



# Survival of human coronaviruses 229E and OC43 in suspension and after drying on surfaces: a possible source of hospital-acquired infections

J. Sizun, M.W.N. Yu and P.J. Talbot

Laboratory of Neuroimmunovirology, Human Health Research Center, INRS-Institut Armand-Frappier, University of Quebec, Laval, Québec, H7V 1B7, Canada

**Summary:** Strains OC43 and 229E of human coronaviruses (HCoV) cause one-third of common colds and hospital-acquired upper respiratory tract HCoV infections have been reported in premature newborns. To evaluate possible sources of infection, virus survival was studied in aqueous suspensions and on absorptive and non-absorptive surfaces representative of a hospital environment. Virus susceptibility to chemical disinfection with standard products was also characterized. Virus survived in saline solution for as long as six days but less in culture medium, with or without added cells. After drying, HCoV-229E infectivity was still detectable after 3 h on various surfaces (aluminum, sterile latex surgical gloves, sterile sponges) but HCoV-OC43 survived 1 h or less. Of the various chemical disinfectants tested, Proviiodine® reduced the virus infectious titre by at least 50%. This study suggests that surfaces and suspensions can be considered as possible sources of contamination that may lead to hospital-acquired infections with HCoV and should be appropriately disinfected.

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## Introduction

Coronaviruses are enveloped RNA viruses of the *Coronaviridae* virus family. Human coronaviruses (HCoV), with two known serogroups designated OC43 and 229E, are responsible for up to one-third of common colds.<sup>1</sup> They may also be involved in more serious diseases. For example, we have previously reported hospital-acquired upper respiratory tract infections in premature newborns with apnoea and bradycardia.<sup>2</sup> The incidence of hospital-acquired HCoV infections was estimated at about 25% in a prospective evaluation of hospitalized

premature newborns with a gestational age of under 32 weeks.<sup>3</sup> Moreover, in a prospective study of elderly people attending a day care centre, coronavirus 229E was found to be a common cause of hospital-acquired lower respiratory tract infection.<sup>4</sup>

Prevention of such hospital-acquired infections is based on knowledge of the mode of transmission. The route of entry of HCoV appears to be the nasal mucosa since common colds can be produced after intranasal inoculation of healthy volunteers.<sup>5,6</sup> Infection via the conjunctival mucosa, as described for respiratory syncytial virus (RSV), has not been demonstrated for HCoV. Horizontal transmission via small particle aerosols is theoretically possible because airborne HCoV-229E has previously been shown to survive for as long as 86 h under controlled temperature and humidity conditions.<sup>7</sup> Hand contamination from environmental

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Author for correspondence: Dr Jacques Sizun, Department of Pediatrics, University Hospital, 29609 Brest, France.  
Fax: (33) 2 98 22 39 86; E-mail: Jacques.Sizun@univ-brest.fr

surfaces is also theoretically possible by analogy with RSV<sup>8</sup> and rhinoviruses;<sup>9</sup> however, survival of HCoV on surfaces has not yet been studied.

In the current study, we report the comparative survival of HCoV-229E and HCoV-OC43 in suspensions and on various environmental surfaces commonly found in hospitals, and report on their susceptibility to common disinfectants.

## Material and methods

### Cells and viruses

The human embryonic lung cell line L132 and the human rectal tumour cell line HRT-18, as well as the HCoV-229E and HCoV-OC43 viral strains, were originally obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). Cells were grown at 37 °C and 5% (v/v) CO<sub>2</sub> in Earle's minimal essential medium: Hank's M199 solution (1:1, v/v), supplemented with 0.13% (w/v) sodium bicarbonate, 50 µg/mL gentamicin (Canadian Life Technologies, Burlington, Ontario, Canada) and foetal bovine serum (FBS) [5% (v/v) for HCoV-229E and 10% (v/v) for HCoV-OC43]. Viruses were cloned twice and grown on L132 (HCoV-229E) or HRT-18 (HCoV-OC43) cells as described previously.<sup>10,11</sup> The third passage of HCoV-229E with a titre of 5.5 × 10<sup>5</sup> tissue culture infective dose 50%/w (TCID<sub>50</sub>/mL) and the fourth passage of HCoV-OC43 with a titre of 5.15 × 10<sup>5</sup> TCID<sub>50</sub>/mL from laboratory stocks kept at -90 °C were used for all experiments.

