Efficacy of some Disinfectant compounds against porcine bacterial pathogens.

Jill R. Thomson, Nichola A. Bell, Meighan Rafferty
Scottish Agricultural College, Veterinary Services, Bush Estate, Penicuik, Midlothian EH26 0QE

Abstract

Seven chemical disinfectants were tested against 10 species of porcine bacterial pathogens that are common in UK pig units, using the British Standard method BS EN 1656:2000. A range of temperature conditions, two contact times and two levels of organic soiling were included. The bacterial pathogens showed widely different susceptibilities to the different disinfectants and none of the compounds was universally effective. *Salmonella enterica* Typhimurium, *Salmonella derby*, *E.coli* (Abbotstown strain) and *Yersinia enterocolitica* generally showed poor susceptibility to disinfectants whereas *Brachyspira hyodysenteriae, Actinobacillus pleuropneumoniae, Bordetella bronchiseptica, Pasteurella multocida, Haemophilus parasuis, Streptococcus suis* and *Staphylococcus hyicus* were all susceptible to multiple disinfectants under low organic matter conditions. Under high organic matter conditions, the efficacy of disinfectant compounds was markedly reduced in most tests. Low temperature and short contact times adversely affected results in some tests.

Introduction

Effective prevention and control of infectious diseases in piggeries rests on a series of key factors including herd biosecurity, management of pig flow in combination with cleaning and disinfection, maintaining a good general level of hygiene, vermin control vaccination, prompt treatment of infectious diseases and isolation of infected animals. The importance of effective cleaning and disinfection, the correct procedure and chemical compounds available have been reviewed by Waddilove and Blackwell (1997). From a series of studies published between 1988 and 1999, the calculated economic benefits from applying improved biosecurity protocols in herds with apparently healthy pigs ranged from £2.10 to £8.80 per pig (Gadd, 2007). Changes in the UK pig industry including the now endemic nature of post-weaning multisystemic wasting syndrome (PMWS) and the ban on using antibiotic growth promoters have heightened awareness of clinical and subclinical endemic diseases and the need for up-to-date information on the most optimal methods of disease control.

Cleaning and disinfection of buildings between batches of pigs is one of the most important critical control points, both in everyday prevention of diseases and in controlling the spread of infection during disease outbreaks. However, there is limited information in the scientific literature on the efficacy of disinfectants against bacterial pathogens that are endemic in the UK pig industry. The Department for the Environment, Food and Rural Affairs (Defra) has in place a statutory mechanism under the Diseases of Animals (Disinfectants Approvals) Order 1978 that allows veterinary disinfectants to be placed on an approved list for control of different diseases if they demonstrate efficacy in laboratory testing (Defra, 2007). Approvals are divided into five categories; foot-and-mouth disease, swine vesicular disease, diseases of poultry, tuberculosis and general purpose use under other Orders made under the Animal Health Act 1981. Testing for approval for general purpose use is by the British Standard BS 6734: 1986 using *Salmonella cholerae suis* (NCTC 10653).

This project aimed to test selected recent bacterial isolates representing a range of common endemic diseases against a number of disinfectancts recommended for agricultural use, thereby contributing to existing information on the range of susceptibilities of bacterial

pathogens to disinfectants. The aim was to optimise the benefits of the cleaning and disinfection procedures on farms and maximise the cost-benefits of these procedures.

Materials and methods

Seven chemical disinfectants were tested against 13 bacterial isolates representing 10 species of porcine bacterial pathogens. The tests comprised a range of conditions including different temperatures, contact times and levels of simulated organic matter soiling.

Bacterial isolates

The bacterial isolates used in the study are shown in Table 1. All pathogens were recent field isolates from commercial pig units in the UK, that had clinical disease problems related to the particular infection. *E.coli* (NCTC 10418) was used as the reference strain.

Disinfectants

The disinfectant agents tested are shown in Table 2. All were tested at concentrations ranging from 1:100 to 1:10,000 using doubling dilutions. This range covered the recommended dilution ranges for all products except B and F that have recommended dilution ranges starting from 1:50.

Procedure

The method used was the British Standard (European Standard) method BS EN 1656:2000 'Chemical disinfectants and antiseptics - quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants used in the veterinary field'. This is a phase 2/step 1 method.

