The Impact Of A Hydrogen Peroxide Vapour Based Decontamination Method On The Infectivity and Detectability of Feline Caliciviruses as Surrogates for Human Noroviruses

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INTRODUCTION

Human noroviruses are a leading cause of gastroenteritis worldwide and effective decontamination following an outbreak is crucial to avoid recurrent outbreaks. Recently hydrogen peroxide vapour has been shown to be a suitable decontamination system. The aim of this study was to test a decontamination device (DCXpert®) in complete rooms, based on hydrogen peroxide micro aerosol fumigation. As noroviruses are caliciviruses we tested the efficacy of this technology on the feline calicivirus (FCV) as surrogate by determining infectivity and detectability via RT qPCR.

RESULTS AND DISCUSSION

Infectivity

Infectivity tests showed that it was difficult to obtain clear cytopathic effects (CPE) when fumigated viruses were used for infection of CRFK cells. It could be seen that the infected cells were damaged in comparison to the not infected controls but they were not as much lysed as when non treated viruses were used (Fig. 1). Fumigated negative controls did not lead to a changed phenotyp of CRFK cells. This indicates that the CPE of the cells infected with vaporised viruses was to due to the viruses and not to residues of vaporization.

$TCID_{50}$- determinations could be made from both types of tiles treated for 60 minutes and the ceramic tile fumigated for 120 minutes. A reduction in infectivity of 2 – 3 log_{10} was observed (see Fig 2). FCVs on PVC tiles that were treated for 120 min did not show CPE. None of the viruses treated with H_2O_2 were able to produce plaques.

Detectability

It was possible to detect viruses in all samples using RT qPCR. However, there was a reduction of 3 log_{10} as a result of the 60 and 120 min treatments. Virus numbers counts on the PVC title carrier fumigated for 120 min decreased by 4 log_{10}.

Detectionlimit

Both PCR and $TCID_{50}$ data show a reduction of virus numbers on the not treated process control tiles of at least 0.5 log_{10} compared to the starting concentration (data not shown). This indicates a reduction of virus numbers during the processing caused by drying, elution and transport.

This and the use of only 100 µl sample for PCR and titer determination led to a detection limit of a maximum reduction of 3 log_{10} using $TCID_{50}$ determination and 4 log_{10} using PCR.

Fig.1: Cells after treatment with fumigated viruses (B). (A) shows cells 'infected' with process negative control and (C) cells after infection with non treated viruses are shown.

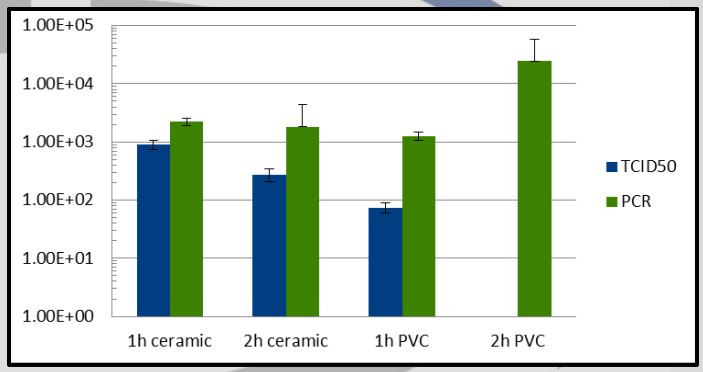

Fig.2: Log10 Reduction of FCV after DCXpert® Treatment. $TCID_{50}$ determinations showed that no infective viruses were observed after two hours fumigation on PVC.

MATERIAL AND METHODS

Experimental procedure

PCV- and ceramic tiles were artificially contaminated by 50 µl suspension of feline calicivirus or PBS as a negative control and left to dry for 30 min in a cabinet. Afterwards the carriers were placed in a room which was decontaminated by the DCXpert® for 60 min and 120 min or left in the cabinet as process controls (all samples in duplicate).

The tiles were washed with 35 ml PBS for 30 min on a shaker to elute the viruses. The washing solution was stored on ice, transported to the laboratory and used directly and in ten-fold dilutions for titer determination and RNA isolation.

Determination of Infectivity

Infectivity of the viruses in the washing solution and ten-fold dilutions of it was tested by $TCID_{50}$-determinations and plaque assays using confluent layers Crandell-Reese feline kidney (CRFK) cells.

Detectability by RT qPCR

To test detectability, 100 µl of the washing solution was used for RNA isolation and subsequent RT-qPCR.

Calculation of virus reduction

Drying, elution and transport can lead to a reduction of viruses. Therefore the effect of the DCXpert® was calculated in comparison to the non fumigated process controls. $TCID_{50}$ values / ml were multiplied with 0.7 the get PFU / ml.

CONCLUSION

While $TCID_{50}$ values and PCR data cannot be compared directly, both methods revealed reduced counts of feline caliciviruses by at least 2 log_{10} following DCXpert® fumigation when titer determinations were done or 3 log_{10} when quantification was done by PCR. These observations are in agreement with other studies which have investigated hydrogen peroxide decontamination and which showed a slightly higher reduction (e.g. Bentely et al. 2012: - 4 log_{10}).