Paper

OPEN ACCESS

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

L. R. Hancox, M. Le Bon, C. E. R. Dodd, K. H. Mellits

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 proteinaceous and fatty, comprising 20–22 per cent and 7–12 per cent of dry matter, respectively; fatty and proteinaceous soiling is insoluble, meaning water-based cleaning methods alone may be inadequate (Eggum and Christensen 1974, Ohta 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'Toole 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, Shafa 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.

Veterinary Record (2013) doi: 10.1136/vr.101392

L. R. Hancox, BVetMed,
M. Le Bon, BSc PhD,
C. E. R. Dodd, BSc PhD,
K. H. Mellits, BSc PhD,
Division of Food Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire , LE12 5RD, UK

E-mail for correspondence: Ken.Mellits@nottingham.ac.uk

Provenance: Not commissioned; externally peer reviewed

Accepted June 17, 2013

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)

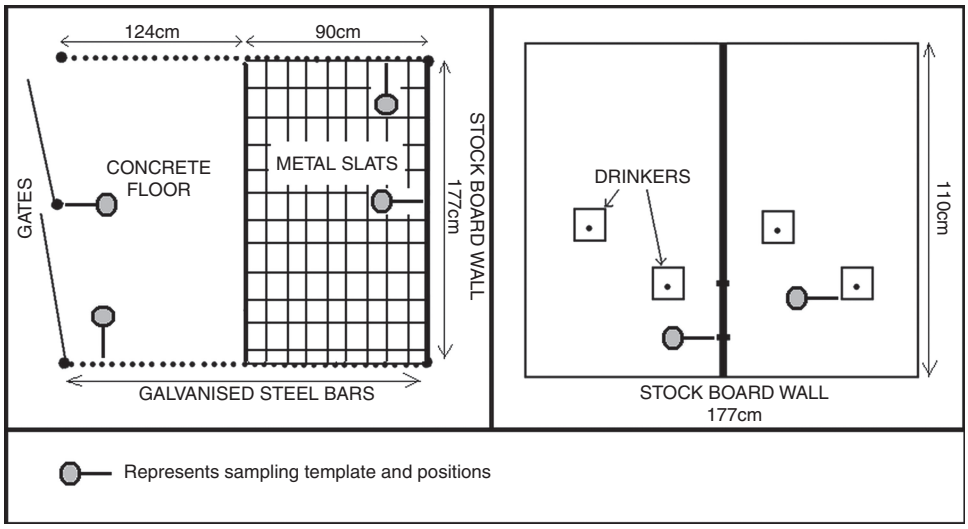

FIG 1: Sampling sites: dimensions and sample sites of the pen floor (metal and concrete surfaces) and stock board wall within each pen

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 pressure washer (Brendick 1500, Brendick, Derby). Immediately after the pig vacated, each pen was scraped to remove all loose faeces 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: Alanine, 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 cm^2 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 and an even pressure applied as the swab was moved from side to side towards the 6 o'clock position to cover the surface contained within the template; the swab was then turned over and the same technique applied placing the swab at the 9 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 repeated on a second site for the same pen material, and the second sponge placed in the stomacher bag with the first to give a combined sample.

Microbial enumeration

Sterile maximum recovery diluent (60 ml; MRD, Oxoid, UK) was added to each stomacher bag (30 ml/sponge 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/100cm^2 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 cm^2 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/cm^2, $P<0.005$) but not ENT; detergent-treated metal had significantly larger reductions in both TAC and ENT (1.5 and 0.4 log cfu/cm^2, 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/cm^2, $P<0.005$ and 0.7 log cfu/cm^2, $P<0.05$, respectively) and stock board (1.1 and 0.6 log cfu/cm^2 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/cm^2, respectively, $P<0.05$) and stock board (0.8 and 1.8 log cfu/cm^2, 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).

TABLE 1: Effect of detergent treatment on bacterial load of livestock pen materials

		Mean cfu/cm²			
		Total aerobic count		*Enterobacteriaceae*	
Surface	Stage	Treatment	Control	Treatment	Control
Concrete	Postscrape	3.34×10^{10}	3.24×10^{9}	7.56×10^{3}	1.29×10^{5}
	Postsoak and wash	6.32×10^{5} ** (0.96)	2.24×10^{6}	8.00×10^{2}	2.13×10^{3}
Metal	Postscrape	6.09×10^{9}	2.12×10^{9}	4.04×10^{3}	8.54×10^{3}
	Postsoak and wash	4.62×10^{5} * (0.99)	4.64×10^{6}	1.16×10^{2} * (0.36)	6.42×10^{2}
Stock board	Postscrape	1.66×10^{8}	5.93×10^{7}	9.71×10^{1}	1.54×10^{2}
	Postsoak and wash	1.30×10^{5}	3.58×10^{5}	2.44×10^{2}	9.49×10^{1}

