Evaluation of the performance of different cleaning treatments in reducing microbial contamination of poultry transport crates

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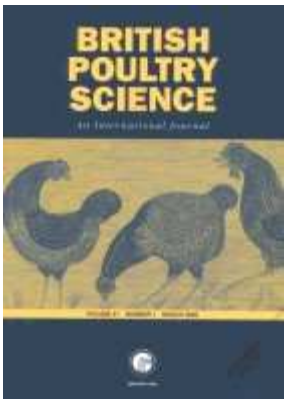

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Journal:	*British Poultry Science*
Manuscript ID:	CBPS-2007-263.R1
Manuscript Type:	Original Manuscript
Date Submitted by the Author:	17-Jan-2008
Complete List of Authors:	Allen, Vivien; University of Bristol, Clinical Veterinary Science Burton, Colin; CEMAGREF Wilkinson, David; Formerly Silsoe Research Institute Whyte, Robin; Formerly Silsoe Research Institute Harris, Jillian; University of Bristol, Clinical Veterinary Science Howell, Mary; Food Standards Agency, Veterinary Public Health Tinker, David; David Tinker & Associates Ltd
Keywords:	Broilers, transport crates, cleaning

Evaluation of the performance of different cleaning treatments in reducing microbial contamination of poultry transport crates

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Accepted for publication 27th January 2008

Abstract 1. The present systems for cleaning the plastic crates (drawers) used to transport live poultry to the processing plant are known to be inadequate for removing microbial contamination.

2. To investigate possible improvements, a mobile experimental rig was constructed and operated in the lairage of a poultry processing plant. The cleaning rig could simulate the conditions of commercial cleaning systems and utilise freshly-emptied crates from the processing plant.

3. The aim of the study was to improve cleaning by enhancing the removal of adherent organic material on the crates and by reducing microbial contamination by at least 4 $\log_{10}$ units.

4. Trials showed that the most effective treatments against *Campylobacter* were either (a) the combination of soaking at 55°C, brushing for 90 s, washing for 15 s at 60°C, followed by the application of disinfectant (Virkon S in this study) or (b) the use of ultrasound (4 kW) at 65°C for 3 – 6 min, with or without mechanical brushing of crates.

5. Both of these treatments also achieved a 4-$\log_{10}$ reduction or more in the counts of *Enterobacteriaceae* but were less effective in reducing aerobic plate counts.

6. It was noted that there was little correlation between the visual assessment of crate cleanliness and microbiological counts.

7. It was concluded that the demonstrated enhanced cleaning could contribute significantly to overall hygiene control in poultrymeat production.

INTRODUCTION

The plastic crates in which live poultry are commonly transported from the farm to the processing plant are known to be a source of contamination and cross-contamination with zoonotic pathogens such as *Salmonella* and *Campylobacter* spp. (Tinker *et al.,* 2005). The problem arises primarily because most of the crate-cleaning systems used commercially do not consistently remove microbial contaminants before the crates are re-used (Humphrey and Allen, 2002). The potential role of contaminated crates in spreading *Salmonella* has been highlighted in Canada by Rigby *et al.* (1980*a, b*) and also reported in other countries in relation to either *Salmonella* or *Campylobacter* (Jacobs-Reitsma and Bolder (1998), Bailey *et al.*

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(2001), Corry *et al.* (2002), Slader *et al.* (2002) and Allen *et al.* (2008)) thus showing that the situation has remained unchanged for at least 20 years. Factors responsible for the poor performance of commercial cleaning systems include the practice of recycling most of the wash-water, which becomes increasingly loaded with microbes and organic debris. However, in the absence of effective disinfection, it is likely that, even with the use of fresh water throughout the process, current cleaning systems would still have only a limited impact on microbial contamination of the crates (Burton *et al.,* 2004).

Because it is evident that improvements in crate cleaning are needed, the present study was carried out to evaluate a number of possible treatment options. These were aimed at removing any adherent organic material present and the removal and /or destruction of microbial contaminants on the crate surface. The trials were based around a mobile experimental rig in which the washing conditions simulated those of a commercial cleaning system and which utilised freshly-emptied crates from a poultry processing plant.

