Danish Veterinary Institute

SYMPOSIUM:

DISINFECTION IN ANIMAL PRODUCTION:

Cleaning and Disinfection projects under the Danish *Salmonella* Programme for Poultry, with a view to virus disinfection

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PROCEEDINGS

Contents

Introduction: the Danish *Salmonella* Control Programme for Poultry, and background to the cleaning and disinfection projects

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Introduction

In the mid-1980's, Danish producers faced a growing problem of *Salmonella* in the poultry industry. This situation was similar to the experience of many other poultry-producing countries, caused by the almost pandemic spread of *Salmonella* in poultry at that time.

In Denmark, the broiler producers as an exporting industry acted quickly and set up a voluntary *Salmonella* control scheme in 1989. This scheme was later followed by a similar voluntary scheme in the table egg-producing sector in 1992.

With the introduction of the Zoonosis Directive 92/117/EEC the voluntary industry scheme was largely replaced in 1994, when the first Danish plan for control of *Salmonella* in poultry was approved by the European Commission as an implementation of the directive.

The National *Salmonella* Control Programme 1996-2002

Due to an increasing number of human cases of salmonellosis related to consumption of eggs and chicken meat the Ministry of Food, Agriculture and Fisheries implemented a comprehensive national surveillance and control plan, including all types of flocks in the broiler and layer sectors in 1996.

The budget of the plan was 188.1 mio. DKK for a 3-year period, of which 30 mio. DKK was contributed by the Danish poultry industry. In 1999, it was decided to prolong the plan for additionally 3 years, due to available funds remaining from the first 3-year period.

The plan adopted an elimination strategy, or zero tolerance, for all *Salmonella* serotypes (except for the host-specific *Salmonella* Pullorum and *S*. Gallinarum that are dealt with in existing Danish legislation). The approach is a top-down approach with financial compensation for removal of infected breeder flocks. The sampling programme combines bacteriological and serological analyses in order to optimize the chance of detecting infected flocks as soon as possible.

From January 1st 2003 onwards, the Danish Poultry Council has become responsible for the administration of the plan under continuous monitoring and control by the Danish Veterinary and Food Administration.

The results of the plan have been encouraging by means of which the original objectives have been satisfactorily attained. From 1997 to 2002, a 78% reduction in the estimated number of human cases of salmonellosis related to consumption of eggs and chicken meat has been achieved (from 3,109 to 677 registered cases annually).

A unique cooperation between governmental authorities and institutions, the poultry industry and farmers has been a fundamental prerequisite of these achievements.

Cleaning and disinfection projects under the National *Salmonella* Control Programme
Although the results in terms of reducing the number of human illnesses have been encouraging, it became increasingly clear during the course of the programme that particularly large poultry operations suffered setbacks in the form of recurrent or persistent *Salmonella* infections.

Many reasons for this persistence have been suggested, e.g. rodents, insects, or lack of hygiene barriers, but an effective cleaning and disinfection scheme is utterly important. Poultry houses often have rough water permeable surfaces with cracks and crevices, and the equipment is often difficult to clean, e.g. in battery cage houses.

However, although several guidelines on cleaning and disinfection of animal houses have been compiled and published, the topic has received little attention in the scientific community. Moreover, there are no official and uniform guidelines available for the approval of disinfectants, and most information on the efficacy of chemical disinfectants can only be obtained from the companies marketing these.

Accordingly, it was agreed that research funds should be spent on documenting the effect of cleaning and disinfection procedures. This resulted in the planning and execution of two research projects during the period 2000-2003:

1. Thermal disinfection as a method for eradicating *Salmonella* infection in poultry houses. Phase 1 consisting of laboratory experiments, and Phase 2 consisting of full-scale field trials that involved the testing of measures proven through laboratory experiments to facilitate the elimination of *Salmonella* bacteria.
2. Development of microbiological monitoring models in broiler houses: an assessment of the influence of cleaning and disinfection procedures on *Salmonella* persistence.

This symposium will outline theoretical and practical aspects of disinfection of microorganisms in animal production. The focus of the programme will be to present the results and conclusions from the cleaning and disinfection projects under the Danish *Salmonella* Control Programme for Poultry. However, as eradication by cleaning and disinfection is also relevant for other sectors of livestock production, the scope of the symposium is expanded to include other conditions and microorganisms in order to address cleaning and disinfection in a broader perspective.

Factors that influence the heat resistance of microbes and methods for detecting sublethally damaged vegetative bacteria – experience from food microbiology

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Although there is a variety of newer methods available for inactivation of pathogenic and spoilage microbes (e.g. irradiation, high pressure) heat is undoubtedly still the most important, economic and widely accepted means of food preservation. There are two types of microbes, with quite different degrees of heat resistance. Spores (which are dormant forms produced by some bacteria, and also some fungi) are much more resistant than the 'vegetative' forms. During canning, the aim is to inactivate all organisms capable of multiplying in the food – to achieve 'commercial sterility'. Studies to produce safe canned foods therefore concentrated on the times and temperatures required to kill the most resistant (spores) – in particular *Clostridium botulinum*, and to a lesser extent the spore-formers causing spoilage. Canned foods are normally stable and safe after storage for years at ambient temperatures. In other foods, a lesser heat treatment is applied, which aims to inactivate pathogenic vegetative bacteria, such as *Salmonella* and *Campylobacter* spp., *Listeria monocytogenes* and pathogenic *Escherichia coli,* but which will not affect spores. These foods normally have to be stored chilled, and have a relatively short shelf life. Examples are pasteurised milk, ready meals and cooked sliced meats. Some foods are preserved by use of mild heat treatment in combination with other treatments, which extend their shelf-life and/or enable them to be stored safely at ambient temperature – e.g. dried milk or egg, some types of sausage (with added salt, preservative(s), reduced water content).

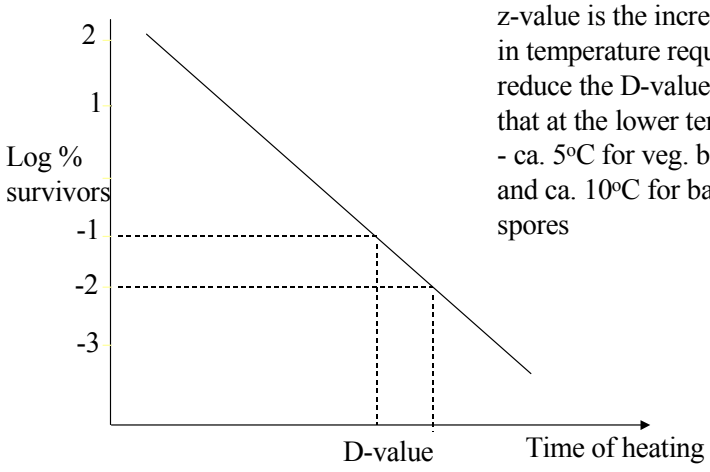
Methods of measuring the relative heat resistance of a variety of spore-formers were developed in the 1920's, and used to determine the times and temperatures required to inactivate spores in canned foods. Survival curves (plots of $\log_{10}$ numbers of survivors against time of heating at a particular temperature) were often straight-line (indicating first order kinetics), and heat resistance could be expressed as 'D-values' – time taken to kill 90% of the initial population at a particular temperature – and 'z-values' – increase in temperature required to reduce the D-value to $1/10^{th}$ of its value at the lower temperature. A '12-D' treatment for *Cl. botulinum* is normally applied to canned foods. Similar principles could be applied to measuring the heat resistance of vegetative microbes, and were used to devise appropriate heat treatment of liquid egg to inactivate contaminating *Salmonella*s.

Laboratory investigations found many factors affected the heat resistance of spores and vegetative cells – phase of growth, initial number of organisms, pH of the heating medium, composition of the medium used to grow the organisms, composition of the heating medium, presence of fats, concentration of NaCl and other preservatives, type and concentration of sugars or other solutes. Reduction in the amount of available water in the heating menstruum – either by direct drying, or by addition of high levels of solutes, was found to have a particularly marked effect on heat resistance. For example, the $D_{71}{}^{o}C$-value of *Salmonella* in chocolate with 2% moisture is 20 hours, and with 4% moisture is 4 hours (Barrile and Cone, 1970), although in high moisture situations they have a D-value of a few seconds only at $71^{o}C$. The *Salmonella*s are able to survive, but not multiply in this and other dried foods – but are still capable of multiplying when cross-contaminated onto other foods and/or infecting consumers.

Besides the effect of conditions before and during heating, it was also apparent that the methods used to recover survivors after heat-treatment were important. Methods of detecting pathogens (and spoilage microbes) often used selective agents to prevent unwanted microbes growing. While undamaged pathogens are resistant to these agents, heat-damaged organisms are frequently sensitive to them. They also often take longer to grow and grow better at lower temperatures than usual. These effects have to be taken into account when examining heat-treated foods for pathogens. Various strategies have been developed for estimation of numbers, which involve a non-selective resuscitation step, followed by selective cultivation. When determining presence/absence (as is usually the case when looking for *Salmonella*s) the techniques are more simple, but care is needed not to allow competitors to outgrow the target organism.

Some questions have not yet been resolved. The lag time before growth of some heat-damaged *Salmonella*s can exceed the normal 18 hour incubation time for non-selective pre-enrichment in traditional methods. Is the 'viable but not culturable' phenomenon real with respect to *Salmonella*s and other bacteria in food and/or on farms (i.e. can bacteria that cannot be recovered on normal laboratory media infect humans or animals)?

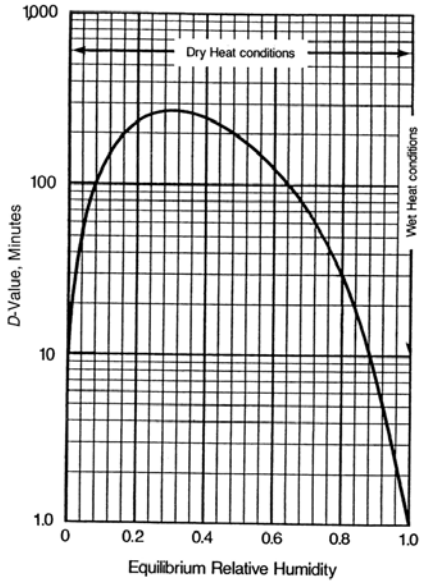
D and z values



z-value is the increase in temperature required to reduce the D-value to 1/10th that at the lower temperature.
- ca. 5°C for veg. bacteria and ca. 10°C for bacterial spores

Heat resistance (D-values in min) at high moisture levels

Vegetative bacteria	D_{60}°C	D_{100}°C
Salmonella/*E. coli*	0.2-1.9	
campylobacter	0.7-1.4	
Enterococcus faecalis	5-20	
Bacterial spores		
Cl. botulinum		7-28
Cl. stearothermophilus		100-1600



Effect of drying on the heat resistance of microbes

Further reading

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Heat as a disinfection method for poultry houses persistently infected with *Salmonella* – an outline of methods and results

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Introduction

Guidelines on cleaning and disinfection of poultry enterprises are based on the assumption that all organic matter can be removed by cleaning, and that the subsequent chemical disinfection will cover all surfaces (Linton *et al.* 1987; Engvall 1993; Anonymous 1993a, 1993b; von Löhren 1994; Meroz and Samberg 1995; Søeberg and Pedersen 1998). This ideal situation is rarely achieved in poultry houses with cracks, crevices, and complex inaccessible equipment. It is especially difficult to clean battery cage houses effectively.

Heat rather than chemical disinfection of contaminated items is recommended whenever possible (Russell 1999). If heat could be evenly distributed in poultry houses and if it could penetrate organic matter without damaging materials it might be useful for dealing with persistent *Salmonella* infections. Moist heat (steam) and formaldehyde treatment has been used in Danish poultry houses for years, but with little evidence of its efficacy. Some poultry farmers have tried to heat poultry houses with dry heat, but it is evident that *Salmonella* remains in these houses, even after mean temperatures around 70 ^{o}C for up to five days (Gradel 2000).

In our laboratory studies, the aim was to perform heat experiments with inoculated organic matter that mimicked worst case scenarios found in badly cleaned empty poultry houses. This was done in order to find a temperature-humidity-time combination that could eliminate *Salmonella*. *E. coli* was used as a possible indicator bacterium that occurs in higher numbers than *Salmonella* in poultry houses, and might be used to verify the effectiveness of the treatment against *Salmonella*.

In our field studies, it was tested if a temperature-humidity-time combination found satisfactory in the laboratory would be equally effective in naturally *Salmonella*-infected poultry houses. This was monitored by *Salmonella*-sampling before and after heating and by placing challenge samples, i.e. organic matter with *Enterococcus faecalis*, enterococci or *E. coli* as possible indicator bacteria, at sites where temperatures were logged continuously.

Laboratory studies, materials and methods

General principles. Survival of three strains of *Salmonella* (*S*. Enteritidis, phage type 8 (SE8), *S*. Typhimurium, definitive type 110 (ST110), *S*. Infantis) and naturally-occurring *E. coli*, was assessed in faeces and pelleted feed in their original state or pre-equilibrated for 10 days to 30% RH. Final heating temperatures were 50, 55, 60, 65 or 70^{o}C and final relative humidity was 16-30 or 100%. Supplementary tests were carried out with one strain of *Salmonella* using egg yolks and finely ground feed as well as faeces and pelleted feed.

Test protocol. Thirty g of test material were put in 5.0 cm diameter beakers, yielding a layer 2-2.5 cm thick. Presence of naturally occurring *E. coli* in each beaker of faeces was checked and all the beakers were inoculated with one of the three strains of *Salmonella*. The beakers containing faeces and feed were put in a climate chamber set to 20 ^{o}C and 30% relative humidity (RH) for 10 days. Lids were put on half the beakers and the other half were left open to equilibrate to the 30% RH, which caused them to dry. After exactly 10 days, some samples ("10-day samples") were weighed and examined for numbers and presence/absence of S*almonella*. Moreover, faecal samples were

examined for presence/absence of *E. coli.* The remaining beakers were transferred to a cooled incubator for the heat tests after removing the lids from the non-dried samples. A PC-software programme was used to increase the temperature from the initial 20°C to the final heating temperature at a rate of increase of 1°C per hour. In order to achieve an atmosphere of 100% RH, some samples were sealed individually in plastic boxes with water in the bottom. Heating was continued for 48 hours, after which 20°C for a further 24 hours was maintained. Samples were weighed and examined as the "10-day samples" (see above) at the beginning of the final heating temperature, after 24 and 48 hours of heating, and 24 hours after heating had ceased. The above procedures were not performed in replicate, so when a satisfactory temperature-humidity-time combination was found the experiment was repeated, but with some modifications. Only dried samples were used (no samples were lidded during the initial 10-day equilibration period), quantitative tests were omitted, and only one serotype (SE8) was used, as the results were very similar for the three strains. Tests were carried out four times in pelleted and ground feed, faeces and egg yolk.

