Efficacy of different disinfectants intended for a pig farm environment.

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Disinfection is a widely accepted element of disease control, although there are many types of product, with differing chemistries, which affects their activity against pathogens such as *Salmonella*.

This study investigated the ability of fifteen disinfectants to eliminate pig-associated *Salmonella*, specifically focusing on monophasic *Salmonella* Typhimurium (*S*. 4,5,12:i:-). The study included three m-cresol, one glutaraldehyde/ formaldehyde, four glutaraldehyde/quaternary ammonium compounds (QAC), two iodine, two peracetic acid and three potassium peroxomonosulphate-based commercial disinfectants.

Eight *Salmonella* serovars; *S.* Typhimurium DT193, two *S.* 4,5,12:i:- with different resistance profiles, *S.* 4,12:i:-, *S.* Derby, *S.* Bovismorbificans, *S.* Kedougou and *S.* Panama, isolated from pigs, were screened against all products using minimum inhibitory and minimum bactericidal concentration testing. There were no significant differences in MIC or MBC values between the serovars.

One *S.* 4,5,12:i:- DT193 strain , resistant to ampicillin, streptomycin and compound sulphonamides, was selected for further testing due to its relevance to recent cases of human s*almonellosis*. All fifteen products were diluted at the Department for Food and Rural Affairs (Defra) Approved Disinfectant General Orders (GO) concentration, half GO and twice GO, in World health Organisation (WHO) standard hard water.

The disinfectants were tested using a faecal suspension model and a surface contamination model to replicate boot dip and animal house cleaning disinfection. All products eliminated *Salmonella* in the faecal suspension model, the majority of the time at GO concentration. Only one glutaraldehyde/QAC-based product and one glutaraldehyde/formaldehyde-based product eliminated *Salmonella* in the surface contamination model at GO concentration.

The type of product chosen can impact on the efficacy of farm disinfection; therefore, clearer guidance is needed to ensure the appropriate product is being used in order to control disease.

Introduction

In Europe in 2013 the largest number of foodborne outbreaks in humans were caused by *Salmonella* with pig meat and pig products the third highest cause (EFSA and ECDC, 2015). *Salmonella* Enteritidis, *S*. Typhimurium and monophasic *S.* Typhimurium 4,[5],12:i:- were the top three serovars isolated, and *S.* Typhimurium and monophasic *S.* Typhimurium 4,[5],12:i:- were the most commonly recorded serovars from pigs in the United Kingdom (ANON, 2013). By the time the pig products reach the consumer they have undergone many processes since leaving the farm, however, if pigs have a nil or low *salmonella* prevalence when being reared, this reduces the possibility of the bacteria ending up in the end product.

One on-farm disease control measure is an effective cleaning and disinfection programme. Disinfectants are used in many different situations, to disinfect animal housing and equipment, to disinfect vehicles before entering the farm and to disinfect personal protective equipment worn by farm personnel.

The aim of this study was to investigate the ability of fifteen disinfectants to eliminate pig-associated *Salmonella* from laboratory replicated farm situations.

Material and Methods

A panel of 15 disinfectants were selected for inclusion in the study, following discussions with pig industry colleagues in Great Britain (GB) and analysis of products available on the open market tailored towards pig housing. The panel included three m-cresol, one glutaraldehyde/ formaldehyde, four glutaraldehyde/quaternary ammonium compounds (QAC), two iodine, two peracetic acid and three potassium peroxomonosulphate-based commercial disinfectants.

Minimum inhibitory concentration (MIC)/Minimum bactericidal concentration (MBC)

Eight *Salmonella* field strains were selected from the most commonly reported serovars in GB pigs between 2010-2013 (Table 1). Overnight cultures were diluted to give an inoculum density of 1 x 10^6. Neat (as bought) disinfectants were diluted 1:25 in WHO hard water. In a 96 well microtitre plate 75µl of Nutrient broth No.2 was added to each well. Disinfectant was added to the first well (column 1) (75µl) and double diluted to column 10. Each *Salmonella* test strain (7.5µl) was added to a separate row, except column 12 (negative control) and incubated for 18h $\pm$ 2h 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 positive growth. MIC value was taken as the last clear well before turbidity was observed.

MBC was determined by aliquotting 10µl from each of the MIC plate wells into 190µl Nutrient Broth No2. and incubating for 18h $\pm$ 2h at 37°C. Turbidity after incubation indicated positive growth; a clear well indicated bactericidal effects.

