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

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ABSTRACT

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

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

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

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

1. Introduction

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

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

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

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sufficient contact time for them to work effectively.

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

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

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

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

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

2. Materials and methods

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

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

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

2.2. Preparation of disinfectants for disinfection model studies

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

2.3. Faecal suspension model

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

2.4. Surface contamination model

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

2.5. Disinfectant stability model

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

Commercially available dip-stick style testing strips were used in