Inclusion of detergent in a cleaning regime and effect on microbial load in livestock housing

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Determining effective cleaning and disinfection regimes of livestock housing is vital to improving the health of resident animals and reducing zoonotic disease. A cleaning regime consisting of scraping, soaking with or without detergent (treatment and control), pressure washing, disinfection and natural drying was applied to multiple pig pens. After each cleaning stage, samples were taken from different materials and enumerated for total aerobic count (TAC) and Enterobacteriaceae (ENT). Soaking with detergent (Blast-Off, Biolink) caused significantly greater reductions of TAC and ENT on metal, and TAC on concrete, compared with control. Disinfection effect (Virkon S, DuPont) was not significantly associated with prior detergent treatment. Disinfection significantly reduced TAC and ENT on concrete and stock board but not on metal. Twenty-four hours after disinfection TAC and ENT on metal and stock board were significantly reduced, but no significant reductions occurred in the subsequent 96 hours. Counts on concrete did not significantly reduce during the entire drying period (120 hours). Detergent and disinfectant have varying bactericidal effects according to the surface and bacterial target; however, both can significantly reduce microbial numbers so should be used during cleaning, with a minimum drying period of 24 hours, to lower bacterial counts effectively.

Introduction

Cleaning and disinfection (C & D) is vitally important in livestock farm management and biosecurity. Implementation of C & D in pig and poultry housing has been shown to reduce pathogens, such as Salmonella and Campylobacter, reducing risk of disease outbreak in resident animals and transfer of zoonotic organisms (Davies and Breslin 2003, Mannion and others 2007).

The aim of C & D is to remove organic matter, using physical and water-based cleaning methods, and to kill remaining micro-organisms using chemical disinfection and natural desiccation (achieved by keeping the area free of livestock to allow drying, ie, rest). Pig faeces may be challenging to remove as they are proteaceous and fatty, comprising 20–22 per cent and 7–12 per cent of dry matter, respectively; fatty and proteaceous soiling is insoluble, meaning water-based cleaning methods alone may be inadequate (Eggum and Christensen 1974, Ōhta and Ikeda 1978, Marriott and Gravani 2006). If C & D is ineffective at removing faeces there will be pathogen persistence and decreased effectiveness of chemical disinfection (Corry and others 2002). Pathogens may also reside and multiply in biofilms; a biofilm is a microscopic community of micro-organisms entrenched in a matrix produced by its own resident organisms (Hood and Zottola 1997, Donlan 2002). Conventional cleaning methods are often ineffective at removing biofilms, however, attempts should be made to disrupt biofilms because, as well as their central role in bacterial survival and persistence, they have been shown to corrode metal, damage concrete and can cause increased resistance to antimicrobials (Mah and O’T oole 2001, Donlan 2002, Dunowska and others 2005, Yang and others 2011). Detergents in cleaning regimes aid physical removal of organic matter, may help to break down biofilms and are bactericidal (Knox and others 1949, Ōhta and Salton 1960, Tanzer and others 1979, Vickery and others 2004). The primary objective of this study was to determine the effect of a detergent soaking period in a cleaning regime by monitoring total aerobic and Enterobacteriaceae counts (TAC and ENT) on different materials in livestock housing. Secondary objectives included determining any relationship between detergent treatment and subsequent disinfection, and the influence of surface type on effectiveness of C & D.

Materials and methods

Study design

The study was carried out during August 2011 in a trial facility livestock building. A commercial farm setting was not selected for the study so as to ensure uniform conditions required for the pens to be technical replicates. The animal housing protocol was approved by the University of Nottingham ethical review process. A power calculation was completed using GenStat 14th Edition (VSN International) to deduce the number of replicates required to demonstrate a significant difference of one log, with an estimated variance of 0.5 log and 70 per cent power; the number of replicates was determined to be four.

