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Evaluation of commonly-used farm disinfectants in wet and dry models of *Salmonella* farm contamination

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Two experimental models of *Salmonella* contamination were used in an attempt to mimic the conditions of disinfectant use on farms. A wet model, for conditions such as boot dips, used disinfectant application to a slurry of poultry faeces inoculated with *Salmonella* Enteritidis or *Salmonella* Typhimurium. A dry model, for disinfectant application to surfaces and equipment with adherent or residual organic material, used *Salmonella*-inoculated poultry faeces that were air-dried onto wooden dowels, immersed in disinfectant solution then left in air at room temperature overnight. All samples were subjected to a disinfectant neutralization step and resuscitation in broth, followed by *Salmonella* culture on semi-solid then indicator media. Disinfectants were tested at 0.5x, 1x and 2x the concentrations specified for the general control of bacterial pathogens on livestock premises in the UK (Defra General Orders rates). Chlorocresol-based disinfectants provided consistently high rates of *Salmonella* killing in both wet and dry tests. Formaldehyde-containing disinfectants showed very high efficacy in the dry test but were less effective in the shorter wet test, whereas the efficacy of glutaraldehyde without formaldehyde was variable between products. Other chemical classes tested (quaternary ammonium compounds, amphoteric surfactants, iodine preparations, peroxygens and a substituted phenol blend) were only moderately effective. They often required concentrations above General Orders rates to eliminate the test salmonellas, and frequently elimination was not achieved even under maximal conditions of concentration and exposure.

Introduction

Disinfection on agricultural premises typically is used in one of two modes: frequent washing down or dipping of equipment such as boots, or intermittent disinfection of accommodation or large items of equipment such as loaders or scrapers. In both scenarios, disinfectant solutions may be applied with or without prior cleaning, and they frequently have to contend with significant amounts of residual organic debris (typically dust, dried or wet faeces and aggregated feed spillage), which, in the case of boot dips, will accumulate in baths of disinfectant solution.

The control of environmentally-robust zoonotic enteric pathogens such as *Salmonella* is an important issue in poultry and pig units, with increasing legal controls on the testing and presence of *Salmonella* in laying hens, broilers and turkeys being imposed in the European Union (EU). Current proposals for a new Animal Health Strategy for the EU (Anonymous, 2007) and new industry guides for good hygiene practices in broiler and layer production (Anonymous, 2008; Anonymous, 2010) include special mention of on-farm biosecurity including cleansing and disinfection (C&D). The effective use of reliable disinfectants for housing, drinkers and feeders is of fundamental importance to these control measures, particularly in all-in-all-out systems.

The chemical characteristics and modes of action, where known, of the disinfectants that are commonly used on livestock units have been reviewed (Denyer & Stewart, 1998; McDonnell & Russell, 1999; Lambert, 2004). Glutaraldehyde and formaldehyde are known to alkylate and create cross-links within protein molecules and to bind to cell wall peptidoglycans. Formaldehyde also forms DNA–protein cross-links. Glutaraldehyde is more stable at acid pH but more microbicidal at alkaline pH (Gorman *et al.*, 1980). It acts quickly, principally via damage to the cell envelope, whilst formaldehyde acts more slowly. Aldehydes, especially formaldehyde, are not readily inhibited by organic material (Gorman *et al.*, 1980).

Halogen-releasing agents expose pathogens to active forms of chlorine or iodine. Chlorine, when presented as hypochlorous acid or hypochlorite anion, causes oxidative damage to bacterial membranes and to DNA. Chlorine dioxide is more active as an inhibitor of protein synthesis. Iodine, typically stabilized with a carrier, kills cells rapidly by reactions with proteins, nucleotides and fatty acids. Halogen compounds are relatively easily inhibited by organic debris (Bessems, 1998). Peroxygens are another group of oxidizing agents, generally using peracetic acid to disrupt lipid membranes, proteins and nucleic acids via attack by reactive species such as the hydroxyl radical $^{\cdot}OH$. Peracetic acid is active in the presence of organic debris (McDonnell & Russell, 1999), although such material reduces the effect of all the

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oxidizing disinfectants owing to consumption of the active chemical species by reaction with organic matrices (Chapman, 2003; Russell, 2004).

Quaternary ammonium compounds (QAC) are cationic surfactants, active on bacterial membranes, although there is some evidence of effects in bacterial cytoplasm (Chapman, 2003), especially at high concentrations (Lambert, 2004). Other microbicidal surfactants presumably have similar targets. Phenols and cresol compounds at low concentrations cause loss of bacterial membrane integrity and, in common with many other disinfectant groups, have coagulative effects on cytoplasm, probably by protein denaturation, at higher concentrations (Russell, 2004).

It is recognized that simple minimum inhibitory concentration data for disinfectant plus pathogen combinations are not useful guides to the agent's effectiveness in the presence of other organic matter and/or when the same pathogens are associated with surfaces of materials, often in a dry environment (Hoff & Akin, 1986; Gradel *et al.*, 2004; Ward *et al.*, 2005; Møretrø *et al.*, 2009). Organic material affects the action of most disinfectants to a greater or lesser extent (Hoff & Akin, 1986; Thomson *et al.*, 2007), and the matrix and surface environment associated with bacteria may be more significant in terms of disinfectant efficacy than temperature, exposure time or initial numbers of viable bacteria (Gradel *et al.*, 2004). Disinfectants that are intended for veterinary applications may be assessed for efficacy by testing using standardized methods of either the suspension or surface type supported by national and international bodies such as the British Standards Institute and the European Committee for Standardization. For the sake of standardization, these methods (e.g. British Standard 6734:2004) do not use the type of matrices commonly found in the farm environment, such as faeces containing a wide range of microbes alongside the target organisms. Typically they employ yeast either as whole killed cells or an extract and/or serum albumin to simulate dirty conditions.

