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ORIGINAL ARTICLE

A comparison of the efficacy of different disinfection methods in eliminating *Salmonella* contamination from turkey houses

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Abstract

Aims: This study aimed to compare the efficacy of different disinfection methods in eliminating *Salmonella* contamination from turkey houses.
Methods and Results: Fifty depopulated turkey houses which had all housed *Salmonella*-positive flocks were visited after cleaning and disinfection. A minimum of 45 swab samples from different surfaces were taken per house and analysed for the presence of *Salmonella*. The sampled surfaces included intact floor surfaces, floor cracks, walls, feeders, drinkers, anteroom, nestboxes and miscellaneous items. Houses were grouped according to the disinfectant which had been used and the efficacy of the different groups of disinfectants was compared. Sixty-eight % of houses tested positive for *Salmonella* after C&D. Out of 4440 samples, 207 tested positive for *Salmonella*, giving an overall sample prevalence of 4·7%. There was no significant difference in the level of residual contamination between breeding, rearing and finishing houses. Products containing a mixture of formaldehyde, glutaraldehyde and quaternary ammonium compounds (QAC) performed significantly better than products containing hydrogen peroxide and peracetic acid. Cleaning and disinfection was least effective in nestboxes and anterooms.
Conclusions: Thorough cleaning and the choice of a suitable disinfectant are crucial if *Salmonella* contamination of turkey houses is to be eliminated.
Significance and impact of the study: This study shows that disinfectants containing a mixture of formaldehyde, glutaraldehyde and QAC perform significantly better under field conditions than oxidising products and should therefore be the first choice for disinfection of turkey premises where *Salmonella* is present.

Introduction

Salmonellosis was the second most commonly reported foodborne zoonosis in the EU in 2006, accounting for 160 649 reported cases (EFSA, 2008a) and is one of the most common causes of infectious gastroenteritis in humans worldwide (WHO, 2005). Although most cases of human salmonellosis are attributed to the consumption of eggs and chicken meat (Humphrey 1990; Poppe *et al.* 1991; de Jong and Ekdahl 2006), turkey meat must be considered a source of infection as well (Baggesen *et al.* 1996).

An EU-wide survey of turkey flocks (SANCO/2083/2006), carried out in 2006/07, found 29·3% of fattening turkey flocks across the EU to be infected with *Salmonella* spp., and the mean EU prevalence was estimated as 30·7%. In the UK, the flock prevalence of *Salmonella* spp. in fattening turkeys was estimated as 32·2%, with 4·6% being positive for either *Salmonella* Typhimurium or *Salmonella* Enteritidis and 28% being positive for serovars other than

S. Typhimurium or *S.* Enteritidis (EFSA, 2008b). However, the number of reported incidents of isolation of *S.* Typhimurium from turkeys in the UK has decreased remarkably over the past 10 years, and no *S.* Typhimurium-positive flock has been identified in the UK between January and September 2009 (VLA surveillance data, unpublished data).

Following the implementation of EU-wide legislation aimed at minimising the prevalence of *Salmonella* in chicken flocks, harmonised measures will be taken to reduce infection in turkeys across the EU (Anonymous, 2003, 2005, 2009a). The community target has been set at a maximum of 1% for both fattening turkey flocks and adult breeding flocks to be positive for either *S.* Enteritidis or *S.* Typhimurium by the end of 2012 (Anonymous, 2008), and in order to monitor this target, a National Control Programme (NCP) for turkeys will be introduced from January 2010 across the UK. This will implement mandatory testing of commercial turkey breeding and fattening flocks for the presence of *Salmonella.*

Interventions to help prevent infection with *Salmonella,* such as vaccination, are limited to certain serovars and may not be cost-effective in commercial flocks, therefore turkey producers normally focus on eliminating any *Salmonella* contamination from the houses by effective cleaning and disinfection (C&D) measures in order to prevent carry-over of infection from one flock to the next. At the same time, prevention of cross-contamination between different houses on a farm and prevention of re-introduction of *Salmonella* through poor biosecurity are important steps to achieve a *Salmonella*-free status (Davies and Wray 1996a; Davies and Breslin 2003; Wales *et al.* 2007; Carrique-Mas *et al.* 2009a). In laying hen holdings, carry-over between consecutive flocks has been shown to be a frequent event and is usually associated with poor C&D standards (Wales *et al.* 2006; Carrique-Mas *et al.* 2009b) and/or the presence of rodents (Carrique-Mas *et al.* 2009a). The presence of rodents does not appear to play an important role in maintaining the life-cycle of *Salmonella* in most commercial turkey flocks in the UK (author's unpublished data).