All dilutions were freshly made on the day of the test, with sterilised standardised hard water used as the diluent. The stated test temperature for the method is $10^{o}C$. Additionally, two further temperature conditions were used in order to simulate a range of potential environmental temperatures for the tests, namely; $4^{o}C \pm 1^{o}C$ (cold winter conditions), $10^{o}C \pm 1^{o}C$ (cool conditions) and $20^{o}C \pm 1^{o}C$ (warm conditions). Test aliquots were equilibrated at the chosen test temperatures before addition of bacterial suspensions.

Bacterial cultures were prepared from 48 hour culture plates (except for *Brachyspira hyodysenteriae* which was prepared from 72 hour cultures owing to the slow growth of this organism). Bacterial suspensions were equilibrated to McFarland standard 5 (approximately 10^{8} c.f.u./ml). The bacterial count of control and respective test suspensions were made by plate cultures on Tryptone Soya Agar.

Two contact times were chosen for the disinfectant tests, namely 30 minutes and 60 minutes.

Two levels of simulated organic soiling were used through the addition of interfering agents, namely; 'low organic matter' simulating well-cleaned conditions, using bovine albumin at 3g/l in the final test solutions, and 'high organic matter' simulating poorly cleaned conditions, using 10 g/l yeast extract and 10 g/l bovine albumin in the final test solutions. The interfering agents were sterilsed before use.

A neutralising agent was used to stop the disinfectant activity at the end of the stated contact times. The neutraliser used for all tests comprised polysorbate 80 (3%), lecithin 3g/L, sodium thiosulphate 5g/L, L-histidine 1g/L and saponin 30g/L. The neutraliser was validated for all disinfectants before use in the tests. Neutralisation was performed at $20^{o}C \pm 1^{o}C$ for all tests.

All tests were performed in duplicate and aliquots for assessing viable bacterial counts were cultured in duplicate. Viable bacterial counts were done using the spread plate technique, with plates incubated at $37^{o}C \pm 1^{o}C$ for 24 hours. *B.hyodysenteriae* growth was assessed following incubation at $42^{o}C$ for 72 hours, and in view of it spreading growth character results were expressed as positive or negative growth, as individual colony counting was not possible.

The test compound was deemed to be effective against the stated organism at the stated dilution and under the specified conditions if it demonstrated a 10^5 or more reduction in bacterial viability.

Results

The results of the tests for each isolate are shown in Tables 3 to 7. The results for the control organism *E.coli* (NCTC 10418) are included in Table 7 for completeness. Results are given as the lowest effective concentration of the product giving at least $1x10^5$ reduction in viable count, against the organism under the stated test conditions. For ease of visualising results, the result fields have been colour coded as follows:

Green shading indicates the concentration of disinfectant product that was found to be effective against the specified pathogen under the stated conditions, and that this result is covered by the recommended dilution range for general disinfection purposes.
Yellow shading indicates that the effective concentration of the disinfectant product was higher than that recommended for general disinfection purposes.
Red shading indicates that the disinfectant product was not effective at the stated concentration.

The bacterial pathogens tested showed widely different susceptibilities to different classes of disinfectants and none of the compounds was universally effective against all of the organisms at the highest concentration tested.

The results for *Actinobacillus pleuropneumoniae, Bordetella bronchiseptica* and *Pasteurella multocida* are shown in Table 3. The *A.pleuropneumoniae* isolate showed good susceptibility to five disinfectant compounds (A, B, C, E and G) under both low and high organic matter conditions. The quaternary ammonium compound (F) was not effective at a concentration of 1:100, and an iodine compound (D) was only effective at a higher concentration than that recommended for general use.
All seven compounds were effective against *Bordetella bronchiseptica* under low organic matter conditions. The quaternary ammonium compound (F) and the peroxygen compound (G) were not effective against *B.bronchiseptica* at a concentration of 1:100 under high organic matter conditions. The iodine compound (D) was only effective at higher temperature and longer contact times under high organic matter conditions.
Two compounds namely acidic-based iodine (A) and quaternary ammonium plus hydrogen peroxide (E) were effective against *Pasteurella multocida* under both low and high organic matter conditions. Three other compounds (B, C and F) showed efficacy under low organic matter conditions though compound C was only effective at 20^0 C.