The limitations of C&D in *Salmonella*-contaminated layer and pig units have been documented (Wales *et al.*, 2006, 2009; Mannion *et al.*, 2007), and in the authors' experience some disinfectants with proven performance in the standard tests do not appear to be as efficacious in field situations. There are many possible reasons for the failure of a disinfectant to perform as desired in a farm environment, and these include: unsuitable dilutions or coverage rates being used, inadequate prior cleaning (Ward *et al.*, 2005, 2006), and difficulties with access particularly in accommodation with complex equipment such as layer hen houses. C&D, or wet cleaning alone, may actually increase the counts of some bacteria, including *Salmonella*, on some surfaces in pig and poultry houses (Davies & Wray, 1995; Mannion *et al.*, 2007).

Different disinfectants will be affected to differing extents by characteristics of the diluting water, the organic debris, the physiological state (including nutrient and moisture stress) of the pathogens and the nature of the surfaces involved (Brown *et al.*, 1991; Davison *et al.*, 1996; Bessems, 1998; Ward *et al.*, 2005). In general, all disinfectants are considered to suffer to some extent from local depletion by reaction with organic debris or uptake by non-target micro-organisms (Hunger, 1990; Russell, 2004). The formation of biofilms with extracellular matrices in areas such as drinker systems is also associated with enhanced bacterial resistance to microbicides (McDonnell & Russell, 1999). Bactericidal performance also needs to take account of the target bacteria, as there are substantial inter-species and strain variations in susceptibility with, for example, salmonellas appearing to be generally more resistant than most other non spore-forming pig pathogens (Davison *et al.*, 1996; Thomson *et al.*, 2007).

In an attempt to test commonly-used examples of disinfectant groups in conditions that would better predict performance in the field against an important and robust pathogen (*Salmonella*) than standardized laboratory tests, we devised two model systems. One was a wet suspension test, the other a dry surface test. Both used faeces and hard water to represent field conditions.

Materials and Methods

Bacterial strains and inocula. Three strains of *Salmonella* were used in the studies: a *Salmonella* Typhimurium (ST) DT104 from a turkey farm (S8978/08) and two strains of *Salmonella* Enteritidis (SE) PT4 from different laying farms (S9574/07 and S711/08). These were chosen from the Veterinary Laboratories Agency archive as representative field strains of the two serovars of major public health significance that are commonly encountered on livestock units, particularly in the poultry sector. The strains, stored on Dorset's egg medium, were subcultured overnight at 37°C on nutrient agar, and then single colony loops were placed, one per tube, in 10 ml nutrient broth No. 2 and incubated aerobically overnight at 37°C. The broth cultures were then allowed to stand at room temperature for 24 h to produce a stationary, quiescent phase of growth before use in the model systems. This aimed to produce a first approximation to the wet, nutrient-limited and cool environment in which recently-excreted *Salmonella* would be found on farm.

Disinfectants. The disinfectant agents used, and their Department for Environment, Food and Rural Affairs (Defra) General Orders (GO) approved concentrations, are detailed in Table 1.

Table 1. *Disinfectant agents used in the present study.*

Name	Microbicidal component(s)	GO dilution[a]
Formalin	Formaldehyde	
GPC 8	Glutaraldehyde	35
Superkill	Glutaraldehyde/formaldehyde	22
Tadcid	Glutaraldehyde/formaldehyde	40
Virocid	Glutaraldehyde + quaternary ammonium compound	200[b]
Ambicide	Quaternary ammonium + tertiary alkylamine	30
Virkon S	Peroxygen	100[c]
Sorgene 5	Peroxygen	200
Hyperox	Peracetic acid	179
Zal Perax	Peracetic acid	256
Tego 2001	Amphoteric surfactant	16
Virudine	Iodine	100
Interkokask	Chlorocresol	50
Farm Fluid HD	Chlorocresol	50
Macroline 500	Substituted phenol blend	103[d]

[a]General Orders (GO) dilution. Figures given are millilitres of water per millilitre of product.
[b]Not approved for GO at time of study, therefore manufacturer's general application rate for wheels and boot dips used.
[c]Millilitres of water per gram of product.
[d]Withdrawn from the Defra Approved list in 2009.

Model systems. Two model systems were used, to simulate, respectively, foot dips (wet) and surface disinfection (dry). Both models used pooled fresh faeces from known *Salmonella*-free layer or turkey farms. An aliquot of each pool was subjected to pre-enrichment in buffered peptone water followed by selective culture, to demonstrate freedom from *Salmonella* contamination. A flow diagram of both models is presented in Figure 1.

Wet model. An aliquot (1 g) of a *Salmonella*-inoculated faecal slurry was added to 9 ml disinfectant under test, diluted in World Health Organisation (WHO) Standard Hard Water to a specified concentration. The disinfectant–slurry combination was mixed, allowed to stand at cool room temperature (approximately 15°C) and shaken briefly after 0.5, 1, 2, 3 and 4 h. At three time points, aliquots were mixed with 10 ml disinfectant neutralizer. Tests were performed at three different concentrations for each disinfectant. To show that the numbers of challenge organisms remained stable within the slurry during the test, 10-fold serial dilutions of the unused slurry were made in nutrient broth 0.5, 2 and 4 h after the disinfectant mixtures had been inoculated.