C&D is costly and laborious and its success depends on the attention to detail with which it is carried out as well as on the right choice and correct application of a suitable disinfectant (Davies and Wray 1996b). Many disinfectants are currently available, but not all are approved by the competent authority and the efficacy varies between different products and product groups. Some products (especially the oxidising products) are readily inactivated in the presence of organic matter and are therefore not suitable for earth floors or for houses where there is a substantial amount of organic matter or biofilm left after cleaning (Davies and Wray 1995; Gradel *et al.* 2004; Russell 2004; Thomson *et al.* 2007). Phenolics have been shown to be more effective in the presence of organic matter than iodophors and quaternary ammonium compounds (QAC) (Berchieri and Barrow 1996), but their use has been limited recently and many products were taken off the market in the UK. A recent study has shown that treatment with 10% formalin, followed by products containing a mixture of formaldehyde, glutaraldehyde and QAC achieved the best results in chicken layer houses (Carrique-Mas *et al.* 2009b), and such products are also becoming more widely used in the turkey industry.

In this study, we analysed data from post-C&D visits to 50 turkey houses which had all housed *Salmonella*-positive flocks before depopulation. The efficacy of the C&D procedure was assessed by collecting swab samples from different surfaces in each house and analysing them for the presence of *Salmonella*. According to the disinfectant which had been used, the houses were grouped into four groups: (A) phenol-based products; (B) products containing a mixture of formaldehyde, glutaraldehyde and QAC; (C) products containing glutaraldehyde, QAC and phosphoric acid; and (D) products containing hydrogen peroxide and peracetic acid. The overall performance of the different products was compared and the houses assigned either the status 'negative for *Salmonella* after C&D' or 'positive for *Salmonella* after C&D'.

Materials and Methods

Farms/turkey houses

Between February 2007 and May 2009, post-cleaning and disinfection (post-C&D) visits to 65 empty turkey houses on 26 farms were carried out. The houses had been cleared of litter and dust and pressure washed prior to disinfection, either by the farm workers or by a contractor. Details about the disinfection procedure were recorded, such as the name of the disinfectant and the concentration at which it was used. Fifteen houses were subsequently excluded because the disinfectant used was unknown, the concentration at which it was applied could not be verified, or the disinfectant was used at a lower concentration than the recommended General Orders concentration. The remaining 50 houses fell into the following production types: 13 breeding houses, 18 rearing houses and 19 finishing houses. Only houses where the disinfectant was used at the approved concentration for *Salmonella* and General Bacteria in animal housing ('General Orders concentration') or at a higher concentration were included in the analysis (Anonymous 2009). For products which were not Defra-approved at General Orders Rate, the 'General Purpose' concentration

as recommended by the manufacturer was considered a suitable concentration for the purpose of this study. The disinfectants were grouped into four categories A to D (see Table 1). A detailed list of the products used as well as a breakdown by production type is given in Table 2.

Sampling and processing of samples

A total of 4440 samples were taken from the 50 houses, giving an average of 89 samples per house, with a minimum of 45 samples taken per house. The sampled surfaces were grouped into the following categories: intact floor, floor cracks (if applicable), walls (including posts, windowsills, beams and ledges, doors and partitions), drinkers, feeders, miscellaneous (including fans, equipment and other variable items) and anteroom (if applicable). In breeding houses ($n = 13$), the nest boxes were also sampled.

Not all sampling categories were represented in all houses, for example not all houses had floor cracks and in some houses, the feeders and/or drinkers were not inside the house at the time of sampling and could not, therefore, be sampled. Floor and wall samples were taken from 50/50 houses, drinkers 43/50 houses, feeders 47/50 houses, floor cracks 14/50 houses, anteroom 33/50 houses, miscellaneous 43/50 houses, nestboxes 12/13 houses.

Hand-held gauze swabs were used to vigorously swab an area of 0·5–1 m^2 of the specific surfaces (depending on the type of surfaces) and then put into 225 ml of buffered peptone water (product code 1.077228.5000; Merck, Darmstadt, Germany). Samples were then transported to the laboratory within 6 h and incubated at 37°C for 18 h.

Selective enrichment of *Salmonella* was carried out using Modified Semi-solid Rappaport-Vassiliadis agar (MSRV) (Difco, 218681), followed by Rambach agar (product code 1.07500.0002; Merck) as described previously (Davies and Wray 1994).

Serotyping was performed according to the Kauffmann–White-Scheme (Grimont and Weill 2007) and phage typing of *S.* Typhimurium was performed according to the HPA (Health Protection Agency) Colindale Scheme (Anderson *et al.* 1977).

