Efficacy of different disinfectants in vitro against porcine circovirus type 2

H. B. Kim, K. S. Lyoo, H. S. Joo

INFECTION with porcine circovirus type 2 (PCV-2) in pigs has been associated with several diseases including postweaning multisystemic wasting syndrome (PMWS). Although PMWS was not clinically apparent until early 2005, it is now considered one of the most economically significant diseases of pigs in the USA. Pig farms with clinical PMWS have high mortality among growing pigs. Recently, the prevention of clinical PMWS has been successful due to the use of commercial PCV-2 vaccines. However, vaccinated pigs can still become infected and shed the virus into the environment.

Prevention of the spread of PCV-2 infection among pigs and between farms can be achieved by the application of strict biosecurity practices. Current practices commonly involve the use of different disinfectants; however, limited information exists regarding the efficacy of disinfectants against PCV-2. Royer and others (2001) reported that exposure of PCV-2 to different disinfectants for 10 minutes resulted in a reduction in virus titre. In their results, however, none of the disinfectants tested showed complete inactivation of PCV-2. The results reported are not sufficient to establish an effective biosecurity programme on a farm, and the efficacy of current commercial disinfectants and disinfection protocols for PCV-2 inhibition need to be re-evaluated. The use of effective disinfectants with proper biosecurity practices is an important step in controlling PMWS and preventing PCV-2 infection on pig farms.

This short communication describes a study to evaluate in vitro the virucidal effect of different commercial disinfectants against PCV-2. In addition, some physicochemical properties affecting the survival of PCV-2 were examined.

The PCV-2 strain used was a Minnesota isolate (GenBank accession number EF452353) (Lyoo and others 2008). In addition, a porcine reproductive and respiratory syndrome virus (PRRSV) (MN1b) (Yoon and others 1992) was used as a comparison in some experiments. PCV-2 was propagated using a porcine kidney cell line (PK-15) free of porcine circovirus type 1 (PCV-1) and PCV-2, and PRRSV was propagated in MARC-145 cells. Cell cultures were maintained in minimal essential medium supplemented with 3 to 5 per cent fetal bovine serum. A one-day-old PK-15 cell monolayer was used for PCV-2 propagation. The PCV-2 infected cell monolayer was treated with 300mM D-glucosamine as described by Tischer and others (1987) to increase virus titre. The median tissue culture infective dose ($TCID_{50}$/ml) was $1 \times 10^{4.5}$ to $1 \times 10^{5.5}$ for stock PCV-2 and $1 \times 10^{5.5}$ for PRRSV.

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H. B. Kim, DVM, MS,
K. S. Lyoo, DVM, MS,
H. S. Joo, DVM, MS, PhD,
Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, 385 Animal Science/Veterinary Medicine Building, 1988 Fitch Avenue, St Paul, MN 55108, USA

Correspondence to Professor Joo,
e-mail: jooxx001@umn.edu

Infectivity titres of PCV-2 were determined by a routine method, and the results were read by an immunofluorescence antibody test as described by Allan and others (1998). Briefly, 0·1 ml of 10-fold serial dilutions of PCV-2 was dispensed into a 96-well microtitration plate, and 0·1 ml of PK-15 cells (1×10^5 to 2×10^5 cells/ml) was added to each well. One day later, the cell culture in each well was treated with 50 µl 300mM D-glucosamine. The cell monolayer was cultured for two more days and then fixed with 50 per cent acetone in ethanol. The immunofluorescence antibody test was performed on the cell monolayer by incubating it with a PCV-2 reference positive pig serum for one hour at 37°C, followed by three washes with phosphate-buffered saline (PBS) (pH 7·2). The monolayer was then incubated with rabbit anti-pig IgG conjugated with fluorescein isothiocyanate for one hour, washed three times with PBS, and examined under a fluorescence microscope. Determination of the infectivity titre for PRRSV was routinely carried out using MARC-145 cells, and the titres were read by examining for cytopathic effects.

