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Effect of ultrasonic treatment during cleaning on the microbiological condition of poultry transport crates

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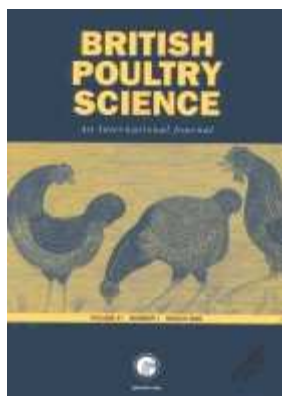
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1 Effect of ultrasonic treatment during cleaning on the microbiological
2 condition of poultry transport crates

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Abstract 1. Small sections cut from commercial crates used to transport live poultry to the processing plant were artificially contaminated with effluent taken from a commercial crate-cleaning system.

2. Laboratory trials, involving the immersion of these sections in an ultrasonic water bath (4 kW energy) showed that aerobic plate counts (APC) and counts of Enterobacteriaceae were progressively reduced as the immersion time was increased from 0 to 120 s and the water temperature raised from 35°C to 58°C.

3. In subsequent trials at a processing plant, using commercially-cleaned crates, there was relatively little effect of ultrasound (or pressure washing) on the biofilm present. However, ultrasonic treatment in combination with an immersion temperature of 60°C reduced counts of Enterobacteriaceae to below the detection limit (\log_{10} 2.3 cfu) within 1 – 3 min, while APC were reduced by $>2 \log_{10}$ units after 3 min.

4. It was concluded that ultrasonic treatment has a possible role in the crate-cleaning process, when used in conjunction with higher immersion temperatures. In this way, it could contribute significantly to hygiene control.

INTRODUCTION

Birds in commercial poultry flocks may be intestinal carriers of zoonotic pathogens, such as *Campylobacter* and *Salmonella* spp. and these organisms can contaminate both carcasses and equipment during processing (Mead, 2004). When the birds are transferred from farm to processing plant, the crates in which they are transported become heavily soiled with faecal material. After the birds are removed at the processing plant, the crates are sent on to another farm for re-loading, either without prior cleaning (Ramesh *et al.*, 2003) or following a

1
2 49 limited washing process at the plant (Tinker *et al.*, 2005). Thus, current cleaning
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4 50 systems can be relatively poor and they are known to be ineffective in removing
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6 51 microbial contamination (Carr *et al.*, 1999). Hansson *et al.* (2005) reported that
7
8 52 *Campylobacter* spp. were isolated from 57% of 122 batches of washed and
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10 53 disinfected transport crates, some of which then contaminated farm-negative
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12 54 flocks during transport to the abattoir. It is evident that poultry transport crates,
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14 55 as handled at present, pose a substantial risk of transmitting any zoonotic
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16 56 pathogens present from one batch of birds to another and may be a source of
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18 57 flock infection on the farm.

20 58 There are no regulations regarding microbiological criteria that relate
21
22 59 to the cleaning of transport crates in the UK. However, there is a statutory
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24 60 requirement (Regulation EC No. 853/2004) for crates, modules and all
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26 61 equipment used for collecting and delivering live animals to the slaughterhouse
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28 62 to be cleaned, washed and disinfected immediately after emptying and if
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30 63 necessary before reuse. Crates and modules must be easy to clean and made of
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32 64 non-corrodible material, while cleaning and disinfection must be undertaken in a
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34 65 separate place with appropriate facilities.

36 66 To improve hygiene control, there is an urgent need for: (a) better
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38 67 cleaning and disinfection of transport crates, which can be hampered by the
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40 68 caking of organic debris (litter, faeces, feathers etc), (b) recycling of part of the
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42 69 wash water and (c) a rapid cleaning process of the crates in order to meet the
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44 70 demands of high-rate processing (Tinker *et al.*, 2005). One approach, involving a
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46 71 period of immersion at 70°C in a solution of hypochlorite (1000 mg/l), was
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48 72 shown to eliminate coliform bacteria (Ramesh *et al.*, 2003), but this is likely to
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50 73 be unsuitable for the less heat-resistant plastic crates currently used in the United
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3 74 Kingdom. Therefore, the present study examined the application of ultrasound in
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5 75 both laboratory- and factory-based trials, because the process has been used
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7 76 commercially for cleaning purposes in other applications, for example, meat
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9 77 trays.

