Title: An improved cleaning system to reduce microbial contamination of poultry transport crates in the UK

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Running title: Poultry transport crate cleaning

ABSTRACT

Aim: Following previous research on improving the cleaning of crates used to transport broiler chickens from the farm to the abattoir, a demonstration project was undertaken to investigate improvements in crate washing on a commercial scale.

Methods and Results: The soak tank of a conventional crate washing system was replaced with a high-performance washer fitted with high-volume, high-pressure nozzles. The wash water could be heated, and a greatly improved filtration system ensured that the nozzles did not lose performance or become blocked. Visual cleanliness scores and microbial counts were determined for naturally-contaminated crates which had been randomly assigned to different cleaning protocols.

Conclusions: When a combination of mechanical energy, heat and chemicals (i.e. detergent and disinfectant) were used, the results showed significant improvements to crate cleaning. Reductions of up to 3.6 and 3.8 $\log_{10}$ CFU per crate base were achieved for *Campylobacter* and Enterobacteriaceae respectively, along with a marked improvement in visual cleanliness.

Significance and Impact of study: Broiler transport crates may become heavily contaminated with faeces and this may contribute to the spread of disease between farms. The results of this trial may be of use in reducing the spread of zoonotic pathogens in the poultry meat supply chain.

KEYWORDS: food safety; *Campylobacter*; Enterobacteria; disinfection, bacteriophages.

Introduction

In modern chicken production, birds reared for meat (broilers) are transported to the processing plant in plastic crates. These crates are often contaminated with faecal matter (Wilkins *et al.* 2003) and this poses a significant biosecurity risk during the partial depopulation (thinning) of flocks. Standard crate washing procedures are largely ineffective in removing pathogens such as *Campylobacter* (Slader *et al.* 2002), partly because of the difficulties of cleaning a complex plastic surface. It has been shown that *Campylobacter jejuni* can survive at least 48h in broiler faeces (Smith *et al.* 2016). Genotypes of *Campylobacter* detected in transport crates can be found in residual flocks after thinning, and also in birds sampled at the abattoir (Agunos *et al.* 2014).

The soiling of transport crates involves adhesion and cohesion of faecal matter and litter. The effectiveness of any subsequent cleaning method is contingent upon factors such as: design of the crates, the surface roughness, biofilms and chemical deposits, the nature of the faecal matter, and feed and water withdrawal duration. These factors lead to high variability in both the degree of soiling and the cleaning forces applied.

At the farm, the modules (containing empty crates), are taken from the truck by forklift, placed inside the poultry shed and filled with birds caught by a dedicated team of catchers. The filled modules are loaded back onto the truck and taken to the processing plant where the modules are placed onto a conveying system. The crates are removed mechanically from the module and the birds are removed manually from the crates. The emptied crates and modules

63 pass through separate washing and sanitising processes before being combined and reloaded
64 onto trucks.

65 The impact of cleaning methods on transport crates have been investigated, both in the US
66 (Bacon *et al.* 2000; Nachamkin 2002; Berrang and Northcutt 2005; Northcutt and Berrang
67 2006) and in the UK (Allen, *et al.* 2008a; Allen, *et al.* 2008b). The major poultry transport
68 systems and practices are different in the two countries and so comparisons should be made
69 with care; however, the results from these studies indicate high variability in the efficacy of
70 cleaning methods. Washing may reduce the bacterial load, but it does not eliminate it on the
71 transport crates or cages. The study by Allen and others led to the draft document, "Best
72 practice for cleaning poultry transport crates", the main findings of which were
73 communicated to the industry in the Food Standards Agency (FSA) Meat Industry Guide
74 (Allen, *et al*. 2008b). These trials found that a reduction of total aerobic counts on the interior
75 base of the crate by 4 $\log_{10}$ units could be achieved by combining the use of hot water
76 containing detergent, vigorous brushing, and applying chemical disinfectants to well-cleaned
77 crates.

78 Poultry transport crates were not originally designed for ease of cleaning, but they are so
79 widely used that it is uneconomic and impractical to redesign and replace them on a large
80 scale. As such, it is appropriate to consider developing a practical solution to crate cleaning
81 rather than redesign of the crates at this stage. The Sinner Circle (Busk Jensen and Friis 2007)
82 states that four factors need to be balanced to achieve satisfactory cleaning: mechanical
83 action, chemical action, temperature and contact time. If one of these factors is reduced, the
84 others will need to be increased to compensate. In addition, an improved washing system
85 needs to maintain near maximum mechanical action throughout the working period, possibly

86 up to 20 hours, without undue manual intervention to clean filters and nozzles during
87 operation.