### Survival of viruses in suspension

Viruses, as described above, were diluted 10-fold in three different media: Dulbecco's phosphate buffered saline (PBS); culture medium with 5 or 10% (v/v) FBS as described above; or culture medium to which 10<sup>5</sup> susceptible cells (L132 for HCoV-229E or HRT-18 for HCoV-OC43) per mL were added to mimic the expected composition of sputum, which could not be used directly because it would come from sick donors and might contain pathogens that would interfere with the current study. Virus suspensions were stored in plastic microtubes (Cryotube Nunc, Intermed, Denmark) left in the laboratory at room temperature (21 °C) and exposed to daylight. At different times, 100 µL samples were collected in triplicate and stored at -70 °C until analysed for infectious viral titres as described below.

### Survival of viruses after drying

Three different surfaces were tested: aluminium (Alcan, Montréal, Québec, Canada); sterile cotton gauze sponges (Johnson and Johnson, Montréal, Québec, Canada); and sterile latex surgical gloves (Smith and Nephew, Massillon, Ohio, USA). Virus survival was tested according to a modification of a previously published protocol.<sup>12</sup> Pieces of aluminium with diameters of 1 cm were washed with tap water and disinfected with 70% (v/v) ethanol for 30 min before heat disinfection. Sterile sponges and gloves were cut just before the experiment. Aliquots (10 µL) of virus suspensions, as described above, were dropped on to each surface and allowed to dry in a laminar flow hood for 15–45 min. Each piece of dried material was placed in a sterile Petri dish. At different times, pieces of material were placed in plastic microtubes (cryotubes; Nunc) containing 990 µL of PBS and then incubated in a sonicating water bath (Branson, Shelton, USA) for 30 s. The eluate was stored at -70 °C until it could be analysed for infectious viral titres as described below. For each experiment, three pieces of each material were tested. The negative control was PBS dried on to each surface. The temperature was kept at 21 °C and humidity between 55 and 70% during all the experiments (White Box, Stamford, Connecticut, USA).

### Chemical disinfection

Various common disinfecting agents were evaluated: 70% (v/v) ethanol; Proviiodine® detergent containing 0.75% free iodine (Rouquier, Chamby, Québec, Canada); freshly prepared 1.5% (v/v) domestic bleach (Morency, St-Léonard, Québec, Canada); and soap (Synergel, Choisy, Louiseville, Québec, Canada). Ten microlitres aliquots of virus suspension, as described above, were mixed with 10 µL of serial 10-fold dilutions of each disinfectant. After a contact time of 5 min, 980 µL of culture medium were added. The mixture was stored at -70 °C until analysed for infectious viral titres as described below, with the additional monitoring of cytotoxic effects that could mask the detection of infectious virus. Such effect of disinfectants on the ability of virus to infect cells was assayed by pre-incubating dilutions of each disinfectant with cell monolayers for 30 min at 33 °C, followed by washing with PBS. A fixed dilution of virus was then added and infectious virus titres were assayed as described below, except that 16 wells were used.

### **Immunoperoxidase for quantitation of infectious virus titres**

Susceptible cells (L132 for HCoV-229E or HRT-18 for HCoV-OC43) at 70% confluence were inoculated with serial 10-fold dilutions of samples in a 96-well Linbro plate (Flow, McLean, Virginia, USA). After five days of incubation in a humidified chamber at 33 °C and 5% (v/v) CO<sub>2</sub>, the cells were washed with PBS and fixed with 0.3% (v/v) hydrogen peroxide (Sigma, St Louis, MO, USA) in methanol for 30 min. After washing with PBS, they were incubated for 2 h at 33 °C and 5% (v/v) CO<sub>2</sub>, with virus-specific monoclonal antibodies produced in our laboratory by standard hybridoma technology.<sup>13</sup> Cells were then washed three times with PBS and horseradish peroxidase-conjugated anti-mouse immunoglobulins (Cappel, Durham, North Carolina, USA) were added and incubated for 2 h at 37 °C without CO<sub>2</sub>. Bound antibodies were detected by incubation with 0.025% (w/v) 3,3'-diaminobenzidine-tetrahydrochloride (Bio-Rad, Richmond, California, USA), 0.01% (v/v) hydrogen peroxide (Sigma) in PBS. The colour reaction was stopped with deionized water. Infectious virus titres were calculated by the Karber method,<sup>14</sup> using four wells per sample dilution. Negative and positive controls consisted of non-infected cells and cells infected at an estimated multiplicity of infection (MOI) of 0.01, respectively.

