Bactericidal efficacy of two disinfectants against *Brachyspira hyodysenteriae* and one feed supplement against *B. hyodysenteriae* and *B. pilosicoli*

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ABSTRACT: *In vitro* tests were used to evaluate bactericidal efficacy of two disinfectants on the basis of peroxygen compounds against one type strain and one field isolate of *B. hyodysenteriae*. Mean bactericidal concentrations (MBCs) of the two products ascertained with and without the load of organic matter of sterile pig faeces were several times lower than the recommended application concentrations. Bactericidal efficacy of an extract of citric seeds (feed supplement) against type strains of *B. hyodysenteriae* and *B. pilosicoli*, with six field isolates of *B. hyodysenteriae* and three field isolates of *B. pilosicoli* was also demonstrated, and its MBCs were determined. It was further determined that after 10 minutes exposure of 10% sterile pig faeces to field isolate and type strain of *B. hyodysenteriae* the efficacy of both disinfectants was 16 times higher than the concentration recommended by the manufacturer. The bactericide effect of citrus fruit extracts was exhibited at 0.05% concentrations after 5 min exposure, which is the same as recommended by the manufacturer.

Keywords: swine dysentery; porcine intestinal spirochetosis; disinfectants

Intestinal infections caused by brachyspiras are a frequent reason for diarrhoeas and reduced weight gains in the post-weaning and fattening periods in pigs. *Brachyspira hyodysenteriae*, which causes the clinically most serious swine dysentery (Harris et al., 1972), and *B. pilosicoli*, which causes intestinal spirochetosis in pigs (Trott et al., 1996), occur with a varying frequency as the causative agents of endemic infections in pig populations in a number of countries (Hampson, 1997; Thomson et al., 2001). The primary source of such infections is faeces of clinically ill and latently infected pigs in which brachyspiras survive for different periods depending on the external conditions (Harris et al., 1999). It follows from the above that the critical areas for the survival of brachyspiras in pig herds are the faeces-covered surfaces in pig barns (pen floor, space below grid, etc.). The cleaning and effective disinfection of surfaces of equipment, transport vehicles, footwear, instruments and the pigs themselves will be a necessity not only in all in-all out operations and eradication programmes, but also in the treatment of dysentery and porcine intestinal spirochetosis. Efficacy evaluation of disinfectants against *B. pilosicoli* has been studied sporadically (Corona-Barrera et al., 2002). There are no experimental data available on the efficacy of disinfectants used to control *B. hyodysenteriae*. At the same time the failures of sanitation may be the cause of the re-infection of pigs from the contaminated environment. This may ultimately facilitate the survival and spreading of *B. hyodysenteriae* strains with reduced sensitivity to antidysenterics, as recently reported from Germany (Karlsson et al., 2002) and the Czech Republic (Cizek et al., 2002).

Plant extracts and metabolites with antibacterial activity as in-feed additives for pigs may be an alternative to antibiotics. Antimicrobial effects of some substances of plant origin against the causative agent of respiratory infections in pigs such as *Pasteurella multocida* and *Actinobacillus pleuropneumoniae* have already been demonstrated (Lyoo et al., 2001). A proper

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screening of such products will be very important to examine for the antidysenteric effects.

In the present study, *in vitro* bactericidal efficacy of two disinfectants on the basis of peroxygen compounds (Virkon S and Chirox) and of an extract of citric seeds (Citrex™) against type and field strains of *B. hyodysenteriae* a *B. pilosicoli* was evaluated.

MATERIAL AND METHODS

Bacterial cultures

The type strains used in the study were *B. hyodysenteriae* ATCC 27164^T and *B. pilosicoli* ATCC 51139^T, and the field strains included 6 field strains of *B. hyodysenteriae* and 3 strains of *B. pilosicoli* isolated in clinical cases of dysentery and intestinal spirochetosis in pigs between 1997 and 2000. The strains were kept in Tryptose soya broth (BBL) with 10% bovine foetal serum at –80°C. The frozen strains of *B. hyodysenteriae* and *B. pilosicoli* were inoculated on Tryptose soya agar (BBL) with 5% ovine blood (TSBA) and incubated in the atmosphere produced by a gas generating kit (BR38, Oxoid) for 3–4 days at 37°C. The cultures obtained in this way were removed from TSBA with a cotton ball, suspended in 2 ml sterile PSB to opacity adjusted on a photometer (Densi-La-Meter, LIAP, Latvia) to 1° at the Mac Farland opacity scale. The working suspension was then prepared by a 100-fold dilution, which corresponded to the 3×10^6 CFU/ml concentration.

Antibacterial substances

Substances of peroxygen compounds Virkon S – mixture of peroxygen salts, organic acids, (Antec International) and Chirox – potassium peroxomonosulphate, tensides and adjuvants, (Bochemie Bohumin, CR) were used to prepare 4% solutions in fresh water (pH 7.2) according to instructions of the manufacturers. The solutions were further diluted with the coefficient 2. Individual specimens were mixed with the same volume of the working suspension of brachyspiras to achieve the final concentration of disinfectants (1.0%, 0.5%, 0.25%, 0.125%, 0.06% and 0.03%). The extract of citric seeds (Citrex™, Citrex, Inc.) used in the bactericidal efficacy tests was diluted in fresh water (pH 7.2) to concentrations of 0.05, 0.025, 0.0125, 0.006 and 0.003%.