The results for *Haemophilus parasuis, Staphylococcus hyicus* and *Streptococcus suis* type are shown in Table 4. The *H.parasuis* isolate showed good susceptibility to five compounds (A, B, E, F and G) under both low and high organic matter conditions. The peroxygen compound (G) was particularly effective. The peracetic acid plus hydrogen peroxide compound (C) and the iodine compound (D) were not effective under high organic matter conditions, and the latter compound was only effective at a higher concentration that that recommended for general use, under low organic matter conditions.
Five compounds (A, B, C, E and F) were effective against *S.hyicus* under low organic matter conditions, though the quaternary ammonium compound (F) was not effective at 1:100 at 4^0 C. Under high organic matter conditions, only the acidic-based iodine compound (A) showed efficacy, though not at 4^0 C.

All compounds were effective against *S.suis* under low organic matter conditions except for the iodine compound (D) which was not effective at the recommended dilution rates at 4^0 C, or at shorter contact times at 10^0 C and 20^0 C. Under high organic matter conditions, compounds A, B, E and F showed efficacy against *S.suis* whereas compounds C, D and G did not.

The results of three *Salmonella enterica* Typhimurium isolates are shown in Table 5. None of the compounds showed efficacy against any of the isolates under high organic matter conditions whereas compounds C and E showed varying efficacy under low organic matter conditions. The peracetic acid plus hydrogen peroxide compound (C) was the most effective, but not for two of the *S.* Typhimurium isolates tested at 4^0 C.

The results for *Brachyspira hyodysenteriae, Salmonella derby* and *Yersinia enterocolitica* are shown in Table 6. *B.hyodysenteriae* was generally susceptible to all compounds except the iodine compound (D) under high organic matter conditions, and at 4^0 C under low organic matter conditions. Under high organic matter conditions, at 4^0 C, the peroxygen compound (G) was not effective.
The enteropathogens *S.derby, Y.enterocolitica* and *E.coli* (Abbotstown strain) (Table 7) all showed poor susceptibility to disinfectants. None were effective against any of these isolates under high organic matter conditions. *S.derby* was susceptible to the peracetic acid plus hydrogen peroxide compound (C) and the quaternary ammonium plus hydrogen peroxide compound (E) under low organic matter conditions, but not at all temperatures and contact times. *Y.enterocolitica* was susceptible to the glutaraldehyde plus quaternary ammonium compound (B) and the quaternary ammonium plus hydrogen peroxide compound (E) under low organic matter conditions, at 20^0 C but not at lower temperatures. The isolate of *E.coli* (Abbotstown strain) was only susceptible to the quaternary ammonium plus hydrogen peroxide compound (E) under low organic matter conditions.

Discussion.

This project has provided useful information of the susceptibilities of recent field isolates of porcine bacterial pathogens against a number of disinfectants suitable for farm use. Testing to the British Standard method (BSEN 1656:2000) proved to be very exacting and time consuming, however a valuable set of data has been obtained. Unfortunately the limit on time and resources did not allow testing of a more diverse range of products or higher numbers of bacterial isolates. Extending the range of disinfectants tested and increasing the number of field isolates tested are the suggested directions for future work. Two of the compounds tested (B and F) have recommended dilution ranges starting from 1:50. This higher concentration was not tested as commercial experience has shown that farmers generally use these products at concentrations of 1:100 or less. In any follow-up study, testing at the higher concentration should be done for completeness.

Some important pathogens eg, *A. pleuropneumoniae*, *B. hyodysenteriae*, *S. suis* and *H. parasuis* showed good susceptibility to a number of disinfectants under a wide range of conditions, providing several options for choice of disinfectants as part of control programmes. Similar findings were generally true for *P.multocida* and *B.bronchiseptica* which are both common respiratory pathogens and are endemic on most pig farms. Conversely, the poor susceptibility of several important enteric pathogens (including some zoonotic organisms) to disinfectants are of concern as disinfection is an important critical control point for effective control of such infections. This is particularly true for salmonella control as part of national control programmes.

The level of organic matter in the test conditions generally had a major impact on the efficacy of disinfectants. Six of the enteropathogenic bacterial isolates were not susceptible to any of the disinfectants tested, at the stated concentrations, under high organic matter conditions.For other bacterial species, the disinfectant efficacies seen under low organic matter conditions were negated under high organic matter conditions, in many instances. This was particularly apparent for *S.hyicus* and *S.suis.* When comparing efficacies of disinfectants under low and high organic matter conditions, most tests showed a considerable difference in effective concentrations between the two conditions. Similar findings have been recorded in previous pig pathogen studies using other disinfectant compounds and test methods (Gutiérrez *et al* 1995, 1999; de la Puente *et al* 1998 and Corona-Barrera *et al* 2004).