Dry model. Wooden dowels (40 mm long × 10 mm diameter) were thoroughly coated with *Salmonella*-inoculated faeces slurry and then placed on greaseproof paper and allowed to air dry for 3 days at room temperature within a standard metal autoclave container with the lid slightly open. For each test, a beaker containing disinfectant product at a specified concentration was freshly prepared with WHO Standard Hard Water, and three coated dowels were then placed in the beaker. After 10 min the dowels were removed from the solution and stored overnight (20 h) at a cool room temperature of approximately 15°C. Each dowel was then vortex-mixed for 10 sec in neutralizer broth. One of the three resulting mixtures was then serially diluted in nutrient broth for semi-quantitative enumeration. Each product was tested at three concentrations and on three separate occasions (runs 1 to 3). To assess the *Salmonella* challenge level, 10 coated dowels were placed in WHO hard water for 10 min and then transferred to Petri dishes and left at room temperature overnight. In the morning, the dowels were immersed for 10 min and then vortexed in 20 ml WHO hard water for 10 sec and serial 10-fold dilutions were made in nutrient broth for semi-quantitative enumeration. "Shedding" controls comprised coated dowels that were immersed either in WHO hard water or in neutralizer broth for 10 min, and then subjected immediately to vortex mixing and enumeration as described above. Five dowels were used for each medium, providing an indication of recoverable organisms released from the dowels.

Bacterial resuscitation and culture. (See Figure 1.) In the wet model, 1 ml of the mixture of slurry and disinfectant in neutralizer was transferred to a resuscitation tube of nutrient broth. In the dry model, 1 ml aliquots of vortexed neutralized test mixture were transferred to resuscitation tubes: from the enumeration dilution series, one aliquot into each of seven tubes; and from each of the two remaining suspensions not subjected to serial dilution, two aliquots into two tubes.

The inoculated resuscitation tubes were incubated overnight, then plated onto modified semi-solid Rappaport-Vassiliadis agar with 0.01% novobiocin (MSRV; Difco 218681). This was incubated at 41.5°C overnight, and a 1 µl loop from the edge of the opaque growth zone was inoculated onto chromogenic Rambach agar. The absence of detectable *Salmonella* at this stage was taken as indicating an effective combination of product and concentration.

Results

For the wet model tests, SE and ST were detected at dilutions of 10^{-8} in prepared slurries at 0.5 h, 2 h and 4 h, indicating a concentration of approximately 10^9 colony-forming units of *Salmonella* per gram of faeces. Given that the resuscitation and culture procedure involved a 10^3-fold dilution in two steps, negative culture findings indicated a reduction in viable *Salmonella* of six or more log cycles.

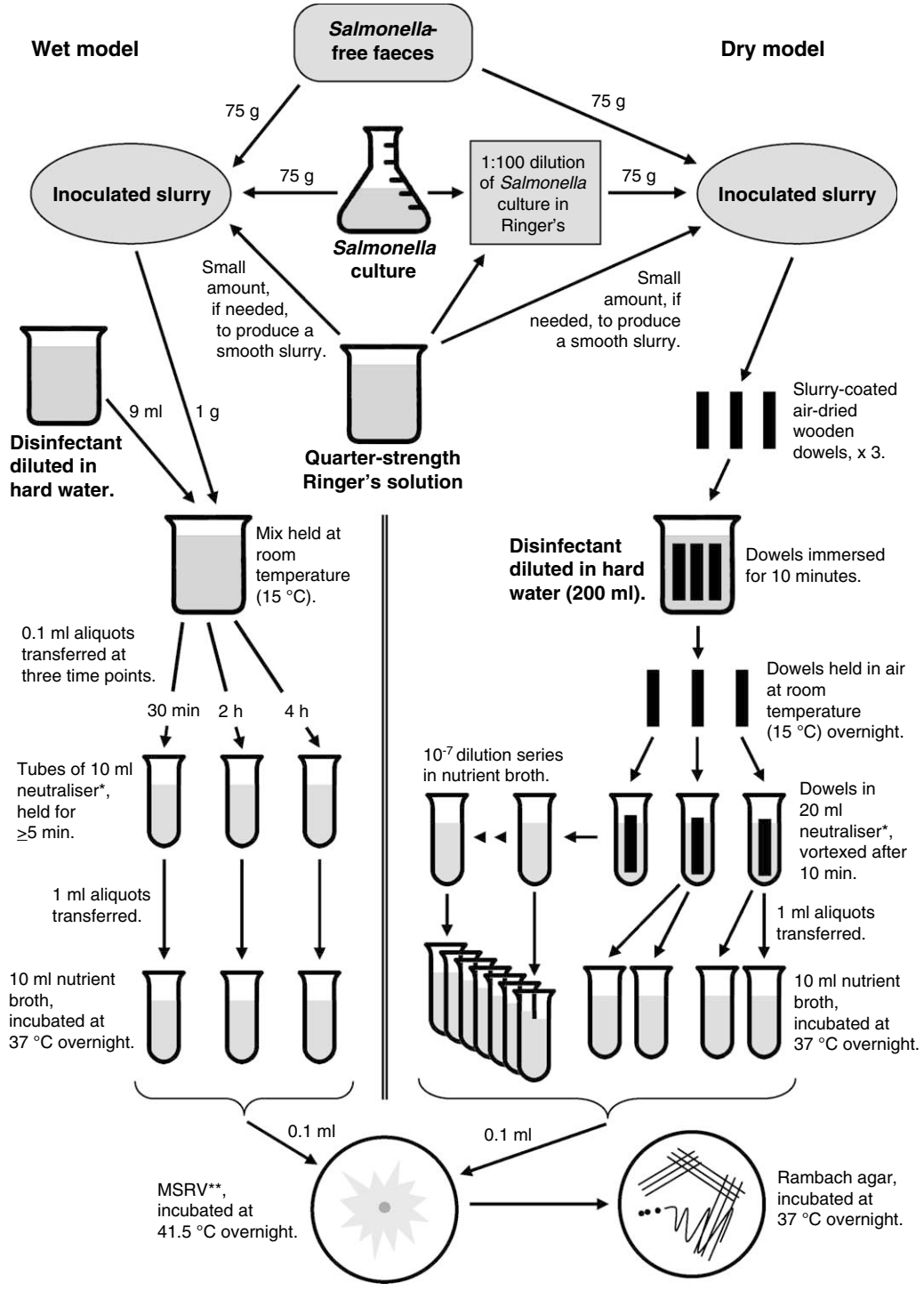
For SE in wet layer faeces (Table 2), Interkokask and Farm Fluid HD were the most effective disinfectants with greater than six log cycle reductions in *Salmonella* at all concentrations and exposure times, followed by GPC8. Poorer performance, with surviving *Salmonella* following shorter exposures at 1x GO concentration, was observed with Superkill, Macroline 500, Virkon S and Tego 2001. TadCid, Virocid and Ambicide had still poorer outcomes, with *Salmonella* growth at 2x GO concentration, whilst Hyperox, Sorgene and Virudine failed to achieve a six log cycle reduction under any experimental conditions of time or concentration.