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 stage compared to control **P<0.005, *P<0.05. Estimated standard error (log_{10}) given in brackets

TABLE 2: Effect of disinfection and rest on bacterial load of livestock pen materials

		Mean cfu/cm²	
Surface	Stage	Total aerobic count	*Enterobacteriaceae*
Concrete	Postwash	1.44×10^{6}	1.47×10^{3}
	Postdisinfection	3.80×10^{4} ** (0.21)	2.72×10^{2} * (0.28)
	24 hours rest	6.51×10^{4}	3.88×10^{2}
	48 hours rest	3.48×10^{4}	6.05×10^{1}
	120 hours rest	6.69×10^{4}	1.33×10^{2}
Metal	Postwash	2.55×10^{6}	3.79×10^{2}
	Postdisinfection	1.00×10^{6}	1.97×10^{2}
	24 hours rest	1.66×10^{4} * (0.16)	1.62×10^{1} * (0.25)
	48 hours rest	1.98×10^{4}	1.34×10^{1}
	120 hours rest	1.35×10^{4}	2.05×10^{1}
Stock board	Postwash	2.44×10^{5}	1.69×10^{2}
	Postdisinfection	1.76×10^{4} * (0.21)	4.59×10^{1} * (0.26)
	24 hours rest	2.79×10^{3} * (0.11)	6.56×10^{-1} * (0.24)
	48 hours rest	2.30×10^{3}	1.42×10^{0}
	120 hours rest	3.36×10^{3}	5.47×10^{0}

Colony forming units (cfu)/cm² after cleaning stages on concrete, metal and stock board. There was no significant difference between counts, or effect of subsequent cleaning steps, on detergent treated and control surfaces after washing, hence, treatment and control datasets have been combined. P values indicate significantly reduced counts from previous cleaning stage **P<0.005, *P<0.05. Estimated standard error (log_{10}) given in brackets

Detergent reduced TAC on concrete and metal by more than one log, but had no effect on stock board. Detergent had little effect on reduction of ENT counts, although there was a statistically significant effect of ENT on metal (0.4 log cfu/cm² reduction) although this small reduction may have low biological relevance; however, since the ENT counts are significantly lower than TAC, large reductions are more difficult to demonstrate (Table 1). The lack of detergent effect on ENT may also be due to the ENT-surface interactions. Bacteria commonly prefer to grow on surfaces rather than in the aqueous phases which surround them (Katsikogianni and Missirlis 2004); after initial attraction and adhesion-specific interactions occur between bacteria and the surface: the strength and durability of these interactions vary according to the surface and bacterial type (Rijnaarts and others 1995). Additionally, a lack of detergent effect on bacterial load on stock board may be due to the surface being vertical, thus, contact time may have been effectively reduced through poor adhesion.

The disinfectant used in this study, Virkon S, has been shown to have reduced action in the presence of organic matter, hence, it was hypothesised that detergent soaking would cause a significant improvement in subsequent disinfectant action due to removal of organic matter (Amass and others 2001, Vohra and Poxton 2011). However, the present results showed no such significant relationship. After pressure washing, the surfaces were visually clean, but this does not discount some level of organic matter remaining; considering the long contamination period, a one-hour soak time may have been insufficient to demonstrate a significant difference in organic matter removal. Detergents also have their own bactericidal properties, and many contain additional biocides (Knox and others 1949, Shafa and Salton 1960, Tanzer and others 1979). The detergent in this study contains quaternary surfactants, non-ionic surfactants, chelating agents and sodium hydroxide. If the one-hour soak period was insufficient to cause significantly greater reductions in organic matter, the significant reduction in counts seen in detergent-treated pens may be partially attributable to biocidal action of the detergent product.