Eight disinfectants, purchased from commercial suppliers, were tested: Virkon S 1:100 (Antec International) (a mixture of potassium peroxymonosulphate and sodium chloride); 1-Stroke Environ 1:256 (Steris) (a phenolic compound); Tek-Trol 1:256 (Bio-Tek Industries) (a phenolic compound); Roccal D Plus 1:256 (Pfizer Animal Health) (a quaternary ammonium compound); DC&R 1:128 (Neogen) (a formaldehyde and quaternary ammonium compound); Synergize 1:256 (Preserve International) (a quaternary ammonium and glutaraldehyde compound); Nolvasan 1:42·7 (Fort Dodge Laboratories) (chlorhexidine); and Clorox Bleach 1:21·3 (Clorox) (sodium hypochlorite). Three per cent sodium hydroxide was also used.

The virucidal effect of each disinfectant was evaluated. Each disinfectant was prepared in distilled water at twice the manufacturer's recommended dilution (or sodium hydroxide at 6 per cent) and equal volumes of the disinfectant and PCV-2 were mixed (giving a final concentration of 3 per cent sodium hydroxide). The PCV-2/disinfectant mixtures were incubated at room temperature for different lengths of time. After incubation, the mixtures were centrifuged through a Sephadex LH-20 bead (GE Healthcare Bio-Sciences) detoxification column, as described by Blackwell and Chen (1970) with some modifications. Briefly, 1 ml of the PCV-2/disinfectant mixture was added to the top of a 5 ml tube containing a 4 ml slurry of 22 per cent Sephadex LH-20 beads. The 5 ml tube had a small hole in the bottom, and a cotton bud was placed to cover the hole. The tube was wedged inside a 15 ml tube that had a 1·5 ml microcentrifuge tube in the bottom, and the 15 ml tube was centrifuged at 1000 *g* for 10 minutes. The detoxified virus passed through the gel and the hole in the 5 ml tube, collecting in the microcentrifuge tube. The infectivity titre was determined for the collected virus as described above.

The susceptibility of PCV-2 to heat and different pH values was studied by routine methods. One part PCV-2 was mixed with nine parts pH buffer (Ricca Chemical) and incubated at room temperature for 30 minutes. Infectivity titres of each mixture were tested after neutralisation. The effect of formalin was also tested using virus-infected monolayer cells. PCV-2 and PRRSV were inoculated on to PK-15 and MARC-145 cells, respectively, in 24-well culture plates. Formalin (0·5 ml/well) at concentrations of 0·05, 0·1, 0·2 and 0·4 per cent was added to the virus-infected cell monolayer, and the monolayer was incubated at room temperature for one hour. The formalin was aspirated and the monolayer was washed three times with PBS. The cell monolayers were then scraped with 0·2 ml PBS per well, and the cell suspension from three wells was pooled. The suspension was frozen and thawed three times and centrifuged, and the supernatant was examined for the presence of virus.

The reduction of PCV-2 infectivity titres following treatment with each disinfectant in shown in Fig 1. For mixtures of virus and Virkon S, Clorox Bleach and sodium hydroxide, a complete reduction in PCV-2