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13 79 MATERIAL AND METHODS

14
15 80 **Laboratory-based trials**

16
17 81 The base of each crate comprised a grid of 25 mm square indentations. Sections
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19 82 of approximately 270 x 150 mm were cut from a new, unused crate and
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21 83 immersed in the soak tank of a crate-cleaning facility at a poultry plant. The
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23 84 water in the tank was heavily contaminated with organic material that had
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25 85 accumulated from the on-going cleaning process. After 24 h, the sections were
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27 86 removed and transferred to the laboratory for the trials described below. As a
28
29 87 result of prolonged immersion, the sections were coated with organic debris.

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31 88 A heated, bench-top sonicator tank of 300 x 150 x 120 mm (Production
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33 89 Line Cleaning Ltd, Diss, Norfolk, UK) was filled with tap water and 2% (v/v)
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35 90 surfactant was added (CB10: Access Chemicals Ltd, Wellingborough, Northants,
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37 91 UK). The water temperature was increased as required from 35°C to 45°C and
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39 92 then to 58°C during the experiments and monitored with a digital thermometer.

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41 93 Each crate section was first washed gently in cold running water to
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43 94 remove any loose material. It was then hung on a wire cradle, lowered into the
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45 95 tank and supported in such a manner that the top row of three squares was kept
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47 96 out of the water. The section was progressively raised: one row of squares was
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49 97 removed from the water at 30 s intervals up to 90 s, to give a series of immersion
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51 98 times at a particular temperature. The same procedure was used for a second
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2 99 crate section, which was subjected to ultrasonic treatment (4 kW). Before doing
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4 100 so, the ultrasound was switched on for 1 min and the water de-gassed by stirring
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6 101 thoroughly. Each trial was replicated at least three times.
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8
9 102 There were three treatments: no immersion (control), immersion alone or
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11 103 immersion plus ultrasonic treatment; following which the surface of each square
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13 104 indentation was sampled individually by the wet and dry swab method. The
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15 105 swabs from each square were transferred to 10 ml of Maximum Recovery
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17 106 Diluent (MRD, CM733, Oxoid Ltd, Basingstoke Hants, UK), agitated with a
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19 107 vortex mixer and the resultant suspension examined microbiologically, as
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21 108 described below.

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23 109 After commercial cleaning, sections of 20 x 20 mm were cut from the
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25 110 base of a randomly-selected, well-used crate and given no further treatment,
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27 111 treated with ultrasound or subjected to pressure washing. For ultrasonic
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29 112 treatment, the section was immersed in water containing surfactant at 50°C for 1
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31 113 min, (after de-gassing the water for 30 min). To assess the effect of pressure
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33 114 washing, the crate section was mounted on a frame and passed through a pressure
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35 115 washer at a speed of 41 mm/s and at a distance of 110 mm from the nozzle. The
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37 116 water was delivered in a 45° fan pattern and at a nozzle pressure of 12 bars.

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39 117 Following treatment, each 20 mm x 20 mm section was mounted on a
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41 118 base plate and plated with gold in an argon/vacuum chamber. The surfaces of the
42
43 119 sections were then examined by scanning electron microscopy (SEM).
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45 **Factory-based trials**

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47 121 The trials were carried out at a commercial processing plant, using crates that had
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49 122 been cleaned routinely. The purpose was to investigate the effects of different
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51 123 additional treatments on the microbiological condition of the crates. In two of the
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3 124 trials, the additional cleaning treatment was repeated after the crates had been re-
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5 125 used and cleaned commercially to determine any cumulative effect.