Disinfectants, such as Virkon S, are chemical agents designed to control biocontamination on inanimate objects (Bridier and others 2011). Significant reductions in bacterial counts were seen on concrete and stock board following disinfection, but not on metal. The lack of significant effect of disinfectant on metal may be due to an interaction between bacteria, surface characteristics and the chemical composition of the disinfectant. Virkon S is chiefly described as an oxidising disinfectant, it causes bacterial death by oxidation of bacterial proteins and lipids impeding enzymes and disturbing integrity of cell walls (De Benedictis and others 2007). Virkon S has several components which contribute to its bactericidal action: potassium peroxymonosulfate, two organic acids (malic and sulfamic), an organic buffer (sodium hexameta-phosphate) and a surfactant. Acid compounds are known to react with metals creating metal salts and hydrogen; this neutralising reaction may impede disinfectant action, as the oxidising compound, triple salt of potassium monosulfate, is most effective at a low pH. Reduction in ENT counts due to disinfection was less than one log; although statistically significant, the biological relevance of such small reductions must be considered. The disparity between product effect on TAC and ENT in our study may be because disinfectants and detergent biocides have a different bactericidal action, according to the bacterial type it is used against (McDonnell and Russell 1999). Moreover, as with detergent treatment, a disinfectant effect may be more difficult to demonstrate considering the relatively lower counts of ENT, especially in the presence of remaining organic matter (Table 2). Although in vitro testing demonstrates Virkon S is significantly effective against a range of bacteria including members of the ENT family and other vegetative bacteria, in the presence of organic matter, Virkon S efficacy drops, as is the case with many other classes of disinfectant (Gasparini and others 1995, Herñndez and others 2000, Amass and others 2001, Møretrø and others 2009, McLaren and others 2011, Vohra and Poxton 2011). During on-farm studies, after physical cleaning, Virkon S did not reduce ENT or aerobic bacteria counts on metal or wooden surfaces within poultry buildings, in agreement with our own findings (Ward and others 2006).

Areas of animal housing are rested between batches to allow decrease of micro-organisms remaining after C & D. Desiccation is thought to be the main cause of microbial death during this rest time; in agreement with our results, particularly regarding ENT, other studies have shown drying to cause higher bacterial mortality than some chemical disinfectants (Asséré and others 2008). The differing microbial death with rest on surfaces may be attributable to the surface characteristics. Concrete is rough and porous giving it a large surface area and ability to adsorb liquids; galvanised steel and stock board are smooth and less porous allowing for easier evaporation, more drying, and hence, more desiccation and significant microbial death.

In conclusion, this study has displayed a disparity between effectiveness of C & D and rest on different surfaces, for example, disinfectant action of Virkon S on metal and the effect of rest on concrete. Effectiveness of detergent and disinfectant was dependant on the

bacterial groups tested. Compared with TAC, there were lower reductions of ENT, a group encompassing important enteric pathogens, suggesting 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.

Acknowledgements

We acknowledge the technical assistance of the Bio-Resource Unit at the University of Nottingham, Oliver Watts, Elizabeth King, Siyu Wu and Yogeshwar Chandelia.

Funding This work was supported by grants from the BBSRC and BPEX, a division of the Agriculture and Horticulture Development Board.

References

AMASS, S., RAGLAND, D. & SPICER, P. (2001) Evaluation of the efficacy of a peroxygen compound, Virkon®S, as a boot bath disinfectant. *Journal of Swine Health and Production* **9**, 121–123

ASSÉRÉ, A., OULAHAL, N. & CARPENTIER, B. (2008) Comparative evaluation of methods for counting surviving biofilm cells adhering to a polyvinyl chloride surface exposed to chlorine or drying. *Journal of Applied Microbiology* **104**, 1692–1702

BRIDIER, A., BRIANDET, R., THOMAS, V. & DUBOIS-BRISSONNET, F. (2011) Resistance of bacterial biofilms to disinfectants: a review. *Biofouling* **27**, 1017–1032

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

DAVIES, R. & BRESLIN, M. (2003) Observations on Salmonella contamination of commercial laying farms before and after cleaning and disinfection. *Veterinary Record* **152**, 283–287

DE BENEDICTIS, P., BEATO, M. S. & CAPUA, I. (2007) Inactivation of Avian Influenza viruses by chemical agents and physical conditions: A review. *Zoonoses and Public Health* **54**, 51–68

DHIR, V. & DODD, C. (1995) Susceptibility of suspended and surface-attached Salmonella enteritidis to biocides and elevated temperatures. *Applied and Environmental Microbiology* **61**, 1731–1738

DONLAN, R. M. (2002) Biofilms: microbial life on surfaces. *Emerging Infectious Diseases* **8**, 881–890

DUNOWSKA, M., MORLEY, P. S. & HYATT, D. R. (2005) The effect of Virkon®S fogging on survival of Salmonella enterica and Staphylococcus aureus on surfaces in a veterinary teaching hospital. *Veterinary Microbiology* **105**, 281–289

EGGUM, B. R. O. & CHRISTENSEN, K. D. (1974) Utilization by rats of proteins in pig faeces relative to Barley and fortified casein proteins. *Agriculture and Environment* **1**, 59–64