Statistical methods

Two statistical analyses were done, the first to compare the proportions of positive houses by disinfectant group and production type and the second to compare the sample prevalences, taking into account in addition the locations of the samples. Preliminary analyses used mixed-effects logistic regression models with farm and house as random nested effects, but the farm components were not significant ($P > 0·05$, likelihood ratio test). Therefore the results presented here are based on simpler logistic regression models with robust variance estimates taking into account the clustering of samples by house only. All comparisons were done by Wald tests with P-values $<0·05$ indicating statistical significance. These analyses used STATA software (Stata Corp. LP, College Station, TX, USA) and descriptive statistics were calculated using MINITAB 15 software (Minitab Ltd, Coventry, UK).

Follow-up investigation on farm A (breeding farm)

As an example of how the choice of a suitable disinfectant influences the outcome of the post-C&D visits, one particular farm was followed up which was always cleaned and disinfected by the same team, using different products at different times. This farm had a history of persistent *Salmonella* – contamination, most likely due to

Table 1 Disinfectants used, grouped into four categories A to D according to their main compounds

Group	A	B	C	D
Compounds	Phenols	Formaldehyde/ Glutaraldehyde/QAC	Aldehyde, QAC and other compounds	Hydrogen peroxide/ peracetic acid
Trade names	Antec Longlife 250S (DuPont (UK) Ltd., Stevenage, Hertfordshire)	Superkill (AFSAnimalcare, Thetford, Norfolk, UK)	SWC Broadol (The Proton Group, Normanton, UK) (Glutaraldehyde, QAC, Phosphoric acid)	Hyperox (DuPont (UK) Ltd, Stevenage, Hertfordshire)
	New Bio Phen Plus (Biolink Ltd., Market Weighton, Yorkshire, UK)	Viroguard (Kilco International Ltd., Lockerbie, UK)	Viragri Plus (Johnson Diversey UK Ltd, Weston Favell Centre, Northhants, UK) (Glutaraldehyde, QAC, Tetrasodium EDTA, Phosphoric acid)	Zal Perax 2 (Johnson Diversey UK Ltd, Weston Favell Centre, Northhants, UK) Sorgene 5 (Sorex Ltd, Widnes, Cheshire, UK)

QAC, quaternary ammonium compounds.

Table 2 Disinfectant groups, number of houses treated, production type (breeding, rearing, finishing house) and products used

Disinfectant group	No. of houses	No. of farms	Product used (no. of houses in brackets)
A	4 (2b, 2f)	3	New Biophen Plus (3), Longlife 250 S (1)
B	12 (2r, 6b, 4f)	8	Superkill (12)
C	6 (6r)	1	Viragri (6)
D	28 (5b, 10r, 13f)	10	Sorgene5 (3), Zal Perax2 (13), Hyperox (2), Hyperox and Formalin fogging (9), Hyperox and Formalin (applied by a specialist company) (1)

b, breeding house; f, finishing house; r, rearing house.

presence of rodents. Post-C&D samples were taken four times over a period of 2 years.

Results

Analysis of overall prevalence

Thirty-four out of 50 houses tested positive after C&D in at least one sample, giving an overall prevalence of 68% contaminated houses. Disinfectant group B performed best with 25% positive houses, and group D performed worst with 86% positive houses (see Fig. 1). Group A (75%) and group C (67%) performed intermediately, but as the number of houses in these groups was low (four houses for group A and six houses for group C), the results for groups A and C have to be treated with care, and more extensive studies might be necessary to evaluate the efficacy of these products. Statistical analysis showed that there was a significant overall effect of disinfectant group ($P = 0{\cdot}019$). Products belonging to disinfectant group B performed significantly better than those belonging to group D ($P = 0{\cdot}002$), but the other individual differences were not significant. There was no significant difference between production types ($P = 0{\cdot}716$). 78% of rearing houses tested positive after C&D, 68% of finishing houses and 54% of breeding houses.

The predicted mean proportions of houses positive and 95% confidence intervals are given in Table 3.

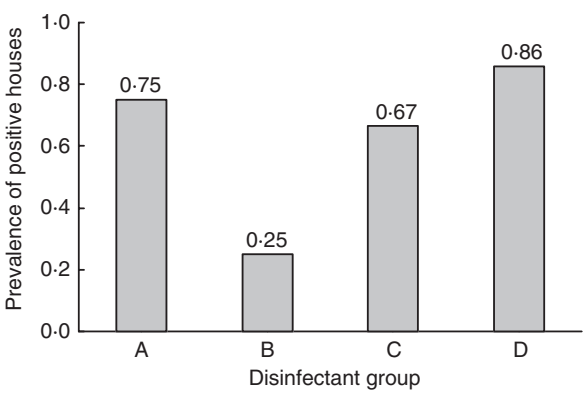

Figure 1 Prevalence of positive houses after C&D by disinfectant group. Group A: $n = 4$; group B: $n = 12$; group C: $n = 6$; group D: $n = 28$. For detailed description of the different disinfection groups see text.

