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Efficacy of disinfectants and detergents intended for a pig farm environment where *Salmonella* is present

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ABSTRACT

Disinfection is a useful component of disease control, although products and chemical groups vary in their activity against different pathogens.

This study investigated the ability of fifteen disinfectants to eliminate pig-associated *Salmonella*. Active compounds of products included chlorocresol, glutaraldehyde/formaldehyde, glutaraldehyde/quaternary ammonium compounds (QAC), iodine, peracetic acid and potassium peroxomonosulphate. Six detergents were also tested for their ability to dislodge faecal material, and interactions with specific disinfectants.

Eight serovars were screened against all products using dilution tests and a monophasic *Salmonella* Typhimurium strain was selected for further testing. The disinfectants were tested using models to replicate boot dip (faecal suspension) and animal housing (surface contamination) disinfection respectively at the Department for Environment, Food and Rural Affairs Approved Disinfectant General Orders (GO) concentration, half GO and twice GO. Stability over time and ability to eliminate *Salmonella* in biofilm was also assessed. The most effective products were then field tested. Most products at GO concentration eliminated *Salmonella* in the faecal suspension model. One glutaraldehyde/QAC and one glutaraldehyde/formaldehyde-based product at GO concentration eliminated *Salmonella* in the surface contamination model. Chlorocresol-based products were more stable in the faecal suspension model. One chlorocresol and the glutaraldehyde/formaldehyde-based product were most successful in eliminating *Salmonella* from biofilms. All products tested on farm reduced bacterial log counts; the glutaraldehyde/QAC based product produced the greatest reduction.

The type of product and the application concentration can impact on efficacy of farm disinfection; therefore, clearer guidance is needed to ensure the appropriate programmes are used for specific environments.

1. Introduction

In 2014, *Salmonella* was the second most common cause of foodborne disease outbreaks in Europe, with *Salmonella* in pig meat reported as a major source by most member states (EFSA and ECDC, 2015). By the time pig meat products reach the consumer they have undergone many processes that may reduce contamination, however, if pigs have low *Salmonella* prevalence when being reared, this reduces the possibility of the organism entering the slaughter chain and contaminating the end product.

One major component of an on-farm disease control programme is an effective cleaning and disinfection (C & D) regimen. Disinfectants are used on farms for two main reasons; firstly, to disinfect cleaned surfaces, including floors, walls and equipment/tools and secondly to prepare boot dips which aim at disinfecting boots on the entry of animal accommodation. Disinfectants are also used to disinfect vehicles as they enter the site and to sanitise water delivery systems.

In Great Britain (GB), the Department for Environment, Food and Rural Affairs (Defra) is required to maintain a list of approved disinfectants that are suitable for use against various disease agents in the case of an outbreak (ANON, 2007). Four Orders cover specific diseases (Avian Influenza, Tuberculosis, Foot and Mouth Disease and Swine Vesicular Disease). A General Orders (GO) test is also included which covers pathogens which do not have their own specific Order. In the case of a disease outbreak, Defra will provide farmers and their private vets with details of approved products and the concentration they should be used at. However, it is often observed that farmers do not accurately measure disinfectants that are used routinely, or allow

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sufficient contact time for them to work effectively.

Disinfectants applied to surfaces often face challenges such as dried organic matter and biofilms which, if not eliminated during the washing process, can inhibit penetration of the disinfectant (Amass et al., 2000). Wet surfaces which have not been allowed to dry between washing and disinfection can dilute the applied disinfectants and reduce their ability to penetrate if the material is still saturated with wash water. This is particularly problematic for wooden surfaces and interfaces between materials.

Not all disinfectants or disinfectant product formulations are equally effective, and some disinfectants are more effective than others in the presence of organic matter, or at low temperatures (Gosling et al., 2016; McLaren et al., 2011). Disinfectants may also have a limited lifespan after the initial dilution is prepared, and in addition to organic matter, other factors such as excessive heat, dilution by rainwater, evaporation and sunlight may reduce their activity (McDonnell and Russell, 1999). The chemical characteristics and modes of action, where known, of the disinfectants that are commonly used on livestock units have been extensively reviewed (Denyer and Stewart, 1998; Lambert, 2004; McDonnell and Russell, 1999; Walton et al., 2008).

The use of a suitable disinfectant in the C & D process has been identified as a factor likely to reduce the risk of *Salmonella* infection in turkey flocks (Featherstone et al., 2010) and *Salmonella* reduction or elimination has occurred in farm settings through effective C & D (Carrique-Mas et al., 2009; Davies and Breslin, 2003; Mueller-Doblies et al., 2010; Payne et al., 2005). However field testing of disinfectants is labour intensive and requires access to suitable contaminated farm buildings.

Disinfectant efficacy has been evaluated *in-vitro* using poultry *Salmonella* isolates in faecal suspension surface contamination models to mimic conditions on chicken and duck farms (Gosling et al., 2016; McLaren et al., 2011). Both studies reported chlorocresol-based products to be the most efficient for eliminating *Salmonella* in a boot dip model, and aldehyde-based disinfectants proved to be superior for disinfection of contaminated surfaces.