## **Results**

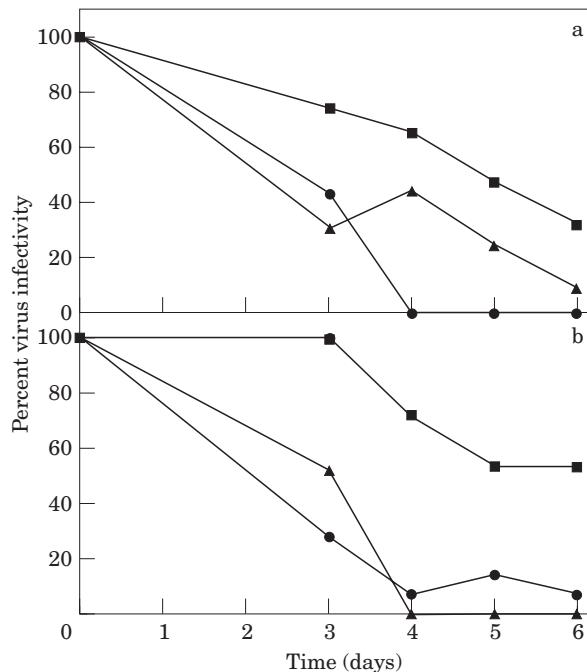
We evaluated the survival of human coronavirus (HCoV) infectivity after various incubations that may reflect clinically relevant situations. Results of these experiments were expressed as the percentage of infectivity recovered after the various treatments compared with the starting infectious titre.

### **Virus survival in suspension**

Figure 1 shows virus survival in aqueous suspensions. Both HCoV-229E and HCoV-OC43 could survive for at least six days in our experimental conditions. The half-life was approximately five days in PBS and between two and three days in the other two media.

### **Virus survival after drying on surfaces**

Figure 2 shows survival after drying, with time zero representing the time just after drying. Rapid loss

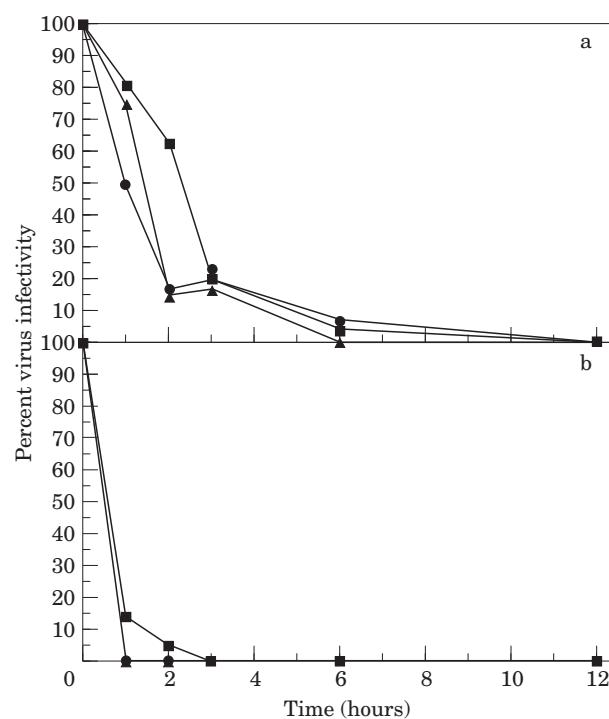


**Figure 1** Infectivity of HCV-229E (a) and HCV-OC43 (b) incubated for various times at 37 °C in suspension in either PBS (■), Earle's minimal essential medium (●), or Earle's minimal essential medium to which suspended cells were added (▲). The remaining infectivity is expressed as a percentage of that at time zero.