Analysis of different sample categories

The percentage of houses remaining positive in one of the following sample categories is shown in Fig. 2. From highest to lowest, 58% of the sampled houses had at least one positive sample in the category 'nestboxes', followed by 'anteroom' (52%), 'walls' (38%), 'floor cracks' (29%), 'miscellaneous' (23%), 'floor' (22%), 'feeders' (19%) and 'drinkers' (14%).

Analysis of the sample prevalence

A total of 4440 samples were tested, and 207 out of those 4440 samples tested positive, giving an overall sample prevalence of 4·7%. The sample prevalence by disinfectant was 4·2% for group A, 1·1% for group B, 2·3% for group C and 6·2% for group D.

The sample prevalences for the different sample categories are shown in Fig. 3 and were, from highest to lowest, anteroom 12·8%, nestboxes 11·8%, floor cracks 5·5%, miscellaneous 4·9%, floor 4·5%, walls 3·2%, feeders 3·2% and drinkers 2·4%.

The logistic regression analysis of the sample prevalence showed significant effects of disinfectant group ($P = 0{\cdot}025$) and sample location ($P < 0{\cdot}001$) but not

Table 3 Predicted mean proportions of houses positive and 95% confidence intervals, analysed by production type and by disinfectant group

	Category	Proportion +ve (95% CI)
Production type	Breeder	0·55 (0·26–0·81)
	Finisher	0·71 (0·43–0·88)
	Rearer	0·82 (0·52–0·95)
Disinfectant group	A	0·75 (0·24–0·97)
	B	0·25 (0·08–0·55)
	C	0·67 (0·26–0·92)
	D	0·86 (0·67–0·95)

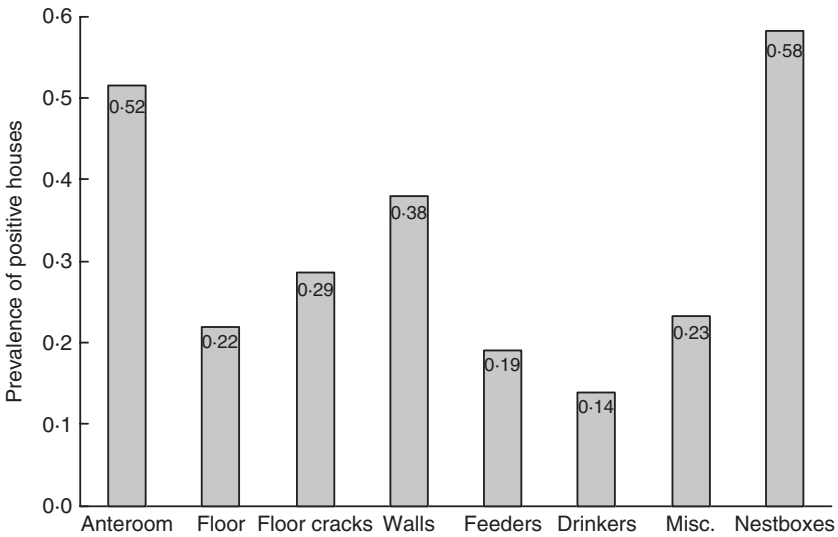

Figure 2 Prevalence of houses which had at least one positive sample in one of the following sample categories.

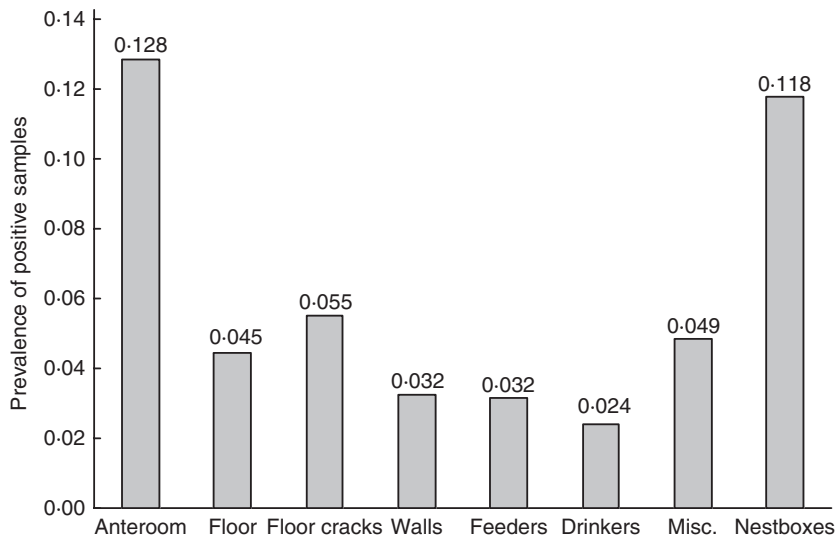

Figure 3 Sample prevalence for the different sample categories.

production type ($P = 0{\cdot}566$). The means and confidence intervals predicted from the regression model are shown in Table 4. The prevalences for the 'anteroom' and 'nestboxes' samples were significantly higher than all the other locations except 'floorcracks'. Among the disinfectant groups, samples from houses treated with group B had a significantly lower prevalence than those treated with D ($P = 0{\cdot}008$) or A ($P = 0{\cdot}038$).