Clear guidelines on effective cleaning procedures including washing out buildings and the use of a farm approved detergent before using disinfectant are available in the literature (Waddilove and Blackwell 1997, Hurnik 2005, Dewulf and Ledoux 2007, Gadd 2007). This should form part of a full biosecurity protocol including all-in, all-out building management, removing equipment and any loose items from pens, washing out all loose organic matter, thorough wetting of surfaces with use of detergent at the recommended concentration, re-washing after a 20 – 30 minute interval, applying disinfectant to surfaces, aerosol fogging, sanitising the water system followed by allowing the building to dry out properly before re-stocking. Vectors such as flies and vermin should all be controlled. The adverse impact of high organic matter conditions has been clearly demonstrated in this study. Failure to complete the vital step of organic matter removal might completely negate the effects of disinfectants and prove to be a complete waste of money and effort. Though time consuming and hard work it is vital that poorly accessible areas, for example, the sides of slats, are adequately cleaned in order to maximise the efficiency of cleaning and disinfecting procedures. The economic benefits of using a full biosecurity protocol has been estimated at £5.50/pig and the benefit to cost ratio of a full biosecurity protocol over a disinfection-only system has been estimated at 12.5:1 (Gadd 2007).

There are many factors that influence the choice of disinfectant compounds used on farms. Knowledge of the likely efficacy of compounds against the target infection and the conditions under which they will be used, are highly important. Additional factors to take into account are environmental temperature (the possible need for higher concentrations of disinfectant under cold conditions) and contact time (allowing 60 minutes or more wherever possible). Most disinfectants are hazardous to operators and the provision of training, protective clothing and equipment are important for health and safety reasons. Factors such as corrosiveness of compounds, environmental impact, fish toxicity and suitability for discharging run-off into drains and water courses all require careful consideration before use.

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Disclaimer

The results presented in this report were those obtained using the stated method and isolates under laboratory conditions at SAC Veterinary Services, Edinburgh. They do not prove or disprove the efficacy of products under field conditions or when tested by alternative methods or laboratories. They do not prove or disprove the efficacy of other products containing the chemical compounds stated, against the bacterial species tested.

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Table 1. Bacterial isolates used in the disinfectant study.

Organism	**Reference number**	**Source of isolate**
E.coli	NCTC 10418	Reference strain used for establishing and standarising techniques
Actinobacillus pleuropneumoniae	P4681/3/06	Porcine lung lesions during pleuropneumonia outbreak
Bordetella bronchiseptica	P50977/3/04	Porcine nasal turbinates during rhinitis/respiratory disease outbreak
Brachyspira hyodysenteriae	P3695/6B/04	Porcine colon from a case of swine dysentery
E. coli (Abbotstown)	P5297/06	Porcine intestine; neonatal colibacillosis outbreak
Haemophilus parasuis	P600194/1/06	Porcine lung; case of Glasser's disease
Pasteurella multocida	P502613/2/06	Porcine lung lesions; pneumonia outbreak
Salmonella derby	P502102/2/06	Porcine intestine; diarrhoea outbreak
Salmonella enterica Typhimurium	P502512/06	Porcine intestine; diarrhoea outbreak
Salmonella enterica Typhimurium	P502485/06	Porcine intestine; diarrhoea outbreak
Salmonella enterica Typhimurium	P502573/2/06	Porcine intestine; diarrhoea outbreak
Staphylococcus hyicus	P502515/1/06	Porcine skin; outbreak of Greasy pig disease.
Streptococcus suis serotype I/II	P50257/2	Porcine meninges; meningitis outbreak
Yersinia enterocolitica	P5341/06	Porcine intestine; diarrhoea outbreak

Table 2. Disinfectant compounds used in the study.