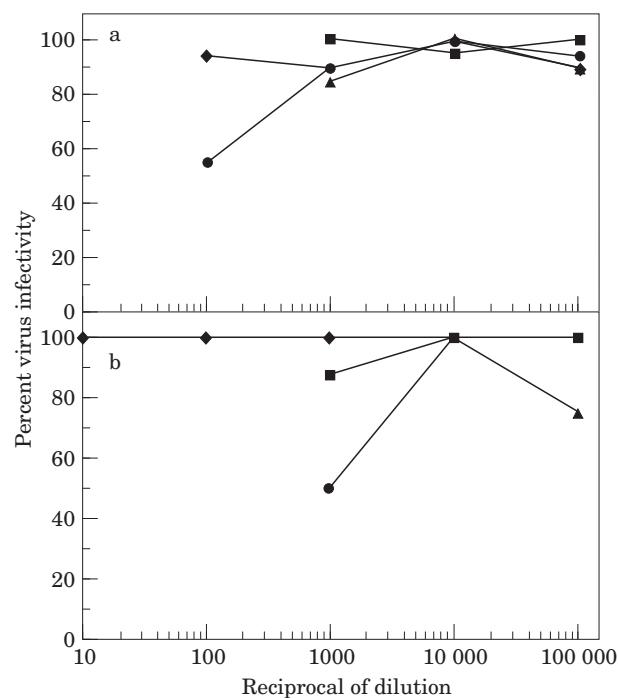
of virus infectivity was remarkable for the two viruses on all surfaces but HCoV-229E infectivity was still detectable after up to 3 h.

### **Chemical disinfection**

Figure 3 describes the infectivity of HCoV after inactivation by chemical disinfectants in suspension at the dilutions of disinfectants that did not interfere with the biological viral titration assay (Table I). A reduction of 50% in infectivity of the two viruses was observed after chemical disinfection with Proviiodine®. However, disinfection of HCoV-229E required ten times more concentrated Proviiodine® solution than HCoV-OC43 for a 50% reduction of virus infectivity. We could not measure a reduction of infectious virus titres after treatments with the other chemical agents (70% ethanol, soap or 5% bleach) because their cytotoxicity (Table I) interfered with the biological viral titration assay at the dilutions of disinfectant that might be expected to be effective in eliminating infectious viruses. This technical limitation may mean that we have underestimated the disinfection efficacy of these products.



**Figure 2** Infectivity of HCV-229E (a) and HCV-OC43 (b) after drying for various times on various surfaces: either aluminum (■), sterile sponges (●) or latex surgical gloves (▲). The remaining infectivity is expressed as a percentage of that at time zero.



**Figure 3** Infectivity of HCV-229E (a) and HCV-OC43 (b) after inactivation by chemical disinfectants: either 1.5% bleach (■), Proviiodine® (●), soap (▲) or 70% ethanol (◆). Only virus-disinfectants dilutions that were not cytotoxic in the infectious virus assay (see Table I) are shown. One hundred percent infectivity represents the infectious titre of virus incubated in PBS in the chemical disinfection assay.

**Table I** Cytotoxic effects of chemical disinfectants

Virus	Disinfectant	Cytotoxicity at 10-fold dilutions of disinfectant					
		0	-1	-2	-3	-4	-5
HCV-229E	5% bleach	**	**	*	+	+	+
	Proviiodine®	**	**	+	+	+	+
	Soap	**	**	**	+	+	+
	70% ethanol	fixed cells†	*	+	+	+	+
	PBS	+	+	+	+	+	+
HCV-OC43	5% bleach	**	**	-	+	+	+
	Proviiodine®	**	**	-	+	+	+
	Soap	**	**	**	-	+	+
	70% ethanol	fixed cells†	+	+	+	+	+
	PBS	+	+	+	+	+	+

\*\*Cytotoxicity of disinfectants observed in all of 16 wells tested;

\*cytotoxicity of disinfectants not observed in all of 16 wells tested;

- , absence of infected cells (direct effect of disinfectant on infection of cells); +, presence of infected cells (no effect of disinfectant on infection of cells); †This treatment caused fixation of the cells.

## Discussion

During viral infections of the respiratory tract, patients shed large amounts of virus into their naso-tracheal secretions and these can contaminate the environment. Respiratory viruses, such as RSV,<sup>8</sup> rhinovirus<sup>9</sup> and parainfluenza virus<sup>15</sup> have been shown to survive for extended periods in suspensions and on surfaces. Contaminated environmental surfaces are considered to represent a significant vector for hospital-acquired viral infections, which occur frequently in paediatric units.<sup>16</sup> Therefore, appropriate disinfection of surfaces is important for the control and prevention of such cross-infections. Although HCoV were also found to be involved in hospital-acquired infections,<sup>3,4</sup> their survival in the environment had not yet been tested in conditions similar to other respiratory viruses. Therefore, we tested the survival of the two known HCoV strains, 229E and OC43, in suspensions and on surfaces, using laboratory conditions designed to reproduce those found in a hospital environment as well as possible.