88 This is a proof of principle study supported by a partnership between the UK's Food
89 Standards Agency and a poultry processor, representing the industry, together with a
90 multidisciplinary team of researchers. The primary objective of this study is to determine the
91 ability of a new poultry transport crate washing system to reduce surface contamination by
92 *Campylobacter*, Enterobacteriaceae and *E. coli* bacteriophage under different conditions in a
93 commercial poultry abattoir.

94

95 **Materials and methods**

96 **Study design**

97 A baseline study was undertaken at two similar poultry processing plants within the same
98 company; one was to have the new washing equipment installed (Plant A) while the other
99 plant would continue to run a similar typical crate cleaning system (Plant B). This
100 arrangement effectively gave two controls, one at the modified plant where the control
101 sampling ceased after the new equipment was installed, and the sister plant where control
102 samples were taken continually.

103

104 **Equipment selection**

105 A schematic diagram showing the original and modified arrangements of the crate and
106 module flow in the processing plants is presented in Figure 1. The pre-existing commercial
107 crate cleaning system had very little mechanical cleaning action, a low water temperature,
108 poor effective chemical concentration control, a high organic load and limited contact time.
109 Additionally, the wash-water was recycled over run-down filters which removed only larger
110 particulate material so that much of the organic debris continued to circulate and accumulate
111 leading to reduced flow and pressure from the spray nozzles. It was not uncommon for flow
112 to stop as nozzles became clogged with debris.

113 The specifications of the new equipment were based on the "Best practice for cleaning
114 poultry transport crates" developed from previous research by Allen and others (Allen, *et al.*
115 2008b). Cost, ease of use and reliability also had to be considered, as the equipment was to be
116 used in a commercial plant processing approximately 8, 500 birds per hour. Undue stoppages
117 were unacceptable, both on commercial and welfare grounds.

118 The equipment selected was a Numafa RWM 800 Combi Washing System with Belt
119 Filtration and Rotary Fine Filtration Units. The washer combined a high flow stage
120 circulating over 130 m^3 per hour at a pressure of 345 kPa (Stage 1) through nozzle bars. This
121 was followed by the high-pressure nozzles operating at 2,000 kPa with a flow of 15 m^3 per
122 hour (Stage 2). A belt filter took the full return flow filtering down to 400 micron and was
123 cleaned continuously by a rotating brush and an air knife. Filtration for the high-pressure
124 section was via the separate Rotary Fine Filter Unit accepting 10 m^3 per hour with a single
125 drum using 80 micron and 130 micron cloth in the two stages. This filter was continually
126 cleaned by a small bleed-off from the fully filtered water return. Heating of the water was by

thermostatically controlled steam. Interlock emergency stops, steam/aerosol extraction and overall control via a Programmable Logic Controller (PLC) were also incorporated.

Keeping the original crate inverter simplified crate handling and it easily removed loosely-bound organic material. The original washer included a re-inverter and both the original washer and re-inverter were left in place to provide a detergent rinse stage and create handling without compromising the performance of the new washer. The previous sanitising applicator and chemical choice formed part of the trials. The existing crate re-loader remained unchanged as did the complete module handling and washing system.

Crate selection and interventions

Crates in the control and treatment groups were randomly removed from the line by abattoir staff at intervals over several hours. The selection process could not be formally randomised because workflow and staff availability varied throughout the study. The crate design (open or closed base) and manufacturer (Anglia Autoflow or Giodano) was recorded and a photograph taken before visual scoring and microbiological sampling (see below). Thirty-seven samples were taken in each trial, which comprised unwashed crates (n=6), washed crates (n=15), modules (n=10), soak tank (n=2), tray wash (n=2) and module run down filter (n=2). The following treatment groups were used in Plant A following installation of the new crate washing system: (I) Use of a disinfectant spray following crate washing (5% Peracetic acid, Holchem Perbac Farm, used at a rate of 0.6-1.0%); (II) Increasing the temperature of the wash water to 55°C and using peracetic acid disinfectant spray; (III) Increasing the temperature of the wash water to 60°C only; (IV) Increasing the temperature of the wash

149 water to 60°C and using peracetic acid disinfectant spray. In addition to the above variations,
150 all trials used a caustic soda detergent (Holchem Caustak) at a nominal 1% v/v (0.63% w/v
151 NaOH) at the start of washing. The duration of the crate cleaning process was approximately
152 17 sec from start to finish for both the original and modified cleaning systems. The contact
153 time with the chemical disinfectant was approximately 5 min prior to sampling.

154 A preliminary study was performed by sampling crates at both plants, prior to the
155 modification of Plant A. This was done to determine whether the average and range of
156 microbial counts on washed and unwashed crates were broadly comparable between the two
157 plants. During collection of this preliminary (pilot) data, both plants used a conventional soak
158 tank cleaning system (Figure 1), with unheated water containing household washing powder
159 and Virkon S disinfectant.