MATERIALS AND METHODS

Test rig

The stages typically involved in crate cleaning are (i) the inversion of the crate, (ii) pre-washing, (iii) soaking, (iv) final wash, (v) crate reversion, and (vi) disinfection. For experimental purposes, a mobile crate-cleaning rig was designed and constructed for independent operation in the lairage/crate washing area of a poultry processing plant. This approach (a) avoided any disruption of the commercial cleaning process, (b) made use of the actual soiled crates as soon as the birds had been removed and (c) allowed a wide range of possible treatments to be evaluated after the crates had been

inverted to remove any loose organic debris. The rig is shown in Figure 1 and its basic features illustrated in Figure 2. With the use of water spray-jets and a soak tank, it was possible to simulate various commercial conditions using cleaning water from the adjacent commercial plant that was naturally contaminated with organic matter from the crate-cleaning operation. Alternatively, the rig could use clean water and included a water heater. The rig operated in a batch-wise manner, cleaning individual crates for specified times corresponding to the measured residence periods in the commercial system.

Crate treatments

Specific treatments studied in conjunction with the rig were as follows.

Use of detergent

For some trials, a detergent was added to the soak tank at the beginning of the trial to facilitate the cleaning process. This was a low-foam, caustic product (Spectak G: Johnson Diversey, Northampton, UK) and it was incorporated in the water at a concentration of 0.1% (v/v).

Crate disinfection

Chemical disinfection of washed crates was carried out with a hand-held spray that delivered a measured amount of disinfectant solution to each crate. The disinfectant chosen was Virkon S (Dupont Animal Health Solutions, Sudbury, Suffolk, UK) as an example of a product commonly used in the industry. Applications were specified as 250 ml of 0.5% (v/v), 500 ml of 1% (v/v) or 500 ml of a 2% (v/v) solution.

Water removal

In order to remove the residual wash-water that could carry a high microbial load, the vibrating tray rig was used in conjunction with the washing trials. An alternative rig

used a system of jets linked to a compressed air supply. This produced an effect close to drying.

Brushing of crates

To simulate mechanical brushing, a cylindrical nylon brush attached to an electric drill was applied manually over the entire surface of the crate base; an operation taking 30 to 90 s. Before each re-use, the brush was thoroughly cleaned.

Steam treatment

Steam was generated from a unit that included a 1.5 kW boiler, an applicator pipe and a hood (100 mm x 75 mm). The interior of the crate was treated for 2 min in total, during which 90 g of steam was applied.

Ultra-violet (UV) treatment

The crate was exposed to a set of four 20 W ultraviolet lamps (Uvitec, Cambridge, UK) located in the hood of the main rig, approximately 0.5 m above the crate base. The exposure time to UVC at 254nm was 1 min.

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Use of ultrasound

An ultrasonic generator (Production Line Cleaning (PLC) Ltd, Diss, Norfolk, UK) was used to provide 4 kW of energy within a separate 700 l stainless steel tank containing water at 45° or 60°C to which 2% (v/v) of a surfactant (CB 10: Access Chemicals Ltd, Wellingborough, Northants, UK) had been added. Each crate was treated for either 3 or 6 min.

Measurement of microbial load on crates

Two different methods were used as follows.

Swab method

Four large cotton-wool swabs with wooden shafts (MW 104J, Medical Wire, Corsham, Wilts, UK) were moistened with Maximum Recovery Diluent (MRD,

CM 733, Oxoid, Basingstoke, Hants, UK) and each was used to sample one quarter of the interior base-area of the crate. Swabbing was carried out in horizontal, vertical and diagonal directions, and all 4 swabs were pooled in 10 ml of MRD.

Sponge method

Using aseptic precautions, a sterile sponge of 103 x 185 x 5.8 mm (cat. No. 95000087, Spongyl 87, Spontex Professionel, Neuilly-Sur-Seine, France) was wetted with a small amount of liquid from 100 ml of MRD and transferred to a sterile plastic bag. When required, the sponge was removed and used to swab the interior base of the crate in horizontal, vertical and diagonal directions from bottom left to top right. The sponge was then returned to the bag and the remainder of the MRD added. Using both hands, the bag was squeezed 60 times to release microbial cells into the diluent. Finally, the sponge was wrung out aseptically by hand and the resultant suspension transferred to a 100 ml screw-capped container. For both sampling methods, samples were transported to the laboratory in a cool box held at around 1°C using ice packs and were examined within 12 h.

Microbiological examination

Aerobic plate counts (APC) and presumptive Enterobacteriaceae

From serial, 10-fold sample dilutions in MRD, 100 µl amounts of each were used to inoculate in duplicate, Plate Count Agar (PCA, Oxoid CM0325) and Violet Red Bile Glucose Agar (VRBGA, Oxoid CM 0485). Plates were incubated at either 30°C for 48 h (PCA) or 37°C for 24 h (VRBGA) and the colonies counted. Characteristically, *Enterobacteriaceae* appear as round, purple colonies 1 – 2 mm in diameter and surrounded by purple haloes. As recommended by the media manufacturer, however, all red colonies were counted as presumptive *Enterobacteriaceae*.