This study investigated the efficacy of fifteen commercial disinfectants using pig-associated *Salmonella* in seven different laboratory models. Boot dip samples were also collected from pig farms and were analysed for total bacteria present and their ability to eliminate *Salmonella*.

2. Materials and methods

A panel of fifteen disinfectants and six detergents were selected following discussions with the pig industry in GB and analysis of products available on the open market tailored towards pig housing. The disinfectants, their active ingredients and concentration are detailed in Table 1; the detergents are detailed in Table S1. All dilutions were made using World Health Organisation (WHO) Standard Hard Water. WHO Hard Water was prepared by dissolving 0.304 g of anhydrous calcium chloride and 0.139 g of magnesium chloride hexahydrate in 1 l distilled water. This provides water with a hardness of 342 mg/L calculated as calcium carbonate.

2.1. Maximum inhibitory dilution (MID)/Maximum bactericidal dilution (MBD)

Eight *Salmonella* field strains were selected from the most commonly reported serovars in GB pigs between 2010 and 2013 (Table S2). Overnight cultures were diluted to 1×10^6 CFU/ml. Neat (as bought) disinfectants were diluted 1:25 in WHO hard water. In a 96 well microtitre Plate 75 µl of Nutrient broth No.2 was added to each well. Disinfectant was added to the first well (column 1) (75 µl) and double diluted to column 10. Each *Salmonella* test strain (7.5 µl) was added to a separate row, except column 12 (negative control) and incubated for 18 h ± 2 h at 37 °C. Plates were prepared in duplicate for each test, and each product was tested three times over a six month period. Visual turbidity after incubation indicated *Salmonella* growth. MID value was taken as the last clear well before turbidity was observed. MBD was determined by adding a 10 µl aliquot from each of the MID plate wells into 190 µl Nutrient broth No2. and incubating for 18 h ± 2 h at 37 °C. Turbidity after incubation indicated positive growth; a clear well indicated bactericidal effects.

2.2. Preparation of disinfectants for disinfection model studies

Each disinfectant was accurately measured and diluted in WHO hard water to 0.5, 1 and 2× Defra General Orders (GO) concentration, as recommended at the time of the study (July 2014).

2.3. Faecal suspension model

Isolate BB (monophasic *Salmonella* Typhimurium) was mixed in equal measures with *Salmonella*-free pig faeces to obtain a smooth slurry with 5×10^6 CFU/g of *Salmonella*. In replicates of three, 1 g of *Salmonella*-spiked faeces was added to 9 ml of each disinfectant concentration. Each sample was mixed thoroughly and held at 4 °C. After 30 min, 2 and 4 h, each tube was agitated and a 100 µl aliquot removed into 10 ml Nutrient broth No. 2 + 5% horse serum with a contact time of at least 5 min. One millilitre was then transferred to 10 ml Nutrient broth No. 2 and incubated for 18 ± 2 h at 37 °C. All tubes were further agitated at 1 and 3 h. After 18 ± 2 h incubation in broth, 100 µl was plated onto Modified Semi-Solid Rappaport-Vassiliadis agar (MSRV) and incubated for 24 h at 41.5 °C. A 10 µl loop of turbid medium was then plated onto Rambach agar and incubated for 24 h at 37 °C. A positive or negative result for *Salmonella* was recorded. Counts on faeces not exposed to disinfectants were performed at 30 min, 2 and 4 h to confirm the continued presence of *Salmonella*.

2.4. Surface contamination model

Wooden dowels (40 mm × 10 mm) were immersed in *Salmonella*-contaminated faecal slurry, using a 1:1 mixture of *Salmonella*-free pig slurry and 5×10^6 CFU/g monophasic *Salmonella* Typhimurium (BB), stirred to achieve thin uniform coating of approximately 1 g/dowel. Dowels were placed in vented autoclave tins to dry at room temperature for three days. In replicates of 3, dowels were then exposed to each disinfectant concentration for 10 min at 15 °C. After exposure, dowels were placed in a petri dish overnight. Residual disinfectant on dowels was then neutralised by immersion in 20 ml Nutrient broth No.2 + 5% horse serum for 10 min before being vortexed for 10 s. Two aliquots of 1 ml were added to fresh Nutrient broth No. 2 and incubated for 18 ± 2 h at 37 °C. *Salmonella* presence was determined for each sample by MSRV and Rambach agar method for *Salmonella* isolation as above.

2.5. Disinfectant stability model

Disinfectants at GO concentration; 100 ml, were added to polypropylene containers and held at 4 °C and 15 °C, in duplicate. In the morning of day 0, 3, 6, 9 and 13, 2 g of *Salmonella*-negative pig faeces were added to half of the containers and stirred. In the afternoon on day 0, 3, 5, 7 and 14, 1 ml of the disinfectant solution was collected and 3 × 0.9 ml aliquots prepared and held at 4 °C. Each aliquot was inoculated with 100 ul 5×10^6 monophasic *Salmonella* Typhimurium. After a 30 min contact time, 100 microlitres was transferred into Nutrient broth No.2 + 5% horse serum; lecithin was used as an alternative neutraliser for products containing QAC. After a 10 min contact time, 1 ml was transferred into fresh Nutrient broth No. 2 and incubated for 18 ± 2 h at 37 °C. *Salmonella* presence was determined for each sample as above.