160

161 **Microbiological sampling**

162 The sampling protocol was based on visual assessment and microbiological examination of
163 samples from the two types of crate currently used (closed and open grid base). Samples from
164 the module top and base were also taken. Sample collection and processing methods follow
165 those used by Allen and others (Allen, *et al.* 2008b) .

166 A sterile sponge of 103 × 185 × 5.8 mm (cat. No. 95000087, Spongyl 87, Spontex
167 Professionel, Neuilly-Sur-Seine, France) was moistened with approximately 10 ml from a
168 100 ml volume of Maximum Recovery Diluent (MRD, CM 733, Oxoid, Basingstoke, UK).
169 The sponge was then used to swab the entire interior base of the crate three times (once each

in horizontal, vertical and diagonal directions). The sponge was then placed into a sterile plastic bag along with the remainder of the 100 ml MRD. The sponge was manually stomached by squeezing the bag containing the sponge with both hands a total of 60 times in order to release microbes into the diluent. The sponge was then wrung out and the suspension transferred to a sterile 150 ml screw-capped container. For the module samples, a sponge (moistened with MRD as above) was used to swab the entire top surface and another sponge was used to wipe the upper surfaces of the supporting frame at the base of the module. The sponges were processed in an identical manner to the crate swabs (above). Samples of water (approximately 20 ml) from the soak tank (prior to modification) and wash water (after modification) were taken at the start and end of crate sampling. All samples were transported to the laboratory in an insulated box held at approximately 4°C using ice packs and were processed within four hours of collection.

Microbiological examination

Decimal dilutions of each stomachate or water sample were prepared in MRD. Volumes (100 µl) of each dilution were spread-plated onto duplicate plates of Violet Red Bile Glucose Agar (VRBGA, Oxoid CM 0485), Plate Count Agar (PCA, Oxoid CM0325) and modified charcoal cefoperazone deoxycholate agar (mCCDA, Oxoid CM0739, SR0155). These plates were incubated aerobically at either 30°C for 48 h (PCA), 37°C for 24 h (VRBGA) or microaerobically (CampyGen gas packs, CN0035A, Oxoid) at 41.5°C for 48 h (mCCDA) prior to enumeration of typical colonies. All colonies were counted on PCA plates while characteristic red colonies with purple haloes were counted on VRBGA as presumptive Enterobacteriaceae. Standard confirmatory tests were performed on presumptive

193 *Campylobacter* colonies. These included Gram staining, the oxidase test and failure to grow
194 aerobically at 25°C. In addition, a selection of colonies were confirmed as *Campylobacter*
195 spp. by a latex agglutination test (*Campylobacter* Test Kit: Oxoid, DR 0150M).

196

197 **Enumeration of bacteriophages**

198 A 1 ml sample of each sponge stomachate or water sample was transferred to a sterile
199 microfuge tube and subjected to centrifugation at 13, 000 *g* for 5 min to remove bulk debris.
200 The supernatant was then filtered through a 0.45 μm pore size filter (16533K, Minisart,
201 Sartorius, Gottingen, Germany) and decimally diluted to 10^{-8} in SM Buffer (50 mM Tris-Cl
202 [pH 7.5], 0.1 mol l^{-1} NaCl, 0.008 mol l^{-1} $MgSO_4.7H_2O$, 0.01% gelatine, Sigma, Gillingham,
203 Dorset). Volumes (10 μl) of each dilution were spotted in triplicate onto the surface of a
204 bacterial lawn. Briefly, 0.1 ml of an overnight culture of *E. coli* K-12 (approx. 10^8 CFU ml^{-1})
205 was added to 5 ml of molten overlay agar (nutrient broth, CM0001; 0.5% w/v bacteriological
206 agar LP0011, Oxoid), gently mixed, then poured on to pre-warmed (37°C, 30 min) nutrient
207 agar plates (CM0003, Oxoid). These plates were incubated at 37°C for 24 h before examining
208 for phage plaques.

209

210 **Visual assessment of crates**

211 A semi-quantitative system of visual scoring was devised in order to determine any
212 correlation between visual cleanliness of the crates and their microbial load. Crates were
213 scored visually for the total amount (g) of contaminating material (faeces, litter etc.) on each

of three sections 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 to be weighed, so the amount present was estimated on the basis that one heaped 5 ml teaspoonful of debris was found to weigh approximately 2 g. Visual scores were calibrated according to the assessment of at least two trained researchers.

Statistical treatment of data

All microbial counts were $\log_{10}$-transformed prior to statistical analysis. The significance of differences between microbial counts, and the quantity of organic matter between unwashed and washed crates was determined using the Mann-Whitney U Test.