To summarise, there was no significant difference between the results of cleaning and disinfection from different production types. Disinfectant group B was the most effective and significantly better than D. Based on the prevalence from the various sample locations, cleaning and disinfection was least effective for 'nestboxes' and 'anteroom'.

Follow-up investigation on farm A (breeding farm)

This farm was visited four times over a period of 2 years and the results are shown in Table 5. On this particular farm, the disinfectant from group B was able to clear the contamination in all three cases where it was used, whereas the disinfectant from group D performed very poorly.

Discussion

In this study, we describe the isolation of *Salmonella* from turkey houses after cleaning and disinfection, and as this was a field study, there are some uncontrolled variables one has to be aware of. The level of infection of the birds

Table 4 Means and confidence intervals predicted from the regression model

	Category	Prevalence (95% C.I.)
Sample location	Anteroom	0·093 (0·053–0·159)
	Drinkers	0·016 (0·008–0·034)
	Feeders	0·023 (0·010–0·049)
	Floor	0·031 (0·018–0·054)
	Floorcracks	0·049 (0·014–0·158)
	Misc	0·033 (0·018–0·058)
	Nestboxes	0·071 (0·043–0·116)
	Wall	0·024 (0·015–0·040)
Disinfectant group	A	0·043 (0·019–0·093)
	B	0·011 (0·004–0·035)
	C	0·026 (0·011–0·059)
	D	0·062 (0·038–0·101)
Production type	Breeder	0·039 (0·017–0·086)
	Finisher	0·033 (0·017–0·062)
	Rearer	0·039 (0·020–0·075)

Table 5 Results of post-C&D-visits to farm 'A'

	Disinfectant group used	Pos. samples/ total no. of samples – house 1	Pos. samples/ total no. of samples – house 2
July 07	D	50/160 (31·3%)	24/160 (15%)
Feb 08	B	0/96 (0%)	Not done
Sept 08	A	8/100 (8%)	2/100 (2%)
May 09	B	0/112 (0%)	0/80 (0%)

and the level of contamination of the houses were not quantified prior to sampling, and the *Salmonella*-load may have been higher in some houses than in others. The fact that different cleaning teams operate to a different standard has to be taken into account as well, however, our aim was to analyse the efficacy of different disinfection methods under field conditions, which automatically implies a higher variability than experimental studies.

Many different factors influence the success of C&D of poultry houses, and choosing the right procedures and the right disinfectant are often a compromise between price, feasibility and convenience.

Cleaning

The first step to a successful outcome is to ensure that the house is carefully cleaned and, after that, properly washed so that as little soiling as possible is left before disinfection starts. Cleaning the floor is an essential step, and, while cleaning is fairly straightforward in houses with an intact concrete floor, it can be a lot more demanding when the concrete floor has deep cracks or when the house has an earth floor. Some disinfectants, in particular oxidising disinfectants (group D in our study), may be inactivated in the presence of organic matter (Russell 2004), resulting in an unsatisfactory C&D result when small amounts of residual organic matter or biofilms are still present after power-washing. This does not only apply to floor and floor cracks, but also to areas which require a lot of attention to detail, such as nestboxes. Our results show, that areas which are more difficult to clean had a higher sample prevalence than the intact floor. Feeders and drinkers had the lowest sample prevalence in our study. These are less likely than other surfaces to be subject to faecal contamination and are usually made of metal and plastic, which is easier to clean than concrete or wooden surfaces.

Fifty-two percent of houses in our study had at least one positive sample collected from the anteroom, indicating that this is an area which may be neglected by the cleaners and might not be considered as important as the inside of the house. However, anterooms usually contain items which are difficult to clean, such as electrical system control panels, and a suitable amount of time must therefore be dedicated to make sure the anteroom is cleaned and disinfected properly.

Choosing the right disinfectant

Various disinfectants are currently used by the poultry industry for terminal disinfection of poultry houses, and the most commonly used products are based on glutaraldehyde/QAC and oxidising disinfectants. In our study, the two main product groups used were the formaldehyde/glutaraldehyde/QAC group (group B) and the hydrogen peroxide/peracetic acid group (group D). A decision on which product is used usually depends on the practicality (how 'user-friendly' the product is), the price and the knowledge of the farmer.