Key	**Active compound**	**Recommended dilution range**
A	Iodine (acidic based)	1:125 – 1:600
B	Glutaraldehyde plus quaternary ammonium	1:50 – 1:190 (SVD 1:250)
C	Peracetic acid plus hydrogen peroxide	1:100 – 1:200
D	Iodine	1:200
E	Quaternary ammonium plus hydrogen peroxide	1:100 – 1:200
F	Quaternary ammonium	1:50 – 1:100
G	Peroxygen	1:100 – 1:200

Table 3. Evaluation of the bacteriocidal activity of seven chemical disinfectants against three respiratory bacterial pathogens of pigs using British Standard Method BSEN 1656:2000 (phase 2 /step 1). Results are given as the lowest effective concentration of product giving at least 10^5 reduction in viable bacterial count under the stated test conditions.

Bacteria	Disinfect ~	Test conditions											
		Low organic matter						High organic matter					
		4^0 C		10^0 C		20^0 C		4^0 C		10^0 C		20^0 C	
		30 min	60min	30 min	60min	30 min	60min	30 min	60min	30 min	60min	30 min	60min
APP^	A	1/1000	1/1000	1/1000	1/1000	1/1000	1/1000	1/800	1/800	1/800	1/800	1/800	1/800
	B	1/200	1/200	1/200	1/200	1/200	1/200	1/100	1/100	1/100	1/100	1/100	1/100
	C	1/1000	1/1000	1/1000	1/1000	1/1000	1/1000	1/800	1/800	1/800	1/800	1/800	1/800
	D	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100
	E	1/10000	1/10000	1/10000	1/10000	1/10000	1/10000	1/1000	1/5000	1/1000	1/5000	1/1000	1/5000
	F	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	G	1/800	1/800	1/800	1/800	1/800	1/800	1/100	1/100	1/400	1/400	1/400	1/400
BB*	A	1/200	1/400	1/400	1/400	1/400	1/800	1/200	1/200	1/200	1/200	1/200	1/200
	B	1/200	1/200	1/200	1/200	1/200	1/200	1/100	1/100	1/100	1/100	1/100	1/100
	C	1/200	1/200	1/200	1/200	1/200	1/200	1/100	1/100	1/100	1/100	1/100	1/100
	D	1/200	1/200	1/200	1/400	1/200	1/400	1/100	1/100	1/100	1/200	1/100	1/200
	E	1/400	1/800	1/400	1/1000	1/800	1/1000	1/100	1/100	1/100	1/100	1/100	1/100
	F	1/200	1/200	1/200	1/200	1/200	1/200	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	G	1/100	1/100	1/100	1/100	1/100	1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
PM#	A	1/1000	1/1000	1/1000	1/1000	1/1000	1/1000	1/400	1/400	1/400	1/400	1/400	1/400
	B	1/200	1/200	1/200	1/200	1/200	1/200	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	C	NE 1/100	NE 1/100	NE 1/100	NE 1/100	1/100	1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	D	NE 1/100	NE 1/100	NE 1/100	NE 1/100	1/100	1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	1/100	1/100
	E	1/1000	1/5000	1/1000	1/5000	1/1000	1/5000	1/100	1/200	1/200	1/200	1/200	1/200
	F	1/100	1/100	1/100	1/100	1/100	1/200	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	G	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100

^*Actinobacillus pleuropneumoniae* - field isolate P4681/3/06. Isolated from porcine lung (pleuropneumonia outbreak)
**Bordetella bronchiseptica* - field isolate P50977/3/04. Isolated from porcine nasal turbinates (rhinitis/respiratory disease outbreak)
#*Pasteurella multocida* - field isolate P502613/2/06. Isolated from pneumonic porcine lung tissue (respiratory disease outbreak)
~ The key for the disinfectant compounds is given in Table 2
NE 1/100 = Not effective at the highest concentration tested (1/100)

Table 4. Evaluation of the bacteriocidal activity of seven chemical disinfectants against three bacterial pathogens of pigs using British Standard Method BSEN 1656:2000 (phase 2 /step 1). Results are given as the lowest effective concentration of product giving at least 10^5 reduction in viable bacterial count under the stated test conditions.

