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Hydrogen peroxide vapour decontamination of surfaces artificially contaminated with norovirus surrogate feline calicivirus

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SUMMARY

Background: Noroviruses are a leading cause of gastrointestinal disease and are of particular concern in healthcare settings such as hospitals. As the virus is reported to be environmentally stable, effective decontamination following an outbreak is required to prevent recurrent outbreaks.

Aim: To investigate the use of hydrogen peroxide vapour to decontaminate a number of surfaces that had been artificially contaminated with feline calicivirus (FCV), a surrogate for norovirus. The surfaces tested were representative of those found in hospital wards.

Methods: FCV was used to contaminate materials representative of a hospital setting (stainless steel, glass, vinyl flooring, ceramic tile and PVC plastic cornering). The carriers were exposed to 30% (w/w) hydrogen peroxide vapour at 5-min intervals over 20 min, after which postexposure viral titres were measured.

Findings: Hydrogen peroxide vapour reduced the viral titre by 4 $\log_{10}$ on all surfaces tested within 20 min of exposure. The reduction in viral titre took longest to achieve on stainless steel (20 min), and the quickest effect was seen on vinyl flooring (10 min). For glass, plastic and ceramic tile surfaces, the desired reduction in viral titre was seen within 15 min of exposure. Hydrogen peroxide vapour allows for large-scale decontamination of areas following outbreaks of infectious organisms.

Conclusion: Hydrogen peroxide vapour is effective against FCV and is active on a range of surfaces. Therefore, it may represent a suitable decontamination system for use following a hospital outbreak of norovirus.

Introduction

Noroviruses are a group of related, non-enveloped, single-stranded RNA viruses, belonging to the *Caliciviridae* family, that are the leading cause of outbreaks of non-bacterial gastroenteritis in humans.[1] Noroviruses are estimated to be responsible for around 85–95% of all non-bacterial gastroenteritis outbreaks worldwide. In recent years, there has been increased reporting of gastroenteritis outbreaks in both Europe and the USA, with norovirus confirmed as the causative agent in a large percentage of cases.[2–4] The virus is highly contagious with an estimated infectious dose as low as 10–100 viral particles. As a result, outbreaks of acute gastroenteritis caused by norovirus are common in hospitals, institutional settings, cruise ships and hotels, where large groups of people are confined in relatively small areas, allowing for rapid spread of the virus.[5–8] Outbreaks commonly result from the consumption of contaminated food or

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water, and because person-to-person spread occurs via faecal–oral and aerosol routes, the stability of the virus in the environment means that contaminated surfaces and fomites also play an important role in transmission.[9–11] Reports of sequential outbreaks in the same setting have been attributed to the survival of virus on surfaces due to a lack of adequate cleaning and disinfection following an initial outbreak.[11]

Currently, *in vitro* methods for the study of human norovirus infectivity are limited to a three-dimensional organoid model of the human small intestine and the use of virus-like particles.[12–14] Whilst the development of a cell culture model is a significant step forward in being able to study and analyse human norovirus, the current complexity of the organoid model does not lend itself to the scope of the disinfection studies described within this article. The use of surrogate viruses to test the efficacy of disinfectants is an established practice recognized in published protocols and standards.[15–19] Feline calicivirus (FCV), another member of the *Caliciviridae* family, is structurally and genomically similar to norovirus, can be cultured *in vitro*, and the development of a plaque assay has provided a simple and reproducible assay for measuring infectivity.[20] As a result, FCV has been widely used as a surrogate virus for testing the efficacy of disinfectants against norovirus.[19,21–23]

The ability of norovirus to survive on surfaces can result in the transmission of virus to uninfected individuals who have had no direct contact with infected individuals.[24] Disruption of this mode of transmission can be achieved using suitable disinfection procedures, thus limiting, or preventing, outbreaks.[25] There are limited published data concerning the survivability of norovirus on the numerous surface types that the virus commonly encounters, although FCV has been used in place of norovirus in some survival studies.[26–28] Hydrogen peroxide vapour (HPV) can be used to decontaminate surfaces in large areas such as hospital wards and laboratories, and has been tested successfully against a range of bacteria.[29–31] However, there are limited published data on the efficacy of HPV systems against viruses.[32,33]

Recent guidelines produced by the US Centers for Disease Control and Prevention have outlined the current situation with regard to the control and prevention of norovirus outbreaks in healthcare settings.[34] When considering environmental cleaning procedures, the need for further research was highlighted in several areas. This included: (1) quantifying the effectiveness of cleaning and disinfecting agents against norovirus or appropriate surrogates; (2) evaluating the effectiveness and reliability of novel environmental disinfection strategies, including vapour-phase hydrogen peroxides; and (3) the comparison of surrogate and human norovirus strains with respect to survival and persistence following cleaning and disinfection. The objective of this study was to investigate the potential of HPV for surface decontamination when norovirus is the suspected causative agent during an outbreak in order to try and address some of these issues.