The livestock building contained 48 identical pens (Fig 1) each having housed a single male pig (Landrace × large white) which had entered and exited the pen at an average weight of 35 kg and 97 kg, respectively (resident for two months). Within the building, 22 pens were selected for the study as they were vacated simultaneously. Adjacent pens were assigned to groups: six groups of three pens and two groups of two pens; groups of the same size were then paired. Each pair of groups was randomly assigned a treatment, control (n=4).
or detergent (n=4); via coin flip performed by an independent person. To prevent cross-contamination, groups with different treatments were separated by a pen not involved in the trial. All participants in the trial were blinded to treatment.

Cleaning protocol
The washing regime was implemented by a single, trained, animal technician. Product application and washing was performed with a power washer (Brendick 1500, Brendick, Derby). Immediately after the pig vacated, each pen was scraped to remove all loose faces and hemp bedding. The following day pens were soaked for one hour with 10 litres of cold water (control) or 10 litres of detergent at the recommended dilution (1:100 Blast-Off, Biolink, UK (Components: Alkaline, N,N-bis (carboxymethyl)-trisodium salt, β-alanine, N-(2-carboxyethyl)-N-dodecyl monosodium salt, Alcohol Ethoxylates, Sodium Hydroxide and Alkyl Dimethyl Benzyl Ammonium Chloride in a water base)). After soaking, each pen was pressure washed with cold mains water for 35 minutes using a consistent and repeatable method. Each pen was disinfected 24 hours later by application of 5 litres of 1 per cent Virkon S (DuPont, UK). Pens were then left to dry (rest) at ambient room temperature.

Sampling
For each pen, swab samples were taken from concrete, metal (galvanised steel slats) and stock board surfaces at several stages throughout the cleaning using a predesignated position identified via consistent landmarks within each pen (Fig 1). Samples were collected using a pre-moistened sterile sponge (Medical Wire & Equipment, UK) and a circular 100 cm² wire template. The sterile sponge was removed from its packaging with a clean gloved hand, placed face down at the 12 o’clock position of the template area, wiping the entire surface using a top to bottom motion, finishing at the 3 o’clock position. Once completed, the sponge swab was placed into a sterile stomacher bag. The technique was repeatable method. Each pen was disinfected 24 hours later by application of 5 litres of 1 per cent Virkon S (DuPont, UK). Pens were then left to dry (rest) at ambient room temperature.

Microbial enumeration
Sterile maximum recovery diluent (60 ml; MRD, Oxoid, UK) was added to each stomacher bag (30 ml/spawn representing a 10⁻¹ dilution), and the samples agitated via a stomacher machine, 230 bpm for two minutes (Seward Stomacher 400 Circulator, Seward, UK). A 10 ml sample of the MRD suspension was removed from the stomacher bag, transferred into a sterile universal container and a 10-fold serial dilution completed in MRD. One millilitre samples of the dilution series were plated, in duplicate, onto ENT and TAC media (Petrifilm 3M, UK). Plates were incubated at 37°C, ENT for 24 hours and TAC for 48 hours, and colonies were manually counted. The calculated limit of detection was 15 cfu/100cm² of recoverable cells per swabbed surface, assuming 100 per cent recovery from the sponge.

For the samples taken after disinfection with Virkon S, the MRD was supplemented with 0.5 per cent sodium thiosulfate to neutralise Virkon S (Dhir and Dodd 1995).

Statistical methods
Counts were converted into colony-forming units per cm² and an arithmetic mean was calculated for each material in each group of replicate pens. A general analysis of variance of the log-transformed reduction in counts after each cleaning step was carried out between treatments, blocking by group, using the statistical program GenStat 14th Edition.

Results
Effect of detergent treatment
When compared with control pens, after washing, detergent-treated concrete had significantly larger reductions in TAC (1.6 log cfu/cm², P<0.005) but not ENT; detergent-treated metal had significantly larger reductions in both TAC and ENT (1.5 and 0.4 log cfu/cm², respectively, P<0.05). There was no significant effect of treatment on stock board (Table 1).