In wet layer faeces containing ST (Table 2), Farm Fluid HD performed very well, similar to the result against SE; and Ambicide performed nearly as well, and was noticeably better than its performance against SE. Macroline 500 and Superkill showed slightly better and slightly worse performances, respectively, against ST than against SE.

Amongst the disinfectants tested in wet turkey faeces against ST (Table 3), six (Virocid, Superkill, TadCid, Tego 2001, Sorgene 5 and Virudine) were moderately effective. In comparison with their performance against SE in layer faeces (Table 2), the first four were similarly effective in both settings whereas the latter two showed more activity against ST than against SE at 2x GO concentrations. One disinfectant (GPC8) had noticeably less effect against ST at the shortest contact time for all concentrations, compared with its activity against SE in layer faeces. Two others (Interkokask and Virkon S) appeared to be a little less effective against ST than against SE in layer faeces, but Interkokask was nonetheless the most effective disinfectant of those tested; indeed it was the only one that was effective at 1x GO concentration for all exposure times.

For the dry model, the challenge controls (10 min in hard water, overnight in air then vortexing) and shedding controls (10 min in hard water or neutralizer then vortexing) both yielded similar ranges of concentrations of viable *Salmonella* in the resulting suspensions, for each strain used (Table 4). This indicates that immersion in water or neutralizer followed by overnight air drying did not in itself appreciably reduce *Salmonella* counts, and these counts were in an appropriate range to test the various disinfectant efficacies encountered. The ST strain (S8978/08) and SE strain S711/08 yielded higher counts overall in the controls than did the other SE strain.

Results of the dry model disinfectant tests are summarized in Table 5 (SE and ST in layer faeces) and Table 6 (ST in turkey faeces). Any attempt to rank the disinfectant performances in the dry model must be more tentative than with the wet model, owing to the variation in *Salmonella* counts revealed by the challenge and shedding controls in the dry model. However, this source of variability is ameliorated by the studies having been performed on three separate occasions, and in the dry model some disinfectants were clearly consistently effective whilst certain others were consistently poorly effective. Four of the five aldehyde-based disinfectants (with the exception of Virocid, a glutaraldehyde + QAC blend) were consistently effective in all faeces plus challenge strain combinations, whereas all of the peroxygen, QAC, iodine and surfactant-based examples were less effective. The tested chlorocresol disinfectant (Interkokask) performed well in all three



* Nutrient broth plus 5 % horse serum. **Modified semisolid Rappaport-Vassiliadis agar + 0.01 % novobiocin

Figure 1. *Schemes for the wet and dry disinfectant testing models.*

challenge strain plus faeces combinations, whereas the substituted phenol mix (Macroline 500) was tested against SE and ST in layer faeces and had limited efficacy.

Discussion

The present studies were undertaken to address the issue of differences between standardized tests of disinfectants and their observed effectiveness against *Salmonella* in the field. Two model systems were used. One tested situations such as disinfectant boot dips, and the other was designed to be representative of surfaces with a thin layer of organic (principally faecal) soil to which disinfectant is applied. WHO Standard Hard Water was used for diluting all disinfectants, to account for the known effect of hard water (principally calcium ions) in inhibiting some disinfectants (Hunger, 1990). The 14 products tested were selected on the basis of replies to EU survey questionnaires and personal observations of products seen to be used commonly in the field.

It is accepted that standardization of faecal material is not practical; however, it was considered that as it is an important element of organic soil in livestock operations, the use of faecal material could help to provide a realistic assessment of *Salmonella* control in the field, particularly

Table 2. *Findings from the wet model using laying hen faeces inoculated with* Salmonella.