Commercially available dip-stick style testing strips were used in

Table 1
Disinfectants used in the present study with active ingredients and Defra General Orders (GO) dilution.

Code	Commercial name and main active ingredients	GO[a]
Glutaraldehyde & Formaldehyde		
C	Intercid – Glutaraldehyde & Formaldehyde	40
Glutaraldehyde & QAC		
B	GPC-8 – Glutaraldehyde, Didecyldimethylbenzyl ammonium chloride	35[d]
A	Bioshield – Gluteraldehyde, Cocobenzyl dimethyl ammonium chloride	35[c]
L	Multides-GA – Quaternary ammonium compounds & benzyl-C12-16-alkyldimethyl & chlorides, Didecyldimethyl ammonium chloride, Glutaral, Propan-2-ol	65.5[e]
M	Virocid – Glutaraldehyde, Alkyldimethylbenzylammoniumchloride, Didecyldimethyl ammonium chloride, Isopropanol	49[d]
Chlorocresol		
I	Bi-oo-cyst – 4-Chloro-3-methylpheno, Alkyl benzene sulphonic acid, Lactic acid	125[b]
J	Farm Fluid HD ROW – Chlorocresol, biphenyl-2-ol, acetic acid, Benzenesulfonic acid, 4-C 10–13-sec-alkyl dervis	60
K	Interkokask – Chlorocresol, Propionic acid, Phosphoric acid	50
Iodine		
N	Fam30 – Sulphuric acid, phosphoric acid, iodine	90
O	Virophor 2.8% – Phosphoric acid, sulphuric acid, iodine	50
Peracetic acid		
G	Hyperox – Peracetic acid, hydrogen peroxide	179
H	Kickstart – Peracetic acid, hydrogen peroxide	256
Potassium Peroxymonosulphate		
E	Virex – Potassium peroxomonosulphate	100[c]
F	VirkonS – Potassium peroxomonosulphate	100[c]
D	Victor – Sodium dichloroisocyanurate	120[ce]

[a] Figures given are millilitres of water per millilitre of product, correct as of July 2014.
[b] Not approved for GO at time of the study but was in use on farms, therefore Poultry Orders rate was used.
[c] Millilitres of water per gram of product.
[d] GO concentration has increased since July 2014.
[e] Product is no longer listed as GO approved.

this study to assess their suitability for use on farms; Quantofix glutaraldehyde strips (Machery-Nagel, Duren) and Peracetic Acid strips (Precision Labs, Arizona) strips were tested. At day 0, 3 and 5 the strips were dipped in the disinfectant solution as per the manufacturer's instructions and the result read using the colour strip provided with each.

2.6. Biofilm formation

Biofilms were formed on wooden toothpicks in continuous culture of 800 ml buffered peptone water (BPW) in two polypropylene containers on a slow rotary shaker. *Salmonella*-negative pig faeces were added to one container, 80 g, and 1 ml of 1×10^7 stationary phase monophasic *Salmonella* Typhimurium broth added to both. At 3 h, 1d, 4d, 8d, 11d, 18d and 25d, three toothpicks were removed and exposed to freshly made disinfectant at GO concentration for 30 min, then placed in a petri dish overnight. The following day, each toothpick was placed into 20 ml BPW and incubated for 18 ± 2 h and *Salmonella* presence was determined as above. At the same time points 50 ml of the solution was removed and replaced with fresh BPW.

2.7. Filter disc method

Whatmann 6 mm filter discs were submerged in an overnight culture of monophasic *Salmonella* Typhimurium for 4–6 h at 4 °C following Model 2 as described by Rabie et al. (2015). Discs were removed and placed in a petri dish. For this method disinfectants were made up using WHO hard water and locally sourced stream water. Disinfectants were prepared at GO concentration. Six discs were placed in each disinfectant for 30 min, removed and individually cultured in 225 ml BPW. These were incubated for 18 ± 2 h at 37 °C and *Salmonella* presence determined as above. This method was also followed for testing on-farm boot dip samples, substituting the prepared disinfectants with those collected on-farm and testing one filter disc per sample.

2.8. On-farm disinfectant product comparison

Four disinfectant products were selected for an on-farm field evaluation. Products B (glutaraldehyde/QAC), K (m-cresol), F (peroxymonosulphate) and O (iodine) from the initial panel were chosen. Five litres of each product was applied at GO concentration to four pens, to point of saturation. Hand swab samples were collected before application, 1 h after application and 24 h after application from floors (4), walls (4), feeders (1) and drinkers (1). Samples were diluted in BPW and 10 ml plated out on blood agar and Violet Red Bile Glucose Agar (VRBG) agar to determine total aerobic bacteria and Enterobacteriaceae counts respectively. Swabs and BPW dilutions were also cultured for *Salmonella* following the method described above.

2.9. Detergent activity

Wooden coupons (90 mm × 60 mm) of marine plywood were coated on one side with 4 g of *Salmonella*-negative faecal slurry, prepared with 1:1 pig faeces and Nutrient broth No. 2. Coupons were dried for a minimum of 96 h at room temperature.

