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Comparative efficacy of several disinfectants in suspension and carrier tests against *Haemophilus parasuis* serovars 1 and 5

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ABSTRACT

The comparative efficacy of 16 active compounds (including the most commonly used chemical groups) and 10 commercial formulations against *Haemophilus parasuis* serovars 1 and 5 was studied. These organisms were tested in suspension and carrier tests in the presence and absence of serum as representative of organic matter. Chloramine-T and half of the formulations from commercial sources (most of them including quaternary ammonium compounds) were effective in both *in vitro* tests, regardless of the presence or absence of organic load. All 26 disinfectants except for an iodophor (0.1% available iodine) resulted in at least 3-$\log_{10}$ reduction in colony-forming units in suspension test, and most of them resulted in the maximal level of detection (>6-$\log_{10}$ reduction). On the other hand, disinfectants were not as effective in carrier test as in suspension test, and the presence of serum considerably reduced the activities of most of the compounds tested, especially in carrier test. These results suggest the importance of selecting suitable disinfection for routine use on surfaces contaminated with *H. parasuis*, particularly when organic matter is present. Chloramine-T and formulations 2 and 7–10 are recommended for a complete inactivation of *H. parasuis* in swine herds.

1. Introduction

Antiseptics and disinfectants are used extensively in modern extensive livestock production. In particular, they are an essential part of infection-control practices and aid in the prevention of clinical and subclinical diseases, because crowding characteristic of intensive production systems have increased substantially the risk of contact spread of infective agents (McDonnell and Russell, 1999). A wide variety of active chemical agents are found in these products, many of which have been used for hundreds of years for antisepsis, disinfection, and preservation (McDonnell and Russell, 1999).

Haemophilus parasuis is a commensal of the upper respiratory tract of healthy pigs (Oliveira and Pijoan, 2004). However, it is also considered an important pathogen and the etiological agent of Glässer's disease, which is characterized by fibrinous polyserositis, polyarthritis and meningitis, and causes significant financial losses worldwide (Oliveira and Pijoan, 2004). To date, 15 serovars have been described using an immunodiffusion test (Kielstein and Rapp-Gabrielson, 1992), along with a large number of non-typable strains depending on geographic region and typing method (Oliveira and Pijoan, 2004).

H. parasuis has recently emerged as one of the major causes of nursery mortality in swine herds, and practices such as early weaning and use of three-site production systems may have influenced the epidemiology of this pathogen within herds, especially regarding the early colonization of pigs by virulent strains of *H. parasuis* and its spread throughout a swine population (Oliveira and Pijoan, 2004). It is well known that this organism may preferentially colonize the nasal mucosa of pigs, but the organism can also be detected in the tonsillar area (Amano et al., 1994) and in other respiratory sites, such as trachea (Oliveira and Pijoan, 2004). In this respect, the reduction of respirable aerosols and disinfection of contaminated surfaces in swine farms would provide a powerful tool to avoid the spread of *H. parasuis*.

To the authors' knowledge, information on the activity of disinfectants against *H. parasuis* is nonexistent; therefore, selection of an appropriate, effective and innocuous product, easy to be applied, remains fundamental. For this purpose, the efficacies of 16 commonly used chemical agents and ten commonly used formulations from commercial sources were investigated by suspension and carrier tests against two of the serovars (1 and 5) showing high virulence (Kielstein and Rapp-Gabrielson, 1992).

2. Materials and methods

2.1. Strains and preparation of initial inocula for suspension and carrier test

H. parasuis serovars 1 (H409) and 5 (Nagasaki) reference strains were used in this study. These organisms, stored at −80 °C, were

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passaged not more than three times on artificial media before testing. They were reconstituted on chocolate agar plates (BioMérieux, France) and were incubated for 24 h at 37 °C. The organisms were then grown under the same conditions on PPLO agar plates (Difco, Detroit, MI) supplemented with 0.025% nicotinamide adenine dinucleotide, 0.01% L-glutamine; 0.026% L-cysteine hydrochloride, 0.001% L-cystine dihydrochloride, 0.1% dextrose and 0.1% Tween 20 (enriched PPLO). The cells were suspended in either sterile saline solution containing 0.05% Tween 20 or sterile serum (Ref. SR0048C, Oxoid, England) to obtain 10^9 colony-forming units (CFU)/ml. These suspensions were used as the initial inocula for both tests. Although porcine nasal secretions would ideally represent the organic load of choice for testing *H. parasuis*, however, serum has been chosen as a good alternative (Best et al., 1990b; Gutiérrez et al., 1995), because of the difficulty in obtaining large amounts of sterile nasal secretions.