Microbiological procedures. Three ml of a stationary phase *Salmonella* 10^{-2} dilution was added to the test material in each beaker, mixing immediately with a sterile spoon, after which the samples were incubated as described above. After incubation, 0.3-0.4 g (faecal samples) or ca. 1.0 g (feed samples) were taken from the middle of each sample, diluted and plated on Rambach agar to determine numbers of CFU. The remaining test materials were examined using a routine presence/absence test. Results from Rambach plates were recorded for both S*almonella* and non-S*almonella* colonies. Faecal samples were also checked for presence/absence of *E. coli.*

Laboratory studies, results

Weight loss of samples. Faeces and feed were 20.0-34.2% and 83.5-87.9%, respectively, of their original weight after equilibration (drying) to 30% relative humidity for 10 days. Owing to malfunction of the chamber, the RH sometimes differed from 30% - see Table 1.

Survival during heating in faeces. Results are shown in Table 1. Overall, very few or no *Salmonella* colonies were detected by direct plating of faeces (data not shown), although enrichment frequently gave positive results. At low humidity, survivors were found in dried faeces at every sampling time and every final heating temperature. In the undried faeces exposed to low humidity and in both the dried and undried faeces exposed to 100% RH *Salmonella* was not detected after 24 hours at 55°C and above.

Survival during heating in feed. At low humidity, *Salmonella* survived in both dried and undried feed, regardless of the final heating temperature. Heating at 100% RH resulted in all *Salmonella* samples testing negative after 24 hours at 60 °C and above; at all temperatures up to 65 °C, *Salmonella* died more rapidly in undried than in dried feed, while no difference was seen at 70 °C. *Salmonella* was frequently detected by direct plating. At low humidity, *Salmonella* was often detected by direct plating at all stages, albeit in low numbers after 24 hours and beyond.

Survival of S. Enteritidis phage type 8 in four substrates, with four replicates, using predried samples and more rigorous tests for Salmonella. Salmonella was not detected in any 24-, 48- or 72-hour sample in this experiment, regardless of the method of detection or type of substrate (pelleted or ground feed, egg yolk or faeces). Thus, 60 °C and 100% RH during a 24-hour period was identified as a suitable standard to be applied in field studies.

Association between occurrence of Salmonella and E. coli. E. coli was detected in all faecal samples prior to the testing. Table 2 shows the association between results for *Salmonella*,

including Rambach agar plate readings, and *E. coli*. There was a high correlation between the occurrence of *E. coli* on one side and *Salmonella* and non-*Salmonella* colonies on the other side. *E. coli* results could statistically predict *Salmonella* status.

Laboratory studies, conclusions

The application of ≥ 60 ^{o}C and 100% RH during a 24-hour period eliminated *Salmonella* and *E. coli* in all samples. *E. coli* could be used as an indicator bacterium for the elimination of *Salmonella*. More details can be read elsewhere (Gradel 2002; Gradel *et al.* 2003a).

Table 1:
Survival of *Salmonella* in experiments at five final heating temperatures (50, 55, 60, 65 or 70 °C) (TEMP) using faeces or feed as organic matter (ORG), three serotypes (*S.* Enteritidis, phage type 8 (SE8), *S.* Typhimurium, definitive type 110 (ST110), *S.* Infantis (Inf)) (TYPE), with samples dried (RH sometimes deviated from 30%) or not dried in a 10-day period prior to the final heat treatment (DRD)

TEMP	ORG	TYPE	DRD	RH(SD)[1]	Heating at 16-30% RH					Heating at 100% RH				
					10d[2]	0[2]	24[2]	48[2]	72[2]	10d	0	24	48	72
50	Faeces	SE8	Yes	30 (0.2)	-[3]	+[4]	+	+	+	+	-	-	-	-
50	Faeces	SE8	Yes	39 (3.12)	ND[5]	ND	ND	ND	ND	-	+	+	-	-
50	Faeces	SE8	Yes	50 (27.8)	ND	ND	ND	ND	ND	+	-	-	-	-
50	Faeces	SE8	No		+	-	-	-	-	+	-	-	-	-
50	Faeces	ST110	Yes	30 (0.2)	+	+	+	+	+	+	+	-	-	-
50	Faeces	ST110	Yes	39 (3.12)	ND	ND	ND	ND	ND	+	+	+	-	-
50	Faeces	ST110	Yes	50 (27.8)	ND	ND	ND	ND	ND	+	+	-	-	-
50	Faeces	ST110	No		+	-	-	-	-	+	-	-	-	-
50	Faeces	Inf	Yes	30 (0.2)	+	+	+	+	+	+	+	-	-	-
50	Faeces	Inf	Yes	39 (3.12)	ND	ND	ND	ND	ND	+	+	-	-	-
50	Faeces	Inf	Yes	50 (27.8)	ND	ND	ND	ND	ND	+	+	-	-	-
50	Faeces	Inf	No		+	-	-	-	-	+	-	-	-	-
50	Feed	SE8	Yes	30 (0.2)	2.8[6]	2.3	0.8	0.6	+	11.0	3.0	1.3	+	+
50	Feed	SE8	Yes	39 (3.12)	ND	ND	ND	ND	ND	2.0	0.2	+	+	+
50	Feed	SE8	Yes	50 (27.8)	ND	ND	ND	ND	ND	0.8	+	+	+	-
50	Feed	SE8	No		5.8	0.8	0.6	0.6	+	0.9	+	+	-	-
50	Feed	ST110	Yes	30 (0.2)	5.3	1.6	0.9	0.5	0.6	8.7	2.1	1.0	1.0	+
50	Feed	ST110	Yes	39 (3.12)	ND	ND	ND	ND	ND	1.5	0.4	+	+	+
50	Feed	ST110	Yes	50 (27.8)	ND	ND	ND	ND	ND	+[7]	+[7]	+	-	-
50	Feed	ST110	No		10.5	1.0	0.8	+	+	+	+	+	-	-
50	Feed	Inf	Yes	30 (0.2)	19.7	13.4	3.3	2.9	3.0	6.0	1.3	0.8	+	+
50	Feed	Inf	Yes	39 (3.12)	ND	ND	ND	ND	ND	1.4	0.3	+	+	+
50	Feed	Inf	Yes	50 (27.8)	ND	ND	ND	ND	ND	0.5	0.1	+	+	-
50	Feed	Inf	No		17.4	2.7	2.3	2.5	2.6	4.2	0.6	+	-	-
55	Faeces	SE8	Yes	30 (0.2)	+	+	+	+	+	+	-	-	-	-
55	Faeces	SE8	Yes	33 (2.7)	ND	ND	ND	ND	ND	+	+	-	-	-
55	Faeces	SE8	Yes	41 (20.9)	ND	ND	ND	ND	ND	+	+	-	-	-
55	Faeces	SE8	No		+	-	-	-	-	+	-	-	-	-
55	Faeces	ST110	Yes	30 (0.2)	+	+	+	+	+	+	-	-	-	-
55	Faeces	ST110	Yes	33 (2.7)	ND	ND	ND	ND	ND	+	+	-	-	-
55	Faeces	ST110	Yes	41 (20.9)	ND	ND	ND	ND	ND	+	+	-	-	-
55	Faeces	ST110	No		+	-	-	-	-	+	-	-	-	-
55	Faeces	Inf	Yes	30 (0.2)	+	+	+	+	+	+	-	-	-	-
55	Faeces	Inf	Yes	33 (2.7)	ND	ND	ND	ND	ND	+	+	-	-	-
55	Faeces	Inf	Yes	41 (20.9)	ND	ND	ND	ND	ND	+	+	-	-	-
55	Faeces	Inf	No		+	-	-	-	-	+	-	-	-	-
55	Feed	SE8	Yes	30 (0.2)	2.4	0.5	0.7	+	0.3	1.7	+	+	-	-
55	Feed	SE8	Yes	33 (2.7)	ND	ND	ND	ND	ND	1.7	+	+	-	-
55	Feed	SE8	Yes	41 (20.9)	ND	ND	ND	ND	ND	19.5	3.0	+	+	+
55	Feed	SE8	No		5.9	0.3	0.5	0.2	0.2	28.3	+	-	-	-
55	Feed	ST110	Yes	30 (0.2)	3.0	2.1	1.1	0.5	0.3	1.8	0.4	+	-	-
55	Feed	ST110	Yes	33 (2.7)	ND	ND	ND	ND	ND	1.7	0.2	+	-	-
55	Feed	ST110	Yes	41 (20.9)	ND	ND	ND	ND	ND	8.1	1.2	+	+	-
55	Feed	ST110	No		10.3	1.8	1.4	1.3	1.2	19.1	+	-	-	-
55	Feed	Inf	Yes	30 (0.2)	6.2	6.1	1.7	1.2	1.7	2.7	0.2	+	-	-
55	Feed	Inf	Yes	33 (2.7)	ND	ND	ND	ND	ND	3.2	0.7	+	-	-
55	Feed	Inf	Yes	41 (20.9)	ND	ND	ND	ND	ND	6.6	0.9	+	+	-
55	Feed	Inf	No		29.1	3.0	2.7	2.6	2.4	31.2	+	-	-	-

To be continued

Table 1, continued

TEMP	ORG	TYPE	DRD	RH(SD)	Heating at16-30% RH					Heating at 100% RH				
					10d	0	24	48	72	10d	0	24	48	72
60	Faeces	SE8	Yes	30 (0.2)	+	+	+	+	+	+	-	-	-	-
60	Faeces	SE8	Yes	33 (2.9)	-	-	+	+	+	+	-	-	-	-
60	Faeces	SE8	No		+	-	-	-	-	+	-	-	-	-
60	Faeces	ST110	Yes	30 (0.2)	+	+	+	+	+	+	-	-	-	-
60	Faeces	ST110	Yes	33 (2.9)	+	+	+	+	+	+	-	-	-	-
60	Faeces	ST110	No		+	-	-	-	-	+	-	-	-	-
60	Faeces	Inf	Yes	30 (0.2)	+	+	+	+	+	+	-	-	-	-
60	Faeces	Inf	Yes	33 (2.9)	+	+	+	-	+	+	-	-	-	-
60	Faeces	Inf	No		+	-	-	-	-	+	-	-	-	-
60	Feed	SE8	Yes	30 (0.2)	2.2	1.1	0.2	0.3	0.3	1.1	+	-	-	-
60	Feed	SE8	Yes	33 (2.9)	0.1	0.1	0.1	0.1	+	0.2	+	-	-	-
60	Feed	SE8	No		1.2	+	+	+	+	3.0	-	-	-	-
60	Feed	ST110	Yes	30 (0.2)	2.6	1.1	0.7	0.7	0.8	1.7	+	-	-	-
60	Feed	ST110	Yes	33 (2.9)	0.1	0.1	+	0.1	+	0.1	+	-	-	-
60	Feed	ST110	No		0.6	0.1	+	0.1	0.1	2.5	+	-	-	-
60	Feed	Inf	Yes	30 (0.2)	6.2	1.6	1.2	1.1	1.1	6.4	1.0	-	-	-
60	Feed	Inf	Yes	33 (2.9)	0.2	0.2	0.1	0.1	+	0.4	+	-	-	-
60	Feed	Inf	No		3.3	0.6	+	+	+	4.4	-	-	-	-
65	Faeces	SE8	Yes	30 (0.2)	+	-	+	+	+	+	-	-	-	-
65	Faeces	SE8	No		-	-	-	-	-	+	-	-	-	-
65	Faeces	ST110	Yes	30 (0.2)	+	+	+	+	+	+	-	-	-	-
65	Faeces	ST110	No		+	-	-	-	-	+	-	-	-	-
65	Faeces	Inf	Yes	30 (0.2)	+	+	+	+	+	+	-	-	-	-
65	Faeces	Inf	No		+	-	-	-	-	+	-	-	-	-
65	Feed	SE8	Yes	30 (0.2)	3.0	0.3	0.2	+	+	0.5	+	-	-	-
65	Feed	SE8	No		9.1	1.0	0.9	+	+	3.6	-	-	-	-
65	Feed	ST110	Yes	30 (0.2)	3.0	1.1	0.7	0.7	+	1.7	+	-	-	-
65	Feed	ST110	No		5.9	1.1	0.9	+	+	5.7	-	-	-	-
65	Feed	Inf	Yes	30 (0.2)	3.0	2.9	0.7	0.6	0.6	2.4	+	-	-	-
65	Feed	Inf	No		16.0	2.2	1.5	1.4	1.3	8.5	-	-	-	-
70	Faeces	SE8	Yes	30 (0.2)	+	+	+	+	+	+	-	-	-	-
70	Faeces	SE8	Yes	31 (1.9)	ND	ND	ND	ND	ND	+	-	-	-	-
70	Faeces	SE8	No		-	-	-	-	-	-	-	-	-	-
70	Faeces	ST110	Yes	30 (0.2)	+	+	+	+	+	+	-	-	-	-
70	Faeces	ST110	Yes	31 (1.9)	ND	ND	ND	ND	ND	+	-	-	-	-
70	Faeces	ST110	No		+	-	-	-	-	+	-	-	-	-
70	Faeces	Inf	Yes	30 (0.2)	+	+	+	+	-	+	-	-	-	-
70	Faeces	Inf	Yes	31 (1.9)	ND	ND	ND	ND	ND	+	-	-	-	-
70	Faeces	Inf	No		+	-	-	-	-	+	-	-	-	-
70	Feed	SE8	Yes	30 (0.2)	1.4	0.3	+	+	+	4.4	-	-	-	-
70	Feed	SE8	Yes	31 (1.9)	ND	ND	ND	ND	ND	0.2	-	-	-	-
70	Feed	SE8	No		4.3	0.8	0.8	+	+	0.2	-	-	-	-
70	Feed	ST110	Yes	30 (0.2)	1.6	0.5	+	+	+	4.9	-	-	-	-
70	Feed	ST110	Yes	31 (1.9)	ND	ND	ND	ND	ND	0.8	-	-	-	-
70	Feed	ST110	No		5.2	0.7	0.7	+	+	1.0	-	-	-	-
70	Feed	Inf	Yes	30 (0.2)	4.6	0.5	0.4	0.3	+	5.8	-	-	-	-
70	Feed	Inf	Yes	31 (1.9)	ND	ND	ND	ND	ND	0.9	-	-	-	-
70	Feed	Inf	No		5.5	0.7	0.4	0.4	+	1.2	-	-	-	-

[1]Mean % relative humidity (standard deviation). [2]10d = 10-day samples, 0, 24, 48, 72 = 0-, 24-, 48-, and 72-hour samples. [3]*Salmonella* not detected in qualitative or quantitative tests. [4]*Salmonella* detected in qualitative but not in quantitative tests. [5]Not done. [6]Percent surviving *Salmonella* in quantitative tests; all detected in qualitative tests. [7]Quantitative results not available, as the initial CFU g^{-1} organic matter could not be calculated.

