Available online at www.sciencedirect.com
ScienceDirect
The Veterinary Journal 177 (2008) 388–393
The Veterinary Journal
www.elsevier.com/locate/tvjl

Virucidal efficacy of nine commercial disinfectants against porcine circovirus type 2

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Accepted 25 June 2007

Abstract

A number of commercially available disinfectants are commonly used on pig breeding farms and are authorised by the French Agricultural Ministry. However, the efficacy of these disinfectants is unknown with regard to the emergent porcine circovirus type 2 (PCV2). The virucidal efficacy of nine disinfectants was evaluated by testing a suspension of PCV2 isolated in France. The assays were performed at 20 °C and the efficacy determined after 30 min contact time between virus and disinfectant. After this time, the mixture was passed through a detoxification column and then diluted to remove compounds toxic to the virus and the porcine kidney cell line. The filtrate was serially diluted and inoculated onto cell culture. The infectivity of PCV2 was determined by an immunoperoxidase monolayer assay.

No reduction in PCV2 titre was demonstrated with iodine and phenolic products. Significant PCV2 titre reductions ($\geqslant 1.61$ $\log_{10}$) were noted for the seven other products. For five disinfectants, namely a product composed of potassium monopersulfate, two products comprising a quaternary ammonium with one or three aldehyde(s), sodium hypochlorite, and sodium hydroxide, the concentration that significantly reduced the PCV2 titre was equal or 1.5–4 times lower than the authorised use concentration. Only two disinfectants, one composed of potassium monopersulfate, the other containing peracetic acid with hydrogen peroxide, reduced the PCV2 titre with a product concentration at best equal or two times higher than the authorised use concentration.

Keywords: Disinfectant test; Disinfection; Virucidal activity; Circovirus; PCV2

Introduction

Porcine circoviruses are the smallest known autonomously replicating animal viruses and are characterised as small non-enveloped viruses with a capsid size of 17 nm containing single stranded circular DNA. Two types of porcine circovirus (PCV) have been isolated and are referred to as porcine circovirus type 1 (PCV1), which is non-pathogenic for swine (Allan et al., 1995; Tischer et al., 1986), and porcine circovirus type 2 (PCV2), which is pathogenic and associated with disease syndromes such as post-weaning multisystemic wasting syndrome (PMWS) (Allan et al., 1999; Allan and Ellis, 2000; Segales and Domingo, 2002; Ghebremarium and Gruys, 2005).

PMWS was first described in western Canada in pig herds (1991) and since then outbreaks of PMWS have been reported worldwide (Chae, 2004). PMWS is now endemic in many swine producing countries and continues to be a major cause of wasting disease. Indeed, a Canadian group recently reported a dramatic increase in PCV2-associated disease between 2004 and 2005 (Carman et al., 2006). PMWS is often seen in combination with other viral or bacterial pathogens. Although PCV2 vaccines have now become available, improvements in hygiene are important in the control of PCV2/PMWS infection. To date, only

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doi:10.1016/j.tvjl.2007.06.016

Table 1
Concentrations of active ingredients in the nine disinfectants tested against PCV2 at the official use concentration

Disinfectant	Class of disinfectant	Product composition	French official use concentration (%)	Active ingredients concentrations at use concentration μg L^{-1}
1	Oxidising agent	Sodium hypochlorite	0.3	3000
2	Alkali	Sodium hydroxide	0.8	8000
3	Iodine	Iodine 1.08%	3	324
4	Phenol	2Benzyl 4chlorophenol (P1) 5% 4Chloro 3methyl phenol (P2) 10%	0.4	600 (P1 + P2)
5	Oxidising agent	Peracetic acid (APA) 4,3% Hydrogen peroxide (HP) 23.5%	1	430 (APA) 2330 (HP)
6	Aldehydes Quaternary ammonium	Formaldehyde (Ald1) 3,15% Glutaraldehyde (Ald2) 4% Glyoxal (Ald3) 3.2% Didecyldimethylammonium chloride (AQ1) 10%	0.5	 515 (Ald1 + Ald2 + Ald3) 500 (AQ1)
7	Aldehyde Quaternary ammonium	Glutaraldehyde (Ald2) 15% *n*-Alkyldimethylbenzylammonium (AQ2) 8%	1.5	2250 (Ald2) 1200 (AQ2)
8	Oxidising agent Acid	Potassium monopersulfate (PMP) 23.1% Sodium dichoroisocyanurate (DCCNa) 5% Sulfamic acid (SA) 15%	0.5	1155 (PMP) 250 (DCCNa) 750 (SA)
9	Oxidising agent Acids	Potassium monopersulfate (PMP) 22.5% Malic acid (MA) 10% Sulfamic acid (SA) 5%	0.5	1125 (PMP) 500 (MA) 250 (SA)

two papers have documented the efficacy of disinfectants against PCV2 suspensions (Royer et al., 2001; Yilmaz and Kaleta, 2004).

In the European Union, Members States are required to ensure that disinfectants are used according to their authorised use concentrations as officially approved by the competent national authority. In France, the efficacy of disinfectants has to be demonstrated according to Guidelines published by the French Agricultural Ministry (Anon., 1957, 1999). Disinfectants are authorised for use against viruses if they can prove their efficacy at a given concentration for use against a resistant Talfan virus, which is the official reference strain. To date, no information exists on PCV2 sensitivity to disinfectants or even whether the virus could be resistant. The objective of the present study was to ascertain the virucidal activity of several disinfectants already authorised by French regulations (albeit not for this specific use) against the emergent PCV2.

Materials and methods

Virus

Virus, porcine kidney cell line (PK15), free of PCV type 1 and type 2, and anti PCV2 porcine serum were provided by the Swine Virology and Immunology Unit (AFSSA, Ploufragan, France).

PCV2 propagation on PK15 cells was performed as previously described (Albina et al., 2001). The infectivity of the PCV2 on PK15 cells was determined by an immunoperoxidase assay (IPMA) (Mahé et al., 2000) and virus titres measured using the median infective dose ($TCID_{50}$) method with Wyshak and Detre tables (Anon., 1989). The protein concentration of the PCV2 stock was 13 mg mL^{-1} as determined by the Lowry method (Lowry et al., 1951). The stock was aliquoted and frozen at −70 °C. The titre was $3.66 \pm 0.12 \log_{10}$ median tissue infective doses ($TCID_{50}$ mL^{-1}).

Disinfectants

Two out of the nine disinfectants (disinfectants 1 and 2) were purchased from a commercial supplier. The other seven products (disinfectants 3–9) were obtained directly from the manufacturers. For each disinfectant, the product composition and the authorised use concentration, expressed as product dilution (%) and equivalent active ingredients (μg L^{-1}), are shown in Table 1.

Product dilutions were undertaken using sterile distilled water. For each disinfectant, a range of concentrations (see Table 2) including the authorised use concentration (see Table 1) was tested.

Procedure for evaluating disinfectant efficacy against PCV2

As described in the French standard NF T 72-180 (Anon., 1989), the virucidal efficacy of a product is validated after tests performed under standardised conditions, but with a weaker viral titre. So, efficacy tests and preliminary studies were performed following the procedure below.

Efficacy tests

To 0.6 mL of PCV2 virus stock, an equal volume of double–strength disinfectant was added to give 1.2 mL of virus–disinfectant mixture. The disinfectant remained in contact with the virus in a water-bath at 20 °C for 30 min, after which time the reaction was stopped by filtration through a detoxification column as described in the NF T 72–180 standard (Anon., 1989). Because of the product's cytotoxicity, the filtration was combined with a dilution (1/2 or more) in cold MEM medium (kept in ice) supple-