Results

Visual cleanliness assessment of crates before and after washing

A summary of the visual cleanliness scores and microbial counts of unwashed and washed crates at the test (A) and control (B) processing plants during the pilot study and main study are presented in Tables 1 and 2 respectively. The pilot data showed differences in visible contamination, with crates from Plant B showing a higher median contamination level than those from Plant A, but this difference was not significant. The visible contamination of washed crates from both plants during the pilot trial was almost identical. For the main trial (Table 2), the difference between the visible cleanliness of unwashed crates in the plants A and B was not statistically significant ($p = 0.052$ before, $p = 0.819$ after installation).

235 The majority (75%) of crates washed using the modified system in Plant A were classified as
236 visually clean compared with 5% for the unmodified system. All the crates were classified as
237 visually clean when they were washed using the modified system with detergent in the rinse
238 washer followed by a disinfectant spray. The reduction in faecal contamination on crates
239 washed in the new system was significantly greater than that observed for crates washed prior
240 to modification ($p < 0.0001$). However, the visual cleanliness scores did not correlate well
241 with microbial counts (Table 2).

242

243 **Comparison of the microbial counts in the soak tank and washer unit**

244 Samples were taken of water recirculating in the crate soak tank prior to modification of the
245 washing equipment, and from the new spray washer unit after modification. Prior to
246 modification, median microbial counts (log_{10} CFU or PFU per ml) were as follows: aerobic
247 plate count (10.2), Enterobacteriaceae (8.7), *Campylobacter* (8.5) and *E. coli* bacteriophage
248 (6.6). The microbial counts in water collected from plant A following modification were up
249 to 1.4 log_{10} CFU lower than counts in water from plant B: aerobic plate count (9.6),
250 Enterobacteriaceae (7.9), *Campylobacter* (7.5) and *E. coli* bacteriophage (5.2). However, the
251 difference in median microbial counts between the unmodified and modified systems was not
252 statistically significant when both systems used unheated water. There was a slightly greater
253 reduction in microbial counts when the temperature of the water in the modified system was
254 raised to 55°C. However, when the temperature was raised to 60°C there was a significant
255 reduction ($p < 0.05$) in all median log_{10} CFU or PFU microbial counts: aerobic plate count
256 (7.6), Enterobacteriaceae (<4.4), *Campylobacter* (<4.4) and *E. coli* bacteriophage (5.1).

257

258 **Microbial counts from samples taken from the crate surface**

259 Median reductions in Enterobacteriaceae and *Campylobacter* spp. counts before and after
260 plant modifications are presented in Table 2. Initially, the modifications made to the Plant A
261 did not result in a significant reduction in microbial counts compared with the unmodified
262 plant. The median reduction for Enterobacteriaceae on washed crates before modification was
263 approximately 1.1 and 1.5 $\log_{10}$ CFU per crate base for open and closed-base crates
264 respectively, compared with 1.0 and 1.1 $\log_{10}$ CFU respectively after modification. For
265 *Campylobacter* spp, the median reduction on washed crates before modification was 0.6 and
266 0.8 $\log_{10}$ CFU compared with 1.1 and 0.9 $\log_{10}$ CFU after modification, for open and closed-
267 base crates respectively.

268 The chemical detergents and disinfectants used at the two plants were nominally the same
269 during the main trial although a different disinfectant had been used at the original plant.
270 During the pilot trials household washing powder had been used in the soak tank and the
271 disinfectant had been Virkon S. Application of detergent and disinfectant was somewhat
272 inconsistent at both plants partly because of replacement water steadily diluting the initial
273 detergent concentration and a poor dosing system for disinfecting the crates at the original
274 plant that was found to be inoperative or empty, on some occasions. Disinfectant at both
275 plants was applied to rapidly moving crates as they exited from the re-inverters.

276 The use of the modified system with unheated water, but with detergent and disinfectant,
277 resulted in a reduction in median counts of Enterobacteriaceae (0.9-1.2) and *Campylobacter*
278 spp. (0.9-1.6), whereas aerobic plate counts and bacteriophage numbers did not decrease

279 appreciably. These reductions were similar to those obtained in the unmodified sister plant
280 during the same time period where Enterobacteriaceae counts were reduced by 1.3 to 1.5
281 $\log_{10}$ CFU and *Campylobacter* by 1 – 1.2 $\log_{10}$ CFU. Increasing the temperature of the water
282 used to wash crates in the modified plant to approximately 60°C without the use of detergent
283 or disinfectant did not result in any further significant reduction in median counts of
284 Enterobacteriaceae (0.8-0.9 $\log_{10}$ CFU), and aerobic plate counts and bacteriophage titres
285 remained relatively unchanged. However, *Campylobacter* counts were reduced significantly
286 ($p<0.001$) by 1.4 – 2.5 $\log_{10}$ CFU, for closed and open-base crates respectively. .. The
287 combination of high temperature water (60°C) and disinfectant resulted in a significant
288 reductions ($p <0.001$) in median microbial populations ($\log_{10}$ CFU/PFU per crate base) of
289 aerobic plate counts on closed-base crates (2.0), bacteriophage on open-base crates (1.0),
290 Enterobacteriaceae (3.5-4.0) and *Campylobacter* (3.2-3.9) compared with the unwashed
291 control crates. Significant reductions in Enterobacteriaceae (2.1-2.4) and *Campylobacter*
292 (3.0-5.1) were also recorded when the crates were washed at 55°C with disinfectant, although
293 the reductions in aerobic plate counts and bacteriophage were more limited.