Table 2:
Results from all faecal samples for *Escherichia (E.) coli* readings on MacConkey agar and qualitative *Salmonella* experiments, including readings on Rambach agar

E. coli detected?	*Salmonella* spp. detected on Rambach agar?				SUM
	Yes		No		
	Pure culture of *Salmonella*	*Salmonella*- and non-*Salmonella*	Growth of non-*Salmonella*	Sterile	
Yes	8	90	10	8	116
No	40	8	3	222	273
SUM	48	98	13	230	389

Field studies, materials and methods
Participating premises. Table 3 shows farms and houses in the field studies.

Table 3
Layer farms and houses studied, with summary of the disinfection treatments and microbiological checks.

Farm	House	House type	Treatment	No. of *Salmonella* samples		Challenge samples[1] used
				Before treatment	After treatment	
A	A1	Barn	Model 1[2]	287	288	Yes[3]
	A2	Battery cage	Model 1	302	303	Yes[3]
B	B1	Barn	Model 2[4]	100	102	Yes
	B2	Barn	Model 3[5]	100	96	No
	B3	Barn	Model 4[6]	100	100	No
C	C1	Battery cage	Model 2	298	308	Yes
D	D1	Battery cage	Model 2	289	290	Yes
E	E1	Battery cage	Model 2	308	308	Yes
F	F1	Battery cage	Model 2	150	150	No

[1]Samples of feed inoculated with *Enterococcus faecalis* or *Escherichia* (*E.*) *coli* or faeces with naturally occurring enterococci and *E. coli*, placed next to temperature probes.
[2]60 °C and 100% RH for 24 h without formaldehyde (Period I), followed by a short heating to ca. 60 °C with formaldehyde, 13 and 22 ppm in House A1 and A2, respectively (Period II).
[3]Challenge samples placed both during Period I, Period II and Period I+II.
[4]60 °C and 100% RH for 24 h with 30 ppm formaldehyde at the beginning of the process.
[5]Pulse-fogging (2.75 l of a branded product consisting of 23% glutaraldehyde and 5% benzalkonium chloride, 0.28 l pH-regulator (KOH and H_3PO_4) and 5.5 l tap water was run for ca. 25 min).
[6]Surface disinfection (same product as in Model 3, using 15 l disinfectant, 1.5 l pH-regulator, dissolved in 500 l tap water) that took ca. 3 h.

Steam treatment procedure. One company that has steam treated poultry houses for years was used for heating all the houses. A hose attached to a steam generator was inserted through an opening, which was then sealed tightly with plastic. All other openings were also sealed tightly. The steam generator heated the water to ca. 160-170 °C within ½-1 hour, after which the insertion of steam commenced. Formaldehyde at 23.4% (v/v) was used for all relevant houses; a dose to yield 30 ppm formaldehyde was calculated from the volume to be heat-treated, i.e. it was not measured.

Salmonella sampling procedures. Sample sites were selected beforehand to provide an even "geographical" distribution in the house and on different types of equipment. All battery cage houses had either five or six tiers, and the first, third and fifth/sixth tiers were used consistently for sampling; each tier was evenly divided into seven sample sites, including one at each end. For all samples, beakers with sterile gauze swabs in 100 ml sterile buffered peptone water (BPW) were used. Each sampling site was swabbed vigorously with the swab, which was then returned immediately to BPW in the beaker. Disposable gloves were used and changed between each sample. All samples were processed in the laboratory using standard *Salmonella* isolation procedures. Results from Rambach agar were recorded for both S*almonella* like and non-S*almonella* like colonies.

Monitoring of temperature and humidity. In all houses that were steam treated, calibrated loggers logged temperature or temperature/relative humidity (RH) at 5-min intervals. After the treatments, all loggers were read in the corresponding PC-programmes. Loggers where placed at six sites per house, one in each corner and two evenly distributed in the middle. In houses where ancillary rooms were included in the heat treatment (Houses A2, C1, D1), one "corner" measurement was removed to the farthest corner of the ancillary room. At each of the six sites there was a "low" measurement, normally logging 2.5 cm above floor level, and a "high" measurement that in most cases logged 2-2.5 m above floor level. RH was measured in some houses not less than 2 m above floor level. In Houses B1, C1, D1 and E1, additional temperature and humidity measurements were taken in holes drilled in the concrete floor to monitor temperatures achieved in cracks and the concrete itself. The holes had different diameters, and some were sealed with a sealant. Temperatures were measured at different heights above the floor to see where 60 ^{o}C was achieved.

Challenge samples. Samples with high numbers of putative indicator bacteria in high amounts of organic matter were placed beside the standard site temperature probes. Bacteria found in the same habitats as *Salmonella* spp., but often in higher numbers, which are at least as heat resistant as *Salmonella* spp., would be suitable indicator bacteria. A strain of *E. coli* and *Enterococcus* (*E.*) *faecalis* were used for spiking feed samples whereas naturally occurring *E. coli* and enterococci were used in faecal samples. Challenge samples were weighed into beakers and dried at ambient temperature (for Farms A, B/C/E and D for 4, 10 and 15 days, respectively). Control samples (three for each combination of bacterial type and organic matter, i.e. 12 per house) remained in the laboratory during the heat treatments. In the animal house, all challenge samples were placed at the same height as and not more than 20 cm from a temperature probe placed at one of the 12 standard sites (see above). In the laboratory, traditional procedures were used for isolating the relevant bacteria.

Field studies, results

Salmonella samples before treatments. All 206 *Salmonella*-positive samples were serotyped as *S.* Enteritidis, which was also the serotype persisting in all eight houses. There were big variations in numbers of *Salmonella*-positive samples between houses (Figure 1). Generally, fewer equipment and surroundings samples, not related directly to floors, were *Salmonella*-positive than surroundings samples related to floors, but there were several exceptions, as samples from droppings belts, drip channels, feed chain "corner" wheels and egg equipment often yielded *Salmonella* (data not shown). There were no conspicuous differences between barn and battery cage houses, and the distribution in all houses with high *Salmonella*-percentages (A1, A2, C1, E1) was "geographically" even (data not shown). On house level, there were significant associations between % *Salmonella*-positive samples and % non-sterile Rambach agar plates ($p=0.013$, Figure 1). However, these associations were not seen when specific sites were compared across houses

with *Salmonella*-positive samples subtracted from the % non-sterile Rambach agar plates (data not shown), i.e. the associations were site specific.

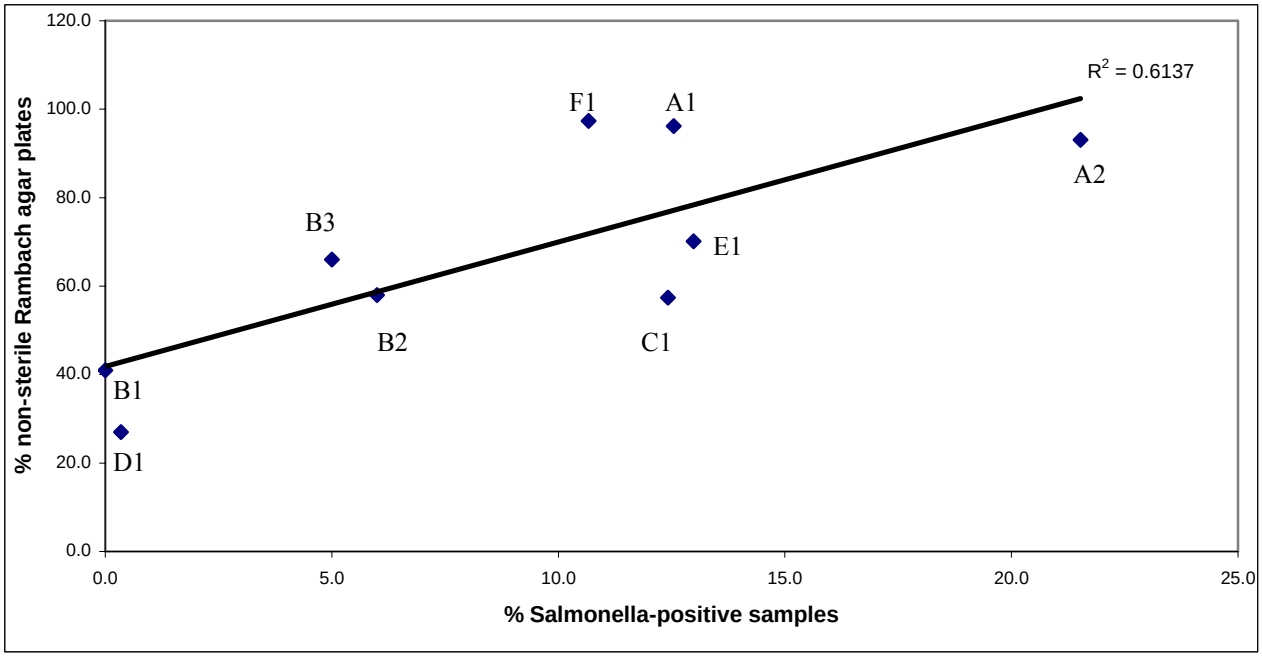

Figure 1: Relationship between percent *Salmonella*-positive samples and percent non-sterile Rambach agar plates before treatment for the eight houses of the study. House designations (cf. Table 3) shown in the diagram.

Salmonella samples after treatments. No *Salmonella* was found in any house except for six samples with *S.* Enteritidis in House A2, all of which were related to floors (data not shown). The reduction in non-sterile Rambach agar plates was highest in the five houses that were steam and formaldehyde treated (Table 4).

Table 4
Non-sterile Rambach agar plates before and after treatment.

House	Before treatment		After treatment		R^1	S^2
	No./total	%	No./total	%		
A1	278/287	96.9	94/288	32.6	3.0	a
A2	284/302	94.0	114/303	37.6	2.5	a
B1	41/100	41.0	3/102	2.9	14.1	b
B2	58/100	58.0	33/96	34.4	1.7	c
B3	68/100	68.0	8/100	8.0	8.5	cd
C1	180/298	60.4	2/308	0.65	92.9	e
D1	78/289	27.0	1/290	0.34	79.4	f
E1	224/308	72.7	9/308	2.9	25.1	d
F1	146/150	97.3	3/150	2.0	48.7	

[1]Reduction factor, i.e. the proportion between % non-sterile Rambach agar plates before and after treatment.
[2]Results from logistic regression. Houses with different letters are significantly different. No statistics made for House F1.

Physical parameters. In the air, 100% RH was accomplished 10-15 min after the steam treatment commenced, and it was maintained throughout the heat treatment period (data not shown). In tightly sealed houses, the temperature that was maintained during the 24-hour period was achieved within

the first hour, and this temperature was generally evenly dispersed both longitudinally and transversely in the house (data not shown). The main difficulty was to achieve the required 60 °C near the floor, but measurements at different heights near the floor indicated that the first ca. 10 cm were the most critical, as 60 °C was achieved at this height and the temperature changes were minor at higher levels (data not shown). In House E1, 60 °C was not achieved, probably because the ridge roof was not tightly sealed, so cold air from the outside was sucked into the house. In the same house, it also took 4-6 h before the target temperature was achieved (data not shown). In concrete holes with little space for the steam, the temperatures were generally ca. 3-5 °C lower than in corresponding measurements 2.5 cm above floor level, while similar temperatures were achieved where more room was left for the steam to enter (data not shown). Moreover, it normally took 9-10 h to achieve a stable temperature in the holes, regardless of their type (data not shown). Sealing of concrete floor holes generally lowered the mean temperature by ca. 10 °C (data not shown).

Challenge samples. The relevant bacteria survived in all the control samples. Nearly all *E. coli* in faecal samples were eliminated regardless of procedure and mean temperature whereas the three other challenge-sample types showed almost uniform reduction tendencies (Table 5).

Table 5
Results (positive/all samples, ≥1 positive sample illustrated by shaded areas) for challenge samples related to mean temperature.

Mean temperature (°C)	Feed								Faeces							
	Enterococcus faecalis				*Escherichia coli*				Enterococci				Escherichia coli			
	PI[1]	PII[2]	PI/II[3]	WF[4]	PI	PII	PI/II	WF	PI	PII	PI/II	WF	PI	PII	PI/II	WF
> 62.5	0/4	2/4	0/4	0/11	0/4	2/4	0/4	0/11	0/4	1/4	0/4	0/11	0/4	0/4	0/4	0/11
]60.0-62.5]	0/8	6/8	0/8	0/8	1/8	3/8	0/8	0/8	2/8	3/8	0/8	1/7	0/8	1/8	0/8	0/8
]57.5-60.0]	1/3	2/3	0/3	0/9	0/3	2/3	0/3	0/9	1/3	2/3	0/3	2/9	0/3	0/3	0/3	0/9
]55.0-57.5]	2/4	4/4	1/4	1/2	1/4	2/4	1/4	1/2	2/4	3/4	0/4	1/2	0/4	0/4	0/4	0/2
]52.5-55.0]				2/5				0/5				1/5				0/5
]50.0-52.5]	0/1	1/1	1/1	0/3	0/1	1/1	0/1	0/3	0/1	1/1	0/1	0/3	0/1	0/1	0/1	0/3
]47.5-50.0]	1/1	1/1	1/1	0/1	1/1	1/1	0/1	0/1	0/1	1/1	1/1	0/1	0/1	0/1	0/1	0/1
]45.0-47.5]	2/2	2/2	2/2	0/2	2/2	2/2	1/2	1/2	2/2	2/2	2/2	1/2	0/2	0/2	1/2	0/2
]42.5-45.0]																
]40.0-42.5]				1/1				1/1				1/1				0/1
]37.5-40.0]				1/1				1/1				1/1				1/1
]35.0-37.5]																
]32.5-35.0]																
≤ 32.5	1/1	1/1	1/1	3/3	1/1	1/1	1/1	1/3	1/1	1/1	1/1	1/3	1/1	0/1	0/1	0/3

[1]Period I (cf. Table 1) for Houses A1 and A2.
[2]Period II (cf. Table 1) for Houses A1 and A2. The mean temperatures in the left column are invalid for Period II.
[3]Period I and II (cf. Table 1) merged.
[4]Heat treatment with 30 ppm formaldehyde for Houses B1, C1, D1 and E1.