Each detergent product was diluted in WHO hard water to the maximum concentration as recommended by the manufacturer (Table 2). Each coupon was weighed and placed into a separate sealable 300 ml plastic container (Plastic Box Shop, Northallerton, UK) with 25 × 12 mm chrome steel ball bearings (Simply Bearings, Manchester, UK) and 50 ml of the diluted detergent; WHO hard water was used as a negative control. Each box was placed on a rotary shaker for 30 min at 120 rpm. Coupons were removed and rinsed under distilled water for 2 s and left to dry at room temperature for a minimum of 7 d in a vented autoclave tin. Coupons were re-weighed and the amount of faeces dislodged calculated as the difference between pre and post weight.

2.10. Interaction of detergent and disinfectants

Four detergents were selected and diluted at the maximum con-

Table 2
Factors analysed with P values for the null hypothesis.

	Faecal suspension model	Surface contamination model	Filter disk test	Biofilm test	Stability over time
Product Group	P < 0.001	P < 0.001	P < 0.001	P < 0.0001	P < 0.001
Product Group within Concentration	0.5 × GO P < 0.001 GO P = 0.261 2 × GO P = 0.067	GO P < 0.001 2 × GO P < 0.001	N/A	N/A	N/A
Concentration	P = 0.300	P < 0.001	N/A	N/A	N/A
Individual Products within Product Groups	Iodine P < 0.001 Chlorocresol P = 0.001 align = "center"	G & Q P = 0.747 Chlorocresol P = 0.051 align = "center"	G & Q P < 0.001	Not analysed	Not analysed
Faeces	N/A	N/A	N/A	Faeces P = 0.05	Faeces P < 0.001
Other factors	Contact time P = 0.305	N/A	Water type P = 0.383	N/A	Temperature P = 0.874

centration as recommended by the manufacturer. Five disinfectants were also selected and diluted to 0.5× GO rate. This rate was chosen as the majority of the disinfectants tested performed well at GO rate and a weaker concentration was required in order to determine any synergistic or antagonistic effects when detergent and disinfectant were combined.

Using the filter disc method previously described, *Salmonella*-inoculated discs were submerged in 5 ml of detergent for 5 min before being removed and submerged in 5 ml disinfectant for 30 min; no rinsing took place between steps. Controls of detergent only and disinfectant only, for 5 and 30 min, respectively, were also included. Discs were then transferred into Nutrient broth No. 2 and incubated and *Salmonella* presence determined.

2.11. Statistical analysis

Statistical analyse was performed using Stata12 (Statacorp, College Station, Texas, USA).

MID/MBD data and dislodge data were analysed using Analysis of Variance.

Data from the faecal suspension, surface contamination and biofilm models were analysed using Fisher's exact tests as many values were small or zero. A ranking trend test was used for ordinal group categories e.g. concentration, time and run that were significant ($p <= 0.05$) on the initial test.

Logistic regression was used for the biofilm data with product as a 14 level factor to assess if all individual treatments had the same proportion positive. The effect of faeces was tested using a Chi Square test.

For the filter model, only GO concentration was used, but the proportion of positive samples by type of water used as a 2 level factor and product group were analysed by Fisher's exact test. Where individual differences within a product group were evident from the observations, a within group test was used to assess differences between the individual products.

The stability study was a repeated measures study with sampling of the same experimental unit at sequential points over 14 days. The total number of days positive and the mean first day positive per group were used as summary measures and compared by a Kruskall Wallis (KW) ranking test for product group, faeces presence or absence, and two levels of temperature as univariate analyses. Since a control group was present, where there was a difference in product group the test was repeated without the control group for between treatment differences. A Poisson regression on the count of days positive per experimental unit with faeces and temperature and product group as factors was used to identify differences between product groups. A Generalised Estimating Equation (GEE) method was also used to assess the probability of detecting a positive sample allowing for the repeated measures, with temperature and faeces as factors and day as a variable, to investigate whether the day of the study affected the probability of a sample being positive.

For the on-farm study, total bacteria and Enterobacteriaceae count data were converted into log values (count +0.1) and analysed using Factorial analysis by Analysis of Variance. Analysis of Variance was a poor fit to the log values at each time point so the difference in log counts between times was used. The pen averages per factor were used to avoid the within-pen correlation.

3. Results

3.1. Maximum inhibitory Dilution/Maximum bactericidal dilution

No significant difference in MID/MBD results was found between the eight different strains of *Salmonella* (p = 0.971), therefore data presented in Fig. S1 are means of all strains tested. All products within the same chemical grouping produced similar results (Fig. S1), with the glutaraldehyde/QAC combinations and peracetic acid-based products demonstrating bactericidal effects when present at less than 0.1%, whereas iodine-based products were effective at 0.4%. The detergents required higher concentrations in order to achieve inhibition or elimination of *Salmonella* (Fig. S2).

The overall results from the main tests presented in this paper are displayed in Table 2. A product effect was observed for all tests, and concentration also had an impact on efficacy. The results for each test are presented below in more detail.