294

295 **Discussion**

296 Allen and others (Allen, *et al.* 2008b) identified the most effective treatments to reduce
297 *Campylobacter* as a combination of soaking at 55°C, brushing for 90 sec, washing for 15 sec
298 at 60°C followed by application of detergent (Spectak G, 0.1% (v/v), Johnson Diversey, UK)
299 and disinfectant (Virkon S, 2% v/v). These treatments were applied in a test rig and achieved
300 a 4-$\log_{10}$ CFU reduction in Enterobacteriaceae per crate base using these conditions but were

301 less effective in reducing aerobic plate counts. Similar reductions of 3.9-4.0 $\log_{10}$ CFU have
302 been achieved for *Campylobacter* and Enterobacteriaceae respectively using the modified
303 washing system described in this study, when wash water was heated to 60°C, and the crates
304 were treated with a detergent rinse and disinfectant spray. The earlier study by Allen and
305 others led to the draft document, "Best practice for cleaning poultry transport crates" (Allen,
306 *et al.* 2008b). The document states the specifications for the new washer used in this study,
307 along with some additional requirements on size, cost, commercial availability and
308 practicality for installation in a commercial poultry processing plant. This study shows that
309 the selected washer met these requirements.

310 Enhancing existing washing systems with the use of high temperature and chemical
311 treatments would be problematic. The newly installed two-stage crate washer has a water
312 capacity of 1, 000 litres but still required about 224 MJ of heat and around 102 kg of steam,
313 for a start-up working temperature of 60°C. A crate washing system based on a soak tank
314 with 43, 500 litres of water would require about 44 times more heat energy just for start-up,
315 even if well insulated. Heat and fog would be produced from a heated soak tank requiring
316 containment and separation from the other areas of the arrival bay and hanging-on area. The
317 enclosed, purpose designed washer had steam extraction units built in for simplicity.
318 Furthermore, without satisfactory mechanical cleaning to remove organic matter from the
319 crates the impact of chemical treatments, particularly the disinfectant, would be limited.

320 The results of visual assessment of crates did not correlate well with microbial load. Visually
321 clean crates ($\leq$0.5 g per crate base) often had aerobic plate counts exceeding 9.0 $\log_{10}$ CFU
322 and Enterobacteriaceae counts exceeding 7.0 $\log_{10}$ CFU. Washing the crates and modules

using the pre-existing system did not reduce either of these counts significantly, and in some cases increased them.

Prior to the modifications, washing appeared to decrease median *Campylobacter* spp. counts on crates 0.6-0.8 $\log_{10}$ CFU. However, reductions in *Campylobacter* counts varied considerably from trial to trial suggesting that reductions in microbial loads are dependent on the condition of the crate washing facilities and efficacy of the chemicals and their application at the time of sampling. Purportedly more robust groups of bacteria, such as the Enterobacteriaceae appear to be less sensitive to such fluctuations, and aerobic plate counts and bacteriophage titres even less so. Similar microbial counts were recorded on modules, before and after washing, as were found on crates. No detergents or disinfectants were used on the modules.

Bacteriophage capable of infecting *E. coli* K12, were recovered from most water and crate surface samples. Bacteriophages in general, and coliphages in particular, have been used as surrogates to indicate the survival of rotavirus (Arraj *et al.* 2005), noroviruses (Dawson *et al.* 2005), polioviruses (Ketratanakul *et al.* 1991) and adenoviruses (Williams and Hurst 1988), in diverse systems were wastewater is to be reused (Verbyla and Mihelcic 2015). The presence of coliphage does not, in itself, indicate the presence of viruses which can infect animals or humans. However, it does indicate that should any contamination of this kind occur, such viruses may remain viable on the crate surface after cleaning and treatment with disinfectant. The poultry transport chain is probabilistically the most important step in the spread of viruses such as Avian Influenza A H7N9 (Zhang *et al.* 2018). It is therefore very important to determine if the washing of the crates is efficient at reducing the viral as well as the bacterial contamination.