		Salmonella cultured (+) or not cultured (–) after exposure for given time								
		2x GO[a] concentration			1x GO[a] concentration			0.5x GO[a] concentration		
Disinfectant	Serovar[b]	30 min	2 h	4 h	30 min	2 h	4 h	30 min	2 h	4 h
GPC8	SE1	–	–	–	–	–	–	+	–	–
Virocid[c]	SE1	+	–	–	+	+	–	+	+	+
Superkill	SE1	–	–	–	+	–	–	+	–	–
	SE2	–	–	–	–	–	–	+	–	–
	ST	+	–	–	+	+	–	+	+	–
Tadcid	SE1	+	–	–	+	–	–	+	+	–
Interkokask	SE1	–	–	–	–	–	–	–	–	–
	SE2	–	–	–	–	–	–	–	–	–
Hyperox	SE1	+	+	+	+	+	+	+	+	+
Virkon S	SE1	–	–	–	+	–	–	+	+	+
Sorgene 5	SE1	+	+	+	+	+	+	+	+	+
Tego 2001	SE1	–	–	–	+	–	–	+	–	–
Virudine	SE1	+	+	+	+	+	+	+	+	+
Farm Fluid HD	SE2	–	–	–	–	–	–	–	–	–
	ST	–	–	–	–	–	–	–	–	–
Macroline 500	SE2	–	–	–	+	+	–	+	+	–
	ST	–	–	–	–	–	–	–	+	–
Ambicide	SE2	+	–	–	+	+	–	+	+	–
	ST	–	–	–	–	–	–	+	–	–
Formalin[d]	SE2	+	–	–	+	–	–	+	–	–
	ST	+	+	–	+	+	–	+	–	–

[a]Defra General Orders (GO) concentrations, as given in Table 1.
[b]SE1, *Salmonella* Enteritidis 9754/07; SE2, *Salmonella* Enteritidis 711/08; ST, *Salmonella* Typhimurium S8978/08.
[c]Not approved for GO at time of study, manufacturer's general application rate for wheels and boot dips used.
[d]Formalin concentrations used were 4%, 2% and 1%.

in the poultry sector. There are also many variables, in the surface model in particular, that are difficult to control. However, by carrying out these tests in triplicate and on three separate occasions, a high level of confidence in the consistency of results could be attained.

For neutralization of disinfectants, a strategy of using marked dilution in a solution containing serum protein and other interfering organic substances was adopted. The wet model used a one in 100 dilution into the neutralizer followed shortly by a further 10-fold dilution into resuscitation broth. In the dry model, the disinfectant originally taken up by the dowel in approximately 0.5 g test solution (at 0.5x to 2x GO concentrations) was dispersed into 20 ml neutralizer, representing a 40-fold dilution over the original concentration. This was followed by a further 10-fold dilution into resuscitation broth, and additional decimal dilutions in the enumeration series.

Culture detection was by pre-enrichment of a 1 ml aliquot of neutralized mix in resuscitation (nutrient) broth, followed by enrichment on semi-solid medium and detection on Rambach indicator medium. The combination of pre-enrichment, semi-solid and indicator media has proved to be sensitive and reliable for detection of *Salmonella* in environmental samples (Carrique-Mas & Davies, 2008).

Table 3. *Findings from the wet model using turkey faeces inoculated with* Salmonella *Typhimurium.*

	Salmonella cultured (+) or not cultured (–) after exposure for given time								
	2x GO[a] concentration			1x GO[a] concentration			0.5x GO[a] concentration		
Disinfectant	30 min	2 h	4 h	30 min	2 h	4 h	30 min	2 h	4 h
GPC8	+	–	–	+	–	–	+	–	–
Virocid[b]	+	–	–	+	–	–	+	+	–
Superkill	–	–	–	+	–	–	+	–	–
Tadcid	+	–	–	+	–	–	+	–	–
Interkokask	–	–	–	–	–	–	+	–	–
Zal Perax	–	–	–	+	+	+	+	+	+
Virkon S	+	–	–	+	–	+	+	+	+
Sorgene 5	–	–	–	+	+	+	+	+	+
Tego 2001	–	–	–	+	–	–	+	+	+
Virudine	–	–	–	+	+	+	+	+	+

[a]Defra General Orders (GO) concentrations, as given in Table 1.
[b]Not approved for GO at time of study, manufacturer's general application rate for wheels and boot dips used.

Table 4. *Semi-quantitative enumeration of* Salmonella *shed from dowels coated in dried laying hen faeces.*

	Number of dowels yielding *Salmonella* at the given maximum log dilution																
	Challenge controls[a]							Shedding controls[b]									
								Neutralizer 10 min					Hard water 10 min				
	1	2	3	4	5	6	0	1	2	3	4	5	1	2	3	4	5
S. Enteritidis S9574/07																	
Run 1	3	7	–	–	–	–	1	–	3	1	–	–	–	3	1	1	–
Run 2	–	–	1	9	–	–	–	–	2	3	–	–	–	2	3	–	–
Run 3	–	–	2	8	–	–	–	–	–	3	1	–	–	1	4	–	–
S. Enteritidis S711/08																	
Run 1	–	–	2	4	4	–	–	–	–	–	2	3	–	–	1	3	1
Run 2	–	–	1	3	6	–	–	–	–	–	2	3	–	–	1	3	1
Run 3	–	–	–	9	1	–	–	–	–	2	1	2	–	–	–	4	1
S. Typhimurium S8978/08																	
Run 1	–	–	–	2	7	1	–	–	–	2	3	–	–	–	–	4	1
Run 2	–	–	–	–	4	6	–	–	–	1	2	2	–	–	–	4	1
Run 3	–	–	–	5	4	1	–	–	–	–	3	2	–	–	3	2	–

[a]*Salmonella* plus faeces-coated dowels immersed in hard water, held in air overnight then vortex mixed in hard water.
[b]*Salmonella* plus faeces-coated dowels immersed in water or neutralizer broth, then vortex mixed in neutralizer broth.