3.2. Faecal suspension model

The challenge inoculum was 5×10^6 CFU/ml therefore a negative result indicates a greater than 6 log reduction. An increasing linear trend was observed for effect of product concentration on *Salmonella* elimination over time, although this was not found to be statistically significant (p = 0.300). Significant differences were observed between chemical groups and concentrations (p < 0.001; Table 3). This was mainly due to differences within the 0.5 × GO concentration study (p < 0.0001), whereas there were no significant differences between chemical groups tested at GO (p = 0.261). All products eliminated *Salmonella* at 2 × GO concentration; the one exception to this was the glutaraldehyde/formaldehyde product (C) where one test failed at the 30 min contact time point. Variation between individual products within each chemical group was observed for iodine and chlorocresol-based products, with further analysis indicating the products to be statistically different (p < 0.0001 and p = 0.001 for iodine and chlorocresol-based products respectively). The peroxymonosulfate group consistently eliminated *Salmonella*, even at 0.5 × GO concentration.

Table 3
Ability of disinfectant product group and concentration to eliminate *Salmonella* when tested using the faecal suspension model.

Disinfectant chemical group	Products	Concentration and *Salmonella* elimination result (samples negative/samples tested)		
		0.5 × GO	GO	2 × GO
Glutaraldehyde/ Formaldehyde	1	24/27	27/27	26/27
Glutaraldehyde/QAC	4	100/108	105/108	108/108
Iodine	2	37/54	51/54	54/54
Peracetic acid	2	35/54	52/54	54/54
Peroxymonosulfates	3	81/81	81/81	81/81
Chlorocresol	3	73/81	80/81	81/81

GO – General Orders concentration, QAC – Quaternary Ammonium Compounds.

3.3. Stability studies

The challenge inoculum was 5×10^6 CFU/ml therefore a negative result indicates a greater than 6 log reduction. The number of *Salmonella*-positive samples increased with the number of days since making the dilution and product efficacy decreased over time for both temperatures (Fig. S3 and S4). Using total days positive and mean first day positive as summary measures over the 5 time points, the control and treatment groups were not the same ($p < 0.001$) by the KW test. Excluding the control group, not all treatment groups were the same, but the main difference was that the chlorocresol group had fewer days positive than the other groups (Figs. S3 and S4). Addition of faeces was a significant factor in increasing the number of days positive ($p = 0.001$) and reducing the time to first day positive ($p < 0.001$), however temperature had no significant effect on the outcome ($p = 0.874$). These conclusions were supported by multivariate Poisson regression and GEE. GEE analysis indicated that the day was a significant factor with increasing probability of a positive sample as the days progressed.

3.4. Surface contamination model

Elimination of *Salmonella* from dried-on faecal contamination on wooden dowels differed between the chemical groups ($p < 0.001$), and effect was concentration dependent ($p < 0.001$). The glutaraldehyde/formaldehyde-based chemical group was the only group to eliminate *Salmonella* consistently at GO. Glutaraldehyde/QAC-based products (B, L & M) were also able to eliminate *Salmonella* at GO concentration, but not consistently, however all products in this group were effective at 2 × GO. All other product groups tested were unable to eliminate *Salmonella* even at 2 × GO concentrations.

3.5. Biofilm disinfection studies

Five products (the peracetic acid-based products, G & H, two chlorocresol-based products, I & J, and one iodine-based product, N) were unable to eliminate *Salmonella* after day 8 of biofilm formation. The gluteraldehyde/QAC-based products were effective against 4–8 day old biofilms, whereas the peroxymonosulfates became ineffective when biofilms were more than 1 day old. The most effective products were one of the chlorocresol-based products, K, the gluteraldehyde/formaldehyde-based product, C, and one of the iodine-based products, O (Fig. 1). The presence of faeces inhibited the activity of the disinfectants ($p = 0.05$).

3.6. Filter disc model

The inoculation dose in the filter discs was 2×10^7 CFU/disc *Salmonella*, therefore a negative result indicates a greater than 7 log reduction in *Salmonella* by the disinfectant tested.

Chemical groups tested using the filter disc method differed in their ability to eliminate *Salmonella* ($p < 0.0001$). This was not affected by the type of water used to prepare the disinfectants ($p = 0.383$), however due to the majority of results being 0 values, the ability to detect a significant effect may be small (Table S3). Some variability in results occurred between products within the glutaraldehyde/QAC chemical group and the Fishers Exact test concluded all the products were not the same ($p < 0.0001$) with product M being substantially less effective than the other products (A,B and L) in this group, which all eliminated *Salmonella*.

3.7. Detergent activity study

In the organic matter dislodging test, none of the products differed from the water control, with the only differences observed between the two alkaline products ($p = 0.033$; Fig. S5). The poorer performing alkaline product was recommended to be used at 10%, which made it a very thick solution, compared to the better performing alkaline product which was recommended for use at 2%. It is likely that the mechanical action applied in the study had the greatest effect at the removal of organic material, with the presence of any liquid helping to soften and loosen the material.