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Enumeration of Campylobacter *spp.*

Sample dilutions (100 µl) were used to inoculate modified charcoal cefoperazone desoxycholate agar, which comprised Campylobacter Blood-free Selective Agar Base (Oxoid, CM 739) and Campylobacter Selective Supplement (Oxoid, SR 155). Plates were incubated at 42°C for 48 h under micro-aerobic conditions from gas-generating packs (Oxoid, CN 0035A), after which colonies were counted. Standard confirmatory tests included a positive oxidase reaction, microscopical appearance of Gram-strained preparations and failure to grow in air at 25°C. Some colonies were confirmed as *Campylobacter* by a latex agglutination method (Campylobacter Test Kit: Oxoid, DR 0150M).

Visual assessment of organic debris

To determine the effect of residual organic debris on the microbiological condition of the crates, tests were carried out on crates cleaned and sampled at the processing plant. The tests involved 12 crates, each of which was also sampled by swabbing the internal surface of the base and obtaining an APC and a count of *Enterobacteriaceae*, as described above. Crates were then scored visually for the total amount of organic debris in grams on each of three parts of the crate: (i) the interior of the base; (ii) the sides, both inside and out, and (iii) the underside. The organic matter could not be completely removed from the crate, so the amount present was estimated on the basis that one heaped 5 ml teaspoon of debris was found to weigh approximately 2 g.

Statistical analysis

Analysis of variance (ANOVA) was undertaken using 'Minitab' software. Because the limit of detection for the organisms being sought was $\log_{10}$ 3 cfu / crate base, values below this level were assumed to be $\log_{10}$ 2.7 cfu for the purpose of analysis.

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RESULTS

Selection of processing plant

Before starting the trials, it was necessary to ensure that the processing plant used in conjunction with the test rig was not atypical with respect to the cleaning of transport crates. Therefore, a comparison was made of plants belonging to three different companies and tests were carried out on crates before and after cleaning to determine APC and incidence of *Enterobacteriaceae* and *Campylobacter*. For this purpose, 12 crates were taken on each occasion and sampled by the swab method. The results shown in Table 1 indicate that the three plants were comparable, especially in relation to APC, but varied markedly with regard to *Campylobacter,* which would have been influenced by the colonisation status of the flocks processed that day. In the two cases where crates were tested before and after the cleaning process, there was little effect of cleaning on APC or *Enterobacteriaceae* counts. The plant selected for the study (Plant B) had sufficient space to accommodate the test rig alongside the commercial crate-cleaning system, with easy access to the supply of used crates and necessary services.

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Significance of visual scores for organic debris

Table 2 gives a comparison of visual scores and the microbiological condition of a random set of factory-cleaned crates. It is clear that the scores show no correlation with APC or counts of *Enterobacteriaceae* and, in each case, microbial contamination remained high after the commercial cleaning process.

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Preliminary trials

Trials were carried out with the test rig and using the sponge method of sampling to determine the efficacy of various treatments in reducing adherent organic matter and numbers of microbes on naturally-contaminated crates (n = 4). These followed 4

different approaches, the aim being to reduce microbial contamination by at least 4 $\log_{10}$ units. This value was chosen as it is the standard often used to assess effective cleaning of food contact surfaces. The first series of trials covered variations in current commercial practices for soaking and washing crates and is designated TA in Table 3. The second approach (TB) was concerned with the removal of contaminated process water from the crates and the third (TC) was devoted to different options for crate disinfection, including the use of a chemical disinfectant (Virkon S), steam, UV light and ultrasonic treatment. The final series of trials, TD covered more vigorous cleaning systems, which involved brushing, use of detergent and increased amounts of disinfectant, and a second wash at the end of the process. The results given in Table 3 are presented in each case as the changes in mean counts on PCA and VRBGA, respectively, relative to those obtained for the uncleaned control crates.

In general, most of the treatments had only a relatively small effect (< 2 $\log_{10}$ units) in reducing crate contamination and, in some cases, the mean counts were slightly higher after treatment, showing the absence of any obvious kill or removal of microbes. However, some treatments resulted in reductions of at least 3 – 5 $\log_{10}$ units. These were mostly related to process options including brushing, soaking or washing at 63^{o}C and using an increased amount of disinfectant. Therefore, a second series of trials were based on selected combinations of the more successful treatments.