Generally, few bacteria survived above 60 °C, a tendency seen especially when 30 ppm formaldehyde was used. The use of 30 ppm formaldehyde seemed to lower the lethal temperature by 2-5 °C. The combination of a 24-hour steam treatment without formaldehyde followed by a short heating with formaldehyde added to the steam seemed to be as effective as adding 30 ppm formaldehyde to the steam at the beginning of the 24-h treatment. A short heating to ca. 60 °C with formaldehyde in the steam (Period II) was generally ineffective, as there were surviving challenge-bacteria at all temperatures.

Field studies, conclusions
In general, the results showed that a steam treatment of ≥60 °C and 100% RH during a 24-h period with the addition of 30 ppm formaldehyde at the beginning of the process was effective for the

elimination of *Salmonella* from naturally infected poultry layer houses and putative indicator bacteria in challenge samples. This temperature could be accomplished in tightly sealed poultry houses ca. 10 cm above floor level. As per October 2003, no *Salmonella* has been detected in any of the heat-treated houses. More detailed descriptions have been or will be published (Gradel *et al.* 2002, 2003b).

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UK experience with cleaning and chemical disinfection of persistently *Salmonella*-infected poultry houses

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Cleaning and Disinfection of Poultry Facilities in UK

Situations in which cleaning and disinfection is used

In most European countries housing for breeding flocks, rearing flocks and commercial meat production birds is operated on an all-in/all-out basis. This involves total clearance of the farm and decontamination of the houses before restocking. Most organic producers do not operate such a batch system and are not permitted to use many conventional disinfectants. Similarly, most cage layer units operate on a continuous production cycle with multiple ages either within individual houses or in different houses on the site. This creates a difficult situation in which to attempt decontamination of farms. In many countries, notably the USA, broiler houses are not cleaned between every batch of birds but only when the litter accumulates to a certain level or at set periods within the production cycle. Organisms such as *Salmonella*, and particularly *Campylobacter*, show a rapid reduction once the house is emptied but lower numbers of *Salmonella* may persist for long periods and may multiply if moisture is added when houses are re-stocked. Additionally, wildlife vectors, especially mice are capable of acquiring and multiplying *Salmonella* infection and harbouring the organism for many months. It is therefore essential to combine effective control of farm pests with cleaning and disinfection or the cost of disinfection will be wasted.

Construction of poultry farms

Most breeding and production buildings are composed of concrete floors, brick or concrete block dwarf walls and wood based cladding, beams, doors, partitions, etc. Older breeder houses have wooden nest boxes but modern farms have automatic nest boxes comprising metal and plastic fittings. Some older farms have 'earth' floors but this does not seem to make them more likely to harbour *Salmonella*. On the contrary, some of the newest, easy to clean metal structure houses appear to have greater problems of continuous contamination. Some older commercial turkey and duck farms have semi-open naturally ventilated buildings but most have been updated to a similar standard as those used for chickens, where flocks are totally enclosed and automated ventilation and lighting is used. This gives better control over the production environment as well as improved bio security. In the commercial broiler sector there is a tendency towards a smaller number of larger houses, requiring less labour. Many older buildings persist, however, because the economic conditions or planning permission problems have not permitted a major active replacement policy. Cage layer farms may be single or double storey and include a range of metal, fabric, wood and plastic fittings. Most houses are mechanically ventilated but some layer producers are successfully using automatically controlled natural ventilation, even in cage layer houses. Houses may be operated on a deep pit or manure belt system. Barn egg and free-range housing tends to be of similar wooden construction to broiler breeder farms, usually employing some form of automatic egg collection in larger flocks.

Effectiveness of cleaning

In general, in poultry meat production the standard of cleaning has improved dramatically in recent years. Moveable equipment is normally dismantled for cleaning outside the house; dust on beams and ledges brushed or blown down and litter or manure removed by mechanical loader. Floors may

then be brushed mechanically before power washing or power washed using plain water or water containing a detergent sanitiser, mild disinfectant such as a quaternary ammonium compound or ammonia. Any repairs should ideally be done before washing. There are still many older houses where the electrical system is not waterproof so cannot be washed. Oxidising disinfectants, chlorines or Chloramine T are used to sanitise water systems. A significant problem is failure to fully drain water systems or completely empty feeding systems prior to washing. Failure to effectively power wash high fittings may be a problem unless suitably robust inspection towers or ladders are used to gain suitable access and visibility. It is common to find that roof mounted air extractor fan chimneys have not been cleaned as baffles were closed when the washing was done. The turbulence involved in restarting fans can dislodge contaminated dust, which can drop down into the cleaned house when fans are restarted. This is a particular problem when open drinkers placed below vents become contaminated by this dust. Another problem is missed areas. The majority of the house surfaces may be well cleaned but small areas of heavy contamination remain. The significance and consequences of this residual contamination depend on its location and the effectiveness of subsequent disinfection. Thus, small areas of missed dust on beams and ledges may never come into contact with birds, so although *Salmonella* is present it may not represent a significant risk. In contrast, *Salmonella* persisting in feeding systems may initially multiply when houses are washed. In most cases levels would then fall due to competitive flora activity but if houses are re-populated quickly after washing then high levels of *Salmonella* may still be found in these environmental niches.

Other areas which are commonly missed are the undersides of doors, frames and ledges, undersides of struts on partitions, gaps in walls where old litter may accumulate, floor cracks and expansion joints and feed pipe reservoirs. It is also common to find substantial amounts of residual dust within stir fans and space heaters and this may also create a risk when such fans are turned on for the new flock.

Although large metal sided clear span houses are easier to clean than traditional wooden houses there are often problems of variable cleaning caused by operator fatigue and the psychological effect of having to clean a huge space with large numbers of fittings before moving on to the next house. Such houses may also have certain fittings which are relatively difficult to clean such as gable end cooling fans or roof exhaust fan baffles which accumulate dust and wash water. Drinker lines and pan feeders are cleaned in situ and it is difficult to ensure a high standard throughout. The pan feeders also accumulate water, which inactivates disinfectant applied subsequently so some farmers have drilled a small drain hole in the base of these.

What is essential is for a named responsible person to thoroughly check the cleaning before the power washing gang moves on. To do this it is necessary to let any mist settle and to carefully inspect the house using a high power torch, as house lights may not be bright enough. Any missed areas should be washed again. Once cleaning contractors get used to the idea that someone will be carrying out this detailed checking they will raise their standard in order to avoid being called back. If such checking is not done or poorly done then the standard may fall further as cleaning teams attempt to gain time and move on to the next farm.

Ante-rooms, Service Areas

It is common for service areas and storerooms to either be missed in the cleaning programme or cleaned to a lower standard. Although these areas are not in direct contact with the birds there is a risk that farm staff will pick up contamination if any *Salmonella* persists from the previous flocks and since hand hygiene has been identified as an important factor in keeping infection out of flocks then it makes sense to keep the service areas as clean as possible.

Equipment in laying houses

In some barn and free-range egg houses automatic nest boxes can be dismantled for cleaning outside the house but it is often not feasible to do this within the timescale so much of the equipment remains in situ. It is extremely difficult to clean thoroughly under these conditions and there is commonly a lot of splashing of contamination on to vertical surfaces when this is carried out. If it is not possible to fully dismantle equipment then washing as thoroughly as possible with an angled lance can be carried out but it is essential that these surfaces are fully dried before disinfection and more concentrated disinfectant than would be used for clean surfaces is used. In the case of cage-houses it is possible, but very time consuming, to fully clean all aspects of the cages, particularly in belt systems where all sides of belts may not be exposed. Where possible final cleaning should be carried out with egg and dung belts running and all aspects of the belts, scrapers, brushes, elevators and winding mechanisms included in the cleaning. A particular problem area is the drinker spillage trough or cups in cages, which accumulates a dusty scum and is difficult to access for cleaning. Power washing can blast out most of the organic matter but it is essential that these reservoirs are allowed to drain fully or completely dry before application of disinfectant.

When egg conveyors and dung belts run between houses there is a risk of contamination being re-introduced into the cleaned house, at least into the service areas. It is essential that staff are aware of this risk and it is best to carry out effective sampling in adjacent houses so that their *Salmonella* status is accurately known and measures to avoid cross-contamination put in place.

General moveable equipment

Equipment such as nest boxes, belting, astroturf, feeders, drinkers, hoppers, etc, is often dismantled and removed from the house for easier washing outside the building. This allows equipment to be manipulated so that all sides can be cleaned more easily but it is essential that this work is co-ordinated with the overall cleaning of the houses or there may be a risk of recontaminating previously cleaned and disinfected areas or equipment by spray drift from elsewhere on site or from outside the building via power washing aerosols. Moveable equipment may receive a preliminary disinfection outside the house but it is essential to incorporate it in the overall house disinfection. Items which tend to be poorly cleaned are the wooden bases of nest boxes in older breeder houses and weighers, where substantial amounts of organic matter may accumulate under weighing platforms and between stabilising weights and framework. An important item of equipment which is moveable but which is usually left in the house is the slave feed hopper and its motor units. It is important to remove all traces of feed from this before washing and to wash thoroughly to remove residual material in lodged in the machine. The cleaning equipment itself, including power washers, brushes, scrapers, ladders and protective clothing can also become contaminated so it is important that these items are included in the cleaning and disinfection effort.

Areas outside the houses

Decontamination of areas outside the house is often overlooked or left until late in the cleaning process at which point it could provide a recontamination hazard. Ideally dust on fan outlets should be vacuumed, blown or washed down from the outside of the house, although this may not be possible in the case of some roof mounted vents on fragile roofs. At the point when houses have been washed litter that has escaped from houses under doors and fan dust, washings solids, etc, should be gathered up and safely disposed of.

Manure pits
Mini-pits in breeder, barn and free-range laying houses are mostly emptied, cleaned and disinfected at the same time as the rest of the house is cleaned. This is not always true of deep pit cage layer houses however and these may be emptied according to needs for manure, such that most pits on site are emptied at the same time regardless of the state of occupancy. Although the material in pits is separated from the birds it may act as a source of contamination for farm pests, which may travel, to the occupied part of the house. It is therefore recommended that when *Salmonella* is present that pits are emptied and disinfected at the same time as the house. A further complication is that flock owners will often not remove all manure from deep pits in order to preserve populations of litter beetles whose larvae ingest fly eggs and first stage larvae. Fly control in deep pit houses is difficult because of the continuously available breeding sites but when *Salmonella* is present it would be preferable to totally clear the manure and litter beetles then bring in some manure from a *Salmonella* and disease-free flock to re-establish the beetle population.

Disinfection of poultry houses

Application of disinfectant
For disinfectants to work they need to come into contact with the target organisms at sufficient concentration for sufficient time to either eliminate the organism or to reduce it to a level which is no longer a risk. In the case of *Salmonella*, which is an organism, which multiplies in the environment under suitable conditions, it is best to aim for total elimination, which is often not achievable in practice. What is essential is to aim for complete coverage of disinfectant, which requires a very thorough approach to ensure that all surfaces have been treated. It is not possible to do this effectively if lighting is poor or if surfaces are already wet so the margin of the disinfected area cannot be easily seen. Foaming disinfectants are sometimes proposed as a solution to this problem but although some of these may be reasonable surface disinfectants the foaming process may reduce penetration of thick organic matter, cracks and crevices and in general it is preferable to use a liquid disinfectant. Similarly, some farms may mix disinfectant with Diesel oil in an attempt to improve its persistence, particularly on earth floors. This practice is counterproductive as some disinfectants, particularly phenols which are the disinfectants most often applied in this way, partition into the oily layer which again reduces penetration into moist material.

For effective disinfection in poultry houses it is usually necessary to allow some drying time between washing and disinfection. Drying is slower in cold weather but house fans and space heaters can be used to speed this up and the time can be used for replacement of washed equipment prior to the main disinfection. Application of spray disinfectant is normally done at medium pressure through a power washer. On some farms an orchard sprayer is driven through the house but this produces a less controllable effect and some shadowing as the spray can only be applied in a limited number of directions. For less accessible areas such as ventilation ducting and automated nest boxes it may be preferable to apply disinfectant at higher pressure so that the rebound effect helps to distribute the spray. Where possible moving equipment such as belting, should be disinfected whilst static and again whilst operating so as to most effectively cover all surfaces. Small items can be dunked in tanks of disinfectant for effective coverage. If structural defects and difficult to clean areas are present these should receive extra disinfectant. Ante-rooms and areas around external entrances to houses should be disinfected also. It is important to start at one end of the house and work systematically through, ensuring that all sides of all fittings and equipment are sprayed to saturation point. It is common to find insufficient volume of disinfectant has been calculated to achieve full saturation so some areas remain lightly treated. It is preferable therefore to work out the required volume for a specific house on an empirical basis, allowing a safety margin, rather than relying on calculated volumes. It is also vitally important to use the disinfectant

at sufficient concentration. Commercial disinfectants come with a range of suggested application concentrations, most of which are too dilute to effectively eliminate *Salmonella*. There is commercial pressure to sell disinfectants, which appear to work at low concentration. Not only do these appear to be financially attractive but may give an impression of effectiveness, which is not justified. In the UK there is an approval procedure for disinfectants for use against avian viruses, general bacteria and tuberculosis (mycobacteria). This approval can be a reasonable guide to the choice of commercial disinfectants, e.g. a disinfectant, which inactivates mycobacteria at low concentration, is likely to penetrate organic matter reasonably well. It is however advisable to seek additional guidance since some disinfectants may perform well in the approved test but not so well under field conditions. In the UK, the Department for the Environment, Food and Rural Affairs (Defra) General Orders concentration is usually recommended for use where *Salmonella* is present, but when surfaces are difficult to clean, building structures are deteriorating or it is not possible to fully dry houses after disinfection then the higher TB Orders concentration is more appropriate. Some farms may use a reduced concentration of disinfectant but carry out two applications in order to try to compensate for missed areas. This is not recommended since when disinfectant of insufficient concentration is applied the associated moisture can encourage the multiplication of *Salmonella*, rather than its destruction.