2.2. Disinfectants

Sixteen active chemical agents (Table 1) and 10 commercial formulations (their brand names, sources and active ingredients are listed in Table 2) were selected. These 26 products were diluted according to instructions of the manufacturers, with sterile tap water at pH 7.0 as the diluent. The in-use concentration of each one is listed in Tables 1 and 2.

2.3. Suspension test

All disinfectant reactions were carried out in 24-well plastic cell culture plates (Nunc, Denmark) as described previously for other organisms (Best et al., 1988, 1990a,b). A 0.1 ml volume of *H. parasuis* suspension (10^9 CFU/ml of sterile saline) was added to 0.9 ml of each disinfectant. After 1 min of contact (this time was chosen because it is the most routinely used in these *in vitro* studies – Best et al., 1988, 1990a,b, 1994; Gutiérrez et al., 1995), 0.1 ml of the reaction mixture was removed and immediately diluted 100-fold in sterile saline solution to stop the disinfectant action. Then, the samples were subjected to further 10-fold dilutions (until 10^{-7}). Controls for each test suspension were mixed with 0.9 ml of sterile tap water instead of disinfectant. Samples (0.1 ml) from each dilution were spread on enriched PPLO agar in triplicate and were incubated as described above. The conditions for suspension tests in the presence of serum were the same, but using 0.1 ml of *H. parasuis* suspended in sterile serum instead of saline.

Each suspension test was done in triplicate using a newly prepared dilution of each disinfectant and a fresh *H. parasuis* suspension adjusted to 10^9 CFU/ml each time. Disinfectant activity was determined by comparing growth on the control and disinfectant plates, and was reported as the mean ± standard deviation (SD) reduction in CFU per ml. Each disinfectant was tested for its capacity to cause up to a 10^6 (6-$\log_{10}$, 99.9999%) reduction in CFU of *H. parasuis* serovars 1 or 5 (maximal level of detection). At least a 10^3 reduction (3-$\log_{10}$, 99.9% reduction in CFU) was considered to be a minimal acceptable effective value, according to previous reports (Best et al., 1988, 1990a,b; Gutiérrez et al., 1995).

2.4. Carrier test

Stainless steel discs (1 cm diameter and 0.75 mm thick) were selected for carrier test. They were placed in the wells of the cell

Table 1
Activities of 16 selected chemical agents against *Haemophilus parasuis*.