Testing of selected best treatment combinations

The results obtained with the most effective treatment combinations are shown in Figure 3 a, b and c for APC, *Enterobacteriaceae* and *Campylobacter*, respectively. Of the three microbial groups, *Campylobacter* was usually the most susceptible and a reduction of 4 $\log_{10}$ units or more was obtained with 5 of the 8 treatments (2, 3, 6, 7 and 8). For all 5, the *Campylobacter* reductions were highly significant (P <0.001)

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when compared with the control group (uncleaned crates). The treatments options investigated included a combination of soaking at 55°C, brushing for 90 s, washing for 15 s in water at 60°C, followed by the application of disinfectant, or the use of ultrasound at 65°C for 3 – 6 min, either with or without mechanical brushing of the crates. Treatment 2 included a double stage of hot soaking, brushing and hot washing. The same treatments were less effective with respect to APC and *Enterobacteriaceae*, but still achieved at least a 4-$\log_{10}$ reduction in the latter ($P <$ 0.001 in all cases).

Of the remainder, treatment 5, the standard simulated factory wash followed by 500 ml of 2% Virkon S, produced significant reductions in APC and *Enterobacteriaceae* ($P < 0.001$), but less so for *Campylobacter* ($P < 0.05$). Similarly, treatment 4, which involved a hot soak and wash prior to disinfectant application, also had a less significant effect on *Campylobacter* ($P = 0.002$). On the other hand, treatment 1, a cold process with brushing, produced significant reductions ($P < 0.001$) for *Enterobacteriaceae* and *Campylobacter*, but had only a marginal effect on APC ($P = 0.05$).

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DISCUSSION

Soiled transport crates are not easy to clean and disinfect properly under the conditions used currently for operating commercial systems. Part of the reason for this lies with the design of the plastic crates themselves. There are many niches present that can trap organic debris and microbes, and, during long-term use, surfaces may become scratched and suffer other minor damage that adds to the problem. Furthermore, there is rapid development of a biofilm, which is a thin layer of adherent organic matter that contains numerous microbes and is extremely difficult to remove

(Burton *et al.,* 2004). Other contributory factors relate to the cleaning process itself, which is often constrained by the space available at the processing plant. One consequence of this is that the residence time of each crate in the washing cycle is greatly limited. Although 'best practices' have been identified from these studies to improve present crate-cleaning procedures (Tinker *et al.,* 2005), these are unlikely to have sufficient effect on microbial contamination to make the cleaning process a critical control point in the processing operation, as proposed by Slader *et al.* (2002). Thus, the present study set out to evaluate a number of possible treatments beyond normal factory conditions that might achieve a significant reduction in microbial contamination. For that purpose, it was necessary to perform the trials in a controlled manner and under conditions that resembled those used commercially. The use of an experimental rig, situated in a processing plant, has avoided the apparent limitations of trials carried out in a purely laboratory setting (Carr *et al.,* 1999).

From the practical viewpoint, complete sterilisation of the crates is not a feasible objective and a reduction of at least 4 $\log_{10}$ units in microbial contamination was considered to be an acceptable target. To achieve this, however, crates would need to be as clean as possible before the application of a disinfectant, since any residual soiling would be expected to neutralise the applied chemical and thus reduce treatment efficacy, as indicated by Corry *et al.* (2002) and Slader *et al.* (2002) from observations on commercial practices, and borne out in the present study. Thus, the treatments studied here have included a number of measures aimed at facilitating the removal of organic debris from the crates. Of these, only mechanical brushing and ultrasonic treatment would require any significant technological changes in the cleaning process. Although the application of ultrasound was aimed primarily at loosening attached debris, it appeared to have a synergistic effect with heat in killing a

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proportion of the microbes present and would merit further investigation, in the context of crate cleaning.

The most effective treatments studied here differed from that recommended by Ramesh *et al.* (2003), in which transport containers with galvanised frames and fibreglass floors were immersed for 2 min in 1000 mg/l of sodium hypochlorite at 70°C. This combination was found to eliminate coliform bacteria and *Salmonella*, when containers were treated in a prototype cleaning system, but it is likely to be less effective when part of the wash water is recycled, due to the build-up of organic matter and the large amounts of chemical. Partial recycling of wash water is a common practice in the United Kingdom and chlorine would be readily inactivated under such conditions. Furthermore, soaking at 70°C would be too severe for the plastic material used in conventional UK crate manufacture - thermosetting plastic which is moulded with a multitude of ridges on a grid framework to provide sufficient reinforcing.

It is clearly possible to modify the existing cleaning process to reduce microbial contamination of the crates and the performance of options studied here would appear to compare favourably with suggestions, such as the use of disposable crate liners (Slader *et al.,* 2002) and drying of cleaned crates before re-cycling them to eliminate *Campylobacter* (Berrang and Northcutt, 2005), both of which are likely to be costly. Not only would the latter require additional space at the plant for drying, but also investment in additional new crates to compensate for the delay in supplying those needed for immediate re-use (Burton *et al.,* 2004).