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7 126 For treatment proposes, each crate was transferred to a sonication tank set
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9 127 up alongside the production facility. This comprised a stainless steel tank (1300 x
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11 128 900 x 1100 mm) containing water at 60°C with 2% (v/v) surfactant (CB 10) and
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13 129 a 4 kW ultrasonic generator (Production Line Cleaning Ltd). The generator
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15 130 directed energy at the vertically-placed crate in a horizontal plane, thus treating
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17 131 the base directly. Before use, the treatment water was necessarily de-gassed by
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19 132 stirring for 10 min with sonication.

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21 133 The 6 trials carried out are summarised in Table 1. In the first two, 12
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23 134 crates per trial were used: one set of 4 was an untreated control, another 4 were
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25 135 immersed for 3 min without ultrasonic treatment and the remainder were
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27 136 subjected to ultrasound for 3 min. In a second pair of trials carried out at a later
28
29 137 date, 5 crates per treatment were used and crates were either immersed alone (1
30
31 138 min) or immersed and treated with ultrasound for 30 s, 1 min or 3 min.
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33 139 Following the 30 s treatment, crates were removed from the tank and brushed
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35 140 mechanically with a cylindrical nylon brush attached to an electric drill. This was
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37 141 applied manually and methodically to the crate base for 1 min.

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39 142 For the two final trials, the crates used had been treated already by
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41 143 sonication during the initial trials described above. They had been tagged and
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43 144 returned to the farm for re-use in transporting birds to the plant. The same crates
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45 145 were then cleaned again by the commercial process and re-treated by sonication.

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47 146 Sampling was confined to the interior of the crate base, this being the part
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49 147 most likely to come into contact with the birds. Using aseptic precautions, a
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51 148 sterile sponge of 103 x 185 x 5.8 mm (cat. No 95000087, Spongyl 87, Spontex
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3 149 Professionel, Neuilly-Sur-Seine, France) was wetted with a small amount of
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5 150 liquid from 100 ml of Maximum Recovery Diluent (MRD, Oxoid Ltd,
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7 151 Basingstoke, Hants, UK) and transferred to a sterile plastic bag. When required,
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9 152 the sponge was removed and used to swab the interior base of the crate in
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11 153 horizontal, vertical and diagonal directions from bottom left to top right. The
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13 154 sponge was then returned to the bag and the remainder of the MRD added. Using
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15 155 both hands, the bag was squeezed 60 times to release microbial cells into the
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17 156 diluent. Finally, the sponge was wrung out aseptically by hand and the resultant
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19 157 suspension transferred to a 100 ml screw-capped crate. Samples were transported
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21 158 to the laboratory in an insulated box containing ice packs and examined within 7
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23 159 h, as described below.

24 160 **Determination of APC and counts of presumptive Enterobacteriaceae**

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26 161 From serial ten-fold sample dilutions in MRD, 100 µl amounts of each were used
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28 162 to inoculate duplicate plates of Plate Count Agar (PCA, Oxoid CM 0325) and
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30 163 Violet Red Bile Glucose Agar (VRBGA, Oxoid CM 0485). Plates were
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32 164 incubated at either 30 °C for 48 h (PCA) or 37 °C for 24 h (VRBGA) and the
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34 165 colonies counted. Characteristically, Enterobacteriaceae appear as round, purple
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36 166 colonies 1 – 2 mm in diameter and surrounded by purple haloes. As
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38 167 recommended by the media manufacturer, however, all red colonies were
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40 168 counted as presumptive Enterobacteriaceae.

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43 169 The results of the trials were analysed by ANOVA using SSPS version 15
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45 170 statistical software.

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49 172 **RESULTS AND DISCUSSION**