3.8. Interaction of detergent and disinfectants

The inoculation dose in the faeces was 5×10^6, therefore a negative result indicates a greater than 6 log reduction in *Salmonella* presence by the detergent/disinfectant combination tested. The gluteraldehyde/formaldehyde-based product (C) failed to eliminate *Salmonella* at 0.5 × GO, and this was not improved when pre-treated with the ammonium chloride detergent. However the use of the acidic detergent improved elimination of *Salmonella* and the use of either alkaline products followed by the gluteraldehyde/formaldehyde-based product eliminated *Salmonella* completely (Table S4). The peracetic acid-based product (G), although effective at 0.5 GO on its own in this test, became ineffective when substrates were pre-treated with one of the alkaline detergents. A similar trend occurred for the iodine-based product (N), which was partially effective at 0.5 GO but was ineffective when the ammonium chloride or one of the alkaline detergent products had been applied. Chlorocresol product (K) was not inhibited by the presence of any of the different detergents tested.

3.9. On-farm disinfectant product comparison

Factorial analysis by Anova looked for statistically significant differences between pre and post treatment and between products, allowing for sample type. Anova was a poor fit to the log values per time so the difference in log counts between times was used. The pen averages per factor were used to avoid the within-pen correlation.

The log reduction of total bacteria recovered before and after treatment varied between the four products tested ($p < 0.001$; Fig. 2). The glutaraldehyde/QAC-based product (B) produced a higher log reduction (2.27) than products K, F and O (1.41–1.24), and when all sample types were combined all log counts were greater than 0 indicating an overall reduction in bacterial counts with treatment.

Log counts for Enterobacteriaceae were much lower (Table S5), with no significant difference between products, nor a significant reduction in log count post treatment. This was also analysed by the proportion of pen samples positive. The proportions positive dropped from 0.23 to 0.14 at 1 h after treatment and there were no positive samples at 24 h post treatment. The effect of time was statistically significant ($p < 0.002$) but there were no product effects.

Salmonella was analysed on binary data only with samples recorded as positive or negative. There was a significant effect of time with both 1 and 24 h being different from time 0 (pre disinfection). The odds ratio was 0.22 which is reflected by the reduction in the proportion positive