Chemical agent (concentration used)	Sero-var	Reduction in CFU/ml in suspension test		Reduction in CFU/ml in carrier test	
		With saline	With serum	With saline	With serum
Chloramine-T (0.4%, w/v)	1	>10^6[a]	>10^6	>10^6	$(3.7 \pm 2.8) \times 10^4$
	5	>10^6	>10^6	>10	$(3.6 \pm 1.0) \times 10^4$
Sodium hypochlorite (0.5%, v/v)	1	$(2.7 \pm 3.5) \times 10^5$	$(0.6 \pm 0.4) \times 10^2$	$(6.7 \pm 3.6) \times 10^2$	2.6 ± 1.5
	5	$(1.9 \pm 0.8) \times 10^4$	$(3.2 \pm 3.8) \times 10^2$	$(5.5 \pm 2.0) \times 10^2$	4.8 ± 2.3
Iodophor (1%, w/v) (0.1% available iodine)	1	5.1 ± 2.3	2.0 ± 0.8	2.7 ± 0.9	1.0 ± 1.0
	5	1.3 ± 2.0	1.1 ± 0.1	2.9 ± 0.7	6.9 ± 0.5
Povidone-iodine (1% available iodine)	1	>10^6	$(3.3 \pm 0.8) \times 10^4$	>10^6	$(1.4 \pm 1.6) \times 10^2$
	5	>10^6	$(2.8 \pm 4.0) \times 10^4$	$(3.5 \pm 0.9) \times 10^5$	$(2.1 \pm 1.0) \times 10^2$
Hydrogen peroxide (3%, v/v)	1	>10^6	$(3.6 \pm 3.4) \times 10^5$	$(3.7 \pm 2.7) \times 10^3$	$(3.4 \pm 1.4) \times 10^2$
	5	>10^6	$(3.8 \pm 0.7) \times 10^5$	$(4.1 \pm 0.8) \times 10^3$	$(2.3 \pm 0.2) \times 10^2$
Potassium permanganate (1%, w/v)	1	$(2.1 \pm 1.4) \times 10^5$	$(3.7 \pm 2.8) \times 10^2$	$(3.3 \pm 1.0) \times 10^2$	$(1.9 \pm 1.0) \times 10^1$
	5	$(6.9 \pm 0.5) \times 10^4$	$(4.2 \pm 2.1) \times 10^2$	$(3.4 \pm 3.5) \times 10^2$	$(3.4 \pm 2.1) \times 10^1$
Benzalkonium chloride (0.02%, w/v)	1	>10^6	$(3.0 \pm 1.4) \times 10^4$	4.1 ± 2.9	5.7 ± 3.8
	5	>10^6	$(7.2 \pm 0.5) \times 10^4$	3.0 ± 1.8	3.6 ± 1.7
Cetylpyridinium chloride (0.1%, w/v)	1	>10^6	$(0.9 \pm 0.4) \times 10^5$	$(5.2 \pm 3.3) \times 10^2$	$(2.1 \pm 0.9) \times 10^1$
	5	>10^6	$(1.6 \pm 0.9) \times 10^5$	$(3.4 \pm 1.8) \times 10^2$	$(3.0 \pm 1.8) \times 10^1$
Ethanol (70%, v/v)	1	>10^6	$(3.6 \pm 3.7) \times 10^4$	$(6.0 \pm 1.8) \times 10^5$	$(1.2 \pm 1.1) \times 10^2$
	5	>10^6	$(2.8 \pm 0.8) \times 10^4$	$(4.8 \pm 1.6) \times 10^5$	$(3.0 \pm 1.7) \times 10^2$
Isopropanol (70%, v/v)	1	>10^6	$(3.3 \pm 2.8) \times 10^5$	>10^6	$(1.3 \pm 1.3) \times 10^2$
	5	>10^6	$(1.9 \pm 0.2) \times 10^5$	$(5.2 \pm 1.4) \times 10^5$	$(2.9 \pm 3.2) \times 10^2$
Chlorhexidine digluconate (2%, v/v)	1	>10^6	$(4.3 \pm 3.2) \times 10^5$	>10^6	$(3.3 \pm 1.9) \times 10^1$
	5	>10^6	$(4.3 \pm 2.9) \times 10^5$	>10^6	$(4.2 \pm 3.0) \times 10^1$
Formaldehyde (3.7%, v/v)	1	>10^6	$(1.8 \pm 1.6) \times 10^5$	>10^6	$(4.2 \pm 1.7) \times 10^1$
	5	>10^6	$(1.9 \pm 1.2) \times 10^5$	>10^6	$(4.3 \pm 2.8) \times 10^1$
Phenol (5%, w/v)	1	>10^6	$(1.9 \pm 0.7) \times 10^5$	>10^6	$(1.8 \pm 1.2) \times 10^2$
	5	>10^6	$(9.8 \pm 9.0) \times 10^4$	>10^6	$(6.1 \pm 1.2) \times 10^2$
Phosphoric acid (0.45%, v/v)	1	>10^6	$(2.3 \pm 1.1) \times 10^5$	>10^6	$(2.1 \pm 2.0) \times 10^1$
	5	>10^6	$(1.5 \pm 1.2) \times 10^5$	>10^6	$(2.0 \pm 1.5) \times 10^2$
Zinc sulfate (0.25%, w/v)	1	$(3.0 \pm 2.6) \times 10^3$	$(5.6 \pm 1.7) \times 10^2$	4.1 ± 2.5	1.6 ± 0.4
	5	$(3.4 \pm 1.8) \times 10^3$	$(1.4 \pm 1.0) \times 10^2$	3.1 ± 0.5	4.4 ± 2.1
Thimerosal (0.1%, w/v)	1	>10^6	$(4.5 \pm 2.6) \times 10^2$	$(5.6 \pm 1.2) \times 10^2$	$(1.2 \pm 1.0) \times 10^1$
	5	>10^6	$(1.5 \pm 1.0) \times 10^2$	$(5.2 \pm 1.4) \times 10^2$	$(3.9 \pm 1.0) \times 10^1$