Formaldehyde has been shown to be the most effective disinfectant to use against *Salmonella* in the presence of organic matter (Berchieri and Barrow 1996; McDonnell and Russell 1999; Whistler and Sheldon 1989; Kumar and Petersen 1991; Allen 1993; Engvall 1993; Davies and Wray 1995; Opitz 1996) but its use has been debated due to health and safety concerns (Anonymous, 1988). Glutaraldehydes/QAC, chlorines, amphoterics and peroxygens appear to be less effective (Taylor *et al.* 1999), but there may differences between different products and the combination of QAC with glutaraldehyde appears to be less effective than glutaraldehyde alone (VLA, unpublished data; Angelillo *et al.* 1998; Davison *et al.* 1996; Hutchison and De Witt 1996). In surface disinfection tests, it was shown that formaldehyde performed better than glutaraldehyde/benzalkoniumchloride and better than

peroxygen compounds (Gradel *et al.* 2004). In layer houses, it has been shown that the use of aldehydes, particularly formaldehyde-based products, results in a greater decrease in *Salmonella* contamination than other products (Wales *et al.* 2006; Carrique-Mas *et al.* 2009b). However, they can be more expensive than some other products and great care has to be taken during application because of the potentially hazardous properties of the ingredients. Hydrogenperoxide/peracetic acid – based products are cheaper than formaldehyde/glutaraldehyde/QAC products and more user-friendly, but they get inactivated easily in the presence of organic matter and should therefore not be used with earth floors or when there is residual organic matter left in the house. Their shelf life is also limited, making it important that the product is not stored for a long time even if the container remains unopened, and they may also be corrosive at effective concentrations. Although turkey rearing and finishing houses are more straightforward to clean than chicken layer houses, the choice of the right disinfectant seems to be equally important to achieve a good result.

The outside temperature (or the temperature of the treated surface) may have an influence on the efficacy of the disinfectant, as most disinfectants are more effective at higher temperatures. Glutaraldehyde for example shows a marked temperature-dependent activity (Russell 2004) and during the winter months it might be worth considering applying higher concentrations to overcome this problem (Russell 2004). The contact time required to achieve an acceptable reduction in surface count can also vary considerably, for example oxidizing disinfectants are generally relatively fast acting whereas aldehydes tend to be slower. However, the contact time should not be a limiting factor under field conditions, as the disinfectant is not rinsed off the treated surfaces but left to dry. Water hardness and cleanliness are also factors that should be taken into account when choosing a product, as most disinfectants are less effective when diluted in hard or dirty water, but there are again differences between substances. Last, and most importantly the disinfectant has to be used at the correct concentration for the agents present and the nature and cleanability of surfaces to be disinfected, and the application rate has to be sufficient, ideally to saturation point.

Salmonella is more resistant to disinfectants in general than many other bacteria and most viruses (Maillard 2004; Thomson *et al.* 2007), so farmers need to be instructed to use disinfectants at the highest concentration recommended by the manufacturer in order to eliminate *Salmonella* contamination from turkey houses.

Development of resistance to disinfectants is a theoretical concern (Paulsen *et al.* 1993; McBain and Gilbert 2001), but seems to be a rare event in gram-negative organisms (McDonnell and Russell 1999) and has not been reported in *Salmonella*.

Level of infection of the previous flock / level of contamination of the house / Production type

The level of infection of the previous flock and the level of contamination of the house may have an influence on the outcome of the C&D, and it appears that farms with a higher burden of *Salmonella* contamination are more likely to have a carry-over from one flock to the next compared to farms with a lower burden (VLA, unpublished data). Younger birds tend to excrete more *Salmonella* than older birds (Gradel *et al.* 2002), however, there was no significant difference between rearing houses and finishing houses in our study. In this study, we did not quantitatively assess the *Salmonella* burden of the previous flock.

The results from the follow-in study of farm 'A' show that, even after a negative post-C&D-visit, there is no guarantee that the farm stays negative for *Salmonella*. There are many ways that a new serotype can be introduced onto a farm, the most common ones being via infected poults, contaminated feed or contaminated bedding. Re-introduction of the same serotype often occurs when the surrounding area of the house is contaminated and sufficient biosecurity measures (such as changing of boots when entering a house and boot dips) are not in place. In the case of farm 'A', a persistent mouse infestation problem was identified as being the most likely cause of repeated introduction of the same serotype into new flocks despite increased efforts of the cleaning team during the cleaning and disinfection procedure.