Bacteria	Disinfect ~	Test conditions											
		Low organic matter						High organic matter					
		4^0 C		10^0 C		20^0 C		4^0 C		10^0 C		20^0 C	
		30 min	60min	30 min	60min	30 min	60min	30 min	60min	30 min	60min	30 min	60min
HP^	A	1/1000	1/1000	1/1000	1/1000	1/1000	1/1000	1/100	1/100	1/100	1/100	1/100	1/100
	B	1/1000	1/1000	1/1000	1/1000	1/1000	1/1000	1/100	1/100	1/100	1/100	1/100	1/100
	C	1/100	1/100	1/100	1/100	1/100	1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	D	1/100	1/100	1/100	1/100	1/100	1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	E	1/400	1/400	1/400	1/400	1/400	1/400	1/200	1/200	1/200	1/200	1/200	1/200
	F	1/800	1/800	1/800	1/800	1/800	1/800	1/200	1/200	1/200	1/200	1/400	1/400
	G	1/5000	1/5000	1/5000	1/5000	1/5000	1/5000	1/1000	1/1000	1/1000	1/1000	1/1000	1/1000
SH*	A	1/400	1/800	1/800	1/800	1/800	1/800	NE 1/100	NE 1/100	1/100	1/200	1/100	1/200
	B	1/100	1/200	1/100	1/200	1/100	1/200	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	C	1/100	1/100	1/100	1/100	1/100	1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	D	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	E	1/100	1/100	1/100	1/100	1/100	1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	F	NE 1/100	NE 1/100	1/100	1/100	1/100	1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	G	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
SS#	A	1/800	1/800	1/800	1/1000	1/1000	1/1000	1/400	1/800	1/400	1/800	1/400	1/800
	B	1/400	1/400	1/800	1/800	1/800	1/800	1/400	1/400	1/400	1/400	1/400	1/400
	C	1/200	1/200	1/200	1/200	1/200	1/400	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	D	1/100	1/100	1/100	1/200	1/100	1/200	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	E	1/1000	1/1000	1/1000	1/1000	1/1000	1/1000	1/400	1/400	1/400	1/400	1/400	1/800
	F	1/200	1/800	1/400	1/800	1/400	1/800	1/100	1/100	1/100	1/100	1/200	1/200
	G	1/100	1/100	1/100	1/200	1/100	1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100

^ *Haemophilus parasuis* - field isolate P600194/1/06. Isolated from porcine lung (from a case of Glasser's disease)

* *Staphylococcus hyicus* - field isolate P502515/1/06. Isolated from porcine skin (greasy pig disease outbreak)

Streptococcus suis - field isolate P50257/2 (serotype I/II). Isolated from porcine meninges (meningitis outbreak)

~ The key for the disinfectant compounds is given in Table 2

NE 1/100 = Not effective at the highest concentration tested (1/100)

Table 5. Evaluation of the bacteriocidal activity of seven chemical disinfectants against three *Salmonella enterica* Typhimurium isolates from pigs using British Standard Method BSEN 1656:2000 (phase 2 /step 1). Results are given as the lowest effective concentration of product giving at least 10^5 reduction in viable bacterial count under the stated test conditions.

Bacteria	Disinfect ~	Test conditions											
		Low organic matter						High organic matter					
		4^0 C		10^0 C		20^0 C		4^0 C		10^0 C		20^0 C	
		30 min	60min	30 min	60min	30 min	60min	30 min	60min	30 min	60min	30 min	60min
ST (1)^	A	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	B	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	C	1/100	1/100	1/100	1/100	1/100	1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	D	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	E	1/100	1/100	1/100	1/100	1/100	1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	F	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	G	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
ST (2)*	A	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	B	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	C	NE 1/100	NE 1/100	1/100	1/100	1/100	1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	D	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	E	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	F	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	G	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
ST (3)#	A	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	B	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	C	NE 1/100	NE 1/100	1/100	1/100	1/100	1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	D	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	E	NE 1/100	NE 1/100	NE 1/100	NE 1/100	1/100	1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	F	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	G	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100

^ *Salmonella enterica* Typhimurium (1) - field isolate P502512/06. Isolated from porcine intestine (diarrhoea outbreak)

* *Salmonella enterica* Typhimurium (2) - field isolate P502485/06. Isolated from porcine intestine (diarrhoea outbreak)

Salmonella enterica Typhimurium (3) - field isolate P502573/2/06. Isolated from porcine intestine (diarrhoea outbreak)

~ The key for the disinfectant compounds is given in Table 2

NE 1/100 = Not effective at the highest concentration tested (1/100)

Table 6. Evaluation of the bacteriocidal activity of seven chemical disinfectants against three enteric pathogens of pigs using British Standard Method BSEN 1656:2000 (phase 2 /step 1). Results are given as the lowest effective concentration of product giving at least 10^5 reduction in viable bacterial count under the stated test conditions.