CFU = colony-forming units.
[a] >10^6, the maximal level of detection was surpassed (absence of growth in the lowest dilution tested in all replicates done).

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Table 2
Activities of ten selected commercial formulations against *Haemophilus parasuis*.

Commercial formulations (active compounds)	Dilution[a]	Sero-var	Reduction in CFU/ml in suspension test		Reduction in CFU/ml in carrier test	
			With saline	With serum	With saline	With serum
(1) 1% benzalkonium chloride plus 0.1875% 2-bromide, 2-nitropropane, 1,3-diol, and 0.0675% 2,4,4′-trichloro, 2′-hydroxydiphenyl ether	Undiluted	1	$>10^{6}$[b]	$>10^{6}$	$>10^{6}$	$(3.3 \pm 3.6) \times 10^{2}$
		5	$>10^{6}$	$>10^{6}$	$>10^{6}$	$(3.4 \pm 3.5) \times 10^{2}$
(2) 10% benzalkonium chloride plus 2.5% glutaraldehyde, 6.8% glyoxal, and 6% formaldehyde	1:5	1	$>10^{6}$	$>10^{6}$	$>10^{6}$	$(2.8 \pm 2.4) \times 10^{4}$
		5	$>10^{6}$	$>10^{6}$	$>10^{6}$	$(2.1 \pm 0.9) \times 10^{3}$
(3) 10% benzalkonium chloride plus 2.5% glutaraldehyde, 6.8% glyoxal, and 6% formaldehyde	1:400	1	$>10^{6}$	$(6.6 \pm 0.6) \times 10^{3}$	$(2.0 \pm 1.6) \times 10^{2}$	$(3.2 \pm 3.1) \times 10^{1}$
		5	$>10^{6}$	$(2.1 \pm 0.9) \times 10^{4}$	$(1.3 \pm 0.2) \times 10^{2}$	$(4.1 \pm 1.0) \times 10^{1}$
(4) 4.5% didecyldimethylammonium chloride	1:400	1	$>10^{6}$	$(2.9 \pm 1.8) \times 10^{4}$	$(1.4 \pm 0.2) \times 10^{2}$	$(5.0 \pm 4.4) \times 10^{1}$
		5	$>10^{6}$	$(8.0 \pm 1.7) \times 10^{4}$	$(3.7 \pm 2.6) \times 10^{2}$	8.3 ± 0.5
(5) 4.5% didecyldimethylammonium chloride plus 5% glutaraldehyde	1:400	1	$>10^{6}$	$(1.7 \pm 1.3) \times 10^{3}$	$(3.9 \pm 2.8) \times 10^{1}$	$(2.5 \pm 3.6) \times 10^{1}$
		5	$>10^{6}$	$(6.6 \pm 0.6) \times 10^{3}$	$(2.4 \pm 0.4) \times 10^{1}$	$(4.1 \pm 1.6) \times 10^{1}$
(6) 5% benzalkonium chloride plus 1.25% glutaraldehyde, and 3.4% glyoxal	1:200	1	$>10^{6}$	$(3.7 \pm 1.1) \times 10^{3}$	3.7 ± 1.1	1.7 ± 1.1
		5	$>10^{6}$	$(5.0 \pm 2.2) \times 10^{3}$	3.7 ± 1.6	2.0 ± 1.0
(7) 10% isopropanol plus 10% *p*-chlorometacresol	1:200	1	$>10^{6}$	$>10^{6}$	$(3.3 \pm 1.0) \times 10^{5}$	$(6.4 \pm 1.3) \times 10^{3}$
		5	$>10^{6}$	$(4.2 \pm 0.8) \times 10^{5}$	$(2.8 \pm 1.9) \times 10^{5}$	$(1.6 \pm 0.4) \times 10^{3}$
(8) 11% *N*-duopropenide (a mixture of benzyldimethyldecadecylammonium, benzyldimethyltetradecylammonium and benzyldimethylhexadecylammonium iodides)	1:400	1	$>10^{6}$	$>10^{6}$	$>10^{6}$	$(4.9 \pm 1.8) \times 10^{3}$
		5	$>10^{6}$	$>10^{6}$	$>10^{6}$	$(2.7 \pm 2.0) \times 10^{3}$
(9) 50% potassium monopersulfate, monopotassium sulphate, and potassium sulphate; plus 5% sulfamic acid, and 15% sodium dodecylbenzenesulfonate	1:200	1	$>10^{6}$	$>10^{6}$	$>10^{6}$	$(2.7 \pm 1.3) \times 10^{4}$
		5	$>10^{6}$	$>10^{6}$	$>10^{6}$	$(5.7 \pm 3.8) \times 10^{4}$
(10) 17% alkyldimethylbenzylammonium chloride plus 7.8% didecylmethylbenzylammonium chloride, 10.7% glutaraldehyde, and 14.6% isopropanol	1:400	1	$>10^{6}$	$>10^{6}$	$>10^{6}$	$(4.7 \pm 2.9) \times 10^{3}$
		5	$>10^{6}$	$>10^{6}$	$>10^{6}$	$(2.1 \pm 2.5) \times 10^{3}$