Choice of disinfectant

There has in recent years been a move away from formaldehyde and formaldehyde based disinfectants because of human health and safety concerns. Much of the adverse publicity has been generated by disinfectant companies who can see formaldehyde as a cheap and effective rival to their compound formulations. Data which demonstrates a real risk to people exposed to formaldehyde is also lacking, for example embalmers, anatomists and others working with formaldehyde have not shown an increased risk of disease. In general formaldehyde, applied as a 2% dilution of commercial formalin solution, has a far superior effect to commercial disinfectants when applied to poultry houses, where some residual organic matter is inevitable. It is claimed that formaldehyde does not work in cold weather but in fact it only works more slowly and still gives a superior effect compared with other disinfectants when left for 24 hours. Part of the excellent activity of formaldehyde is due to its superior penetration and resistance to inactivation by organic matter and part to its fuming effect, which can reach more inaccessible areas in the house. The fumes are what is also most problematic with formaldehyde since exposure of workers may occur by this route. Formaldehyde spray must therefore always be applied by trained operators wearing appropriate protective clothing and breathing apparatus, but it can be applied safely by thermal fogging following spray disinfection with another disinfectant. There are also combination products containing formaldehyde, glutaraldehyde and quaternary ammonia compound (QAC) which can be handled without such stringent precautions. Phenols are the next most active class of disinfectants, and these are widely used in the UK. There are however increasing concerns about the use of phenols and their possible escape into the environment which may limit their use in future but when carefully applied to dry surfaces there is little run-off and this can be easily contained. There are also objections to using phenols because of their smell and discolouration of white surfaces but there are more highly purified products which are more pleasant to work with.

In moving away from formaldehyde and phenols the choice of disinfectant for effective *Salmonella* control becomes more problematic. Other commonly used products are based on glutaraldehyde, glutaraldehyde/QAC, peroxygen compounds (typically blends of hydrogen peroxide and peracetic acid), chlorine generators such as chlorine dioxide or Chloramine T, or QACs. All of these are relatively easily inactivated by organic matter, so depend on a good clean. Chloramine T and some of the peroxygen products can be used in water lines and work well in this context. Chloramine T has also been used as a mist in occupied poultry houses to reduce airborne microbial contamination.

Since these products can be inactivated by organic matter it is usually necessary to apply them at higher concentration to help compensate for this. This obviously increases the cost but also in the case of the peroxygen adds significantly to their corrosiveness and consequent oxidative damage to steel based equipment.

Since resistance to disinfectants is not an issue in *Salmonella* it is not necessary to rotate disinfectants in order to minimise this. It is much more important to maintain an effective disinfection programme so that organisms do not survive to be perpetuated in sequential flocks or disseminated more widely. When *Salmonella* is not present then it is possible to operate a disinfection programme, which is more orientated towards viral pathogens, which are more easily eliminated than *Salmonella*. If *Salmonella* is found on a farm then it will often be found too late to influence the disinfection carried out at the end of the infected flock but with timely results, eg. from mid-crop sampling in broiler production, it should be possible to step up disinfection and monitoring after disinfection and restocking to ensure that decontamination has been successful.

When considering choice of disinfectants then, in the absence of *Salmonella,* a glutaraldehyde based regime would be acceptable, except that glutaraldehyde may in reality be more risky to work with than formaldehyde because it is not so noxious. A peroxygen disinfectant can be used but this may be excessively corrosive when used over consecutive crop cycles. Chloramine T is a reasonable choice if phenols cannot be used. Where *Salmonella* has become established then it is best to make a decision to use highly effective chemicals at an effective concentration at the start of the problem. In non-egg layer houses then primary disinfection with a phenolic disinfectant followed by fogging with formaldehyde works well. Comprehensive spraying with formaldehyde, carried out by a specialist contractor, also works well, as does careful application of a formaldehyde, glutaraldehyde, QAC combination disinfectant. In the case of egg laying flocks phenols cannot normally be used because of the possibility of egg taint and relying on fogging, even with formaldehyde, does not work because of shadowing preventing thorough and comprehensive application of the disinfectant. Laying houses should therefore preferably be treated by application of formaldehyde spray.

In making the choice of disinfection programmes for entire poultry companies it is surprising that little in-house testing is used and the choice is highly influenced by promotional material. All commercial poultry companies experience some *Salmonella* contamination from time to time and this offers an excellent opportunity to evaluate the performance of proposed disinfectants in a real-life setting. It is also relatively easy to set up simple surface contamination tests in a commercial laboratory to compare products in parallel. This too would give a much more informed decision on choices of disinfectant products since the currently used approval tests do not adequately simulate conditions necessary to eliminate *Salmonella* from surfaces coated with biofilms or residual organic matter.

Fogging or fumigation

Fumigation involves the release of a vapour, normally formaldehyde, by the activity of potassium permanganate on formalin solution or by heating paraformaldehyde prills. This is a very effective way of decontaminating items in a small air space such as an egg fumigation chamber in a hatchery but is not suitable for large buildings. Thermal fogging, which is often confused with fumigation uses a micromist generator to create a mist of airborne particles which can disperse throughout the house and coat surfaces with disinfectant as they settle. As with spray disinfection there should be a drying period between disinfection and fogging and the volume of fogging agent should be designed to re-saturate surfaces. Fogging is popular in UK as a means of applying a second disinfectant as insurance against errors in the primary disinfection and to increase the diversity of

organisms covered. Unfortunately, fogging is often carried out immediately after spray disinfection so has little additional benefit and there may even be antagonism between certain products if they are both applied virtually at the same time. In some cases washing, disinfection and fogging are all carried out on the same day, litter laid the next day and new birds in the following day. This is a situation where *Salmonella* is most likely to persist between flocks as even the drying stages alone produce a significant reduction in organism counts.

There are distinct limitations to fogging because of shadowing effects so inaccessible surfaces may be insufficiently treated, especially if non-fuming products such as peroxygen compounds are used. The fuming effect of formaldehyde achieves a greater coverage but sufficient volumes and concentrations must be applied. This is problematic in large houses since a single fogging machine may produce insufficient coverage and fogging alone, as occurs in some cage layer houses, even with formaldehyde is unlikely to achieve the penetration needed to eliminate *Salmonella* in residual organic matter. One common fogging error is to seal the house by closing exhaust vents without fully cleaning and disinfecting behind the vents, which means that these areas are not disinfected. It is however often possible to fog in many houses where electrical systems are not fully waterproof, which is advantageous in older houses.

Recontamination of disinfected houses

During cleaning and disinfection there is usually a large amount of activity to schedule against tight deadlines. Under these circumstances it is not uncommon to find breeches in biosecurity of the disinfected house, which can result in recontamination. Examples of this are failure to replace and use disinfectant footdips or dedicated protective clothing at completion of disinfection; feed left in feeding systems in several areas of the house or repairs carried out to the house after disinfection. This latter may release contaminated material from previously unexposed areas, e.g. beneath cladding or within casings, or may introduce new contamination on contractors' equipment which may have been used in other poultry houses. We have found the bases of contractors' toolboxes and footwells of their vehicles to be regularly contaminated with *Salmonella*. Residual contamination in service areas and stores may be carried back into the house on workers' hands or underneath equipment and *Salmonella* in pockets of litter and washings outside the house may contaminate arthropod vectors as they enter the house from outside. Any vehicles used for delivery of chicks which enter the house can re-introduce contamination, both from other farms and by driving through contaminated material left outside the house. The extent of this contamination may not always be apparent until wet weather when *Salmonella* can emerge from niches in ground surfaces to contaminate standing water.

On continuously occupied organic and multi-age laying farms it is difficult to control recontamination since infected flocks may remain close to newly introduced flocks and transfer by dust or farm pests may occur even if there is no direct connection between houses. Many laying farms have shared egg conveyor and dung belts, which may circulate contamination in the service areas of the houses and act as a convenient route for mice and other rodents. In this situation it is very difficult to break the cycle of infection and re-contamination without much higher cleaning, disinfection and biosecurity standards than those currently in place. In this situation vaccination for important *Salmonella* serotypes such as *S.* Enteritidis has a part to play as it can increase the infection threshold so that infection of flocks from indirect contamination, such as dust or low-level environmental contamination is less likely. Vaccination therefore can assist with elimination of *S.* Enteritidis from farms, and this has been particularly successful at broiler parent level when all-in/all-out production and reasonable cleaning and disinfection programmes with sufficient 'downtime' are usual. There is a danger however that in continuously occupied laying farms vaccination may merely suppress excretion of *S.* Enteritidis so that it is more difficult to detect by

the monitoring schemes currently in place. If farm owners are unaware that infection is present it is unlikely that they will apply sufficiently rigorous cleaning, disinfection and pest control routines to eliminate *Salmonella* and will not take sufficient precautions to avoid spreading infection between houses. Ideally each house should have its own dedicated boots, overalls and gloves, which are always used for, work in that house. There should also be good facilities in each house to wash and disinfect hands or as a minimum use an alcohol based hand sanitiser as contaminated hands are probably underestimated as a source of contamination. Such measures are time-consuming however and it may be preferable on a risk basis to totally eliminate *Salmonella* from the whole farm and then concentrate on improved monitoring and enhancing the bio security barrier between the farm and its environment and suppliers. In the case of Campylobacter, however, each house must be treated as an individual bio secure unit.

The role of farm pests

Mice

Numerous publications from the USA and UK have demonstrated the role of mice in maintenance of *Salmonella* on poultry farms. Some evidence suggests that in some instances the appearance of mice may be associated with breakdowns in *S.* Enteritidis in previously negative broiler parent or broiler flocks, but it has been difficult to prove this conclusively.

It is not possible to control all mice all of the time and the occasional transient mouse may be seen. These are usually not a significant risk but when a breeding population of mice is allowed to build up what may be a short-term low-level infection in an individual mouse develops into a chronic infection of the breeding colony in which a high proportion of individuals are infected at any one time. These may excrete individual faecal pellets, which contain up to a million *Salmonella* organisms and, although *Salmonella* levels vary in individual pellets, over 100 of these may be produced per day. Mice also dribble urine continuously and it is possible that this too may contain *Salmonella* in systemically infected individuals. Mouse faecal pellets are quite attractive to birds and will be readily consumed when present in a feeder. This combination of high levels of excretion plus the ability to contaminate feeders with faecal pellets containing *Salmonella* makes mice the most dangerous source of persistent infection and transmission between closely related houses. It is also likely that *Salmonella*, which has been passaged through infected mice, may have virulence genes up regulated, and so be more infectious than organisms persisting in the environment.

Control of mice depends on careful observation for signs of infestation such as droppings, tracks in the dust on ledges, urine pillars, grease marks and disturbed bait. Canary seed based bait is usually most readily consumed but it must be replenished at least weekly as it soon becomes dusty, stale and unattractive. Gloves should also be used when handling baiting points so the 'human' smell is reduced. It is also essential to have enough baiting points well distributed around the interior of the house, particularly in areas where birds do not go such as storage and service areas. Mouse problems are particularly marked where there are deep-pit or mini-pit systems and bait should be placed in these pits either manually or, where there is no direct access, by 'posting' envelopes of bait between slats. it is also worth spending time sitting quietly in the house outside working hours to observe patterns of mouse activity so that baiting can be focussed in problem areas of the house. It is also very important to do this in empty houses as signs of rodents can be much more easily seen when birds have been removed. Where there are large populations of mice to deal with then measures to supplement traditional baiting such as contact rodenticides, traps and water bait can be used. Contact rodenticide tubes are also useful in situations where baiting is difficult amongst birds.

When rodents are present the most important time to intensify baiting is at depopulation. All feed should be removed as soon as possible and baiting points placed close to feeders. After cleaning and disinfection clean baiting points should be replaced and a careful check made with a powerful torch for signs of rodent activity.

Rats

Rats may be a problem on laying farms but are a less potent source of *Salmonella* than mice as they are less easily infected and do not harbour systemic infection for so long. Also, faecal pellets are less frequently deposited in feeders. If large populations of rats build up they can be involved in *Salmonella* recycling however and so it is essential to control their numbers. Rats respond best to a rotation of baiting formulations since this stimulates their natural inquisitiveness. In the USA cats are used within cage laying houses to deter rodents and this can be quite successful but cats may also harbour and transmit pathogens so are difficult to recommend in European bio security programmes.

Arthropods

There have been numerous publications discussing the capability of arthropod pests such as litter beetles, cockroaches, flies and red mite in relation to carriage of *Salmonella*. Experimentally these can carry the organism for varying periods of time and can be shown to be capable of transmitting high levels of artificial contamination to birds. In field situations high levels of *Salmonella* can be found in flies in heavily infected cage layer houses but significant contamination of litter beetles is unusual. It is difficult to differentiate contamination of red mites from general environmental contamination but the continual presence of mites or litter beetles in previously infected houses does not seem to have prevented clearance of *Salmonella* when cleaning and disinfection and rodent control has been adequate. Populations of litter beetles are deliberately maintained in deep-pit houses to assist with fly control and when properly managed this probably represents a lesser risk than large fly populations, which may more readily move between houses. As with all farm pests the priority is to avoid the build up of populations to critical levels by appropriate hygiene and insecticide measures.

Conclusions

Effective cleaning and disinfection of poultry farms depends on attention to detail, both in assessing the level of risk by effective monitoring of *Salmonella* status and ensuring that correct decisions are made in terms of the choice of disinfection methods which are fit for purpose, and on their correct application.