Effect of disinfection
After disinfection, there was no significant difference in reduction of bacteria between detergent-treated and control pens, therefore, to assess the effect of disinfection on surface types, results were analysed as a single sample set. There were significant reductions in both TAC and ENT after disinfection of concrete (1.6 log cfu/cm², P=0.005 and 0.7 log cfu/cm², P<0.05, respectively) and stock board (1.1 and 0.6 log cfu/cm², respectively, P<0.05), but no significant change in TAC or ENT on metal (Table 2).

Effect of rest
During resting there was no significant difference in reduction of bacteria in detergent-treated and control pens, therefore, to analyse the effect of rest, results were again analysed as a single sample set. There was a significant reduction in TAC and ENT after 24 hours of rest on metal (1.8 and 1.1 log cfu/cm², respectively, P<0.05) and stock board (0.8 and 1.8 log cfu/cm², respectively, P<0.05); counts at 48 or 120 hours were not significantly lower than those recorded at 24 hours. No significant reduction in counts occurred on concrete during the entire rest period (Table 2).

Discussion
Within this study, detergent has shown a differential ability to reduce microbial counts according to material and bacterial type (Table 1).
Detergent reduced TAC on concrete and metal by more than one log, but had no effect on stock board. Detergent had little effect on Metal (0.4 log, but had no effect on stock board. Detergent had little effect on Detergent reduced TAC on concrete and metal by more than one log.

<table>
<thead>
<tr>
<th>Surface</th>
<th>Stage</th>
<th>Treatment</th>
<th>Control</th>
<th>Treatment</th>
<th>Control</th>
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<td>Concrete</td>
<td>Postscrape</td>
<td>3.44 × 10⁴</td>
<td>1.47 × 10⁴</td>
<td>3.24 × 10⁴</td>
<td>7.56 × 10⁴</td>
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<td>24 hours rest</td>
<td>6.51 × 10⁴</td>
<td>3.88 × 10⁴</td>
<td>2.13 × 10⁴</td>
<td>8.54 × 10⁴</td>
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<td></td>
<td>48 hours rest</td>
<td>3.48 × 10⁴</td>
<td>6.05 × 10⁴</td>
<td>4.62 × 10⁴</td>
<td>6.42 × 10⁴</td>
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<td>Metal</td>
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<td>1.33 × 10⁵</td>
<td>6.09 × 10⁴</td>
<td>3.79 × 10⁵</td>
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<tr>
<td></td>
<td>Postdip</td>
<td>2.55 × 10⁴</td>
<td>1.97 × 10⁴</td>
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<td>1.98 × 10⁴</td>
<td>1.34 × 10⁴</td>
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<td>120 hours rest</td>
<td>3.36 × 10⁴</td>
<td>5.47 × 10⁴</td>
<td>5.47 × 10⁴</td>
<td>5.47 × 10⁴</td>
</tr>
</tbody>
</table>

Colony forming units (cfu/cm²) after cleaning stages on concrete, metal and stock board. ‘Treatment’ soaked with a detergent product for one hour prior to washing, ‘control’ soaked with cold water for one hour prior to washing. P values indicate significantly larger reduction in counts from previous cleaning stage compared to control **P<0.005, *P<0.05. Estimated standard error (log₅) given in brackets.
bacterial groups tested. Compared with TAC, there were lower reduc-
tions of ENT, a group encompassing important enteric pathogens, sug-
uggesting that other combinations of approved C & D products need to be tested. This study showed no significant synergistic, or additive, effect between detergent and disinfectant; despite this, it is still broadly
recommended to apply both during cleaning of animal housing to use the individual significant bactericidal actions of each product, ensuring the product choice is suitable for intended surface and target
micro-organisms. Producers should be aware of the influence of building
material on the success of their cleaning and disinfection regimes,
both product effectiveness and ease of drying.

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