(**1**) CR-36 Mural, José Collado, SA, Barcelona, Spain. **(2)** Darodor 9000, José Collado, SA, Barcelona, Spain. **(3)** Limoseptic, José Collado, SA, Barcelona, Spain. **(4)** Limoseptic plus, José Collado, SA, Barcelona, Spain. **(5)** Limoseptic SF, José Collado, SA, Barcelona, Spain. **(6)** Limoseptol, José Collado, SA, Barcelona, Spain. **(7)** Poliformo, José Collado, SA, Barcelona, Spain. **(8)** Totalcide, Bio-Genetic Laboratory, Madrid, Spain. **(9)** Virkon S, Bayer Healthcare, Barcelona, Spain. **(10)** Virocid, Bayer Healthcare, Barcelona, Spain.
[a] Dilutions according to the instructions of the manufacturers.
[b] $>10^{6}$, the maximal level of detection was surpassed (absence of growth in the lowest dilution tested in all replicates done).

culture plates as needed. Twenty microliter of each bacterial suspension (10^{9} CFU/ml of sterile saline) was placed on the carrier surface and was allowed to air dry for 1 h in a class-II biological safety cabinet. The contaminated area was then covered with 20 μl of disinfectant. After 1 min of contact, 980 μl of the diluent (sterile saline + 0.05% Tween 20) was added to each well to dilute the disinfectant and elute the bacteria from the steel carrier disc. The eluates were immediately subjected to 10-fold dilutions, and samples (0.1 ml) from each dilution were plated on enriched PPLO agar similar to that in suspension test. The conditions for the tests in the presence of serum were the same, but using 20 μl of *H. parasuis* suspended in sterile serum instead of saline. Controls were also similar to those in suspension test. Each carrier test was performed in triplicate and the disinfectant activity was determined as described above for suspension test.