Table 1. *Salmonella* isolates selected for inclusion in MIC/MBC testing

	Serovar	Pig production type of Salmonella origin	AMR* profile
A	*S.* Typhimurium	Outdoor	Sensitive to all
B	*S.* 4,5,12:i:-	Outdoor	AM,S,SU
C	*S.* 4,5,12:i:-	Indoor	AM,S,SU,SXT,T
D	*S.* 4,12:i:-	Outdoor	No approved sensitivity
E	*S.* Derby	Indoor	No approved sensitivity
F	*S.* Bovismorbificans	Indoor	AM,APR,C,CN,N,S,SU,SXT,T
G	*S.* Kedougou	Indoor	SU,SXT,T
H	*S.* Panama	Outdoor	Sensitive to all

* Antimicrobial resistance, AM Ampicillin, APR Apramycin, C Chloramphenicol, CN Gentamycin, N Nalidixic acid, S Streptomycin, SU Compound sulphonamides, SXT Sulphamethoxazole/Trimethoprim, T Tetracycline

Preparation of disinfectants for faecal suspension and surface disinfectant ion models

Each disinfectant was accurately measured and diluted in WHO Hard Water to 0.5, 1 and 2 x Defra General Orders concentration, as correct at time of the study (July, 2014).

Faecal suspension model

Isolate B (monophasic *Salmonella* Typhimurium) was mixed in equal measures with *Salmonella*-free pig faeces to obtain a smooth slurry with 5x10^6 CFU/g of *Salmonella.* To 9ml of each disinfectant concentration,

held at 4°C, 1g of *Salmonella*-spiked faeces was added and agitated, in 3 replicates. After 30 minutes, 2 and 4 hours, each tube was agitated and a 100µl aliquot removed into 10ml nutrient broth no.2 + 5% horse serum for at least 5 minutes. One 1ml was then transferred to 10ml nutrient broth no.2 and incubated for 18 ± 2h at 37°C. All tubes were further agitated at 1 and 3 hours. Counts on spiked faeces at 30 minutes, 2 and 4 hours were carried out to confirm challenge present.

After 18 ± 2h incubation in broth, 100µl was plated onto Modified Semi-Solid Rappaport-Vassiliadis agar and incubated for 24h at 41.5°C. A 10µl loop of growth was then plated onto Rambach agar and incubated for 24h at 37°C. A positive or negative result for *Salmonella* was recorded.

Surface contamination model

Wooden dowels (40mm x 10mm) were immersed in *Salmonella*-challenged slurry, 1:1 mixture of *Salmonella*-free pig faeces and monophasic *Salmonella* Typhimurium, stirred to achieve a visually determined uniform coating of approximately 1g/dowel and with $5x10^8$ CFU/g of *Salmonella*. Dowels were then placed in autoclave tins to dry at room temperature for three days. In replicates of 3, dowels were exposed to each disinfectant concentration for 10 minutes at 15°C. After exposure dowels were placed in a petri dish overnight. Dowels were then neutralised in 20ml nutrient broth No.2 + 5% horse serum for 10 minutes before being vortexed for 10 seconds and 2 aliquots of 1ml taken and added to fresh nutrient broth No. 2 and incubated for 18 ± 2h at 37°C. *Salmonella* presence was determined or each sample by MSRV and Rambach method for *Salmonella* isolation as above.

Results

Minimum Inhibitory Concentration/Minimum Bactericidal Concentration

No significant difference in MIC/MBC results was found between the different strains of *Salmonella* (p = 0.971), therefore data presented in Figure 1 are mean averages of all strains tested. All products within the same chemical grouping produced similar results (Figure 1), with the glutaraldehyde and QAC combinations and peracetic acid-based products demonstrating bactericidal effects when present at less than 0.1%, whereas iodine-based products were required at more than 0.4%.

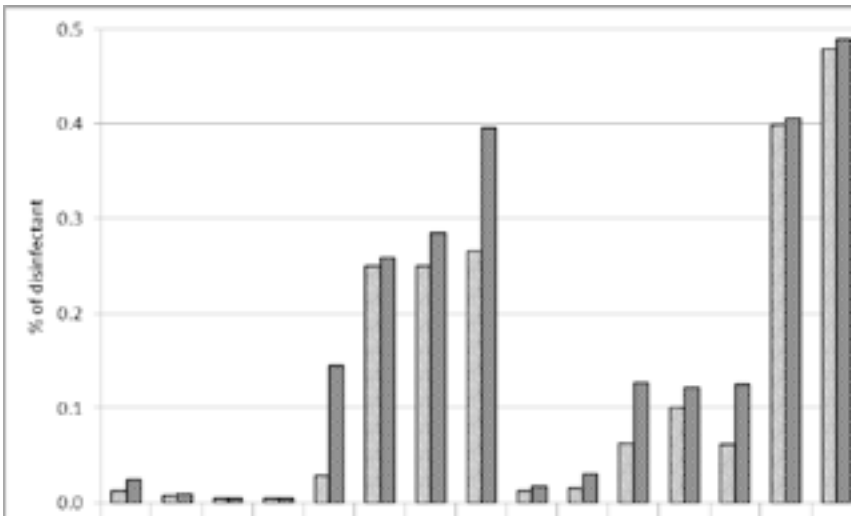

Figure 1. Percentage of disinfectant required for inhibition and bactericidal effects against *Salmeonlla*
G – Glutaraldehyde, QAC – Quaternary Ammoinium Compounds, F – Formaldehyde, POMS – Peroxymonosulfates, PA – Peracetic acid.