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Whatever the most effective changes to the system, a successful means of reducing microbial contamination of transport crates could contribute significantly to overall hygiene control in poultrymeat production and may also play a part in

controlling some diseases that are of economic concern to the Industry. However, total elimination of pathogens on crates may not be possible and it is unclear if such a reduction will be effective in controlling a particular hazard.

Acknowledgement

The work described in this paper formed part of a project funded by the UK Food Standards Agency. The authors are indebted to the management and technical staff of the poultry companies who collaborated in this research and to PLC Ltd for their advice and loan of the ultrasonic equipment.

REFERENCES

ALLEN, V.M., WEAVER, H., RIDLEY, A. M., HARRIS, J. A., SHARMA, M., EMERY, J., SPARKS, N., LEWIS, M. AND EDGE, S. (2008) Sources and spread of thermophilic *Campylobacter* spp. during partial depopulation of broiler chicken flocks. *Journal of Food Protection*,

BAILEY, J.S., STERN, N.J., FEDORKA-CRAY, P., CRAVEN, S.E., COX, N.A., COSBY, D.E., LADELY, S. & MUSGROVE, M.T. (2001) Sources and movement of *Salmonella* through integrated poultry operations: A multistate epidemiological investigation. *Journal of Food Protection,* **64:** 1690 – 1697.

BERRANG, M.E. & NORTHCUTT, J.K. (2005) Use of water spray and extended drying time to lower bacterial numbers on soiled flooring from broiler transport coops. *Poultry Science,* **84:** 1797 – 1801.

BURTON, C.H., WHYTE, R.T., ALLEN, V.M. CORRY, J.E.L. & DAVIES, R. (2004) *Reducing Microbial Contamination of Poultry Transport Crates by Improved Cleaning and Disinfection Systems Based on Better Water Use: A State-of-the-Art Report on Crate Washing.* Silsoe, UK, Silsoe Research Institute.

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CARR, L., RIGAKOS, L., CARPENTER, G., BERNEY, G. & JOSEPH, S. (1999) An assessment of livehaul poultry transport container decontamination. *Dairy, Food & Environmental Sanitation,* **19:** 753 – 759.

CORRY, J.E.L., ALLEN, V.M., HUDSON, W.R., BRESLIN, M.F. & DAVIES, R.H. (2002) Sources of *Salmonella* on broiler carcasses during transportation and processing: modes of contamination and methods of control. *Journal of Applied Microbiology,* **92:** 424 – 432.

HUMPHREY, T.J. & ALLEN, V.M. (2002) *Poultry Crate Hygiene: Final Report (FSA Project ZB 00033).* Bristol, UK, University of Bristol.

JACOBS-REITSMA, W.F. & BOLDER, N. (1998) The role of transport crates in *Campylobacter* contamination of broilers, in: Lastovica, A.J., Newell, D.G & Lastovica, E.E. (Eds) Campylobacter, Helicobacter *and Related Organisms: Proceedings of the Ninth International Workshop* pp. 379 – 380 (Capetown, S.A. Capetown Unversity).

RAMESH, N., JOSEPH, S.W., CARR, L.E., DOUGLASS, L.W. & WHEATON, F.W. (2003) Serial disinfection with heat and chlorine to reduce microorganism populations on poultry transport containers. *Journal of Food Protection,* **66:** 793 – 797.

RIGBY, C.E., PETTIT, J.R, BAKER, M.F., BENTLEY, A.H., SALOMENS, M.O. & LIOR, H. (1980a) Sources of *Salmonella* in an uninfected commercially processed broiler flock. *Canadian Journal of Comparative Medicine,* **44:** 267 – 274.

RIGBY, C.E., PETTIT, J.R, BAKER, M.F., BENTLEY, A.H., SALOMENS, M.O. & LIOR, H. (1980b) Flock infection and transport as sources of salmonellae in

broiler chickens and carcasses. *Canadian Journal of Comparative Medicine,* **44:** 323 - 337.

SLADER, J., DOMINGUE, G., JØRGENSEN, F., McALPINE, K., OWEN, R.J., BOLTON, F.J. & HUMPHREY, T.J. (2002) Impact of transport crate reuse and of catching and processing on *Campylobacter* and *Salmonella* contamination of broiler chickens. *Applied and Environmental Microbiology,* **68:** 713 – 719.

TINKER, D.B., BURTON, C.H. & ALLEN, V.M. (2005) Catching, transporting and lairage of live poultry, in: Mead, G.C. (Ed) *Food Safety Control in the Poultry Industry* pp. 153 – 173 (Cambridge, UK, Woodhead Publishing).

