to result in reductions of egg-related foodborne illness, though their size is uncertain and will depend on the uptake by industry.

Recommendations

The following issues were identified as part of this project and recommendations are made on how these could be address.

Issue:
Due to the rapid commercialisation of large scale egg washing machines the ability to measure the water temperature at the egg shell surface has become very difficult, especially where brushes have been introduced between sprays and eggs. Also the drive to recycle water has made it very difficult to control chemical application. These problems can result in ineffective washing and sanitising.

Recommendations:
These issues can be addressed by

- Developing an industry fact sheet on more effective egg washing (will be undertaken as part of this project)
- Incorporate findings from this project into industry training programs, e.g. through AECL
- Producing further training materials, such as short videos, and additional in-plant assistance

Issue:
Inadequate agitation of chemical solution on the egg due to blocked spray nozzles and brushes that are ineffective or interfere with sprays.

[3] Assuming an average retail price of $4.43 per dozen (AECL, 2010)

Recommendation:

- Raise industry awareness about the importance of proper agitation and unblocking of spray nozzles through an industry fact sheet and updated training materials.
- Encourage further investigation of chemicals and systems to prevent blockages and methods for determining brush effectiveness

Issue:
It can be difficult to monitor and adjust mechanical agitation, chemical concentration in the wash and sanitiser water and the temperature at the egg surface due to washer designs.

Recommendation:

- Provide feedback to manufacturers of egg washers to allow them to modify their washers to assist in the monitoring and adjusting of critical process criteria.

Issue:
Washing and grading equipment that has not been thoroughly cleaned can result in contamination of eggs by faecal bacteria, including pathogens, after washing.

Recommendation:

- Raise industry awareness about the importance of proper cleaning of equipment through an industry fact sheet and updated training materials and development of an industry standard.

Issue:
Washing of eggs is water, energy and chemical intensive – chemicals can also result in calcium precipitation which can block spray jets.

Recommendations:

- Investigate or develop chemical detergents and sanitisers that can be effectively used at lower temperatures, with less water and result in less calcium precipitation.
- Identify opportunities for improved water recycling – these may be plant dependent.

Issue:
Changes in egg production to non-cage systems are likely to result in a higher proportion of dirty eggs with at least some of those dirty eggs being contaminated with *Salmonella*. Hence, there may subsequently be increases in salmonellosis associated with eggs. However, effective washing can result in substantial reduction of *Salmonella* on the egg shell, though cleanliness of equipment is critical to avoid re-contamination post washing.

Recommendation:

- Developing an industry fact sheet on effective egg washing (will be undertaken as part of this project), that also focuses on the importance of cleaning of equipment;
- Incorporate information into industry training programs and development of an industry standard;
- Provide feedback to manufacturers of egg washers to allow them to modify their washers to assist in the monitoring and adjusting of critical process criteria.

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Appendix 1: Visual Assessment of Eggs Following In-Plant Trials

Plant 1

Circhlor

 Unwashed Egg	 Unwashed Egg
 Egg washed twice with Chemical 1	 Egg washed four times with Chemical 1
 Egg washed with Chemical 1 and sent through grading line	 Egg washed with Chemical 1 and sent through grading line

Asepto LF

 Unwashed Egg	 Unwashed Egg
 Egg washed twice with Chemical 2	 Egg washed four times with Chemical 2
 Egg washed with Chemical 2 and sent through grading line	 Egg washed with Chemical 2 and sent through grading line

Plant 2

Circhlor

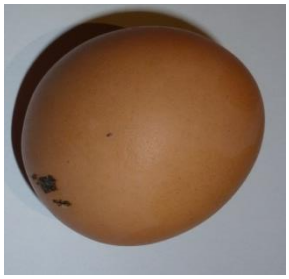
 Unwashed Egg	 Unwashed Egg
 Egg washed three times with Chemical 1	 Egg washed four times with Chemical 1
 Egg washed with Chemical 1 and sent through grading line	 Egg washed with Chemical 1 and sent through grading line

Asepto LF

 Unwashed Egg	 Unwashed Egg
 Egg washed twice with Chemical 2	 Egg washed four times with Chemical 2
 Egg washed with Chemical 2 and sent through grading line	 Egg washed with Chemical 2 and sent through grading line

Plant 3

Circhlor

 Unwashed Egg	 Unwashed Egg
 Egg washed twice with Chemical 1	 Egg washed four times with Chemical 1
 Egg washed with Chemical 1 and sent through grading line	 Egg washed with Chemical 1 and sent through grading line

Asepto LF

 Unwashed Egg	 Unwashed Egg
 Egg washed twice with Chemical 2	 Egg washed four times with Chemical 2
 Egg washed with Chemical 2 and sent through grading line	 Egg washed with Chemical 2 and sent through grading line

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Plain English Compendium Summary

Sub-Project Title:	**Egg washing: Improving efficacy and safety to optimise profitability**
Poultry CRC Sub-Project No.:	3.2.1
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Sub-Project Overview	The aims of this project were to improve the recovery of dirty eggs and to reduce microbial contamination on the egg shells. These aims were achieved through a series of laboratory and in-plant trials.
Background	The annual Australian production of eggs totalled 345 million dozen in 2009/10. The majority of eggs are washed prior to packing to remove dirt and faecal material and to reduce the microbial contamination of the egg shell. An estimated 2% of non-cage eggs are 'black eggs' and these are currently discarded because they are considered unrecoverable. Food Standards Australia New Zealand estimates that egg-related salmonellosis cases of cost the Australian economy $44 million per year.
Research	This research project consisted of three laboratory trials and three in-plant trials. In the first laboratory trial two detergent/sanitiser and suitable wash/sanitise temperatures combinations were identified based on their ability to clean artificially dirtied eggs. These combinations were found to be effective for recovering black eggs and reducing *Salmonella* from inoculated egg shells in lab-based trials. During in-plant trials multiple washing with either of the chemical combinations resulted in up to 85% recovery of black eggs after four washes. The efficacy varied between plants and was affected by plant specific issues such as ability to accurately dose chemicals, blocked spray jets, brushes interfering with sprays, ability to measure and maintain the water temperature at the egg surface and recycling of water. Cleaning of washing and grading equipment is often difficult. Detection of microbes of faecal origin from grading machinery indicates the potential for re-contamination of eggs following washing. Appropriate guidance surrounding plant hygiene can assist in reducing this risk and subsequent potential for foodborne illness.
Implications	Based on the 2009 production volumes and findings from this work, 2.14 million dozen black eggs could potentially be recovered, totalling a retail value of $9.48M. These figures are expected to increase as non-cage production gains market share. Reducing microbial contamination of egg shells and preventing re-contamination during grading will help reduce the potential for foodborne outbreaks.
Publications	None.