Faecal suspension model

. A linear trend was observed in the presence of concentration and *Salmonella* elimination, although not with contact time; significant differences were observed between chemical groups and concentrations ($p<0.001$; Table 2). This was mainly due to differences within the 0.5xGO concentration group ($p<0.0001$), whereas there were no significant differences between chemical groups in the 1xGO ($p=0.261$) and all products eliminated *Salmonella* at 2xGO concentration. Variation between individual products within each chemical group was observed for iodine and m-cresol, however further analysis indicated the products were statistically similar ($p<0.0001$ and $p=0.001$ for iodine and m-cresol respectively). The peroxymonosulfate group consistently eliminated *Salmonella*, even at 0.5xGO concentration.

Table 2. Effect of chemical group and concentration on the ability to eliminate *Salmonella* from faecally contaminated disinfectant (n=27 – 3 replicates of 3 contact times per disinfectant)

		Concentration and result*					
		0.5xGO		1xGO		2xGO	
	No. products	Elim.	Surv.	Elim.	Surv.	Elim.	Surv.
G & F	1	24	3	27	0	26	1
G & QAC	4	100	8	105	3	108	0
Iodine	2	37	17	51	3	54	0
PA	2	35	19	52	2	54	0
POMS	3	81	0	81	0	81	0
m-cresol	3	73	8	80	1	81	0

GO – General Orders concentration, G – Glutaraldehyde, QAC – Quaternary Ammoinium Compounds, F – Formaldehyde, POMS – Peroxymonosulfates, PA – Peracetic acid.
*Elim. = *Salmonella* elimination, Surv. = *Salmonella* survival

Surface contamination

Chemical groups of disinfectants were significantly different from each other ($p<0.001$) in the ability to eliminate *Salmonella* from a dried-on faecal contamination, and effect was concentration dependent ($p<0.001$; Table 3). The glutaraldehyde/formaldehyde-based chemical group was the only group to eliminate *Salmonella* consistently at 1xGO, although the glutaraldehyde and QAC-based products were able to eliminate Salmonella at 2xGO concentration.

Table 3. Effect of chemical group and concentration on the ability to eliminate *Salmonella* from faecally contaminated disinfectant (n=3 per disinfectant)

		Concentration and result*					
		0.5xGO		1xGO		2xGO	
	No. products	Elim.	Surv.	Elim.	Surv.	Elim.	Surv.
G & F	1	0	3	3	0	3	0
G & QAC	4	0	12	7	5[a]	12	0
Iodine	2	0	6	0	6	1	5
PA	2	0	6	0	6	0	6
POMS	3	0	9	0	9	0	9
m-cresol	3	0	9	0	9	3	6

GO – General Orders concentraion, G – Glutaraldehyde, QAC – Quaternary Ammoinium Compounds, F – Formaldehyde, POMS – Peroxymonosulfates, PA – Peracetic acid.
*Elim. = *Salmonella* elimination, Surv. = *Salmonella* survival
[a] Survival of Salmonella across the different products

Discussion

The m-cresols, peroxymonosulfates and glutaraldehyde and formaldehyde based products were all effective at eliminating *Salmonella* in the faecal suspension model, as were three out of the four glutaraldehyde and QAC products tested. These findings are comparable to previous data using laying hen faeces as the faecal matrix (McLaren et al., 2011). McLaren et al (2011) reported that when turkey faeces had been used only the m-cresol product was effective at eliminating *Salmonella* in the faecal suspension model. This highlights the variations in product ability and suitability when applied to a farm environment. In the surface contamination model many of the products, glutaraldehyde and formaldehyde, glutaraldehyde and QAC, peroxymonosulfate and iodine-based products also performed as previously reported (McLaren et al., 2011), however the m-cresols were not as effective in the present study at eliminating *Salmonella* from dried on surface contamination. Again this highlights the need for an appropriate product to be selected for the task, and reinforces the need to remove organic matter prior to disinfection.

Acknowledgements
This work was supported by Defra project OZ0344.

References

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