Preparation of sterile pig faeces (SPV) – (20%)

20 g of sterile pig faeces was mixed with 80 ml sterile deionised water and properly homogenised by vortexing. Next the suspension was left at the room temperature for 30 min. until sedimentation of solid segments of faeces. After that the supernatant was divided into 2 ml aliquots in the sterile plastic tubes. After mixing of 2 ml SPF with 2 ml of the tested disinfectant, 10% concentration of SPF was obtained. The importance of SPV is in the formation of ballast situation for disinfectants.

Bactericidal efficacy tests

Bactericidal efficacy was tested on the *B. hyodysenteriae* ATCC 27164 type strain and the *B. hyodysenteriae* Bh 902 field strain, with and without the load of 10% suspension of sterile pig faeces (SPF) at the laboratory temperature. Two ml of working suspension of brachyspiras were mixed with the same volume of individual dilutions of the two disinfectants. For the bactericidal efficacy tests under load, a 20% SPF suspension in distilled water was made first. Equal volumes of the 20% SPF suspension and of disinfectant dilutions were then mixed together, and brachyspira suspensions were added to each specimen in an amount of 40 µl to the 1.5×10^6 CFU/ml concentration of brachyspiras in each specimen. All the specimens were thoroughly vortexed, and 20 µl of each specimen were pipetted on the surface of Wilkins-Chalgren anaerobic agar (CM 619, Oxoid) with 5% ovine blood (WCABA) at 0, 1, 2, 4, 5, 10, 15 and 20 minute exposition time. After a three-day incubation in anaerobic atmosphere at 37°C, the minimum bactericidal concentration (MBC) of the peroxygen compounds was recorded as the lowest concentration that killed the *B. hyodysenteriae* strain tested, which was defined as the absence of growth and haemolysis in the inoculated spot. Specimens with no tested peroxygen compounds were used as controls.

The two type and 9 field brachyspira strains were tested in a diluted extract of citric seeds including controls with no extract at a laboratory temperature at brachyspira concentration of 3×10^6 CFU/ml in specimens. The specimens were thoroughly vortexed, and 20 µl of each specimen were inoculated on the WCABA surface at times of 0, 5, 10, 15, 20, 25, 30 and 40 minutes and 24 hours. Bactericidal

efficacy was evaluated as described above. All experiments were repeated at least once.

RESULTS

The bactericidal efficacy of the tested peroxygen compounds was expressed as MBC values at different exposure periods in aqueous environment with and without the SPF load. The computed values are in Tables 1 and 2. The data clearly show that MBCs of the two disinfectants were not different when a comparison between the tested strains was made. Differences, however, were found in a comparison between MBC values of the two disinfectants and MBC values with and without the SPF load. The differences between MBCs with and without the SPF load increased with the increasing exposure period, and the difference was particularly noticeable in Chirox.

Bactericidal efficacy of the extract of citric seeds against 11 strains of brachyspiras is shown in Table 3. MBC values decreased with an increasing exposure period, and the highest sensitivity to the extract was found in the *B. hyodysenteriae* 625 field strain. No marked differences in MBC values between other brachyspira strains were found.

DISCUSSION

The environment of pig farms contaminated with faeces of clinically ill and latently infected pigs with *B. hyodysenteriae* and *B. pilosicoli* is an important place for the survival of other species of intestinal infections (*Salmonella, Lawsonia*, etc.). The farmers cannot therefore exist without regular cleaning and disinfecting that reduce the risk of infection of the animals on the farm. This is particularly true about the all in – all out system (Strauch, 1987; Hinton and

Table 1. Bactericidal effect of Chirox against *B. hyodysenteriae* strains with and without SPF load

Strain	SPF load	MBC of Chirox (%) at various exposure periods (min)							
		1	2	4	5	10	15	20	PC
Bh ATCC 27164	no	0.25	0.125	0.06	0.06	0.06	0.06	≤0.03	+
	yes	0.25	0.125	0.125	0.125	**0.125**	0.125	0.125	+
Bh 902	no	0.25	0.25	0.06	0.06	0.06	≤0.03	≤0.03	+
	yes	0.125	0.125	0.125	0.125	**0.125**	0.06	0.06	+

SPF = sterile pig faeces
MBC = minimal bactericidal concentration
Bh = *Brachyspira hyodysenteriae*
PC = positive control growth of strain in an aqueous sample without Chirox

Table 2. Bactericidal effect of Virkon S against *B. hyodysenteriae* strains with and without SPF load

