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Survival on uncommon fomites of feline calicivirus, a surrogate of noroviruses

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Background: Norovirus (NoV) transmission occurs mainly through food and fomites. Contaminated human fingers can transfer the virus to inanimate objects, which may then spread the virus to susceptible persons. However, no information is available on the survival of NoVs on fomites, which may be of importance in the transmission of NoVs in institutional settings such as hospitals and nursing homes.

Methods: In the absence of any in vitro cultivation system for NoVs, feline calicivirus (FCV) was used as a surrogate. Several fomites such as computer mouse, keyboard keys, telephone wire, telephone receiver, telephone buttons, and brass disks representing faucets and door handle surfaces were artificially contaminated with known amounts of FCV. Samples were taken at regular time intervals, and virus was titrated in feline kidney cells to determine its survival on these surfaces.

Results: Survivability of FCV varied with fomite type. The virus survived for up to 3 days on telephone buttons and receivers, for 1 or 2 days on computer mouse, and for 8 to 12 hours on keyboard keys and brass. The time for 90% virus reduction was <4 hours on computer keys, mouse, brass, and telephone wire; 4 to 8 hours on telephone receiver; and 12 to 24 hours on telephone buttons.

Conclusion: The results of this study confirm that FCV (and perhaps NoV) can survive on fomites such as computers, telephones, and faucets and may be transmitted to humans using these contaminated materials. This may necessitate regular cleaning or disinfection of these items, especially in hospitals and nursing homes and after known outbreaks of NoVs. (Am J Infect Control 2006;34:41-3.)

Noroviruses (NoVs; genus *Norovirus*, family *Caliciviridae*), previously known as *Norwalk* and *Norwalk-like* viruses, are a group of related, nonenveloped viruses that contain single-stranded RNA.¹ They are the most frequent cause of acute gastroenteritis in humans and cause up to 96% of nonbacterial outbreaks of gastroenteritis in the United States.^{1,2} These viruses are excreted in high numbers in the feces and vomitus of symptomatic and asymptomatic infected individuals³ and are highly contagious, and as few as 10 to 100 virus particles have been reported to cause infection.¹ Recently, there has been an increase in NoV outbreaks in institutional settings; 253 confirmed outbreaks of NoVs between July 1997 and June 2000 were recorded in hospitals and nursing homes.¹

Hospital-associated nosocomial infections are a cause of great morbidity, involving as many as 2 million persons annually in the United States.^{4,5} The patient population in a hospital is at risk of nosocomial infections because they are at various extremes of ages (from neonates to elderly persons), and some of them are immunocompromised or are under stress.⁶ Such persons may not only get infected easily but can also become a source of infection to others. The NoVs are transmitted by person-to-person contact and by airborne and foodborne routes. Fecally contaminated environmental surfaces are a potential vehicle for transmission of NoVs.⁷ Fomites such as carpets and fabrics have been reported to be a vehicle for NoV infections.^{8,9} Uncommon fomites such as computer keyboards and faucet handles have been found to be significant vehicles for the transmission of *Staphylococcus aureus*,¹⁰ indicating that everyday "safe" activities such as using computers, telephones, and door handles and turning on water faucets can become a vehicle for the spread of pathogens. Whether such surfaces can become a source for spread of NoVs is not known. The potential for a fomite to serve as a vehicle for virus transmission will depend partly on the length of virus survival on that fomite.

Survivability of a number of organisms has been evaluated on porous and nonporous surfaces; however,

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information on caliciviruses is scarce. This study was undertaken to determine the length of calicivirus survival on some uncommon fomites. Because NoVs cannot be propagated *in vitro*, we used feline calicivirus (FCV) as a surrogate for NoVs as has been done in several other studies.¹¹⁻¹⁷ Although FCV is a respiratory pathogen (unlike NoV, which is an enteric pathogen), it has the same resistance to desiccation as the human NoVs and appears to be a better surrogate of NoV than enteric caliciviruses.¹⁸

METHODS

Cells and virus

Feline calicivirus (strain F9) was propagated and titrated in Crandell-Reese feline kidney (CRFK) cells (ATCC, CCL No. 94). The cells were grown in Eagle's minimal essential medium (Celox, St. Paul, MN) supplemented with 8% fetal bovine serum, penicillin (100 U/mL), streptomycin (100 µg/mL), and fungizone (1 µg/mL).

Uncommon fomites

The items used in this study included computer mouse, keyboard keys, brass disks as a representative for water faucets or door knobs, telephone wire, telephone receiver, and telephone buttons. Computer mouse, telephone receiver, and telephone buttons were broken with a hammer into small pieces. Pieces that were approximately 1 cm² in size were used.