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3 173 Initially, pre-soiled crate sections were used to compare the effects on microbial
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5 174 contamination of water immersion alone at different temperatures and a
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7 175 combination of immersion and ultrasonic treatment (Figure 1). It was found that
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9 176 aerobic plate counts (APC) and counts of Enterobacteriaceae were reduced
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11 177 progressively as the immersion time was increased from 0 to 120 s and as the
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13 178 water temperature was raised from 35 °C to 58 °C. Overall there was an effect
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15 179 ($P<0.001$) of treatment time on APC and Enterobacteriaceae counts recovered at
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17 180 both 45°C and 58°C. However a linear model fitted to the decline in each type of
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19 181 count showed a steeper slope at 58°C ($P=0.005$). There was also an effect in each
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21 182 case ($P<0.001$) when ultrasonic treatment was compared with immersion alone at
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23 183 45°C. At 58°C, Enterobacteriaceae were only recovered from 1/3 treated sections
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25 184 (limit of detection \log_{10} 1.4 cfu / cm²) after immersion for 30 s, whether or not
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27 185 ultrasound was used, and were undetectable in all cases after 1 min. By
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29 186 comparison, APC were obtained from 2/3 sections after immersion alone for 1
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31 187 min or 90 s, but no organisms were recovered after 90 s when ultrasound was
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33 188 applied. Thus, the combination of immersion at 58°C and ultrasonic treatment for
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35 189 90 s reduced microbial contamination to below the detection limit for both
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37 190 categories of organism. When studying conveyor belt materials, Tolvanén *et al.*
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39 191 (2007) also found that raising the treatment temperature enhanced the efficacy of
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41 192 ultrasonic cleaning, although the reduction obtained in *Listeria monocytogenes*
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43 193 was significantly greater on stainless steel than on plastic materials.

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45 194 In relation to cleaning of crates, Ramesh *et al.* (2003) reported the
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47 195 presence of a biofilm, which forms during repeated re-use under commercial
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49 196 conditions. This was confirmed in the present study by the examination of a
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51 197 section from a commercially-cleaned crate, using SEM (Figure 2a). Neither
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2 198 treatment with ultrasound at 50°C for up to 1 min (Figure 2b) nor pressure
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4 199 washing (Figure 2c) dislodged the material, which varied in thickness across the
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6 200 crate sample. The exact nature of the matrix and the identity of the component
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8 201 organisms have not been determined.
9

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11 202 Despite persistence of the biofilm, ultrasonic treatment for 1 – 3 min at 60
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13 203 °C consistently reduced mean counts of Enterobacteriaceae to below the
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15 204 detection limit (\log_{10} 2.3 cfu per crate base), as shown in Table 1. When crates
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17 205 were subjected to either immersion alone or ultrasonic treatment for 3 min (trials
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19 206 A and C) microbial contamination was reduced in both cases, but sonication had
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21 207 the greater effect ($P=0.002$ and $P<0.001$ for APC and Enterobacteriaceae
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23 208 respectively). Although immersion alone produced some marked reductions in
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25 209 both types of count, exposure to ultrasound for only 30 s (trial B2) still resulted
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27 210 in a further 1.5 \log_{10} unit reduction in APC ($P=0.006$). By contrast, this trial
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29 211 produced less change in the counts of Enterobacteriaceae, but the subsequent use
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31 212 of mechanical brushing caused a reduction ($P=0.008$).
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33 213 In all trials, the combined effect of soaking at 60 °C and ultrasonic
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35 214 treatment for 1-3 min were less for APC than for Enterobacteriaceae which may
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37 215 indicate the removal of faecal matter rather than the biofilm. However, the
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39 216 combined treatments always produced the lowest APC, which were $>2 \log_{10}$ units
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41 217 less than those achieved with the commercial cleaning process, when the extra
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43 218 treatment time was 3 min. The beneficial effects observed under laboratory
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45 219 conditions were less apparent in the factory trials and there was no significant
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47 220 cumulative effect from intensive cleaning when crates were subjected to two
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49 221 cleaning cycles. Whether or not there would be an improvement after many
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51 222 successive cycles remains to be investigated.
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3 223 The need to improve the cleaning and disinfection of poultry transport
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5 224 crates has long been recognised and could contribute to better hygiene control in
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7 225 the processing plant (Slader *et al.*, 2002). This study has highlighted a possible
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9 226 role for ultrasonic treatment in conjunction with a water temperature of 60°C as a
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11 227 means of reducing microbial contamination of crate surfaces and thereby the
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13 228 microbial hazards associated with transporting live poultry to the processing
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15 229 plant.