Table 1. *Microbiological examination of crates before and after factory cleaning at three different processing plants (all counts: mean log_{10} cfu per base, with standard deviation).*

Company	Processing stage	APC	*Enterobacteriaceae*	*Campylobacter*
A	Before cleaning	7.80 ± 0.37	6.87 ± 1.02	6.91 ± 0.85
B	Before cleaning	7.90 ± 0.73	7.56 ± 0.72	5.60[1]
C	Before cleaning	ND	ND	ND
A	After cleaning	7.57 ± 0.37	6.06 ± 0.34	5.66 ± 0.01
B	After cleaning	7.93 ± 0.52	7.35 ± 0.62	2.93 ± 0.86
C	After cleaning	7.73 ± 0.33	5.96 ± 0.22	5.34 ± 0.06

[1] Only one crate positive. Number of treatments = 12.

APC Aerobic Plate Count
ND Not determined.

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Table 2. *Comparison of visual assessment of residual organic debris on cleaned crates with extent of microbial contamination.*

Sample number	Visual score [1]	APC [2]	*Enterobacteriaceae* [2]
1	3	8.10	6.70
2	<1	7.81	6.63
3	2	7.81	6.33
4	1	8.18	6.87
5	8	8.02	6.67
6	<1	8.18	6.77
7	<1	8.04	6.62
8	1	7.98	7.23
9	2	8.60	6.85
10	1	8.25	7.12
11	1	7.98	6.82
12	<1	8.24	7.14

[1] Weight (g) of material per crate base.

[2] Log_{10} cfu per crate base.

APC Aerobic Plate Count

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Changes in the relative aerobic plate counts and Enterobacteriaceae in preliminary trials using different treatments tested using the experimental rig.

Table 3.

Code	**Description of treatment**	Change in count relative to uncleaned control (log_{10}) [1]	
		APC	Enterobact-eriaceae.
SWC	15 s pre-wash, 30 s soak, 15 s main wash (standard clean control)	+0.2	+0.5
TA3	No pre-wash, 30 s soak, 300 s main wash	-1.0	-1.8
TA4	300 s pre-wash, 30 s soak, 300 s main wash	-0.8	-1.8
TA5	15 s pre-wash, 300 s soak (40^{o}C), 15 s main wash	-0.6	-1.1
TA7	15 s pre-wash, 300 s soak (60^{o}C), 15 s main wash	-1.6	-1.5

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TB8	15 s pre-wash, 30 s soak, 15 s vibration, 60 s air-dry, 15 s wash (60°C)	-0.4	-0.5
TC1	15 s pre-wash, 30 s soak, 15 s main wash, 15 s wash with clean water (60°C), 60 s air-dry, 60 s exposure to UV	-0.4	-0.3
TC2	15 s pre-wash, 30 s soak, 15 s main wash, 15 s wash with clean water (60°C), 120 s steam	-0.6	-1.2
TC3	15 s pre-wash, 30 s soak, 15 s main wash, 15 s wash with clean water (60°C), 250 ml 0.5% Virkon S	-1.6	-1.7
TC4	15 s pre-wash, 30 s soak, 15 s main wash, 15 s wash with clean water (60°C), 60 s air-dry, 250 ml 0.5% Virkon S	-1.4	-1.7
TC5	15 s pre-wash, 30 s soak, 15 s main wash, 15 s wash with clean water, 60 s air-dry, 250 ml 0.5% Virkon S	-1.8	-1.6
TC6	15 s pre-wash, 30 s soak, 15 s wash with clean water, 120 s ultrasonic treatment at 45°C (control)	+0.2	-1.9
TC7	15 s pre-wash, 30 s soak, 15 s wash with clean water (60°C), 120 s ultrasonic treatment at 2 kW and 45°C	+0.3	-2.0
TC8	15 s pre-wash, 30 s soak, 15 s wash with clean water (60°C), 120 s ultrasonic treatment at 4 kW and 45°C	-0.3	-2.3
TD1	15 s pre-wash, 30 s soak (52°C), 300 s brush, 20 s main wash (63°C)	-2.4	-3.8
TD2	15 s pre-wash, 30 s soak, 15 s main wash (63°C), 500 ml 1% Virkon S	-0.8	-1.4

TD3	15 s pre-wash, 30 s soak, 15 s main wash (63°C), 500 ml 2% Virkon S	-2.1	-3.6
TD4	15 s pre-wash, 30 s soak, 120 s brush, 15 s main wash (63°C), 500 ml 2% Virkon S	-2.8	-5.4
TD5	15 s pre-wash, 30 s dirty-water soak (55 – 60°C), 15 s clean wash (63°C)	-1.3	-2.0
TD6	15 s pre-wash, 30 s soak in clean water with 0.1% detergent (55 – 60°C), 15 s clean wash (63°C)	-2.7	-3.2
TD7	15 s pre-wash, 30 s soak in clean water with 0.1% detergent (55 – 60°C), 15 s clean wash (63°C), wash repeated	-3.6	-4.1
TD8	15 s pre-wash, 30 s soak, 15 s wash in clean water (55°C)	-1.5	-1.7

[1] Based on a comparison of mean counts. Number of treatments = 5.