Experimental design

The above fomites' pieces of approximately 1 cm² size were placed in beakers containing 10 mL of 70% ethanol followed by washing in sterilized distilled water and later placed under a laminar flow hood to dry. The dry pieces were placed in 24-well cell culture plates, 20 µL FCV (10⁷ TCID₅₀/mL) was applied on their surfaces, and the plates were incubated at room temperature. A 3% solution of beef extract in 0.05 mol/L glycine (pH 8.5) was added to the wells at 0, 4, 8, 12, 24, 48, 72, 96, 120, and 144 hours to elute the residual virus. At each given time after adding the eluent, the appropriate plate was placed on a rotating shaker at 140 rpm for 30 minutes to recover the residual virus from fomites. All experiments were done in triplicate, and all samples dilutions were assayed in triplicate wells of CRFK.

Virus titration

Serial 10-fold dilutions of the eluates were prepared immediately in plain minimum essential medium. All dilutions were inoculated in 1-day-old CRFK cells in 96-well microtiter plates. Each dilution was inoculated

Table 1. Rate of FCV reduction on uncommon fomites

Fomites (hr)*	Time to 90% reduction in virus titer (hr)*	Time (hr) to undetectable virus
Keyboard keys	0-4	8-12
Computer mouse	0-4	24-48
Brass	0-4	8-12
Telephone buttons	12-24	48-72
Telephone receiver	4-8	48-72
Telephone wire	0-4	24-48

*The test organism feline calicivirus (FCV) was sampled at 0, 4, 8, 12, 24, 48, 72, 96, 120, and 144 hours.

in 3 wells. The plates were incubated at 37°C and examined daily for the appearance of cytopathic effects (CPE). The final CPE was read 96 hours postincubation, and virus titers were calculated by the Reed and Muench¹⁹ method. The results of 3 experiments on each fomite were averaged and are presented as 90% reduction in virus titer and total time of virus survival on these fomites.

RESULTS

The results are summarized in Table 1. FCV survived on telephone buttons and telephone receivers for a maximum of 3 days (48-72 hours). However, the rate of 90% virus reduction was higher on telephone receivers (4-8 hours) as compared with that on telephone buttons (12-24 hours). On computer mouse and telephone wire, the virus survived for 24 to 48 hours, and 90% virus reduction occurred in less than 4 hours. On brass and computer keyboard keys, the virus survived for 8 to 12 hours only, and 90% virus reduction time was <4 hours. Overall, FCV survival was longer on telephone-related items than on computer keyboard and brass.

DISCUSSION

Norovirus outbreaks of acute gastroenteritis are a matter of concern in the general community as well as in institutional settings. One critical factor for the transmission of microorganisms from person-to-person or environment-to-person is its ability to survive on environmental surfaces. Survival studies have been carried out on both enveloped and nonenveloped viruses. Mbithi et al²⁰ evaluated the viability of hepatitis A virus (HAV) on human hands and were able to recover 16% to 30% of the applied virus from hands after 1 hour of drying. In another study, survival of nonenveloped enteric viruses was found to vary according to the surface tested.²¹ Of the viruses tested, HAV and rotaviruses were more stable than polio-1 and adenoviruses on paper, cotton cloth, aluminium, china, glazed tiles, and latex gloves.

Sizun et al²² evaluated the survivability of enveloped RNA virus (human corona virus 229E and OC 43) in liquid media as well as after drying on aluminium, latex gloves, and sterile gauze. They detected residual corona virus for up to 6 days in saline solution, and it survived for only 1 to 4 hours after drying on porous and nonporous surfaces. In comparison with viruses, bacteria and fungi survive longer (1-90 days) on environmental surfaces.²³⁻²⁵ Several other studies on survival of bacterial (gram negative and positive) and fungal agents on environmental surfaces indicate that the survival of microorganisms is highly variable, ranging from minutes to days.²⁶⁻²⁸

The results of this study indicate that FCV can survive for 8 to 72 hours, which is longer than the reported survival of poliovirus and HAV.^{20,21} In our study, FCV survived significantly more on telephone receiver and telephone buttons as compared with other fomites (48-72 hours). However, the rate of 90% virus reduction was different on these 2 fomites. The level of FCV declined faster on receivers (4-8 hours) than on buttons (12-24 hours), indicating that the nature of the surface will have an impact on the survival of the virus. These results are in agreement with those of Abad et al,²¹ who found that survivability of HAV, rotavirus, poliovirus, and adenoviruses was variable on various porous and nonporous surfaces. The results of this and past studies indicate that both nonenveloped and enveloped viruses can survive on environmental fomites for a few hours to days,²⁰⁻²² which may be sufficient time for the virus to be acquired by a susceptible individual. These results emphasize the need to evaluate effective means to disinfect such surfaces in case of possible contamination in addition to following standard hygienic practices such as frequent handwashing.

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