To conclude, our data show, that 68% of the houses had at least one *Salmonella* -positive sample after cleaning and disinfection. Nestboxes and anterooms were the sample categories most likely to test positive for *Salmonella* after cleaning and disinfection. Disinfectants containing a mixture of formaldehyde, glutaraldehyde and QAC performed best in clearing *Salmonella* contamination in turkey houses. This applied for breeding houses, rearing houses and finishing houses.

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References

Allen, P.C. (1993) Effects of formaldehyde fumigation of housing on carotenoid pigmentation in three breeds of chickens. *Poult Sci* **72**, 1040–1045.

Anderson, E.S., Ward, L.R., Saxe, M.J. and de Sa, J.D. (1977) Bacteriophage-typing designations of *Salmonella* typhimurium. *J Hyg (Lond)* **78**, 297–300.

Angelillo, I.F., Bianco, A., Nobile, C.G. and Pavia, M. (1998) Evaluation of the efficacy of glutaraldehyde and peroxygen for disinfection of dental instruments. *Lett Appl Microbiol* **27**, 292–296.

Anonymous (1988) *Occupational Safety and Health Guideline for Formaldehyde*. http://www.cdc.gov/niosh/docs/81-123/pdfs/0293.pdf, accessed 25 August 2009.

Anonymous (2003) Regulation (EC) No. 2160/2003 of the European Parliament and of the Council of 17 November 2003 on the control of Salmonella and other specified food-borne zoonotic agents.

Anonymous (2005) Commission Regulation (EC) No. 1003/2005 of 30 June 2005 implementing Regulation (EC) No. 2160/2003 as regards a Community target for the reduction of the prevalence of certain Salmonella serotypes in breeding flocks of Gallus gallus and amending regulation (EC) No. 2160/2003.

Anonymous (2008) Commission Regulation (EC) No. 584/2008 of 20 June 2008 implementing Regulation (EC) No. 2160/2003 of the European Parliament and of the Council as regards a Community target for the reduction of the prevalence of Salmonella enteritidis and Salmonella typhimurium in turkeys.

Anonymous (2009a) Commission Regulation (EC) No. 213/2009 of 18 March 2009 amending Regulation (EC) No. 2160/2003 of the European Parliament and the Council and Regulation (EC) No. 1003/2005 as regards the control and testing of Salmonella in bredding flocks of Gallus gallus and turkeys.

Anonymous (2009b) *Test Methodologies on the Approval of Disinfectants for the Purposes of the Animal Health Act 1981*. http://www.defra.gov.uk/vla/services/docs/ser_disinfectants.pdf. accessed 17 November 2009.

Baggesen, D.L., Wegener, H.C. and Christensen, J.P. (1996) Typing of *Salmonella* enterica serovar Saintpaul: an outbreak investigation. *APMIS* **104**, 411–418.

Berchieri, A. and Barrow, P.A. (1996) The antibacterial effects for *Salmonella* Enteritidis phage type 4 of different chemical disinfectants and cleaning agents tested under different conditions. *Avian Pathol* **25**, 663–673.

Carrique-Mas, J.J., Breslin, M., Snow, L., McLaren, I., Sayers, A.R. and Davies, R.H. (2009a) Persistence and clearance of different *Salmonella* serovars in buildings housing laying hens. *Epidemiol Infect* **137**, 837–846.

Carrique-Mas, J., Marin, C., Breslin, M., McLaren, I. and Davies, R.H. (2009b) A comparison of the efficacy of cleaning and disinfection methods in eliminating *Salmonella* spp. from commercial egg laying houses. *Avian Pathol* **38**, 419–424.

Davies, R. and Breslin, M. (2003) Observations on *Salmonella* contamination of commercial laying farms before and after cleaning and disinfection. *Vet Rec* **152**, 283–287.

Davies, R.H. and Wray, C. (1994) Evaluation of a rapid cultural method for identification of *Salmonellas* in naturally contaminated veterinary samples. *J Appl Bacteriol* **77**, 237–241.

Davies, R.H. and Wray, C. (1995) Observations on disinfection regimens used on *Salmonella* enteritidis infected poultry units. *Poult Sci* **74**, 638–647.

Davies, R.H. and Wray, C. (1996a) Persistence of *Salmonella* enteritidis in poultry units and poultry food. *Br Poult Sci* **37**, 589–596.

Davies, R.H. and Wray, C. (1996b) Studies of contamination of three broiler breeder houses with *Salmonella* enteritidis before and after cleansing and disinfection. *Avian Dis* **40**, 626–633.

Davison, S., Benson, C.E. and Eckroade, R.J. (1996) Evaluation of disinfectants against *Salmonella* enteritidis. *Avian Dis* **40**, 272–277.

EFSA (2008a) *The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2006*. http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178671312912.htm, accessed 26 July 2008.

EFSA (2008b) Report of the Task Force on Zoonoses Data Collection on the Analysis of the baseline survey on the prevalence of *Salmonella* in turkey flocks, part A. *EFSA J* **134**, 1–91.