In the wet model there was an anomalous result (*Salmonella* isolated after a longer exposure time when a shorter exposure time had been *Salmonella*-negative) in only two of 93 exposure-time series at a given disinfectant concentration (Macroline 500, Table 2; and Virkon S, Table 3). This suggests that the wet model provided a consistent guide to disinfectant performance within the ranges of time and concentration used in the experiments. Therefore, the substantial variation that was seen in several cases between the efficacy of a disinfectant against SE in layer faeces and its efficacy against ST in layer or turkey faeces was likely to be a genuine effect. It is not possible with the present data to attempt to separate the contributions of serovar and faeces type to this variation in efficacy, as the choice of disinfectants for each test was guided by common usages and therefore most disinfectants were not tested against all faeces and serovar combinations. However, variations in susceptibility to disinfectants have been observed between *Salmonella* strains (Sander *et al.*, 2002) and even within the Typhimurium serogroup (Thomson *et al.*, 2007).

In the findings from the dry model, an anomalous result (*Salmonella* recovered from a higher disinfectant concentration after no isolation from a lower concentration of the same disinfectant) was seen in only two of 99 runs (both Virocid, Table 6). This is despite the variation in levels of challenge and shedding from the dowels revealed by the controls, and it suggests that this second model also provided a reasonably consistent measure of disinfectant efficacy. By contrast with the wet model, the qualitative performance of a disinfectant in the dry model appeared to be similar, regardless of the serovar and faeces combination it was tested against.

Semi-quantitative results were also obtained from the dry model. These showed, unsurprisingly, that disinfectants which were highly efficacious in the qualitative assessment were associated with undetectable counts (probably <1 colony-forming unit/ml) in the neutralizer broth; for example, Superkill in Table 5 and 6. Perhaps more interestingly, it is also evident that for many of the poorly-efficacious disinfectants, there was little or no evidence of a trend of reducing counts with increasing disinfectant concentration. Moreover, the counts obtained from many of these disinfectants (positive at dilutions of up to 10^6, Table 5 and 6) were similar to those obtained from the untreated control dowels (Table 4). This suggests that the inherent resistance of the *Salmonella* in these dried, wood-adsorbed preparations to some of the disinfectants was high. Furthermore, it appears that even 2x GO concentrations of such disinfectants were below a threshold where an increasing concentration would result in a useful increase in the bactericidal effect.

Any comparisons of disinfectant efficacy between the wet and dry models need to be made cautiously, because the exposure times and conditions were substantially different between the models. However, as the model conditions were designed to mimic some aspects of use in the field, comparisons between results in the two models may have value for predicting situations in which certain disinfectants will be highly (or poorly) effective. The aldehyde-containing products (especially Superkill and diluted formalin, but with the exception of Virocid) appeared to be more effective under the conditions of the dry model than the wet model at the dilutions tested. Conversely, Farm Fluid HD appeared more effective in the wet model, and Interkokask performed well in both models. In the dry model, only formaldehyde-containing preparations were fully effective at all concentrations in layer faeces. The formaldehyde-containing compound disinfectant Superkill was the only similarly effective preparation for ST in turkey faeces.

These differences in disinfectant performance between the models are probably a consequence of several effects. One important factor is likely to be the physiological state of the *Salmonella* in the dried versus the wet preparations, as a consequence of adaptive responses by the bacterial cells in conditions of low water activity (Russell, 2004) and reduced nutrient availability (Hoff & Akin, 1986). It has been observed that susceptibility of members of the Enterobacteriaceae to certain antiseptics and disinfectants, including QAC and substituted phenols, may increase or decrease depending on cell density, growth rate and the limiting nutrient (Cozens & Brown, 1983; Brown *et al.*, 1990; Bjergbæk *et al.*, 2008).

Table 5. *Findings from dry model using laying hen faeces inoculated with* Salmonella.