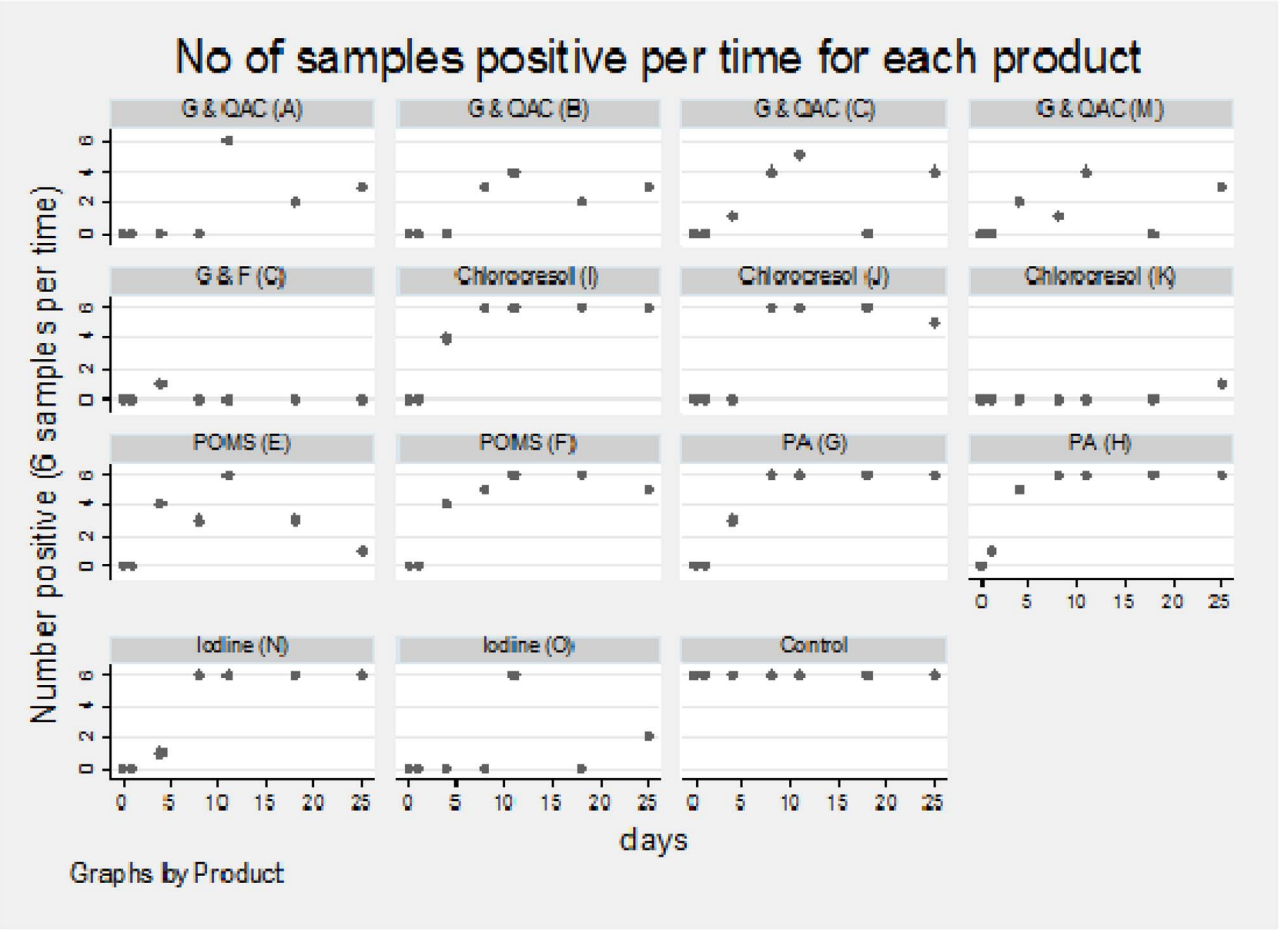

Fig. 1. Ability of disinfectants to eliminate *Salmonella* from biofilms at different time points.G – Glutaraldehyde, F – Formaldehyde, QAC – Quaternary Ammonium Compound, POMS – Peroxymonosulfate, PA – Peracetic acid

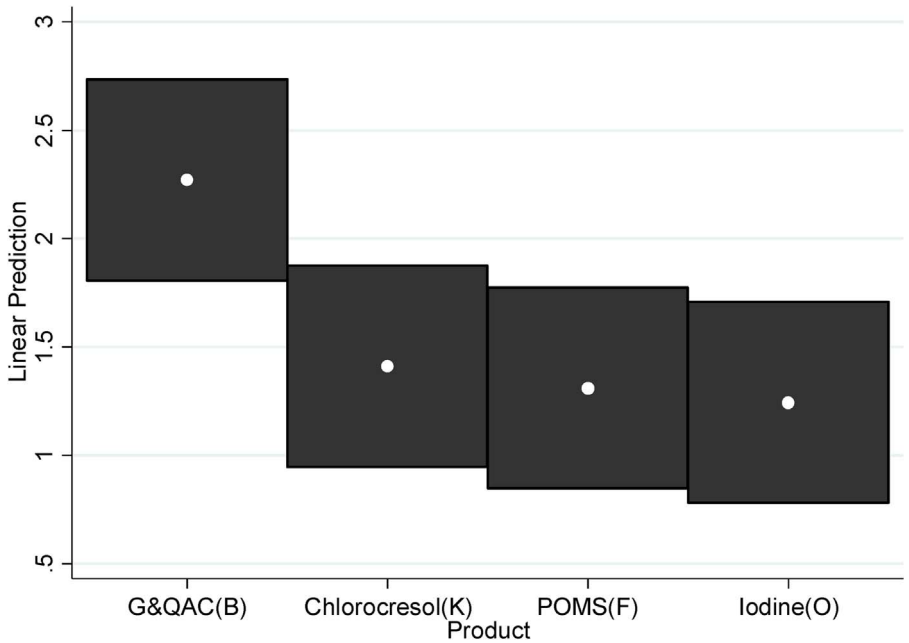

Fig. 2. Box plot for reduction of total bacterial log counts with each product between pre and post disinfection sampling. The plot shows the margins by product with wide overlapping 95% CIs.G – Glutaraldehyde, QAC – Quaternary Ammonium Compound, POMS – Peroxymonosulfate

from 23% at time 0 to 6% at 1 h and also at 24 h. No discernible effect of extending the time from 1 to 24 h was noticed, and no significant difference between products was observed.

No effect of sample type was observed when assessing reductions in *Salmonella* (Table S6) or total bacterial counts (Table S7) however floor swabs were more likely to be positive for Enterobacteriaceae before treatment, compared to the other sample types collected. This would be expected as the floor has the most direct contact with faecal material. The variation in sample types found positive over time does highlight the difficulty in effectively disinfecting equipment such as feeders and drinkers.

4. Discussion

This study has demonstrated differences in efficacy between disinfectant products when tested in a range of laboratory conditions. Boot dips are used frequently, with potentially contaminated organic matter being added with every use and the faecal suspension model was designed to assess the activity of the product in the boot-dip rather than the activity of the disinfectant on the boot. All products performed well in the faecal suspension model when used at the recommended GO concentration. However when a lower concentration was used the iodine and peracetic acid-based products were less effective at eliminating *Salmonella*. These findings are comparable to previous findings (McLaren et al., 2011) where laying hen faeces were used as the faecal matrix, however when turkey faeces were used only one chlorocresol product (product K in this study) was effective at eliminating *Salmonella* in the faecal suspension model. The results supported those reported from duck faeces (Gosling et al., 2016), with almost all products being effective at GO concentration. Payne et al. (2005) previously reported potassium peroxymonosulfate-based products to be effective at reducing *Salmonella* counts in *Salmonella*-contaminated soil samples. Iodine-based products were the least effective at 0.5 GO, demonstrating the importance of using the correct concentration.

Data from the stability study showed that the chlorocresol-based products were able to eliminate *Salmonella* for up to 14 days after dilution of concentrate, even when faeces were present, thus maintaining efficacy over time, and at different temperatures. Interestingly, temperature had no effect on efficacy in this study. The effect of temperature on the efficacy of a product is usually thought to be an important consideration for use on farms, especially for disinfectants which remain exposed to the elements, such as boot dips and wheel washes.