Strain	SPF load	MBC of Virkon S (%) at various exposure periods (min)							
		1	2	4	5	10	15	20	PC
Bh ATCC 27164	no	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	+
	yes	0.06	0.06	0.06	≤0.03	**≤0.03**	≤0.03	≤0.03	+
Bh 902	no	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	+
	yes	0.06	0.06	0.06	0.06	**≤0.03**	≤0.03	≤0.03	+

SPF = sterile pig faeces
MBC = minimal bactericidal concentration
Bh = *Brachyspira hyodysenteriae*
PC = positive control growth of strain in an aqueous sample without Virkon S

Table 3. Bactericidal effect of Citrex against 11 strains of *B. hyodysenteriae* and *B. pilosicoli*

Strain	MBC of Citrex (%) at various exposure periods (min)								
	0	**5**	10	15	20	25	30	40	PC
Bh ATCC 27164^T	>0.05	**0.025**	0.025	0.0125	0.0125	0.0125	0.006	0.006	+
Bp ATCC 51139^T	>0.05	**0.05**	0.05	0.025	0.025	0.025	0.025	0.0125	+
Bh 600	>0.05	**0.0125**	0.0125	0.006	0.006	0.006	0.006	0.006	+
Bh 634	>0.05	**0.05**	0.025	0.025	0.025	0.006	0.006	0.006	+
Bp 98	>0.05	**0.05**	0.025	0.025	0.025	0.0125	0.0125	0.0125	+
Bh 601	>0.05	**0.0125**	0.0125	0.025	0.025	0.006	0.006	0.006	+
Bh 625	>0.05	**0.0125**	0.006	0.006	0.003	≤0.003	≤0.003	≤0.003	+
Bh 630	>0.05	**0.025**	0.0125	0.0125	0.0125	0.0125	0.006	0.006	+
Bh 622	>0.05	**0.05**	0.05	0.025	0.025	0.025	0.025	0.0125	+
Bp 145	0.05	**0.05**	0.025	0.025	0.025	0.025	0.025	0.0125	+
Bp 157	>0.05	**0.05**	0.025	0.025	0.025	0.0125	0.0125	0.0125	+

The efficacy of Citrex^{TM} on the field isolate SH 902 was not defined because it was isolated after this examination
MBC = minimal bactericidal concentration
Bh = *B. hyodysenteriae*, Bp = *B. pilosicoli*
PC = positive control growth of strain in an aqueous sample without Citrex
SPF = sterile pig faeces
+ = growth and haemolysis

Bale, 1991). A rational choice of disinfectants for this purpose should be based on the data on their efficacy *in vitro* and in the field. Tests of the biocidal activity of peroxygen compounds under such conditions demonstrated their very good efficacy in killing bacteria, fungi, viruses and spores (Gasparini et al., 1995; Gutierrez et al., 1999; Hernandez et al., 2000; Amass et al., 2001).

The present study demonstrated very good efficacy of two disinfectants with a mixture of peroxide salts (Virkon S) and potassium peroxomonosulphate (Chirox) as their active ingredients against both tested strains of *B. hyodysenteriae*. The difference between MBCs of the two disinfectants found in the study may have been caused by different levels of additional substances that are an important part of the preparations. After a 10-minute exposure under the load of organic matter in 10% SPF suspension, their effective concentrations expressed as MBCs were 0.125% in Chirox and <0.03% in Virkon S and it was approximately 16 times lower than the recommended working dilutions (Chirox 2%, Virkon S 1%), which is very important from the practical aspect of their use on farms. Comparable results were also obtained by Corona-Barrera et al. (2002), who tested the efficacy of Virkon S and another six disinfecting and cleaning agents against *B. pilosicoli* strains. The benefits of the use of disinfectants based on peroxygen compounds in pig herds include minimum negative impacts on the environment as well as administration at the presence of the animals. For these advantages and their high biocidal efficacy against pathogenic brachyspiras demonstrated in the present study, they can be recommended for disinfection purposes in programmes of the control and eradication of swine dysentery and intestinal spirochetosis in pigs.

It is unlikely that antibiotics for the prevention of infections will be allowed as in-feed additives for pigs in future. In the case of swine dysentery and intestinal spirochetosis in pigs, alternative approaches must therefore be found to reduce the number of carriers that are generally accepted as very important factors in the spreading of intestinal infections. A targeted use of plant in-feed additives might be one of the possible alternatives. Recently published studies confirmed the presence of substances, the oil from the leaves of *Citrus reticulata* and grapefruit (*Citrus paradisi*) peel extract with antibacterial effects against Gram-negative and

Gram-positive bacteria (Negi and Jayaprakasha, 2001; Pachori, 2002).

In our study the efficacy of an extract of citric seeds against *Brachyspira hyodysenteriae* and *B. pilosicoli* was demonstrated in a concentration of 0.05% after 5-minute or in a concentration of 0.025% after 10-minute exposition time. It is recommended by the manufacturer to use the extract for prevention of diarrhoeic infection of pigs in a concentration of 0.05%. Therefore we can assume that the tested extract will be an alternative for the elimination of *B. hyodysenteriae* and *B. pilosicoli* carriers instead of antibiotics in preventive dosages.

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