		Number (out of three) of *Salmonella*-positive dowels in three runs at given Defra General Orders (GO) disinfectant concentration			Maximum log dilution yielding *Salmonella* from neutralizer broth from one dowel after disinfectant exposure in each of three runs[b]		
Disinfectant	Serovar[a]	2x GO	1x GO	0.5x GO	2x GO	1x GO	0.5x GO
GPC8	SE1	0, 0, 0	0, 0, 0	1, 2, 3	–, –, –	–, –, –	–, –, 1
Virocid[c]	SE1	2, 0, 3	3, 0, 3	3, 3, 3	–, –, 2	1, –, 3	2, 2, 3
Superkill	SE1	0, 0, 0	0, 0, 0	0, 0, 0	–, –, –	–, –, –	–, –, –
	SE2	0, 0, 0	0, 0, 0	0, 0, 0	–, –, –	–, –, –	–, –, –
	ST	0, 0, 0	0, 0, 0	0, 0, 0	–, –, –	–, –, –	–, –, –
TadCid	SE1	0, 0, 0	0, 0, 0	0, 0, 0	–, –, –	–, –, –	–, –, –
Interkokask	SE1	0, 0, 0	0, 0, 0	0, 2, 1	–, –, –	–, –, –	–, –, 1
	SE2	0, 0, 0	0, 0, 0	3, 0, 0	–, –, –	–, –, –	1, –, –
	ST	0, 0, 0	0, 0, 0	0, 0, 0	–, –, –	–, –, –	–, –, –
Virkon S	SE1	3, 3, 2	3, 3, 3	3, 3, 3	1, 1, 3	2, 2, 4	2, 2, 4
Sorgene5	SE1	3, 3, 3	2, 3, 3	3, 3, 3	1, 2, 3	–, 2, 4	1, 2, 4
Tego2001	SE1	2, 3, 3	3, 3, 3	3, 3, 3	–, 2, 2	2, 2, 4	1, 3, 4
Virudine	SE1	2, 3, 3	3, 3, 3	3, 3, 3	–, 2, 4	3, 3, 4	3, 3, 4
Hyperox	SE1	3, 3, 3	3, 3, 3	3, 3, 3	2, 2, 3	2, 3, 4	2, 4, 4
Farm Fluid HD	SE2	0, 0, 0	3, 3, 0	3, 3, 2	–, –, –	2, 2, –	2, 2, –
	ST	0, 0, 0	0, 1, 1	3, 3, 3	–, –, –	–, 1, –	3, 1, 1
Macroline 500	SE2	2, 2, 0	3, 3, 3	3, 3, 3	–, –, –	3, 2, 3	3, 3, 2
	ST	1, 0, 0	3, 3, 2	3, 3, 3	–, –, –	2, 4, 1	4, 4, 2
Ambicide	SE2	1, 2, 1	3, 3, 1	3, 3, 3	4, 5, 3	4, 5, 3	6, 5, 3
	ST	1, 1, 1	3, 1, 1	3, 2, 3	5, 3, 3	2, 3, 2	5, 5, 4
Formalin[d]	SE2	0, 0, 0	0, 0, 0	0, 0, 0	–, –, –	–, –, –	–, –, –
	ST	0, 0, 0	0, 0, 0	0, 0, 0	–, –, –	–, –, –	–, –, –

[a]SE1, *Salmonella* Enteritidis 9754/07; SE2, *Salmonella* Enteritidis 711/08; ST, *Salmonella* Typhimurium S8978/08.
[b]One dowel from each set of three in a run was used for enumeration. –, no isolation of *Salmonella* at any dilution.
[c]Not approved for GO at time of study, manufacturer's general application rate for wheels and boot dips used.
[d]Formalin concentrations used were 4%, 2% and 1%.

These effects may be mediated by some or all of: alterations to the cell envelope (Brown *et al.*, 1990; McDonnell & Russell, 1999), the "SOS" response enhancing the repair and replication of damaged DNA (Farr & Kogoma, 1991; Erill *et al.*, 2007), and the related oxidative stress response including enzymatic degradation of oxidative radicals (Farr & Kogoma, 1991; Dukan *et al.*, 1996). It may be that *Salmonella* organisms can mount particularly robust oxidative protection as a consequence of its adaptation to survive as an intracellular parasite of macrophages (Hebrard *et al.*, 2009). There is also evidence that for some disinfectants at lower concentrations, bactericidal effects may involve autocidal processes within target bacteria (Denyer & Stewart, 1998). The significance and potency of such processes will probably be substantially dependent on the physiological state of the bacteria when exposed to the disinfectant.

In consequence of the above considerations, certain types of chemical disinfectant attack may be inhibited when applied to dried faeces containing metabolically stressed *Salmonella* cells, whilst the effects of other disinfectants may be unaffected, or possibly enhanced. Møretrø *et al.* (2009) similarly found substantial variation in disinfectant effectiveness against *Salmonella* in dried deposits on stainless steel surfaces, compared with more uniform results from suspension tests.

Salmonella may additionally be protected from contact with an optimal concentration of disinfectant to differing extents with different models and disinfectants. This may be by physical barriers to penetration, or by chemical neutralization provided by elements of the faeces or of the wooden substrate in the case of the dry model (Hunger, 1990; Russell, 2004).

The generally high effectiveness of the aldehyde-based and some chlorocresol-based disinfectants in the dry model is reflected in the author's experiences of C&D in poultry houses, with formaldehyde appearing to have the most reliable effects on *Salmonella* in the field (Davies & Wray, 1995; Wales *et al.*, 2006; Carrique-Mas *et al.*, 2009; Mueller-Doblies *et al.*, 2010; R. Davies, unpublished observations). The more limited performance of formaldehyde preparations in the wet model may reflect a slower action, unsuited to the exposure times in this model. Variability in the effectiveness of glutaraldehyde in the field and in the present models may partly be because its microbicidal activity is strongly pH dependent. Preparations often use synergistic combinations of glutaraldehyde with, for example, QAC that ameliorate the pH effect (Gorman *et al.*, 1980) but that will introduce more variation in efficacy in complex, soiled environments. Evidence on the effects of organic soil upon glutaraldehyde activity is inconsistent (Russell, 2004). It is interesting to note that the least consistently effective glutaraldehyde-based disinfectant in the present study (Virocid) has, subsequent to the work described herein, been given a GO dilution rate of 1:49, which is substantially more concentrated than the manufacturer's recommendation used in the study.