346 The modification of the test plant improved the percentage of visually clean crates from 5%
347 to 75% which allowed the manual re-washing of crates in the test plant to be halted.
348 However, this modification alone did not lead to a significant reduction in the microbial
349 numbers recovered from the inside base of the crates compared with the control plant.
350 Further, individual measures such as adding detergent or disinfectant or raising the
351 temperature of the wash water did not, by themselves, result in a significant reduction in
352 microbial counts. Recently, other authors have shown that using compressed air foam
353 systems with a cleaner (peracetic acid or chlorinated) may be used to successfully reduce
354 aerobic bacteria in poultry transport crates (Hinojosa *et al.* 2015, 2018). However, on those
355 studies, the crates were artificially contaminated and the efficacy of the cleaning methods in
356 reducing viral contamination was not addressed.

357 The results presented here show that raising the temperature of the water used in the main
358 spray washer, followed by a detergent rinse and a final disinfectant spray, resulted in a highly
359 significant reduction in median counts of both Enterobacteriaceae (3.5-4.0) and
360 *Campylobacter* spp. (3.2-3.9), with all crates appearing visually clean. Under these
361 conditions, the numbers of both these groups of bacteria were below the limit of detection in
362 the re-circulating water; reducing the level of cross-contamination.

363 The results of this study showed that the installation of a new poultry transport crate washing
364 system, in combination with a higher wash water temperature, chemical disinfectants and
365 detergents can significantly reduce the numbers of key bacterial pathogen groups in wash
366 water and on the washed crate surface. In turn, this may reduce the risk bacterial infection of
367 poultry flocks on the farm, particularly with respect to *Campylobacter* and pathogenic
368 members of the Enterobacteriaceae. The modified crate washing system was more efficient

with water and energy use and similar reductions in microbial counts are unlikely to be achieved using conventional crate washing facilities due to cost and practical considerations. These considerations are likely to become more important as issues such as climate change push businesses to use energy and other resources more efficiently.

Acknowledgements

The work described in this paper was funded predominantly by the UK's Food Standards Agency project MS0005. The authors are grateful to the assistance received from their colleagues and those of other partners that collaborated in this project namely C. Burton, and D. Harrison.

Conflict of interest

The authors declare that no conflict of interest exists.

383

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Plant	Crate type and treatment	Number sampled	median visible contamination score (g)	Microbial population counts (median log10 CFU per crate base [median absolute deviation])			
				Enterobacteriaceae	Aerobic Plate Count	Campylobacter	Bacteriophage
A	Open base, unwashed	9	3.0	8.1 [0.5]	9.2 [0.6]	7.5 [0.1]	5.0 [0.8]
	Open base, washed	17	1.0	7.9 [0.5]	9.3 [0.4]	6.2 [0.2]	6.6 [0.2]
	Closed base, unwashed	9	4.0	7.6 [0.1]	9.4 [0.2]	7.5 [0.2]	5.3 [1.5]
	Closed base, washed	28	1.0	7.8 [0.7]	10.7 [1.3]	7.5 [1.0]	6.9 [0.4]
B	Open base, unwashed	10	6.0	7.9 [0.5]	10.3 [1.3]	6.9 [0.7]	4.5 [0.9]

	Open base, washed	25	0.5	6.4 [0.3]	8.6 [0.9]	5.0 [0.9]	4.6 [0.7]
	Closed base, unwashed	8	5.0	8.5 [0.5]	11.4 [0.1]	8.0 [0.7]	4.5 [0.5]
	Closed base, washed	20	1.0	6.9 [0.3]	9.0 [0.3]	4.7 [1.9]	4.3 [0.8]

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Plant	Crate type and treatment	Number sampled	median visible contamination score (g)	Microbial population counts (median log_{10} CFU per crate base [median absolute deviation])			
				Enterobacteriaceae	Aerobic Plate Count	Campylobacter	Bacteriophage
A	Open base, unwashed	12	3.5	8.0 [0.5]	9.2 [0.2]	6.9 [0.8]	4.2 [0.7]
	Open base, washed	18	0.5	6.9 [0.3]	8.6 [0.3]	6.3 [0.7]	4.1 [0.4]
	Closed base, unwashed	12	4.0	8.6 [0.4]	9.8 [0.5]	7.4 [1.0]	4.9 [0.4]
	Closed base,	42	1.0	7.1 [0.3]	9.3 [0.2]	6.6 [0.7]	4.7 [0.3]

	washed						
B	Open base, unwashed	12	3.0	7.4 [0.5]	9.2 [0.4]	7.4 [0.3]	4.2 [0.6]
	Open base, washed	25	1.5	6.7 [0.2]	8.5 [0.4]	6.4 [0.2]	4.4 [0.6]
	Closed base, unwashed	12	4.0	8.2 [0.4]	9.7 [0.5]	8.1 [0.3]	4.8 [0.5]
	Closed base, washed	35	1.0	7.2 [0.3]	9.3 [0.2]	6.6 [0.2]	4.5 [0.3]
Plant	Crate type and	Number	median	Microbial population counts (median log10 CFU per crate base [median absolute			