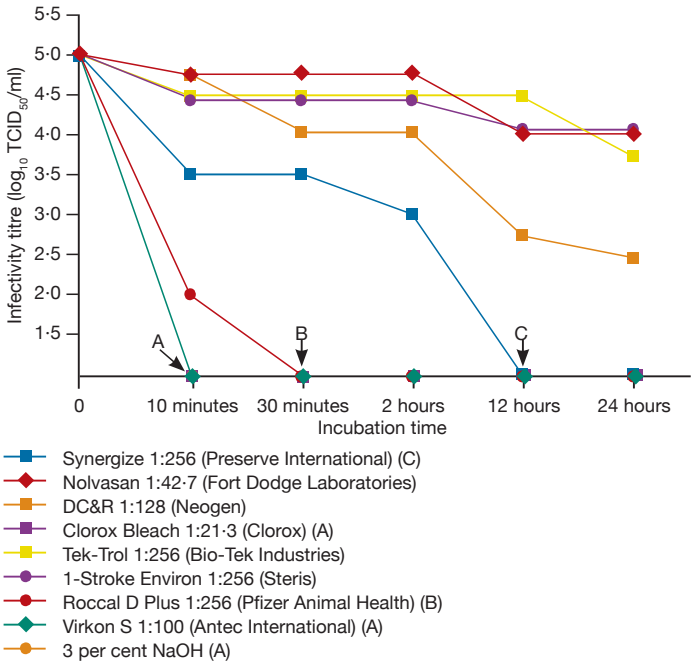

FIG 1: Reduction of porcine circovirus type 2 (PCV-2) infectivity following treatment with eight different disinfectants, at a final dilution in line with manufacturers' recommendations, or 3 per cent sodium hydroxide. Arrows indicate the time of complete inactivation of PCV-2 by certain disinfectants

infectivity occurred within 10 minutes Roccal-D Plus and Synergize caused substantial reduction of PCV-2 infectivity when the incubation time was increased to 30 minutes. DC&R reduced the infectivity from $1 \times 10^{5.0}$ to $1 \times 10^{2.5}$ $TCID_{50}$/ml after 24 hours of incubation time. The remaining disinfectants reduced infectivity only slightly. In another experiment using Virkon S, Clorox Bleach Roccal-D Plus and Synergize at a 1:5 dilution, with 12 hours incubation, PCV-2 was reduced from $1 \times 10^{4.5}$ $TCID_{50}$/ml to $1 \times 10^{2.25}$, $1 \times 10^{1.75}$, $1 \times 10^{3.5}$ and $1 \times 10^{3.75}$ $TCID_{50}$/ml, respectively. For PRRSV there was no detectable virus after all PRRSV disinfectant mixtures had been incubated at room temperature for 10 minutes.

The virus titres remained unchanged after incubation at 56°C for one hour, and were reduced from $1 \times 10^{5.5}$ $TCID_{50}$/ml to $1 \times 10^{3.5}$ after incubation at 56°C for six hours and to $1 \times 10^{3.0}$ $TCID_{50}$/ml after incubation at 56°C for 24 hours. Infectivity was reduced from $1 \times 10^{5.5}$ $TCID_{50}$/ml to $1 \times 10^{2.5}$ $TCID_{50}$/ml after incubation at 70°C for one hour, and no infectivity was detected following exposure to 70°C for six hours. When PCV-2 ($1 \times 10^{5.5}$ $TCID_{50}$/ml) was exposed to different pHs for 30 minutes, the infectivity titres were $1 \times 10^{2.7}$ at pH 2, $1 \times 10^{3.2}$ at pH 3, $1 \times 10^{3.3}$ at pH 4, $1 \times 10^{5.5}$ at pH 7, $1 \times 10^{2.8}$ at pH 10, $1 \times 10^{1.3}$ at pH 11 and $1 \times 10^{0.3}$ at pH 12. When PCV-2 and PRRSV were treated with formalin, there was no detectable virus following treatment with 0·2 or 0·4 per cent formalin for one hour on each virus-infected cell monolayer. However, following treatment with 0·05 or 0·1 per cent formalin for one hour, PCV-2 was detected, whereas PRRSV was not.

PCV-2 is ubiquitous and extremely resilient in many environments (Segales and others 2005). A related virus, PCV-1, is resistant to inactivation at pH 3 and in lipid solvents, and is stable at 70°C for 15 minutes (Allan and others 1994), but little is known about the stability of PCV-2 at different pH and temperatures. The faecal-oral route of virus transmission is common in pigs, and a number of disinfectants are used on pig farms. To implement effective biosecurity measures within and between pig farms, and to be confident that transportation vehicles are thoroughly cleaned, it is crucial to understand the effectiveness of each disinfectant against PCV-2. Once farms use only the most effective disinfectants, PCV-2 control will be possible, and economic losses due to disease associated with the virus will be reduced.