Chemical disinfectants commonly used in the poultry sector and their impact on *Salmonella* – an outline of methods and results

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Introduction

The conditions involved in the efficacy of chemical disinfection can be grouped into three main categories: the micro-organism, the disinfectant, and the surroundings. Within each of and between these, a plethora of conditions determines the outcome, e.g. type of micro-organism, temperature, disinfectant concentration, amount and type of organic matter, just to name a few. Most disinfection research has been related to conditions encountered in food enterprises and hospital wards, but results from these studies are difficult to extrapolate to the agricultural sector where other conditions prevail. Here, there is a conspicuous lack of scientific research, and the main source of information is often commercial.

In the Danish poultry sector, *Salmonella* persistence is at present the main problem (Gradel and Rattenborg 2003) as the *Salmonella* control programmes have successfully reduced the number of new infections (Wegener *et al.* 2003). This persistence can be due to many factors, e.g. rodents, insects or lack of hygiene barriers, but elimination of *Salmonella* in the house itself is of paramount importance. The two projects reported here evaluated this topic from different angles. A few types of disinfectants are intensively used in Danish poultry houses. Theoretically, this could favour the development of resistance to disinfectants, which could be contributory to *Salmonella* persistence. However, such studies alone may be unrealistic, as they do not simulate conditions encountered by microorganisms in real-life situations. Therefore, surface disinfection studies mimicking real-life situations were also performed.

Resistance to disinfectants, aims

There were three main aims of this study:

To find minimum inhibitory concentrations (MICs) of five disinfectants for "non-persistent" and "persistent" *Salmonella* serotypes commonly isolated from Danish broiler houses and to relate these to serotype, persistence and use of disinfectants.
To find MICs of five disinfectants for other *Salmonella* serotypes mainly isolated from poultry enterprises.
To perform adaptation and de-adaptation studies with the five disinfectants for selected isolates having high or low MICs to see if disinfectant resistance was developed and maintained.

Resistance to disinfectants, materials and methods

Bacteria. Table 1 shows the bacterial isolates used in the study, and Table 2 illustrates the epidemiological background in Danish broiler houses, as the somewhat arbitrary designations "persistent" and "non-persistent" serotypes (cf. Table 1) could not be used for statistical evaluations, so more strict definitions were needed. Both "non-persistent" and "persistent" serotypes that had persisted in several or in a few crops, respectively, were included in the study. From most of the houses shown in Table 2, two or more isolates were selected, representing both the beginning and the end of the persistence period; a total of 67 and 21 broiler houses were represented with two or more than two isolates of the same serotype, respectively.

Table 1: Sources of bacterial isolates

Country	Type	No.	Source and description
Denmark	*Salmonella* (*S.*) Enteritidis	34	Danish broiler houses, "non-persistent type"
Denmark	*S*. Typhimurium	39	Danish broiler houses, "non-persistent type"
Denmark	*S*. Tennessee	24	Danish broiler houses, "non-persistent type"
Denmark	*S*. 4.12:b:-	81	Danish broiler houses, "persistent type"
Denmark	*S*. Infantis	61	Danish broiler houses, "persistent type"
Denmark	*S*. Indiana	17	Danish broiler houses, "persistent type"
Denmark	*S*. Senftenberg	13	Poultry sector
UK	*S*. Choleraesuis NCTC 10653	1	Strain used in English disinfection tests
UK	*S*. Typhimurium, DT104	8	Pig and broiler farms, b/a[1] disinfection with phenol, formaldehyde or peroxygen
UK	*S*. 4.12:d:-	4	Feed mill and hatchery, b/a disinfection with formaldehyde
UK	*S*. Senftenberg	4	Hatchery, b/a disinfection with formaldehyde, glutaraldehyde or QAC[2]

[1] Before or after.[2] Quaternary ammonium compound.

Table 2: *Salmonella* serotypes from Danish broiler houses used in this study: numbers of houses and crops with the serotype (period 3/1/92-2/10/01)

Salmonella serotype	Number of crops with the same *Salmonella* type								
	1	2	3	4	5	6-10	11-20	21-30	> 30
Enteritidis		5[1]	6	4		2			
Typhimurium	2	7	4	3	1	2	2		
Tennessee		4	4	1	1	2			
4.12:b:-	1	3	2	2	4	6	4	5	1
Infantis		9	2	3	3	6	4		
Indiana	1	1	2	1	1	3			

[1] *Numbers of broiler houses.*

Disinfectants. A glutaraldehyde/benzalkonium chloride compound, formaldehyde and an oxidising compound were used most commonly for disinfection of broiler houses (38.8, 32.4 and 14.9%, respectively). In the UK, phenols were used commonly in poultry houses, whereas iodophors were used mainly for water systems, foot dips and for general disinfection (R. Davies, pers. comm.). Therefore, the following three "Danish" and two "English" disinfectants were chosen for this study: a glutaraldehyde (23% v/v) and benzalkonium chloride (5% v/v) compound (Bio Komplet® Plus and the corresponding pH-regulator (KOH and H_3PO_4)), formalin (24.5% v/v formaldehyde), an oxidising compound (Virkon® S), a high boiling tar acid phenol compound (Farm Fluid S®) and an iodophor (FAM 30®).

MIC-tests. On the day of performing the MIC-tests, disinfectant solutions in sterile ionised water were prepared and used for double dilutions. Vials of double strength agar were melted, mixed with disinfectant dilutions and poured into Petri dishes. From each isolate, 20 µl broth with an overnight stationary culture and 180 µl physiological saline were mixed in a micro titre well. A multiple-point inoculator was used to inoculate the agar plates incubating 18-24 hours at 37 ^{o}C. Growth was interpreted as at least 90% of full growth (as seen on a control agar plate). For all *Salmonella* isolates, the tests were performed at least in duplicate on different days. *E. coli* control strains were included in each batch to check for deviations between these.

Adaptation and de-adaptation tests. Six isolates, three with high and three with low MICs, were used for adaptation and de-adaptation tests which were performed in duplicate, each involving one of the five disinfectants. Initially, 0.10 ml broth with the isolate grown to stationary phase was passaged to broth with a disinfectant concentration half the lowest recorded MIC incubating overnight at 37 ^{o}C. Each consecutive day, the disinfectant concentration in LB broth was increased by a factor 1.5, and a 0.10 ml inoculum from the LB broth grown the previous day was passaged to

this. The passages ceased when no turbidity and no growth on blood agar (plated from the broth) were observed. Then, MIC-tests were performed as described above. Afterwards, broth was passaged to broth without disinfectant during six consecutive days, after which the MIC-tests were repeated.

Resistance to disinfectants, results

MIC-tests. The results are seen in Table 3.

Table 3: Microbial inhibitory concentrations (MICs) of isolates

Country/ isolate[1]	Formaldehyde				Bio Komplet Plus					Virkon S				Farm Fluid S				FAM 30			
	MIC (x 10^{-3})[2]				MIC (x 10^{-3})					MIC (x 10^{-3})				MIC (x 10^{-3})				MIC (x 10^{-3})			
	4	8	15	30	15	30	60	125	250	30	60	125	250	15	30	60	125	60	125	250	500
DK/Ent		34[3]					14	20			6	22	6	1	12	21		4	9	21	
DK/Typ		39					20	17	2		12	24	3		19	20		7	15	17	
DK/Ten		9	8	7			6	16	2		1	7	16		4	20			2	22	
DK/4.12:b:-		66	15				12	68	1		1	58	22		17	64		1	13	66	1
DK/Inf	1	60					18	43			6	37	18		29	31	1		17	37	7
DK/Ind		16	1				11	6			7	9	1		16	1			14	3	
DK/Sen			12	1			5	8				10	3		1	12			10	3	
UK/Chol		1						1			1				1				1		
UK/DT104		8						7	1			8				8			2	6	
UK/4.12:d:-		4						4				4				4				4	
UK/Sen				4				4					4			4					4

[1] *Cf. Table 1.*
[2] *ml/100 ml, except g/100 ml for Virkon S.*
[3] *No. of isolates. Within each disinfectant, serotypes with bold/underlined numbers have significantly higher/lower MICs than the other serotypes (MICs merged for statistical analyses).*

Among the isolates from Danish broiler houses, *S.* Tennessee had significantly higher MICs to formaldehyde, Virkon S and FAM 30, and *S.* 4.12:b:- had to Bio Komplet Plus, Farm Fluid S and FAM 30. Thus, higher MICs were found for both a "non-persistent" and a "persistent" serotype and included "English" disinfectants. *S.* Senftenberg, both from DK and the UK, generally had high MICs, except for Danish isolates to FAM 30. The MICs of all the English isolates were the same before and after disinfection.

Disinfectants were compared pairwise to deduce putative associations for isolates. Only Bio Komplet Plus vs. FAM 30 (χ^2 = 1.96, Cohen's kappa = 0.59) and Farm Fluid S vs. FAM 30 (χ^2 = 0.31, Cohen's kappa = 0.60) showed significant associations (data not shown).

For each of the 67 and 21 broiler houses with two or more than two isolates, respectively, increases and decreases in MICs during the persistence period were recorded. For all five disinfectants merged there were 93 increases and 82 decreases, i.e. no significance, and none of the five disinfectants deviated significantly from this (data not shown).

For the three "Danish" disinfectants (formaldehyde, Bio Komplet Plus, Virkon S), cross-tabulations between their use in the preceding download period and merged MICs (see above) were made, but no significant differences in MICs were seen between crops using or not using the actual disinfectant (data not shown).

Adaptation and de-adaptation tests. In broth, growth ceased at concentrations up to ca. 13 x MIC. This was, however, not reflected in similar high MICs on agar after adaptation, where all isolates except one were within one double dilution compared to the MICs of the parent isolates (data not

shown). After de-adaptation, no changes beyond one double dilution compared to the MICs of the parent isolates were seen (data not shown).

Resistance to disinfectants, conclusions
There were generally little variations in MICs to commonly used disinfectants, and these could not be related epidemiologically to *Salmonella* persistence or use of disinfectants. Adaptation to or de-adaptation from these disinfectants did not alter the MICs significantly. More detailed literature exists (Gradel 2003; Gradel and Randall 2003).

Surface disinfection tests, materials and methods
General principles. Surface disinfection tests that mimicked worst-case scenarios encountered in badly cleaned poultry houses, often at low temperatures, were performed. High numbers of stationary phase bacteria (*Salmonella* (*S.*) Enteritidis, *S.* Senftenberg or *Enterococcus* (*E.*) *faecalis*) were spiked in organic matter (feed, fat or egg yolks), which was either smeared on the surface of the materials (feed on concrete flags) or the materials were immersed in the organic matter with bacteria (feed chain links, wooden dowels, jute egg belts). Materials with spiked organic matter were then dried for 24 hours at a set temperature. Thereafter, they were immersed in a 1% disinfectant solution (formaldehyde, glutaraldehyde/benzalkonium chloride or oxidizing compound) or water (control) at exact time periods after which they were re-dried 25 hours at a set temperature. For the bacterial detection, all materials with organic matter were immersed in enrichment broth (10^0 dilution) used for 10-fold dilutions until 10^{-4}. During the incubation period, all materials were kept in the 10^0 dilutions. Traditional bacteriological procedures were made for all dilutions, i.e. a most probable number (MPN) method was used.

Bacterial strains. *S.* Enteritidis, phage type (PT) 8, was the most common type in Danish persistently *Salmonella* infected table egg houses. Moreover, this isolate had relatively low MICs (cf. above). *S.* Senftenberg was chosen because this serotype had relatively high MICs (cf. above). *Enterococcus* (*E.*) *faecalis* was used in some series with the worst conditions to test its use as an indicator organism for future field studies.

Poultry house materials and organic matter. Four types of materials (concrete flags, non-stainless steel feed chain links, wooden dowels, jute egg belts) and three types of organic matter (feed for layers, rapeseed oil, egg yolk) were used. These materials are often found in poultry houses, and they have rough surfaces that are difficult to clean and disinfect properly. Only some combinations of materials and organic matter (concrete flags/feed, feed chain links/fat, feed chain links/feed, wooden dowels/fat, wooden dowels/feed, jute egg belts/egg yolk) were included in the study.

Preparation of spiked organic matter. Isolates were grown until stationary phase in broth and diluted 1:10 in buffered peptone water, which was further diluted in sterile ionised water, 1:10 or 1:100 to obtain high or low cfu, respectively, in the organic matter. Feed for layers was added to this mixture in the proportion 1:2 (w/v). The same mixture was used for spiking egg yolks. For each sample, two egg yolks were mixed with an equal volume of the mixture. Rapeseed oil with a melting point of 42 ^{o}C was mixed directly (300:1) with the bacterial broth.

Inoculation of materials with spiked organic matter. All materials were sterilized before each test series. For concrete flags, 10 or 20 g spiked organic matter was distributed evenly with sterile scrapers on an area of 20 x 20 cm. All other materials were immersed in the relevant spiked organic matter, shaken and left for varying time periods, after which they were placed for drying on stainless steel trays.

Incubation before and after disinfection procedures. Before and after disinfection, all materials with spiked organic matter were placed in a cabinet, set at pre-determined temperatures. All materials remained in the cabinet exactly 24 and 25 hours before and after the disinfection procedures, respectively.

Disinfection procedures. Formalin (24.5% v/v), Bio Komplet Plus, Virkon S (cf. MIC-studies, see above), and water (control) were chosen for the study. Standardised hard water was used both for controls and for disinfectant solutions, all of which were 1% and made on the day of use. Two hundred ml disinfectant solutions were used for one concrete flag, three wooden dowels or one jute egg belt piece, whereas a 250 ml solution was used for two feed chain link pairs, except in one series (cf. Table 5). All materials with spiked organic matter were immersed in disinfection solutions at exact time periods (cf. Tables 4-7) before being re-dried.

Microbiological procedures. All materials with spiked organic matter were immersed individually in pre-enrichment broth, *Salmonella* in buffered peptone water (BPW) and *E. faecalis* in Enterococcus broth. Materials were left in the broth for varying time periods (30-120 min, depending on material/organic matter combination) before dilution so organic matter could be dispersed in the broth. After these time periods, tenfold dilutions were made until 10^{-4}, using BPW and Enterococcus broth for *Salmonella* and *E. faecalis*, respectively. All pre-enrichment broths were used for traditional bacterial isolation procedures.

Statistical analyses. The point system (cf. Table 4) was used for pairwise comparisons in t-tests with unequal variances (two-sided p). Moreover, categorical data for 2x2 tables were obtained by using different cut-off values, focusing on 0 vs. >0, as this reflects traditional bacteriological tests. Ninety-five per cent significance limits were used in all tests.