3. Results and discussion

In all tests, control reactions not containing disinfectant resulted in complete recovery of the initial inoculum. The drying process and resuspension with the eluent in carrier test were found to hardly reduce the CFU of inocula: the lowest count obtained was 0.8×10^{7} CFU when the initial inocula were 2×10^{7} CFU, that is, 20 μl of 10^{9} CFU/ml. As the results obtained for any of the 26 products compared were basically the same for serovars 1 and 5 (a 1-$\log_{10}$ difference could be seen only in the 8.3% of all tests done), they will be shown hereafter at a species level, irrespective of the serovar.

Chlorine- and iodine-releasing agents are the most significant microbicidal halogens used for disinfectant purposes. Chloramine-T, a bactericidal compound that releases hypochlorous acid more slowly and is less irritating than hypochlorites (McDonnell and Russell, 1999), was found to be extremely effective in both tests causing the maximal level of inactivation (at least a 6-$\log_{10}$ reduction) without organic matter; however, the presence of serum contaminating the steel carrier discs slightly reduced its disinfectant capacity (a 4-$\log_{10}$ reduction after contact time) (Table 1). This result is in agreement with that reported for other gram-negative organisms (Gutiérrez et al., 1995). Sodium hypochlorite was effective when suspended in saline, resulting in at least 3-$\log_{10}$ reduction in CFU after contact time, but its disinfectant capacity was substantially reduced by serum, yielding only a 2-$\log_{10}$ reduction (Table 1). This effectiveness was similar to that described for *Actinobacillus pleuropneumoniae* (Gutiérrez et al., 1995). Among iodine-releasing agents, iodophor (0.1% available iodine) was completely ineffective, rather different from the result obtained for the povidone-iodine solution (containing a 10-fold higher concentration of available iodine than that of iodophor), which was effective except for carrier test with serum. This fact indicates that more than 0.1% available iodine is required for inactivating *H. parasuis*. The results concerning these two disinfectants were in general agreement with those obtained for other gram-negative organisms (Wang et al., 1983; Girardo et al., 1989; Gutiérrez et al., 1995).

Among oxidant agents, hydrogen peroxide was found effective except for carrier test with serum, which is in accordance with previous studies on *Haemophilus influenzae*, but using a completely different methodology on human patients (Miyasaki et al., 1986; Pericone et al., 2000) as well as on *A. pleuropneumoniae* using the two same *in vitro* methods (Gutiérrez et al., 1995). Potassium permanganate was only effective in suspension test in the absence of

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serum (Table 1), whereas in other investigation *A. pleuropneumoniae* resulted in a 4-$\log_{10}$ reduction when using this test with organic matter (Gutiérrez et al., 1995). Formulation No. 9, composed of several potassium derivatives, considerably improved its activity compared to potassium permanganate alone, and it was hardly reduced by serum (Table 2). This greater efficacy could be due to the higher potassium concentration in the commercial product and the combination with other active chemical agents, such as sulfamic acid and sodium dodecylbenzenesulfonate.

Quaternary ammonium compounds (QACs) are widely used as bactericidal agents and for disinfection of environmental surfaces. The two disinfectants examined alone (benzalkonium chloride and cetylpyridinium chloride) were only effective in suspension test (Table 1), suggesting interference between physical properties of stainless steel surfaces and their activity; in addition, serum slightly reduced the efficacy of both disinfectants in suspension test. Similar results for other gram-negative organisms have been shown (Gutiérrez et al., 1995; Avrain et al., 2003). Of the eight QAC-based formulations compared, all were effective in suspension test, although the activity of some of them was slightly reduced by serum (Table 2). This effect was more marked when using carrier test, in such a manner that the disinfectant capacity of formulation Nos. 1 and 3–6 was completely abolished by this organic material. Even so, our study suggests that some of QAC-based formulations (Nos. 8 and 10) are extremely effective at high dilutions (1:400), thus improving the results shown by the QACs used alone. Previous reports conducted against the human species *H. influenzae* (Pilloni et al., 1999) or the avian species *Avibacterium paragallinarum* (formerly *H. paragallinarum*) (Huberman et al., 2005) have resulted in high efficacies of different QACs using, respectively, other *in vitro* and *in vivo* tests. In other investigation, formulation No. 8 was extremely effective against other *Pasteurellaceae*: *P. multocida* subsp. *multocida* (Gutiérrez et al., 1999).