Soaking and washing were in cold water unless stated otherwise.

Figure 1. *The test rig set up on a trailer to enable periodic use in the lairage/crate washing areas of the processing plant.*

Figure 2. *Schematic diagram of the test rig, showing the basic components.*

Figure 3. *Effects on microbial contamination of crate treatments selected from preliminary trials: (a) aerobic plate counts; (b) Enterobacteriaceae; (c) Campylobacter.*

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Key	
Before cleaning	Freshly-emptied crates
Standard clean	15 s pre-wash, 30 s soak and 15 s wash in dirty water
TE1	15 s pre-wash, 30 s soak, 90 s brush, 15 s wash with clean water 90 s brush
TE2	15 s pre-wash, 30 s soak (55°C) with 0.1% detergent, 90 s brush, 15 s wash in clean water (60°C); soak, brush and wash repeated; 500 ml 2% Virkon
TE3	15 s pre-wash, 30 s soak (55°C) with 0.1% detergent, 90 s brush, 15 s wash in clean water (60°C), 500 ml 2% Virkon
TE4	15 s pre-wash, 30 s soak (55°C) with 0.1% detergent, 15 s wash in clean water (60°C), 500 ml 2% Virkon
TE5	Standard clean, 500 ml 2% Virkon
TE6	Standard clean, 30 s brush, 3 min ultrasound (65°C)
TE7	Standard clean, 30 s brush, 6 min ultrasound (65°C)
TE8	Standard clean, 6 min ultrasound (65°C)
n = 5 for all treatments	