The present results, that Virkon S, Clorox Bleach and sodium hydroxide were the most effective virucidal agents against PCV-2, agree with those of Royer and others (2001). Following longer exposure, Roccal D Plus and Synergize successfully inactivated PCV-2. With a longer exposure time at a dilution of 1:5 of the manufacturer's recommended dilution, Virkon S and Clorox Bleach also showed some effectiveness in inactivating PCV-2. The virucidal effects of Virkon S were very good, but this disinfectant is known to be relatively expensive. Clorox Bleach, an inexpensive product with a broad-spectrum disinfecting ability, worked well. Even with a weaker dilution (1:100) of Clorox Bleach, a marked reduction in virus titres was evident if the treatment period was extended to 24 hours or more. Therefore, Clorox Bleach may be recommended for use against PCV-2 on pig farms; however, there may be associated problems such as skin irritation and metal corrosion.

Regarding the physical and chemical characteristics of PCV-2, its infectivity was somewhat decreased in an acid buffer, but PCV-2 maintained viability even under a strong acid of pH 2. The infectivity was markedly decreased at a high of pH of 11 or 12. Survivability of PCV-2 was demonstrated at high temperatures, indicating that the virus would be likely to survive in any contaminated areas during the hot summer season.

The results of the present study indicate that PCV-2 is inactivated by formalin treatment, providing important information in the face of speculation among some veterinarians that PCV-2 may not be completely inactivated following formalin treatment during vaccine preparation. Formaldehyde vapour was also effective for inactivating PCV-2 if the virus was fumigated for longer than 12 hours (data not shown). Disinfection of a closed space may be achieved by fumigation with formaldehyde vapour, assuming optimal humidity and temperature.

Data from the present study concerning the efficacy of various disinfectants and some physicochemical properties of PCV-2 will be useful for pig veterinarians and researchers in developing effective biosecurity practices. Further research under field conditions should also be conducted to provide practical information on the effectiveness of each disinfectant against PCV-2.

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References

ALLAN, G. M., PHENIX, K., TODD, D. & MCNULTY, M. (1994) Some biological and physico-chemical properties of porcine circovirus. *Journal of Veterinary Medicine B* **41,** 17-26

ALLAN, G. M., MCNEILLY, F., KENNEDY, S., DAFT, B., CLARKE, E. G., ELLIS, J. A., HAINES, D. M., MEEHAN, B. M. & ADAIR, B. M. (1998) Isolation of porcine circovirus-like viruses from pigs with a wasting disease in the USA and Europe. *Journal of Veterinary Diagnostic Investigation* **10,** 3-10

BLACKWELL, J. H. & CHEN, J. S. H. (1970) Effects of various germicidal chemicals on H.Ep. 2 cell culture and herpes simplex virus. *Journal of the Association of Official Analytical Chemists* **53,** 1229-1236

LYOO, K. S., KIM, H. B. & JOO, S. H. (2008) Evaluation of a nested polymerase chain reaction assay to differentiate between two genotypes of porcine circovirus-2. *Journal of Veterinary Diagnostic Investigation* **20,** 283-288

ROYER, R. L., NAWAGITGUL, P., HALBUR, P. G. & PAUL, P. S. (2001) Susceptibility of porcine circovirus type 2 to commercial and laboratory disinfectants. *Journal of Swine Health and Production* **9,** 281-284

SEGALES, J., ALLAN, G. M. & DOMINGO, M. (2005) Porcine circovirus diseases. *Animal Health Research Reviews* **6,** 119-142

SEGALES, J., ALLAN, G. M. & DOMINGO, M. (2006) Porcine circovirus diseases. In Disease of Swine. 9th edn. Eds B. E. Straw, J. J. Zimmerman, S. D'Allaire, D. J. Taylor. Blackwell Publishing. pp 299-307

TISCHER, I., PETERS, D., RASCH, R. & POCIULI, S. (1987) Replication of porcine circovirus: induction by glucosamine and cell cycle dependence. *Archives of Virology* **96,** 39-57

YOON, I. J., JOO, H. S., CHRISTIANSON, W. T., KIM, H. S., COLLINS, J. E., CARLSON, J. H. & DEE, S. A. (1992) Isolation of a cytopathic virus from weak pigs on farms with a history of swine infertility and respiratory syndrome. *Journal of Veterinary Diagnostic Investigation* **4,** 139-143