Engvall, A. (1993) Cleaning and disinfection of poultry hens. Proceedings of the NVI/WHO Conference on Salmonella control in animal production and products. pp. 155–183.

Gradel, K.O., Andersen, J. and Madsen, M. (2002) Comparisons of sampling procedures and time of sampling for the detection of *Salmonella* in Danish infected chicken flocks raised in floor systems. *Acta Vet Scand* **43**, 21–30.

Gradel, K.O., Sayers, A.R. and Davies, R.H. (2004) Surface disinfection tests with *Salmonella* and a putative indicator bacterium, mimicking worst-case scenarios in poultry houses. *Poult Sci* **83**, 1636–1643.

Grimont, P.A.D. and Weill, F.-X. (2007) *Antigenic Formulae of the Salmonella Serovars*. Paris, France: WHO Collaborating Centre for Reference and Research on Salmonella.

Humphrey, T.J. (1990) Public health implications of the infection of egg-laying hens with *Salmonella* enteritidis phage type 4. *World's Poult Sci J* **46**, 5–13.

Hutchison, T.W. and De Witt, W.F. (1996) Quaternary ammonium compound toxicity in broiler chickens. *Can Vet J* **37**, 482.

de Jong, B. and Ekdahl, K. (2006) Human salmonellosis in travellers is highly correlated to the prevalence of *Salmonella* in laying hen flocks. *Euro Surveill* **11**, E060706.1.

Kumar, M.C. and Petersen, M.K. (1991) Field trials on disinfectants in turkey houses. Proceedings of the 40th Western Poultry Diseases Conference. pp. 149–151.

Maillard, J.-Y. (2004) Viricidal activity of biocides. In *Russell, Hugo and Ayliffe's Principles and Practice of Disinfection, Preservation and Sterilization* ed. Fraise, A.P., Lambert, P.A. and Maillard, J.-Y. pp. 272–323. Victoria, Australia: Blackwell Publishing.

McBain, A.J. and Gilbert, P. (2001) Biocide tolerance and the harbingers of doom. *Int Biodeterior Biodegradation* **47**, 55–61.

McDonnell, G. and Russell, A.D. (1999) Antiseptics and disinfectants: activity, action, and resistance. *Clin Microbiol Rev* **12**, 147–179.

Opitz, M. (1996) Disinfecting poultry houses requires attention to detail. *Poult Dig* **55**, 26–31.

Paulsen, I.T., Littlejohn, T.G., Radstrom, P., Sundstrom, L., Skold, O., Swedberg, G. and Skurray, R.A. (1993) The 3' conserved segment of integrons contains a gene associated with multidrug resistance to antiseptics and disinfectants. *Antimicrob Agents Chemother* **37**, 761–768.

Poppe, C., Irwin, R.J., Messier, S., Finley, G.G. and Oggel, J. (1991) The prevalence of Salmonella enteritidis and other Salmonella sp. among Canadian registered commercial chicken broiler flocks. *Epidemiol Infect* **107**, 201–211.

Russell, A.D. (2004) Factors influencing the efficacy of antimicrobial agents. In *Russell, Hugo and Ayliffe's Principles and Practice of Disinfection, Preservation and Sterilization* ed. Fraise, A.P., Lambert, P.A. and Maillard, J.-Y. pp. 98–127. Victoria, Australia: Blackwell Publishing.

Taylor, J.H., Rogers, S.J. and Holah, J.T. (1999) A comparison of the bactericidal efficacy of 18 disinfectants used in the food industry against *Escherichia coli* O157:H7 and *Pseudomonas aeruginosa* at 10 and 20 degrees C. *J Appl Microbiol* **87**, 718–725.

Thomson, J.R., Bell, N.A. and Rafferty, M. (2007) Efficacy of some disinfectant compounds against porcine bacterial pathogens. *Pig J* **60**, 15–25.

Wales, A., Breslin, M. and Davies, R. (2006) Assessment of cleaning and disinfection in Salmonella-contaminated poultry layer houses using qualitative and semi-quantitative culture techniques. *Vet Microbiol* **116**, 283–293.

Wales, A., Breslin, M., Carter, B., Sayers, R. and Davies, R. (2007) A longitudinal study of environmental Salmonella contamination in caged and free-range layer flocks. *Avian Pathol* **36**, 187–197.

Whistler, P.E. and Sheldon, B.W. (1989) Comparison of ozone and formaldehyde as poultry hatchery disinfectants. *Poult Sci* **68**, 1345–1350.

WHO (2005) Drug-resistant Salmonella. http://www.who.int/mediacentre/factsheets/fs139/en/ (accessed 11 January 2010).