Bacteria	Disinfect ~	Test conditions											
		Low organic matter						High organic matter					
		4^0 C		10^0 C		20^0 C		4^0 C		10^0 C		20^0 C	
		30 min	60min	30 min	60min	30 min	60min	30 min	60min	30 min	60min	30 min	60min
BH^	A	1/400	1/400	1/800	1/800	1/800	1/800	1/200	1/200	1/200	1/200	1/400	1/400
	B	1/800	1/800	1/800	1/800	1/800	1/800	1/200	1/200	1/200	1/200	1/200	1/200
	C	1/800	1/800	1/1000	1/1000	1/1000	1/1000	1/400	1/400	1/800	1/800	1/800	1/800
	D	1/100	1/100	1/200	1/200	1/400	1/400	1/100	1/100	1/100	1/100	1/100	1/100
	E	1/200	1/200	1/800	1/800	1/800	1/800	1/200	1/200	1/400	1/400	1/800	1/800
	F	1/800	1/800	1/800	1/800	1/1000	1/1000	1/200	1/200	1/200	1/200	1/200	1/200
	G	1/200	1/200	1/200	1/200	1/400	1/400	NE 1/100	NE 1/100	1/100	1/100	1/100	1/100
SD*	A	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	B	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	C	NE 1/100	1/100	NE 1/100	1/100	NE 1/100	1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	D	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	E	NE 1/100	NE 1/100	1/100	1/100	1/100	1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	F	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	G	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
YE#	A	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	B	NE 1/100	NE 1/100	NE 1/100	NE 1/100	1/200	1/200	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	C	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	D	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	E	NE 1/100	NE 1/100	NE 1/100	NE 1/100	1/100	1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	F	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	G	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100

^ *Brachyspira hyodysenteriae* - field isolate P3695/6B/04. Isolated from porcine colon (from a case of swine dysentery)

* *Salmonella derby* - field isolate P502102/2/06. Isolated from porcine intestine (diarrhoea outbreak)

Yersinia enterocolitica - field isolate P5341/06. Isolated from porcine intestine (diarrhoea outbreak)

~ The key for the disinfectant compounds is given in Table 2

NE 1/100 = Not effective at the highest concentration tested (1/100)

Table 7. Evaluation of the bacteriocidal activity of seven chemical disinfectants against *E.coli* (Abbotstown strain) and E.coli NCTC 10418 (Type strain) using British Standard Method BSEN 1656:2000 (phase 2 /step 1). Results are given as the lowest effective concentration of product giving at least 10^5 reduction in viable bacterial count under the stated test conditions.

Bacteria	Disinfect ~	Test conditions											
		Low organic matter						High organic matter					
		4^0 C		10^0 C		20^0 C		4^0 C		10^0 C		20^0 C	
		30 min	60min	30 min	60min	30 min	60min	30 min	60min	30 min	60min	30 min	60min
EC (A)^	A	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	B	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	C	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	D	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	E	1/200	1/200	1/200	1/200	1/100	1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	F	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
	G	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100
EC (T)*	A	1/400	1/400	1/400	1/400	1/400	1/400	1/100	1/100	1/100	1/100	1/100	1/100
	B	1/800	1/800	1/800	1/800	1/800	1/800	1/200	1/200	1/200	1/200	1/200	1/200
	C	1/1000	1/1000	1/1000	1/1000	1/1000	1/1000	1/200	1/200	1/200	1/200	1/200	1/200
	D	1/200	1/400	1/200	1/400	1/400	1/400	NE 1/100	NE 1/100	NE 1/100	NE 1/100	1/100	1/100
	E	1/400	1/800	1/800	1/800	1/1000	1/1000	1/400	1/400	1/400	1/400	1/400	1/400
	F	1/800	1/800	1/800	1/800	1/1000	1/1000	1/100	1/100	1/100	1/200	1/200	1/200
	G	NE 1/100	1/100	1/100	1/100	1/100	1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100	NE 1/100

^ *E.coli* (Abbotstown strain) - field isolate P5297/06. Isolated from porcine intestine (diarrhoea outbreak)

* *E.coli* NCTC 10418 (Type strain)

~ The key for the disinfectant compounds is given in Table 2

NE 1/100 = Not effective at the highest concentration tested (1/100)