	condition	sampled	visible contamination score (g)	deviation])			
				Enterobacteriaceae	Aerobic Plate Count	Campylobacter	Bacteriophage
A (M)	Open base, unwashed	11	3	7.8 [0.2]	8.7 [0.2]	7.2 [0.2]	5.0 [0.4]
	Open base, washed	25	0	6.8 [0.2]	8.5 [0.2]	6.1 [0.3]	4.7 [0.5]
	Closed base, unwashed	13	5	8.4 [0.3]	9.4 [0.5]	7.5 [0.4]	4.8 [0.6]
	Closed base, washed	35	0	7.3 [0.1]	9.1 [0.2]	6.6 [0.3]	4.6 [0.6]

B	Open base, unwashed	9	4	8.1 [0.2]	8.6 [0.4]	7.5 [0.4]	4.9 [0.5]
	Open base, washed	22	2	6.6 [0.4]	7.9 [0.4]	6.5 [0.5]	5.2 [0.3]
	Closed base, unwashed	9	5	8.1 [0.1]	9.0 [0.4]	7.5 [0.4]	5.4 [0.1]
	Closed base, washed	23	2	6.8 [0.2]	8.7 [0.4]	6.3 [0.5]	5.2 [0.3]
A (M, D)	Open base, unwashed	6	4	8.1 [0.5]	9.4 [0.2]	7.8 [0.5]	5.5 [0.0]
	Open base,	12	0	7.2 [0.3]	8.6 [0.2]	6.9 [0.5]	5.0 [0.2]

	washed						
	Closed base, unwashed	6	6	8.4 [0.2]	9.4 [0.1]	8.2 [0.5]	5.5 [0.0]
	Closed base, washed	18	0	7.2 [0.4]	9.1 [0.2]	6.6 [0.7]	5.2 [0.2]
A (M, D) 55°C	Open base, unwashed	6	6	7.9 [0.1]	8.8 [0.3]	7.7 [0.2]	5.4 [0.1]
	Open base, washed	12	0	5.5 [0.3]	8.6 [0.9]	4.7 [1.5]	5.1 [0.0]
	Closed base, unwashed	6	5.5	8.2 [0.3]	9.6 [0.4]	7.4 [0.2]	5.4 [0.1]

	Closed base, washed	18	0	6.1 [0.4]	8.6 [0.2]	2.3 [2.3]	5.0 [0.2]
A (M) 60°C	Open base, unwashed	5	4	7.3 [0.0]	8.6 [0.2]	7.3 [0.1]	5.5 [0.1]
	Open base, washed	10	0	6.5 [0.2]	8.6 [0.4]	4.8 [0.7]	5.2 [0.1]
	Closed base, unwashed	7	5	8.1 [0.2]	9.3 [0.1]	7.3 [1.1]	5.4 [0.0]
	Closed base, washed	20	0	7.2 [0.2]	8.6 [0.1]	5.9 [0.4]	5.1 [0.2]
A (M, D)	Open base,	3	5	8.2 [0.0]	8.9 [0.1]	7.5 [0.1]	5.5 [0.0]

60°C	unwashed						
	Open base, washed	5	0	4.7 [0.7]	8.0 [0.3]	3.6 [0.3]	4.4 [0.2]
	Closed base, unwashed	3	6	8.8 [0.0]	10.1 [0.1]	8.0 [0.1]	5.5 [0.1]
	Closed base, washed	10	0	4.8 [1.5]	8.1 [0.2]	4.8 [0.3]	4.7 [0.2]