Surface disinfection tests, results
Tables 4-7 show the results.

Table 4: Results for concrete flags inoculated with feed for layers (disinfection conditions deteriorating towards the bottom of the table)

cfu[1]	GPF[2]	TB[3]	TA[4]	DT[5]	*S.* Enteritidis				*S.* Senftenberg			
					F[6]	B[6]	VS[6]	WHO[6]	F	B	VS	WHO
Low	10.0-10.5	20.2 (±0.2)	10.9 (±0.5)	30	0[7]	0	0	1	0	0	0	2
Low	20.0-20.3	20.2 (±0.2)	10.9 (±0.5)	30	0	0	4	ND[8]	0	0	0	2
Low	20.0-20.3	10.9 (±0.5)	10.9 (±0.5)	30	0	0	3	3	0	0	2	2
Low	20.0-20.3	5.9 (±0.5)	5.9 (±0.5)	30	0	1	3	5	0	0	1	1
Low	20.0-20.3	5.9 (±0.5)	5.9 (±0.5)	15	0	0	2	4	0	0	2	1
High	20.0-20.3	5.9 (±0.5)	5.9 (±0.5)	15	0/0	0/1	4.5[9]/4	5/5	0	0	2	2
High	20.0-20.3	5.9 (±0.5)	5.9 (±0.5)	5	0	2	4	5	0	1	2	5

[1]low = ca. 4 x 10^5-6 x 10^6 g^{-1} organic matter; high = ca. 4 x 10^6-6 x 10^7 g^{-1} organic matter (Gradel et al., 2003).
[2]g organic matter per flag. [3]Mean temperature (± deviations merged for all series) (^oC) during 24-h period before disinfection. [4]Mean temperature (± deviations merged for all series) (^oC) during 25-h period after disinfection. [5]Disinfection time (minutes). [6]F = formaldehyde; B = Bio Komplet® Plus, VS = Virkon S®, WHO = WHO standard hard water (control). [7]0 = no growth in any dilution (in the range from 10^0 to 10^{-4}); 1 = only growth in 10^0; 2 = only growth in 10^0 and in 10^{-1}, etc. Replicate results written in the same row, separated by /. [8]Not done because MSRV plates crystallized during incubation; no growth was seen either on Rambach agar plates when streaking from MSRV plates. [9]x.5: no growth in dilution x-1, but in dilution x.

Table 5: Results for feed chain links inoculated with either fat or feed for layers (all feed with high cfu, cf. Table 2) (for each type of organic matter: disinfection conditions deteriorating towards the bottom of the table)

Organic matter	TB	TA	DT	*S.* Enteritidis				*S.* Senftenberg				*Enterococcus faecalis*			
				F	B	VS	WHO	F	B	VS	WHO	F	B	VS	WHO
Fat	30.0 (±0.3)	30.0 (±0.3)	30	4.5/3	3/2	0/0	5/5					1/2	1/1	0/0	3/2
	5.9 (±0.5)	5.9 (±0.5)	30	5/5	5/5	5/5	5/5					3/5	3/5	4/4	4/4
Feed	10.9 (±0.5)	10.9 (±0.5)	30	0/0	5/5	5/5	2/3	0/0	1/0	5/5	5/5				
	5.9 (±0.5)[1]	5.9 (±0.5)	30	0/0	1/0	5/5	4/5	0/0	5/5	5/5	5/5				
	5.9 (±0.5)	5.9 (±0.5)	30	0/0	4/1	5/3	5/5	0/0	1/5	5/5	5/5				
	5.9 (±0.5)	5.9 (±0.5)	15	0/0	1/1	5/5	5/5	0/0	1/1	5/5	5/2				
	5.9 (±0.5)	5.9 (±0.5)	5	0/0	4/4	5/5	5/4	0/1	0/4	4/5	5/4				

[1]The tests in this row were performed with only one feed chain link pair per 250 ml disinfectant (the tests in the other rows were performed with two chain link pairs per 250 ml disinfectant, cf. text).
Legend, cf. Table 4.

Table 6: Results for wooden dowels inoculated with either fat or feed for layers (all feed with high cfu, cf. Table 2) (for each type of organic matter: disinfection conditions deteriorating towards the bottom of the table)

Org. matter	TB	TA	DT	*S.* Enteritidis				*S.* Senftenberg				*Enterococcus faecalis*			
				F	B	VS	WHO	F	B	VS	WHO	F	B	VS	WHO
Fat	30.0 (±0.3)	30.0 (±0.3)	30	2/1/0	3/2/2	0/2/0	4/3/3	0/0/0	1/0/0	0/0/0	2/0/1	2/3/3.5	1/2/2.5	1/0/0	3/2/2
	5.9 (±0.5)	5.9 (±0.5)	30	5/3/3	4/3/3	5/5/5	5/4/5	5/4/2	3/3/3	3/≥2[1]/2	4/5/5	4/4/2	4/1/3	3/3/2	3/4/5
Feed	10.9 (±0.5)	10.9 (±0.5)	30	0/0/0	1/1/0	1/1/1	1/1/1	0/0/0	0/0/0	1/0/0	1/2/2	0/0/0	1/1/1	2/3/2	5/4/5
	5.9 (±0.5)	5.9 (±0.5)	30	0/0/0	0/0/0	1/1/1	4/4/3	0/0/0	0/0/0	0/0/0	2/1/1	1/2/2.5	1/0/0	1/1/2	4/5/3
	5.9 (±0.5)	5.9 (±0.5)	15	0/0/1	0/1/0	1/1/1	2/3/2	0/0/0	0/0/0	0/0/0	1/1/1	0/1/2	0/0/1	1/1/2.5	5/4/5
	5.9 (±0.5)	5.9 (±0.5)	5	2/1/0	0/1/0	2/2.5/2	3/3/3	0/0/0	1/0/0	2/1/2.5	1/3/3	1/1/2	1/1/1	3/3/4	5/5/5

[1]Dilution 10^{-1} overturned; therefore no dilutions were made in the range from 10^{-2} to 10^{-4}.
Legend, cf. Table 4.

Table 7: Results for jute egg belt pieces inoculated with egg yolk (disinfection conditions deteriorating towards the bottom of the table)

cfu[1]	TB	TA	DT	*S.* Enteritidis				*S.* Senftenberg				*Enterococcus faecalis*			
				F	B	VS	WHO	F	B	VS	WHO	F	B	VS	WHO
Low	10.9 (±0.5)	10.9 (±0.5)	30	0/0/0	1/2/2	2/4/4	4/5/5	0	1	1	4				
Low	5.9 (±0.5)	5.9 (±0.5)	30	0	0	4	5	0	1	1	3				
Low	5.9 (±0.5)	5.9 (±0.5)	15	0	1	2	5	0	0	0	3				
High	10.9 (±0.5)	10.9 (±0.5)	30	0	2	5	5	0	2	4	5	2/1	4/3	5/5	5/5
High	5.9 (±0.5)	5.9 (±0.5)	15	0	3	5	5	0	2	2	5				
High	5.9 (±0.5)	5.9 (±0.5)	5	3	5	5	5	0	2	4.5	5	1/0	5/5	5/5	5/5

[1]Low = ca. 2.9 x 10^5-4.6 x 10^6 g^{-1} organic matter; high = ca. 2.9 x 10^6-4.6 x 10^7 g^{-1} organic matter (Gradel et al., 2003).
Legend, cf. Table 4.

For *S.* Enteritidis (SE) and *S.* Senftenberg (SS), the same tendencies were seen (using 95% significance limits), as formaldehyde (F) was more effective than Bio Komplet Plus (B) (SE: $p = 0.026$; SS: $p = 0.010$), Virkon S (V) (SE: $p = 6.3 \times 10^{-7}$; SS: $p = 5.6 \times 10^{-5}$) and WHO water (W) (SE: $p = 6.4 \times 10^{-12}$; SS: 1.9×10^{-9}), B was better than V (SE: $p = 0.002$; SS: $p = 0.023$) and W (SE: $p = 6.2 \times 10^{-7}$; SS: 8.9×10^{-6}), whereas V was statistically as ineffective as W (SE: $p = 0.057$; SS: $p = 0.051$).
For *E. faecalis* (EF), there were no differences between the three disinfectants when these were compared pairwise, but they were all more effective than W (p in the range from 0.003 to 0.03). However, when 0 was used as the cut-off value, there were no differences between the three disinfectants on one side and W on the other (F: $p = 0.051$; B and V: $p = 0.11$).
Pairwise comparisons between SE and SS showed that V was less efficient against the former ($p = 0.028$). SS also seemed to be more susceptible to W, albeit on a non-significant level ($p = 0.061$). Moreover, V was also less effective against SE than SS when 0 was used as the cut-off value ($p = 3.3 \times 10^{-3}$). When SS and EF were compared, SS was generally more susceptible (F: $p = 1.9 \times 10^{-4}$;

B: $p = 5.6 \times 10^{-3}$; V: $p = 2.6 \times 10^{-3}$, all using 0 as the cut-off value). Overall, SE and EF were equally susceptible, except that F was more effective against the former when comparing 0 vs. > 0 points ($p = 0.044$). EF generally had higher mean points than both SE and SS, regardless of statistical significance or not.
The above tendencies were general for all disinfection series, with one notable exception: when testing feed chain links and fats at 30 °C before and after disinfection, V was better than F, B and W, both for SE and EF (Table 5). We observed during this disinfection that only the rapeseed oil in V seethed, which is probably a result of V's corrosive properties on certain metals, maybe exacerbated by these being rusty. When disinfecting wooden dowels under the same conditions, no seething was observed in any of the disinfectants, and the results for V were more similar to those for F, B and W (Table 6).
As several conditions differed between series, poultry house materials and organic matter could not be compared statistically. However, there seemed to be differences between fats and feed (cf. Tables 5-6), as the former were generally more protective. For each disinfectant and for every combination of poultry house materials and organic matter, there were generally little variations related to the other conditions (cfu, amount of organic matter on concrete flags, temperatures before and after disinfection, and disinfection times), though a somewhat higher survival was seen for the most effective disinfectants when conditions deteriorated (Bio Komplet Plus in Table 4, formaldehyde for *S*. Enteritidis in Table 6, and both these disinfectants for *Salmonella* in Table 7).

Surface disinfection tests, conclusions
Under worst-case scenario surface disinfection studies, mimicking conditions encountered in badly cleaned poultry houses, mainly at low temperatures, the general efficacy of the disinfectants was (best first) formalin > Bio Komplet Plus > Virkon S > water, with the exception that Virkon S seemed most effective when disinfecting feed chain links with fats at 30 °C. The higher efficacy of formalin compared to Bio Komplet Plus at 6 °C is conspicuous, as the former is alleged to be effective only above ca. 16 °C, whereas the latter is effective down to ca. 5 °C (Anonymous 2002). The bad results for Virkon S are less surprising, as oxidising compounds are highly susceptible to organic matter (Russell and Chopra 1996). With regard to bacteria, there were no differences between *S*. Enteritidis and *S*. Senftenberg for the effective disinfectants (formaldehyde and Bio Komplet Plus), whereas the weaker disinfectant Virkon S and water (the control) were more lethal to the latter. This is in contrast to the MIC-results, where the *S*. Senftenberg isolate had a four times higher MIC to Virkon S than *S*. Enteritidis. ***E. faecalis*** was equally or more difficult to eradicate than the two *Salmonella isolates*, indicating it could be a useful indicator bacterium.

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Chemical and heat treatment of premises following an outbreak of a notifiable disease

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Introduction

With increasing trade in animals and animal products the likelihood of disease outbreaks increase. In countries which are currently free from many of the most serious the economic losses from an outbreak of Foot and Mouth disease (FMDV) for example would be catastrophic. Farms are potentially major centres for the dissemination of disease and animal by-products can prove a vector for further spread.

Therefore an outbreak of a notifiable exotic viral disease can have major welfare and economic consequences. In many countries, including the UK, it requires the major slaughter of all animals on the affected farms and the decontamination of the premises. Traditionally this is done by cleaning, dilution with water, and finally chemical disinfection with either a proprietary disinfectant or a generic chemical. However in recent years a move to have more environmentally friendly approaches has prevailed. We were asked by the UK's Department of Environment, Food and Rural Affairs (DEFRA) to evaluate the effectiveness of either chemical of heat treatments to the decontamination of an infected premises and the associated slurry tanks.

The aim of the project was to evaluate either chemical or heat treatment of slurries contaminated with a notifiable disease. The following notafiable diseases were chosen.

Office International Des Epizooties List A pig diseases.

Foot and Mouth Disease (FMDV)
Swine Vesicular Disease (SVDV)
African Swine Fever (ASFV)
Classical Swine Fever (CSFV)

List B

Aujeszkys Disease (ADV)

Foot and Mouth Disease Virus (FMDV)

Foot and Mouth disease virus (FMDV) is a member of the Picornaviridae family of viruses, genus Apthovirus. The disease causes foot lesions accompanied my lameness and may be sub-clinical. FMDV is a highly contagious disease of cloven-hoofed animals (cattle, sheep, pigs etc.) It is generally transmitted through the air or by direct contact with infected animals, with the disease being known to have been transmitted more than 150 miles by wind. Bovines are very susceptible due to the large volume of air inhaled by the animal, whilst virus production is much greater in pigs, which can release huge amounts of virus through exhaling. Experimental evidence has shown that pigs can excrete up to 10^8 infectious units per day in aerosols at the height of viraemia. Economically it is very important as it can spread rapidly producing a large number of infections. Foot and Mouth disease virus is known to be very resistant to heat inactivation in milk and other media.

Swine Vesicular Disease Virus (SVDV)
Swine Vesicular Disease virus (SVDV) is a member of the picornaviridae, is icosahedral in shape and lacking an envelope. It is relatively stable over a wide pH range (pH 2-12), and can survive many days without the loss of infectious virus titre and is resistant to many forms of inactivation. SVDV whiles not usually a fatal disease is highly contagious and produces clinical signs that are indistinguishable from Foot and Mouth disease and is therefore considered a serious problem for the differential diagnosis of the two diseases.