Ethanol and isopropanol, at optimal concentrations in the 60–90% range, are the most widely used alcohols especially for both hard-surface disinfection and skin antisepsis (McDonnell and Russell, 1999). Our results confirm their excellent effectiveness against serovars 1 and 5 of *H. parasuis* (except under the most disadvantageous condition: carrier test with serum, Table 1), as well as that of formulation Nos. 7 and 10 (composed, respectively, of a 7-fold lower concentration of isopropanol plus a cresol derivative and of an about 5-fold lower concentration plus two QACs and glutaraldehyde) under all conditions (Table 2). A previous report using 70% ethanol, as in our study, yielded similar results against other gram-negative organism, *Campylobacter jejuni*, but this antiseptic was not studied in the presence of organic matter (Wang et al., 1983). Using the same methodology, practically identical results were shown one more time against *A. pleuropneumoniae* (Gutiérrez et al., 1995).

Chlorhexidine digluconate, a cationic bisbiguanide, is commonly used in antiseptic products because it is mild and non-toxic (McDonnell and Russell, 1999). Although its activity is considered to be greatly diminished by organic matter (Russell and Day, 1993), however, this effect was only observed in carrier test (Table 1), in agreement with that previously described for other *Pasteurellaceae* (Gutiérrez et al., 1995). Chlorhexidine digluconate alone (Grap et al., 2004) or combined with a QAC (Reverdy et al., 1997) was also proven to be efficacious against human species of genus *Haemophilus*.

Formaldehyde, phenol and phosphoric acid were completely ineffective in carrier test with serum (Table 1); unlike our results, those of an earlier study using a lower concentration of formaldehyde but having a longer contact time indicated a good activity against *A. pleuropneumoniae* (Machavariani, 1988). On the contrary, in a latter report on this same gram-negative organism (Gutiérrez et al., 1995), the results obtained for these three disinfectants were almost identical to those obtained for *H. parasuis*. Anyway, the strong odor and toxicity of some of these compounds could make it unsuitable for use in swine practice.

Among metallic compounds, thimerosal was considerably more effective than zinc sulfate; nevertheless, both disinfectants yielded satisfactory results only in suspension test without serum (Table 1). This is in disagreement with a previous study using these two compounds against *A. pleuropneumoniae* under the same conditions (Gutiérrez et al., 1995), in which zinc sulfate was active in suspension test regardless of the presence of organic matter, and thimerosal was also in carrier test with saline.

To conclude, only chloramine-T and formulation Nos. 2, and 7–10 were able to inactivate *H. parasuis in vitro* under all the conditions tested and, consequently, only these compounds might be helpful in the adoption of environmental control measures against this organism.

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References

Amano, H., Shibata, M., Kajio, N., Morozumi, T., 1994. Pathologic observations of pigs intranasally inoculated with serovars 1, 4 and 5 of *Haemophilus parasuis* using immunoperoxidase method. Journal of Veterinary Medical Science 56, 639–644.

Avrain, L., Allain, L., Vernozy-Rozand, C., Kempf, I., 2003. Disinfectant susceptibility testing of avian and swine *Campylobacter* isolates by a filtration method. Veterinary Microbiology 96, 35–40.

Best, M., Sattar, S.A., Springthorpe, V.S., Kennedy, M.E., 1988. Comparative mycobactericidal efficacy of chemical disinfectants in suspension and carrier tests. Applied and Environmental Microbiology 54, 2856–2858.

Best, M., Kennedy, M.E., Coates, F., 1990a. Efficacy of a variety of disinfectants against *Listeria* spp. Applied and Environmental Microbiology 56, 377–380.

Best, M., Sattar, S.A., Springthorpe, V.S., Kennedy, M.E., 1990b. Efficacies of selected disinfectants against *Mycobacterium tuberculosis*. Journal of Clinical Microbiology 28, 2234–2239.