In both the present and previous studies, the activity of phenolic (including cresol) disinfectants against Gram-negative bacteria has been observed to vary substantially between formulations and to outperform many other disinfectants in models of wet, soiled

Table 6. *Findings from the dry model using turkey faeces inoculated with* Salmonella *Typhimurium.*

	Number (out of three) of *Salmonella*-positive dowels in three runs at given Defra General Orders (GO) disinfectant concentration			Maximum log dilution yielding *Salmonella* from neutralizer broth from one dowel after disinfectant exposure in each of three runs[a]		
Disinfectant	2x GO	1x GO	0.5x GO	2x GO	1x GO	0.5x GO
GPC8	0, 0, 0	0, 0, 0	1, 2, 2	–, –, –	–, 1, –	–, 1, –
Virocid[b]	0, 3, 1	2, 3, 0	0, 3, 2	–, 2, –	–, 3, –	–, 4, 2
Superkill	0, 0, 0	0, 0, 0	0, 0, 0	–, –, –	–, –, –	–, –, –
TadCid	0, 0, 0	0, 0, 0	0, 1, 0	–, –, –	–, –, –	–, 1, –
Interkokask	0, 0, 0	0, 0, 0	2, 2, 3	–, –, –	–, –, –	–, 2, 2
Virkon S	2, 2, 0	1, 2, 3	1, 3, 3	–, –, –	–, –, 1	–, 2, 3
Sorgene 5	3, 3, 3	3, 3, 3	3, 3, 3	6, 2, 2	6, 3, 3	6, 3, 5
Tego 2001	2, 2, 1	3, 2, 3	3, 3, 3	4, 1, 2	5, –, 1	5, 1, 4
Virudine	3, 2, 3	3, 3, 3	3, 3, 3	3, –, 3	6, 4, 4	6, 5, 4
Hyperox	–	3, 1, 3	3, 3, 3	–	5, 2, 4	5, 2, 5
Zal Perax	3, 3, 2	2, 3, 2	3, 3, 3	3, 4, 3	4, 5, 3	4, 4, 4

[a]One dowel from each set of three in a run was used for enumeration. –, no isolation of *Salmonella* at any dilution.
[b]Not approved for GO at time of study, manufacturer's general application rate for wheels and boot dips used.

environments (Lucchini *et al.*, 1990; Davies & Wray, 1995; Soliman *et al.*, 2009; Stringfellow *et al.*, 2009). Indeed, as a chemical group the phenolic disinfectants were most consistently effective in the wet model, albeit not uniformly so. The present evidence suggests that activity of this chemical group against dried deposits is less predictable, consistent with variations between formulations also reported elsewhere (Sander *et al.*, 2002).

The subject of bacterial resistance to biocides has been addressed and discussed in recent years, alongside concerns about potential links between antibiotic and biocide resistance. It is generally accepted that exposure to farm disinfectants at recommended concentrations has a gross lethal effect, affecting many cellular targets, and the development of resistant mutants under these conditions is unlikely (Karatzas *et al.*, 2007). However, the data presented here and elsewhere clearly show that in many field applications enough bacterial cells are protected from adequate biocide concentrations (by organic matter, substrates, biofilms, incorrect application, etc.) to apply a selective pressure, and to make even modest decreases in susceptibility potentially significant in terms of surviving population numbers.

Studies of archived farm isolates have shown that there is a range, sometimes wide, in the susceptibility of *Salmonella* isolates to various disinfectants (Chuanchuen *et al.*, 2008), and that *Salmonella* may show a higher intrinsic tolerance of some disinfectants (e.g. QAC and chlorhexidine) than *Escherichia coli* and Gram-positive organisms (Aarestrup & Hasman, 2004). Differential susceptibilities to disinfectants among *Salmonella* isolates appear to correlate with serovar but not with a strain's history of persistence in premises or with likely previous exposure to any particular disinfectant (Gradel *et al.*, 2005). No evidence for a separate population of disinfectant-resistant isolates was found in a survey of 569 Danish farm isolates, including 156 salmonellas (Aarestrup & Hasman, 2004). Evidence for variation in the tendency of *Salmonella* to develop tolerance for different farm biocides is scanty, and relates to studies performed in the laboratory on cells grown in planktonic suspension or on solid media, neither of which accurately mimic farm microenvironments. Single passages of ST on biocide-containing agar yielded a low frequency of isolates with modest increases in minimum inhibitory concentrations, with little difference between oxidizing, aldehyde, QAC plus surfactant, and tar oil phenolic disinfectants (Randall *et al.*, 2007). On the existing limited evidence, it would appear that the selection of farm disinfectants should be governed principally by anticipated application environments and that mutational bacterial resistance is likely to be a secondary concern compared with issues of cleaning and correct application. Currently, it would appear that resistance is more of an issue in medical and food handling environments.

In summary, two models were devised to test the efficacy of disinfectants against *Salmonella*. These produced results that were coherent and repeatable for each disinfectant, and which simulated certain field conditions relevant to poultry and pig units in particular. Disinfectants often performed quite differently in the two model systems, and efficacy was often low, even at Defra GO concentrations and above. Some disinfectants at these recommended concentrations showed almost no effect against *Salmonella* in dried faeces deposits, when compared with water alone. There were also substantial differences even within the same model between the effects of disinfectants in the same chemical class. This reflects field experiences, where the efficacy of a disinfectant cannot be easily predicted by reference either to a single standardized test or to another disinfectant of the same chemical family. Peroxygen, QAC and amphoteric surfactants are often preferred by disinfectant manufacturers owing to their favourable operator and environmental safety characteristics. However, the comparatively low efficacies of peroxygen, QAC and iodine-based disinfectants in the present studies suggest that these may be better suited to situations where frequent cleaning prevents the build-up of organic soil or biofilms.

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