Materials and methods

Cells and virus

Crandell-Reese feline kidney (CRFK) cells were maintained in minimal essential medium (MEM) plus GlutaMAX (Invitrogen, Paisley, UK) supplemented with 10% fetal bovine serum (FBS) (Invitrogen, Paisley, UK) at 37 °C and 5% CO_2 until 90–100% confluent. Cells were passaged every five to seven days.

FCV strain F9 virus stock was generated by infection of confluent monolayers of CRFK cells at a multiplicity of infection of 0.1. Following a 1-h adsorption period at 37 °C and 5% CO_2, MEM + 2% FBS was added to the cells which were then returned to the incubator (37 °C, 5% CO_2). Cytopathic effect was noted after 18–24 h, and virus was harvested by three freeze–thaw cycles followed by centrifugation at $1400 \times g$ for 5 min to remove cell debris. Supernatant was aliquoted in 1-mL volumes (Nunc, Loughborough, UK) and stored at −80 °C until use.

FCV plaque assay analysis

Viral titres were determined using a modified version of the plaque assay.[20] Briefly, plaque assays were performed in flat-bottomed 12-well cell culture plates (Iwaki, Tokyo, Japan). Each well was seeded with 1 mL of cell suspension in growth medium at a density of 5×10^5 cells/mL. Confluent monolayers were achieved following 18–24 h at 37 °C and 5% CO_2. Growth medium was aspirated from each well and 100 μL of virus was added to duplicate wells. Viral dilutions were performed in serum-free MEM. Plates were incubated for 1 h at 37 °C and 5% CO_2 with gentle rocking every 20 min. Overlay medium, consisting of equal volumes of 2 x MEM (Invitrogen, Paisley, UK) and 3% agarose plus 2% v/v FBS, was prepared immediately before use, and 2 mL of overlay was added to each well. Plates were left for 15–20 min in a Class II cabinet and then incubated at 37 °C and 5% CO_2 for 48 h. Cells were fixed by the addition of 1 mL of 10% formaldehyde in PBS to each well for 1 h. Subsequently, the formaldehyde was discarded, agarose plugs were removed and cells were stained with 0.1% (w/v) crystal violet solution for 15–20 min. Plaques were counted and viral titres were expressed as plaque-forming units per mL.

Carrier preparation

The following materials were used in this study: stainless steel (M-Tech Diagnostics Ltd, Warrington, UK), glass (Marienfield Micro Slides, Paul Marienfeld GmbH, Lauda-Koenigshofen, Germany), vinyl flooring (self-adhesive floor tiles with adhesive removed, Marley Floors, Maidstone, UK), ceramic tile (KAI, Isperih, Bulgaria) and PVC plastic cornering strip. Materials were cut into approximately 1-cm^2 pieces, soaked in a 5% Decon 75 solution for 5 min then rinsed continuously in tap water for 5 min. Carriers were subsequently rinsed in five 100-mL volumes of distilled water followed by five 100-mL volumes of 70% absolute alcohol in distilled water. Carriers were left to dry at room temperature and, with the exception of the vinyl flooring, were autoclaved and dried in an oven for at least 18 h. The vinyl flooring, which could not be autoclaved, was subjected to HPV fumigation as described below to ensure sterility prior to use.

Fumigation studies

The virucidal effect of exposure to HPV was tested against the range of surface materials at 5-min intervals over a 20-min period. Carriers were inoculated with 10 μL of viral suspension, or PBS as a control, and left to dry for 1 h in a Class II cabinet. Triplicate discs were inoculated for each time point. Discs were transferred to a Class III cabinet connected to a Bioquell Clarus L HPV generator (Bioquell, Andover, UK) with a hydrogen