Best, M., Springthorpe, V.S., Sattar, S.A., 1994. Feasibility of a combined carrier test for disinfectants: studies with a mixture of five types of microorganisms. American Journal of Infection Control 22, 152–162.

Girardo, P., Reverdy, M.E., Martra, A., Fleurette, J., 1989. Determination of bactericidal mininum concentrations of 3 antiseptics and 1 disinfectant on 580 gram-negative bacilli. Pathologie Biologie 37, 605–611.

Grap, M.J., Munro, C.L., Elswick Jr., R.K., Sessler, C.N., Ward, K.R., 2004. Duration of action of a single, early oral application of chlorhexidine on oral microbial flora in mechanically ventilated patients: a pilot study. Heart Lung 33, 83–91.

Gutiérrez, C.B., Rodríguez Barbosa, J.I., Suárez, J., González, O.R., Tascón, R.I., Rodríguez Ferri, E.F., 1995. Efficacy of a variety of disinfectants against *Actinobacillus pleuropneumoniae* serotype 1. American Journal of Veterinary Research 56, 1025–1029.

Gutiérrez, C.B., Álvarez, D., Rodríguez Barbosa, J.I., Tascón, R.I., de la Puente, V.A., Rodríguez Ferri, E.F., 1999. *In vitro* efficacy of *N*-duopropenide, a recently developed disinfectant containing quaternary ammonium compounds, against selected gram-positive and gram-negative organisms. American Journal of Veterinary Research 60, 481–484.

Huberman, Y.D., Bueno, D.J., Terzolo, H.R., 2005. Evaluation of the protection conferred by a disinfectant against clinical disease caused by *Avibacterium paragallinarum* serovars A, B, and C from Argentina. Avian Diseases 49, 588–591.

Kielstein, P., Rapp-Gabrielson, V.J., 1992. Designation of 15 serovars of *Haemophilus parasuis* on the basis of immunodiffusion using heat-stable antigen-extracts. Journal of Clinical Microbiology 30, 862–865.

Machavariani, E.M., 1988. Disinfection for *Haemophilus pleuropneumoniae* in pigs. Veterinariya (Moscow) 8, 22–23.

McDonnell, G., Russell, A.D., 1999. Antiseptics and disinfectants: activity, action, and resistance. Clinical and Microbiological Reviews 12, 147–179.

Miyasaki, K.T., Genco, R.J., Wilson, M.E., 1986. Antimicrobial properties of hydrogen peroxide and sodium bicarbonate individually and in combination against selected oral, gram-negative, facultative bacteria. Journal of Dental Research 65, 1142–1148.

Oliveira, S., Pijoan, C., 2004. *Haemophilus parasuis*: new trends in diagnosis, epidemiology and control. Veterinary Microbiology 68, 71–75.

Pericone, C.D., Overweg, K., Hermans, P.W., Weiser, J.N., 2000. Inhibitory and bactericidal effects of hydrogen peroxide production by *Streptococcus pneumoniae* on the inhabitants of the upper respiratory tract. Infection and Immunity 68, 3990–3997.

Pilloni, A.P., Buttini, G., Giordano, B., Iovene, M.R., di Salvo, R., Buommino, E., Tufano, M.A., 1999. The *in vitro* effects of cetyltrimethylammonium naproxenate on oral and pharyngeal microorganisms of various ecological niches. Journal of Periodontal Research 34, 473–477.

Reverdy, M.E., Martra, A., Fleurette, J., 1997. Bactericidal activity determination of Biseptine, combination of chlorhexidine, benzalkonium chloride and benzylic alcohol, on 124 hospital bacterial strains. Pathologie Biologie 45, 331–335.

Russell, A.D., Day, M.J., 1993. Antibacterial activity of chlorhexidine. Journal of Hospital Infection 25, 229–238.

Wang, W.L., Powers, B.W., Luechtefeld, N.W., Blaser, M.J., 1983. Effects of disinfectants on *Campylobacter jejuni*. Applied and Environmental Microbiology 45, 1202–1205.

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