Figure 1

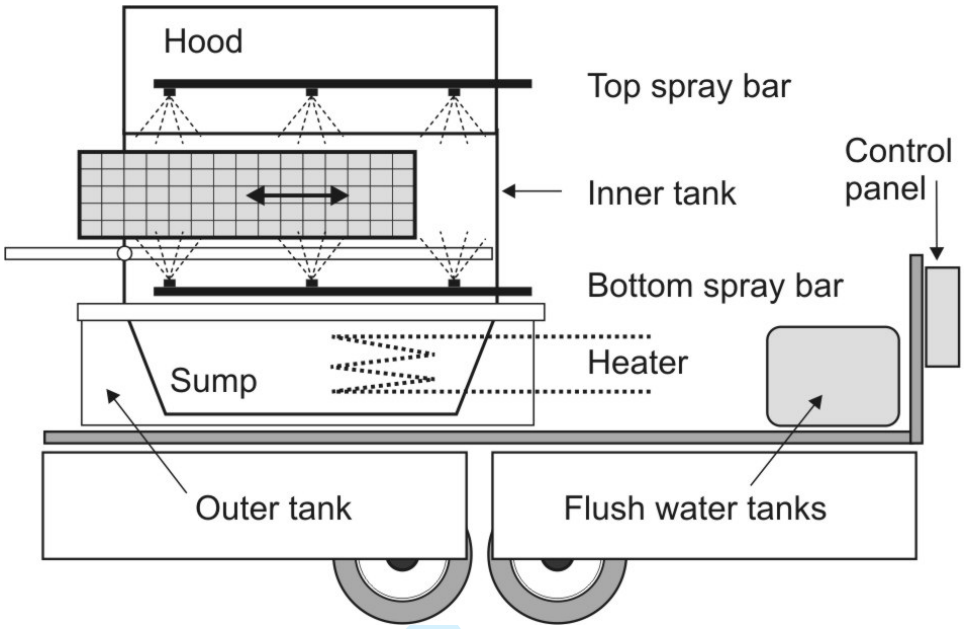
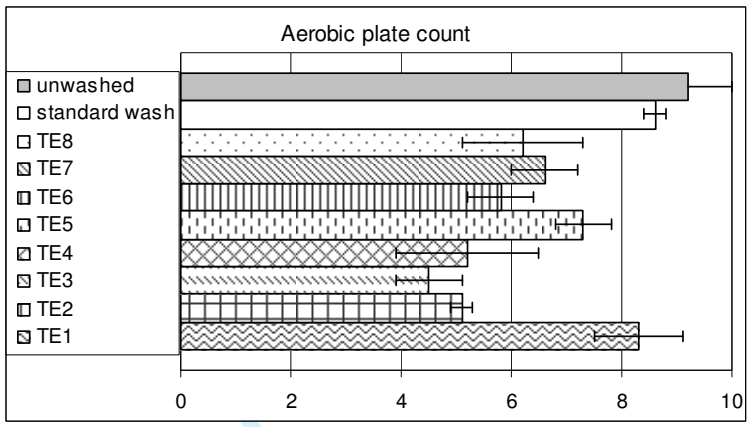
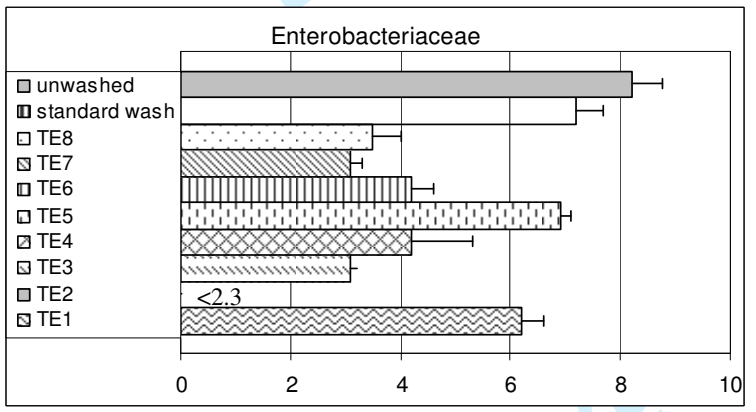
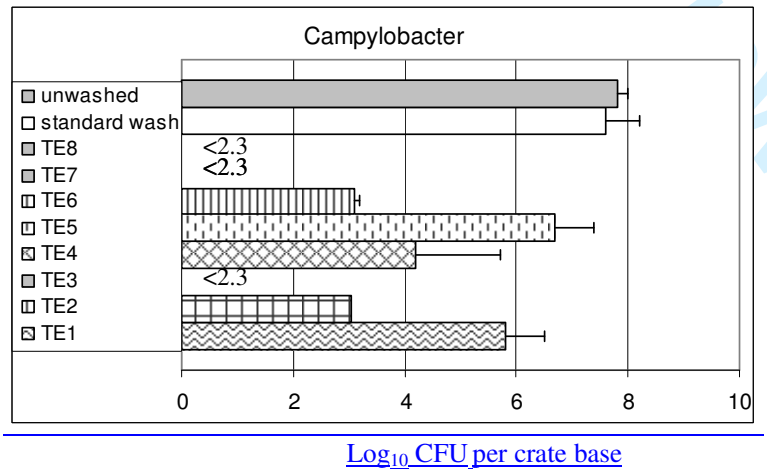


Figure 2







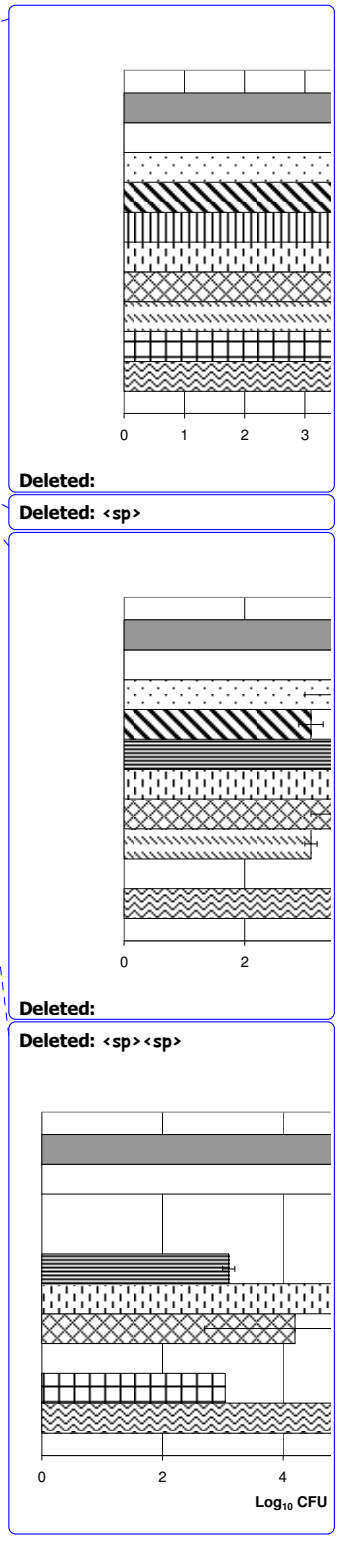


Figure 3