African Swine Fever Virus (ASFV)
African Swine Fever (ASF) is a highly contagious viral disease of wild and domestic pigs. In endemic areas, such as sub-Sahara Africa, wild pigs may show no symptoms of the disease, however, in the domestic pig the situation is quite different. ASF can be very serious with some strains of the virus causing 100% mortality, although since spreading from Africa to Europe the virulence of some isolates of ASF has decreased with a consequent reduction in mortality rates. This has led to the virus being carried by some apparently healthy recovered animals, which may pose a risk to healthy pig populations. ASF virus was present in southern Europe from its arrival in Portugal from Africa in 1957 until it was eradicated from Portugal in 1993 and from Spain in 1995. It is still present in Sardinia. ASFV can produce as much as 10^9 HAD_{50}/ml in blood. ASF is a large enveloped, icosahedral DNA virus. It is generally quite resistant to pH changes and a proportion of the population of some isolates can survive pH 4 and pH 13. It is however very sensitive to drying and is readily inactivated by lipid solvents due to its envelope.

Classical Swine Fever Virus (CSFV)
Classical Swine Fever virus (CSF) is a member of the family Flaviviridae (genus pestivirus) and only affects pigs. CSF is a Haemorrhagic disease and is very variable in its effects. It can be very mild but, at its most severe it has high morbidity and mortality. The spread of CSFV is due to direct contact with infected pigs, ingestion of products from infected pigs or more significantly through the movement of infected pigs. Problems may arise because pigs incubating the acute form of the disease can shed virus before showing clinical signs.

Aujeszkys Disease Virus (ADV)
Aujeszkys disease virus (ADV), which is also known as pseudorabies virus, is a member of the herpesviridae family of viruses. It causes a disease of the central nervous system and respiratory system in domestic and wild animals but is of greatest economic consequences in pigs, which are the only host for the virus. In pigs a mild respiratory disease occurs in adults, although mortality is high in piglets. Cases may occur in cattle, sheep, goats, dogs, cats, mink, foxes, deer, rabbits, mice and, rats but this is rare. Mortality is high in species other than pigs. Humans are unaffected. The rise in intensive pig farming has seen a corresponding increase in the presence and spread of the disease probably because of the increased density of animals and farms.

Treatment Options – Chemical Processes
Chemical treatment is a widely used method of decontamination of buildings and manure's where there has been an outbreak of a notifiable disease. It involves the mixing of sanitising agents (e.g. formaldehyde, sodium hydroxide, or lime) into the resulting manure/slurry tanks and ensuring a significantly long contact time, typically around four days. Where chemicals are added in a continuous stirred tank reactor process (CSTR) it is necessary to ensure adequate contact times owing to the wide residence time distribution (RTD) or there is a likelihood of a significant proportion of the slurry being disposed of without decontamination. The system design can be modified to reduce this effect such as the use of baffles or several mixed vessels in series. In the

case of batch processes (for instance chemicals being pumped into a slurry lagoon) the contents of the store must be continually mixed for at least the minimum time period. The problem is one of ensuring that the chemical is equally distributed and is the same concentration throughout. The addition of chemicals to large open surfaces such as slurry lagoons, present particular hazards of gaseous emissions which would be more easily solved in a closed system.

Treatment Options – Heat Treatment

Previous work had demonstrated that Heat Treatment was the most suitable option. This is because it is a generic treatment, likely to be suitable for the inactivation of viral pathogens, and also many bacteria. It is relatively easy to scale up and has been demonstrated to be one of the cheaper options for large-scale decontamination. In addition the fact that no chemicals were added to the slurry meant that it is safe to dispose of the effluent onto land after treatment.

Heat treatment is often carried out in a batch or semi-continuous systems. The advantage of this is the high degree of control and confidence in total decontamination. However for large volumes of potentially contaminated livestock wastes, running into thousands of tonnes, the logistics of batch treatment rapidly becomes impractical. Continuous processing enables high throughputs, a consistent treatment and lower costs via savings from heat recovery.

Conclusions

Given the disadvantages of treating slurry with chemicals:

In some cases a large quantity of chemical is required this increases waste volumes.
Health and safety risk to staff and animals.
Large emissions of odour and ammonia.
Toxic effects on the environment.

As with the benefits of treating slurry with heat treatment:
Thermal decontamination is a relatively simple method
There is a high level of confidence for the inactivation of pathogens
It is suitable for a wide range of wastes.
They have a limited environmental impact in terms of pollutants compared with chemical treatments.

It was therefore decided to develop a pilot plant based on heat. Previous laboratory work had demonstrated the range of temperatures required to inactivate the pathogens.

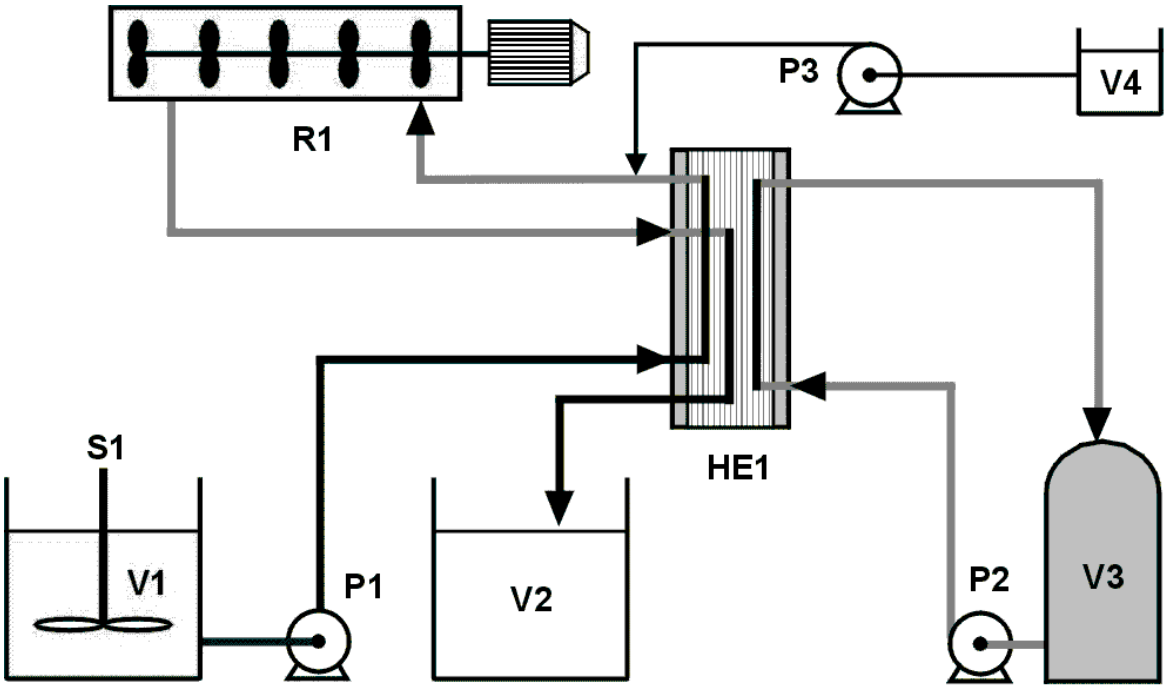
Laboratory Scale Results

Heat inactivation occurred within 5 minutes in slurry at:

56^{o}C for African Swine Fever
60^{o}C for Swine Vesicular Disease
60^{o}C for Classical Swine Fever
62^{o}C for Aujeszkys Disease
67^{o}C for Foot and Mouth Disease

A pilot plant was constructed (See below)

Experimental Continuous Thermal Treatment Plant for the Inactivation of a range of viruses in pig slurry



Key
HE1 = Heat Exchanger
P1 = Slurry feed Pump
P2 = Hot water Pump
P3 = Virus suspension closing pump
R1 = Thermal Reactor
S1 = Stirrer
V1 = Raw slurry
V2 = Treated Slurry
V3 = Hot water supply
V4 = Virus Concentrate

Cold untreated slurry is pumped by the exit flow of treated slurry (heat recovery) and secondly raised to the required temperature by the hot water flow from V3. The heated slurry is passed through the reactor where it was maintained at the required temperature for the required period of time. The treated slurry is then passed through the other side of the heat exchanger (to warm the cold, incoming slurry) and was finally collected in tank V2.
Previous laboratory scale experiments on the survival of selected viruses had concluded that a treatment period of five minutes was the minimal resistance time for adequate decontamination. A reduction in virus titre by four $\log_{10}$ units was adopted as the criterion for effective decontamination. This 10^4 reduction in titre is that required in tests for commercial disinfectant efficacy prior to licensing by the UK Department of the Environments, Food and Rural Affairs (DEFRA) and this study used procedures and protocols for monitoring virus inactivation. This implied that at least 99.99% of slurry needed to be subjected to a minimum treatment time of five minutes.

Results of Pilot Scale Thermal Treatment of Inoculated Pig Slurry
An extensive range of trials in a bio-secure area was carried out. Infectious virus, prepared in medium or slurry, was injected into the slurry prior to the retention vessel to provide a starting

material of known high titre rather than seeking sources of infected slurry. A typical dosing rate for the virus and slurry or water mix was 5% of the flow rate. Trials were performed with each virus in water as well as slurry to establish whether virucidal properties of slurry (e.g. presence of free ammonia) would enable lower inactivation temperatures. The process rate was 100 litres per hour with trials lasting three hours. Towards the end of this period steady-state conditions were assumed, samples taken and temperatures recorded. Inactivation of the viruses in raw pig slurry using the thermal decontamination plant occurred as follows:

Classical Swine Fever Virus at 51^{o}C
Swine Vesicular Disease Virus at 55^{o}C
African Swine Fever Virus at 55^{o}C
Aujeszky's Disease Virus at 58^{o}C
Foot and Mouth Disease Virus at 62^{o}C

Inactivation of the viruses in the absence of free ammonia (i.e. water or acidified slurry) required higher temperatures.

Swine Vesicular Disease Virus at 60^{o}C
Aujeszkys Disease Virus at 62^{o}C
Classical Swine Fever Virus at 63^{o}C
Foot and Mouth Disease Virus at 66^{o}C

Ammonia was hence shown to be virucidal and contributed to the decontamination. Therefore the temperature required for inactivation in livestock slurries where free ammonia is expected is lower because of the synergistic effect of heat and ammonia. At a lower pH ammonia content of slurry varies considerably depending on the diet of the animal, environmental conditions and other factors. It is strongly recommended that in the event of an outbreak of one of these viral diseases, slurry is heated to above the temperatures required for inactivation to provide a safety margin. The following temperatures are thus recommended for this system.

Classical Swine Fever Virus 65^{o}C
Swine Vesicular Disease Virus 65^{o}C
African Swine Fever Virus 60^{o}C
Aujeszky's Disease Virus 65^{o}C
Foot and Mouth Disease Virus 70^{o}C

In conclusion
An on farm process to inactivate important viral pathogens has been developed and tested.
A separate scale up project that with heat recovery the cost of treating a tonne of slurry can be around 1 Euro.
Temperature inactivation was lower than expected.
This generic process might be suitable for other pathogens following trials.

Discussion and conclusions

Many resources are spent on disinfection in the agricultural sector, and it is an important means in the eradication of various diseases. Therefore, many farmers and contractors have gained much experience related to disinfection procedures in animal houses. Often, a thorough disinfection combined with other measures, such as rodent control and a strict biosecurity, are efficient in the elimination of the microbes. Still, there are also several cases in which an infection persists in spite of apparantly efficient disinfection procedures. In these latter cases, it is pertinent to obtain advice on procedures in order to be able to pinpoint possible reasons for a failure.

The scientific, non-commercial research on disinfection in the agricultural sector is sparse. In this context, it is advantageous to apply general principles from the food microbiology where much more research has been performed. Most principles apply generally regardless of the research sector, e.g. fats (and hence often a low moisture) protect bacteria from heat and disinfectants regardless of whether they occur in an animal house or in chocolate. The main problem is probably that sheer data can rarely be extrapolated, as there is an unlimited number of conditions which can vary, and these all influence the outcome.

Heat as a means of disinfection is widely used in food processing, e.g. pasteurisation of milk. It is important to find a temperature-time balance in which the detrimental microbes are killed without altering flavour and texture of the foodstuffs. Similar considerations apply to heating of animal houses where equipment can be damaged by too high temperatures. Monitoring is also an important aspect of heating, as the desired temperature should be evenly distributed, e.g. in the animal house, food or slurry.

In the agricultural sector, buildings and surroundings are often more difficult to clean and disinfect properly than in food premises. It is, however, advantageous that disinfectants, which seem to be more effective, can be used in the former sector. Again, specific tests are needed because of the difficulties of extrapolating sheer data.

The most widely used disinfection tests are performed in test tubes in the laboratory, e.g. the European Suspension Test. Such tests are easy to standardise, relevant controls can be included, and specific factors can be evaluated, e.g. related to genetically well-characterised bacterial strains. However, only a few countries have applied such tests for the endorsement of disinfectants, and even between these conditions differ, e.g. with regard to media and microbes, and this can influence the results significantly. A first important step would be international agreement on the use of one specific suspension test for the endorsement of disinfectants, which would enable comparisons between these. The main drawback, however, of suspension tests is that they do not simulate real-life conditions where microbes are found commonly on surfaces, often imbedded in organic matter.

Key aspects of the topics reported in this symposium have been worst-case scenarios and the application to real-life conditions. These types of tests are performed with the factors encountered under specific conditions and environments being relevant in the actual cases. They are more difficult to standardise, and controls often have to be omitted, especially in field studies. Therefore, it is probably even more unrealistic to expect a standardisation of such tests. For specific microbes, it could, however, be advantageous to determine a set sample scheme, e.g. for *Salmonella* where several studies have indicated some worst-case critical control sites to sample. In field studies, a special problem may be the low occurrence of the relevant microbe, meaning huge resources are needed to sample and detect these. In this connection, indicator microbes (i.e. such that are at least as resistant as the relevant microbe and often occur in higher numbers) could be relevant. Although

the disinfection and control procedures *per se* can be difficult to standardise, this is probably easier with specific organic matter spiked with relevant well-characterised strains of indicator bacteria.

A generally accepted standard monitoring system for disinfection procedures in the field, preferably with the inclusion of relevant indicator microbes, would be beneficial for farmers and contractors. Such monitoring systems should be an inherent part of certification systems run by neutral bodies. Moreover, they should be based on sound scientific studies, and not merely on commercial sources. The latter may be one of the obstacles, as few scientists are involved in this research area, and more realistic studies are needed to recommend procedures that specifically apply to various conditions encountered in real-life situations.

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