In the surface contamination model, the glutaraldehyde/formaldehyde-based product was the most effective at eliminating *Salmonella*. Using a glutaraldehyde/QAC-based product at twice the recommended concentration was also effective; however this may not be a cost-effective or safe option. The superior efficacy of this product group was also confirmed by the field study that was carried out on a commercial pig farm.

All products except the chlorocresols performed as previously reported by McLaren et al. (2011). In the present study chlorocresol-based products were less effective against *Salmonella* in the presence of pig faeces than when they had been tested previously using laying hen faeces and duck faeces (Gosling et al., 2016; McLaren et al., 2011). It should be noted that one of the chlorocresol products was tested using the Poultry Orders concentration in the present study as the product was not approved at General Orders at the time of testing, however this did not impact on the efficacy of the product and the results within the chlorocresol group were all comparable. Formaldehyde-based products have been reported to be more effective for disinfecting a range of surfaces found in poultry houses than glutaraldehyde/benzalkonium chloride-based products, which in turn performed better than a peroxygen compound (Gradel et al., 2004). Mueller-Doblies et al. (2010) also reported that products containing a mixture of formaldehyde, glutaraldehyde/QAC performed significantly better in reducing *Salmonella* in turkey houses than products containing hydrogen peroxide and peracetic acid. The glutaraldehyde/formaldehyde product in the present study was also effective at eliminating *Salmonella* from biofilms that were up to 25 days old. This was similar to activity demonstrated by one of the chlorocresol-based products, with presence of faecal material not appearing to be inhibitory. The majority of other products tested began to fail when biofilms were more than 4 days old. The removal of biofilms is another important factor in the control of disease as *Salmonella* is able to form biofilms on a range of surfaces (De Oliveira et al., 2014). A key area for biofilm formation on pig farms is in the water lines and feed troughs as well as areas which remain moist for extended periods of time.

The filter disc method was included as it was considered to potentially provide a more stable testing method over time, with the disc acting as the organic matter matrix rather than the addition of faecal material. The bacterial load on the discs was high, at 2×10^7 CFU/disc, however almost all products were able to eliminate the *Salmonella*, demonstrating the increased challenge presented by the addition of faecal material in the other test models. This supports previous studies (Gosling et al., 2016; Stringfellow et al., 2009). Further development is needed if a more stable test is to be developed without the use of a faecal matrix, this could involve a shorter contact time, or inclusion of additional interfering substances to increase the level of challenge. Alternative options for an interfering substance are available (Araujo et al., 2013) and although usually targeted at a food hygiene environment these could be explored further for assessment of farm disinfectants in association with the filter paper matrix.

The presence of organic matter is another key factor to consider when applying laboratory results to on-farm disinfection and Wales et al. (2006) observed that it was more important that the cleaning and disinfection process was carried out to a high standard rather than relying on applying the 'best' disinfectant at the end of a poor process. This was demonstrated by the surface contamination model where the use of a disinfectant alone in the presence of a similar thin layer of organic matter to what is commonly found in areas of animal housing that have been less well cleaned, was rarely effective.

As well as being affected by the presence of organic matter and, potentially, temperature, the activity of disinfectants can also be affected by pH, with the activity of phenols/cresols, hypochlorites and iodides reported to decrease as environmental pH increases, and activity of QACs and aldehydes reported to increase (Maillard, 2013). The pH of a surface can be altered by the use of a detergent prior to application of a disinfectant. During normal cleaning and disinfection, the recommended protocol for farmers is to allow drying time between the use of a detergent before a disinfectant is applied, however in practice there is not always sufficient time before the next batch of pigs are due to arrive to enable the process to be followed fully, therefore the testing conducted in this study investigated the impact on *Salmonella* elimination by using a disinfectant immediately after washing with water containing detergent. Interestingly, the impact on *Salmonella* elimination was not consistent between the two alkaline detergents when followed by an iodine-based or peracetic acid detergent, although it is possible the level of pH change varied between the two and detailed chemistry of the test products was not investigated. None of the detergents had a negative impact on the efficacy of the chlorocresol product, and all had an additive effect in combination with the glutaraldehyde/QAC-based product.

5. Conclusion

This study has demonstrated that there are differences between disinfectants in their ability to inhibit or eliminate *Salmonella* in laboratory tests designed to mimic on-farm situations. It has also highlighted differences in efficacy depending on the situation the product is being used in, e.g. boot dips or surface disinfection. The most stable product in the faecal suspension model was one of the

chlorocresol-based products. The iodine and peracetic acid-based products performed poorly in the faecal suspension model compared to the other products tested, indicating that they would not be suitable products to be used in a boot dip aimed at *Salmonella* control, unless the level of contamination of boots was expected to be low. The only product that was consistently effective at GO concentration in the surface contamination model was the glutaraldehyde/formaldehyde-based product. It is therefore vital to consider both intended use of the disinfectant and its concentration when designing a disinfection programme. If detergents are used in wash water used for cleaning animal housing, they should be compatible with the disinfectants that are applied subsequently, or washed away with plain water before disinfection. It is particularly important to use the correct concentration of disinfectant when bacterial pathogens are present, and to ensure a high standard of cleaning prior to disinfection.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vetmic.2017.04.004.

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