The current study demonstrates the survival of a PBS suspension of HCoV for up to six days, with no difference between the two HCoV strains. Culture medium with susceptible cells was tested to mimic human respiratory secretions, without possible interference from contaminating pathogens that might have been found in sputum. These laboratory conditions have been used in previous studies with the same goals as our own.<sup>8,15</sup> Survival was apparently shorter for these two viruses when incubated in culture medium with or without susceptible cells, when compared with PBS. Presumably, the pH stability of PBS could explain this observation: infectivity of HCoV-229E was previously shown to be optimal at pH 6.0 and to decrease rapidly at pH <5 or >8, while the virus was found to be very stable to cycles of freezing and thawing.<sup>17</sup>

We also tested HCoV survival after drying, according to a published protocol,<sup>12</sup> modified in order to test different environmental surfaces which are found in hospitals. In particular, we tested porous and non-porous surfaces since differences in survival according to these types of surfaces were described for other respiratory viruses.<sup>8,15</sup> As expected, virus survival after drying was shorter than in suspension, without any differences between surfaces or virus strains. Variations according to temperature or humidity were not tested.

Our study indicates that HCoV are able to survive for several days in suspensions and for a few hours after drying. These results are consistent with the possibility of person-to-person virus transmission via hand contamination from surfaces, as described for other respiratory viruses. For example, RSV was recoverable from counter tops for up to 6 h and from rubber gloves for up to 1.5 h.<sup>8</sup> Human rhinovirus type 14 could survive for more than 20 h when incubated in suspension in tryptose phosphate broth, and for a shorter time in bovine mucin or in nasal secretions.<sup>12</sup> Also, parainfluenza virus type 2 could be recovered from non-absorptive surfaces for as long as 10 h if the contaminated site remained moist but only 2 h if it was dry. On absorptive surfaces, survival varied between 2 and 4 h, depending on the material tested.<sup>15</sup>

On skin, infectivity of parainfluenza virus decreased rapidly but remained detectable for up to 1 h.<sup>15,18</sup> On the other hand, RSV could be recovered from a skin surface for not more than 20 min<sup>8</sup> and rhinoviruses could survive for 1–3 h.<sup>9</sup> We have not tested survival of HCoV on human skin because of ethical concerns regarding the risk of acute

respiratory tract infection and possible neurological damage.<sup>19,20</sup>

To prevent or reduce the possibility of person-to-person transmission of viruses via surfaces, their disinfection is important. Therefore, we tested the disinfecting activity against HCoV of four classes of disinfectants that are widely used in hospitals. We observed that HCoV-OC43 was more sensitive to Proviiodine® than HCoV-229E since it was neutralized with a lower concentration of this chemical disinfectant. The virucidal activity of domestic bleach and soap could not be established because of their inherent cytotoxicity, despite dilution of virus-disinfectant mixtures in PBS or medium. This dilution (1/100) reduced our initial virus titre to  $10^3$  TCID<sub>50</sub>/mL, so that a 10<sup>4</sup>-fold reduction in the infectious viral titre, as recommended in the German DVV and French Afnor guidelines for assessment of efficacy of disinfectants,<sup>21</sup> was impossible to observe. For RSV, Krilov and Harkness demonstrated a 10<sup>3</sup>-fold reduction with providone-iodine at a 10<sup>-3</sup> dilution.<sup>22</sup>

The use of 70% ethanol did not allow the detection of virucidal activity since it fixed the virus-susceptible cells in the immunoperoxidase assay. The presence of lipids makes enveloped viruses such as coronaviruses more sensitive to disinfection or damage by lipid solvents. Thus, the use of soap and ethanol should be effective since alcohol and detergents destabilize the lipid bilayer of enveloped viruses.

There are no official guidelines for the prevention of HCoV transmission, despite reports of hospital-acquired coronavirus infections.<sup>2,3</sup> Possible prolonged survival of these viruses in suspension and the efficacy of a common disinfectant support the use of measures developed to prevent RSV and other respiratory virus cross-infections:<sup>23</sup> hand-washing; disinfection of surfaces; and staff education.

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