GASPARINI, R., POZZI, T., MAGNELLI, R., FATIGHENTI, D., GIOTTI, E., POLISENO, G., PRATELLI, M., SEVERINI, R., BONANNI, P. & DE FEO, L. (1995) Evaluation of in vitro efficacy of the disinfectant Virkon. *European Journal of Epidemiology* **11**, 193–197

HERÑNDEZ, A., MARTRÓ, E., MATAS, L., MARTíN, M. & AUSINA, V. (2000) Assessment of in-vitro efficacy of 1% Virkon®S against bacteria, fungi, viruses and spores by means of AFNOR guidelines. *Journal of Hospital Infection* **46**, 203–209

HOOD, S. K. & ZOTTOLA, E. A. (1997) Adherence to stainless steel by foodborne microorganisms during growth in model food systems. *International Journal of Food Microbiology* **37**, 145–153

KATSIKOGIANNI, M. & MISSIRLIS, Y. F. (2004) Concise review of mechanisms of bacterial adhesion to biomaterials and of techniques used in estimating bacteria-material interactions. *Cells and Materials* **8**, 37–57

KNOX, W. E., AUERBACH, V. H., ZARUDNAYA, K. & SPIRTES, M. (1949) The action of cationic detergents on bacteria and bacterial enzymes. *Journal of Bacteriology* **58**, 442–452

MAH, T.-F. C. & O'TOOLE, G. A. (2001) Mechanisms of biofilm resistance to antimicrobial agents. *Trends in Microbiology* **9**, 34–39

MANNION, C., LYNCH, P. B., EGAN, J. & LEONARD, F. C. (2007) Efficacy of cleaning and disinfection on pig farms in Ireland. *The Veterinary Record* **161**, 371–375

MARRIOTT, N. G. & GRAVANI, R. B. (2006) Principles of food sanitation. In: HELDMAN, D. R. ed. Food Science Text Series. 5th edn. ASPEN. pp 142

MCDONNELL, G. & RUSSELL, A. D. (1999) Antiseptics and disinfectants: activity, action, and resistance. *Clinical Microbiology Reviews* **12**, 147–179

MCLAREN, I., WALES, A., BRESLIN, M. & DAVIES, R. (2011) Evaluation of commonly-used farm disinfectants in wet and dry models of Salmonella farm contamination. *Avian Pathology: Journal of the W. V.P.A* **40**, 33–42

MØRETRØ, T., VESTBY, L. K., NESSE, L. L., STORHEIM, S. E., KOTLARZ, K. & LANGSRUD, S. (2009) Evaluation of efficacy of disinfectants against Salmonella from the feed industry. *Journal of Applied Microbiology* **106**, 1005–1012

OHTA, Y. & IKEDA, M. (1978) Deodorization of pig feces by actinomycetes. *Applied and Environmental Microbiology* **36**, 487–491

RIJNAARTS, H. H. M., NORDE, W., BOUWER, E. J., LYKLEMA, J. & ZEHNDER, A. J. B. (1995) Reversibility and mechanism of bacterial adhesion. *Colloids and Surfaces B: Biointerfaces* **4**, 5–22

SHAFA, F. & SALTON, M. R. J. (1960) Disaggregation of bacterial cell walls by anionic detergents. *General Microbiology* **23**, 137–141

TANZER, J. M., SLEE, A. M., KAMAY, B. & SCHEER, E. R. (1979) In vitro evaluation of seven cationic detergents as antiplaque agents. *Antimicrobial Agents and Chemotherapy* **15**, 408–414

VICKERY, K., PAJKOS, A. & COSSART, Y. (2004) Removal of biofilm from endoscopes: evaluation of detergent efficiency. *American Journal of Infection Control* **32**, 170–176

VOHRA, P. & POXTON, I. R. (2011) Efficacy of decontaminants and disinfectants against Clostridium difficile. *Journal of Medical Microbiology* **60**, 1218–1224

WARD, P. J., FASENKO, G. M., GIBSON, S. & MCMULLEN, L. M. (2006) A microbiological assessment of on-farm food safety cleaning methods in broiler barns. *The Journal of Applied Poultry Research* **15**, 326–332

YANG, L., LIU, Y., WU, H., SONG, Z., HØIBY, N., MOLIN, S. & GIVSKOV, M. (2011) Combating biofilms. *FEMS Immunology & Medical Microbiology* **65**, 146–157

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

L. R. Hancox, M. Le Bon, C. E. R. Dodd and K. H. Mellits

Veterinary Record 2013 173: 167 originally published online July 9, 2013
doi: 10.1136/vr.101392