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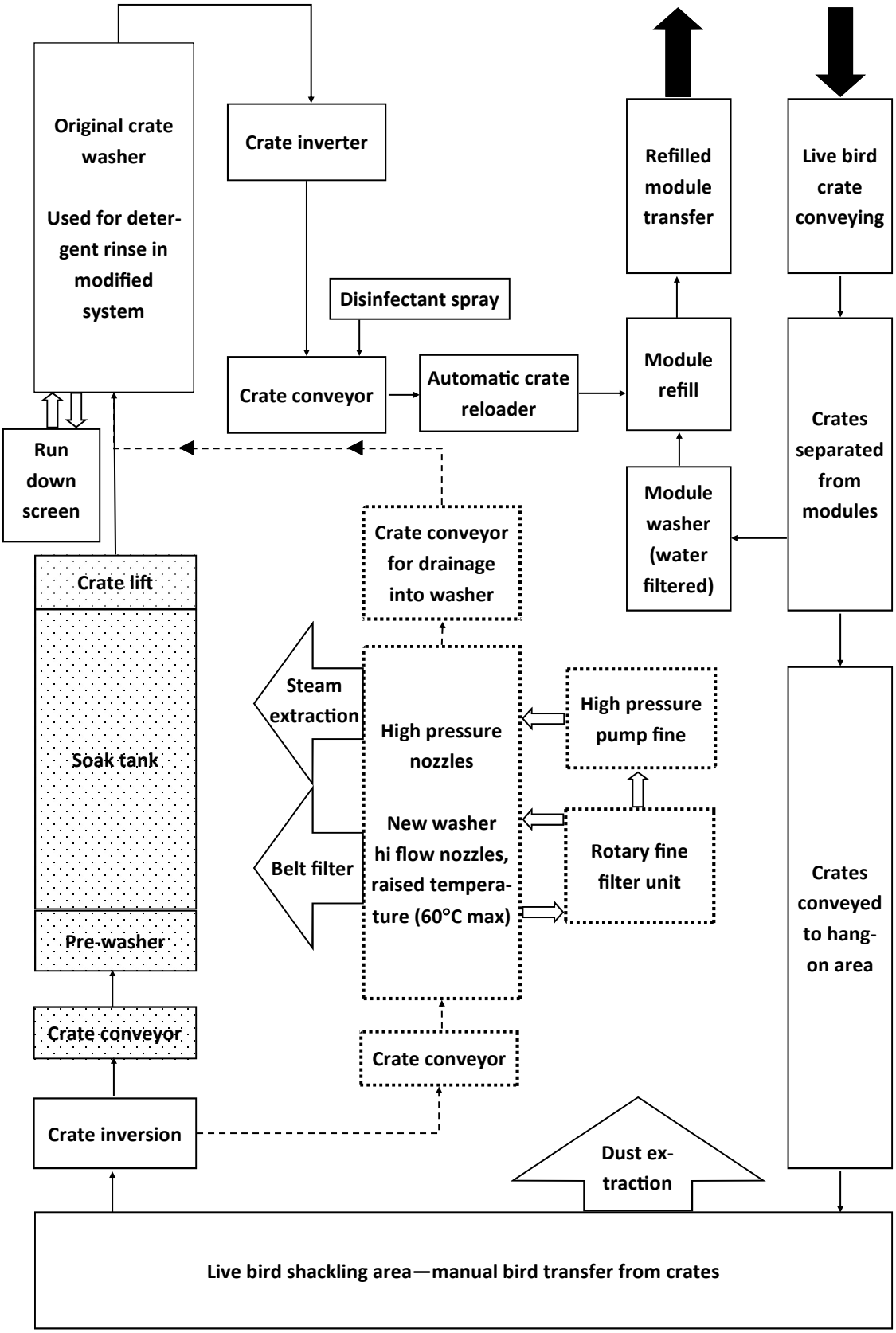
Table 1: Results of poultry transport crate washing pilot trials at plants A and B prior to modification of Plant A. Median counts of aerobic microbes (Aerobic Plate Count), Enterobacteriaceae, *Campylobacter* and coliphage are given along with the median absolute deviation. The visible faecal contamination score for each category of crate is given as median grams of faecal contamination per crate base. The open and closed base refers to whether the floor of the crates are based on a grid (open) or solid (closed) design.

Table 2: Visible contamination scores and microbial counts from poultry transport crates before and after installation of a modified washing system in Plant A. The top of the table shows results from plants A and B prior to modification of Plant A. The bottom of the table shows the results after modification of plant A, and contemporaneous results from the unmodified sister plant (Plant B). Median counts of aerobic microbes (Aerobic Plate Count), Enterobacteriaceae, *Campylobacter* and coliphage are given along with the median absolute deviation. The visible faecal contamination score for each category of crate is given as median grams of faecal contamination per crate base. The open and closed base refers to whether the floor of the crates are based on a grid (open) or solid (closed) design. M = modified Plant A, D = crates were sprayed with disinfectant following washing. The temperature (°C) indicates where the water used to wash the crates was experimentally increased for the trial.

Figure 1: Schematic diagram of the Arrival Bay of a poultry processing plant showing typical flow and processes of poultry transport modules and crates (white boxes, solid arrows) and modified crate washing system (dashed boxes). Removed items in the modified system are shown with a dot fill.


Original crate washer
Used for detergent rinse in modified system
Crate inverter
Refilled module transfer
Live bird crate conveying
Disinfectant spray
Crate conveyor
Automatic crate reloader
Module refill
Crates separated from modules
Run down screen
Module washer (water filtered)
Crate conveyor for drainage into washer
Crate lift
Steam extraction
High pressure nozzles
High pressure pump fine
Soak tank
New washer hi flow nozzles, raised temperature (60°C max)
Rotary fine filter unit
Belt filter
Crates conveyed to hang-on area
Pre-washer
Crate conveyor
Crate conveyor
Crate inversion
Dust extraction
Live bird shackling area—manual bird transfer from crates