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23
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TABLE. *Effects of commercial cleaning followed by various immersion treatments on the microbial contamination of transport crates (log₁₀ mean cfu / crate base and standard deviation. n=4 for trials A and C and n=5 for trials B)*

Trial	Post-cleaning immersion treatment at 60°C	APC	Enterobacteriaceae
A1	none	7.4 (0.8)	5.5 (0.5)
	3 min	7.2 (1.1)	3.7 (0.4) ^x
	3 min with ultrasound	5.1 (0.6) ^{xy}	< 2.3 ^{xy}
A2	none	7.4 (0.2)	5.5 (0.4)
	3 min	5.5 (0.3) ^x	3.1 (0.1) ^x
	3 min with ultrasound	4.9 (0.1) ^{xy}	< 2.3 ^{xy}
B1 [†]	1 min	5.4 (0.1) ^x	3.3 (0.5) ^x
	1 min with ultrasound	5.0 (0.2) ^{xy}	< 2.3 ^x
B2 [†]	30 s	7.5 (0.6)	5.7 (0.6)
	30 s with ultrasound	6.0 (0.7) ^{xy}	4.8 (1.1)
	30 s with ultrasound and 1 min brushing	5.8 (0.1) ^{xy}	3.6 (0.6) ^{xy}
C1 ^a	none	7.9 (0.3)	6.8 (0.1)
	3 min	5.5 (0.3) ^x	< 2.3 ^x
	3 min with ultrasound	5.3 (0.1) ^x	< 2.3 ^x
C2 ^b	none	8.0 (0.3)	5.2 (1.6)
	3 min	5.5 (0.2) ^x	< 2.3 ^x
	3 min with ultrasound	5.3 (0.2) ^x	< 2.3 ^x

^x Significantly different ($P < 0.05$) from control crates

^y Significant difference ($P < 0.05$) between immersion with and without ultrasound

[†] Control group same as trial A2

^a Crates re-used from trial A1 having passed through one cycle: factory to farm to factory.

^b Crates re-used from trial A2 having passed through one cycle: factory to farm to factory.

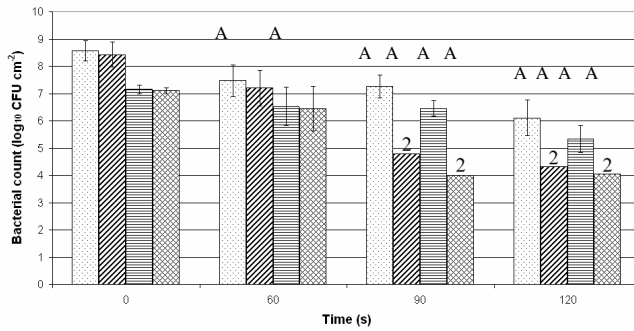
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3 266 **FIGURE 1a.** Comparison of immersion treatment alone and immersion with
4 267 ultrasound for reducing microbial contamination of crate sections at 35°C
5 268 b. Comparison of immersion treatment alone and immersion with ultrasound
6 269 for reducing microbial contamination of crate sections at 45°C
7 270 c. Comparison of immersion treatment alone and immersion with ultrasound
8 271 for reducing microbial contamination of crate sections at 58°C
9 272 Numbers denote counts from either (1) or (2) crate sections out of 3 examined.
10 273 Other sections harboured bacteria below the detection limit of \log_{10} 1.42 cfu / cm²
11 274 * microbial contamination was below the limit of detection (\log_{10} 1.42 cfu / cm²).
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277 **FIGURE 2a.** Magnification by SEM of a fissure in the matrix on an unwashed
278 crate (x 5500)
279 b. Appearance of the matrix after ultrasonic treatment for 60 s at 50°C (x 4000)
280 c. Appearance of the matrix after pressure washing at 12 bar (x 4000)

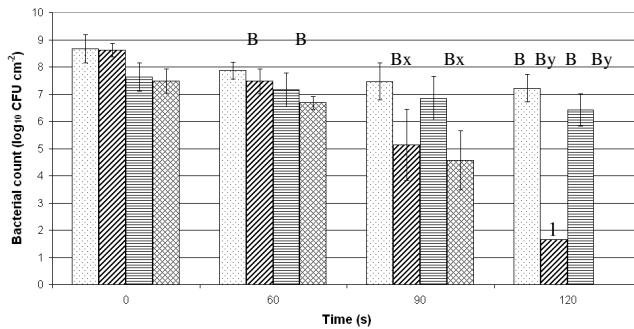
A, B, C = Significant effect ($P < 0.05$) of time at given temperature

^{x, y} = Significant difference ($P < 0.05$) between immersion with and without
ultrasound at given time

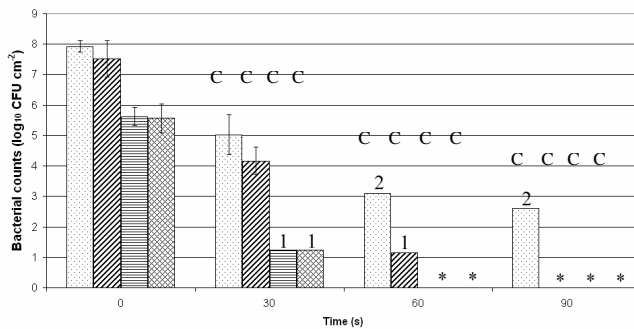
(a)



(b)



(c)



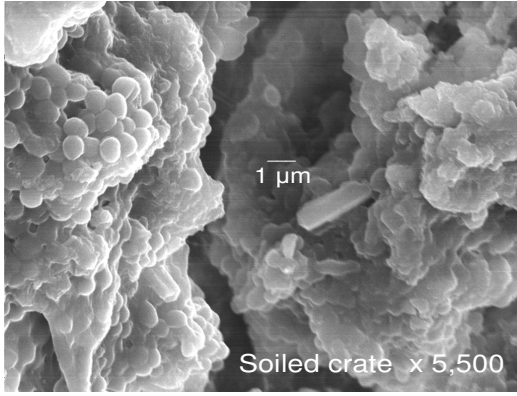
Figs 1a – 1c. Key: APC immersion; APC sonication; Enterobacteriaceae immersion; Enterobacteriaceae sonication. Numbers denote counts from (1) or (2) crate sections out of 3 tested (other sections harboured bacteria below the detection limit of 1.42 log₁₀ CFU cm⁻². * = all crate sections tested harboured bacteria below detectable levels.

Figure 1

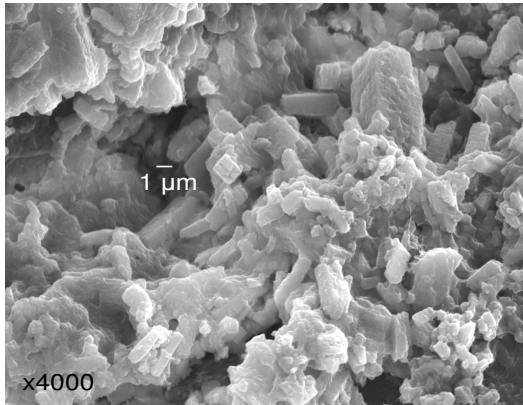
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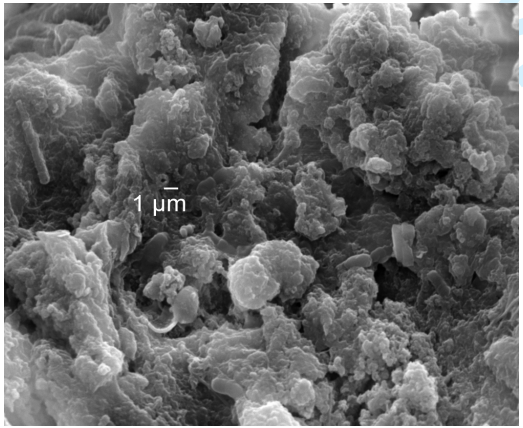
288 (a)



289
290 (b)
291



292
293 (c)
294



295
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Figure 2

Review Only