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Survival and Detection of Rotaviruses on Environmental Surfaces in Day Care Centers

BRUCE H. KESWICK,* LARRY K. PICKERING, HERBERT L. DuPONT, AND WILLIAM E. WOODWARD

Program in Infectious Diseases and Clinical Microbiology, University of Texas Medical School, Houston, Texas 77030

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Previously, we demonstrated that children in day care centers commonly experience diarrhea due to rotavirus, giardia, and bacterial pathogens. Multiple agents frequently coexist, and the environment is heavily contaminated with enteric bacteria during outbreaks. A study of environmental surface contamination with rotavirus was performed during three non-outbreak periods. Of 25 samples collected from environmental surfaces and teachers hands at a day care center, 4 (16%) were positive for rotavirus antigen when a fluorescence assay was used. We also examined the survival of two animal viruses, rotavirus SA-11 and poliovirus type 1, and bacteriophage f2 on similar environmental surfaces in a laboratory. Poliovirus type 1 and bacteriophage f2 were more resistant to drying than rotavirus SA-11 and could be recovered after a 90-min exposure on a dry surface. Rotavirus SA-11 could be detected for 30 min. All three viruses survived longer when they were suspended in fecal material than when they were suspended in distilled water. These data suggest that several agents, including rotavirus, can remain viable on contaminated surfaces long enough to be transmitted to susceptible children. This finding helps explain why rotavirus shows a mode of spread like that of parasitic and bacterial agents within day care center settings.

Rotavirus is a leading cause of diarrhea in children less than 2 years old in day care centers (DCC) (12). It is estimated that up to 10^{10} particles per g of feces may be shed by children with diarrhea, and shedding also may occur in asymptomatic children (1, 5). Since youngsters have poor personal hygiene, it is likely that rotavirus could be spread throughout the DCC environment and transmitted via objects shared between children. Previously, we have shown that fecal coliforms and salmonellae can be detected in DCC on environmental surfaces, including toys, diapering areas, and hands of teachers (E. Ekanem, H. L. DuPont, L. K. Pickering, and B. J. Selwyn, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, Q23, p. 213). In this paper we describe the identification of rotavirus from environmental surfaces in a DCC and the comparative survival of rotavirus SA-11, poliovirus type 1, and bacteriophage f2 on environmental surfaces in a laboratory.

MATERIALS AND METHODS

Virus preparation. Rotavirus SA-11 (grown in our laboratory) and poliovirus type 1 (LSc2ab) were grown in MA-104 and BGM cells, respectively. The titers of animal viruses were determined by a plaque assay. Bacteriophage f2 was grown and titers were determined on *Escherichia coli* Hfr host cells by a plaque assay (2). Animal virus preparations consisted of fluorocarbon extracts of 10-fold concentrates, whereas bacteriophage f2 suspensions were prepared by low- and high-speed centrifugation.

Survival studies. Viruses were diluted to densities of 10^3 to 10^5 PFU/ml in sterile distilled water or in a solution of water containing 10% rotavirus-negative stools and were misted onto counter tops with a household sprayer. The sampling areas (10 by 25 cm) were delineated by grids drawn on the counters. Each area was thoroughly swabbed with a sterile cotton-tipped swab which had previously been dipped in minimal essential medium (MEM) containing 1% antibiotic solution (Irvine Scientific). The swab was then immersed in a tube containing MEM supplemented with antibiotics and squeezed dry against the side of the tube. The titer of the virus-containing medium was determined by a plaque assay.

Environmental sampling. A total of 25 samples from 3 days were collected in a single DCC room housing 6- to 18-month-old children. The adjacent toilet and kitchen also were sampled. All samples were collected during non-outbreak periods. Sterile cotton-tipped swabs were dipped into a tube containing MEM supplemented with antibiotics and wiped over a variety of objects and surfaces in the room. The swabs were placed into a MEM-containing screw-capped tube, placed on ice, and returned to the laboratory within 30

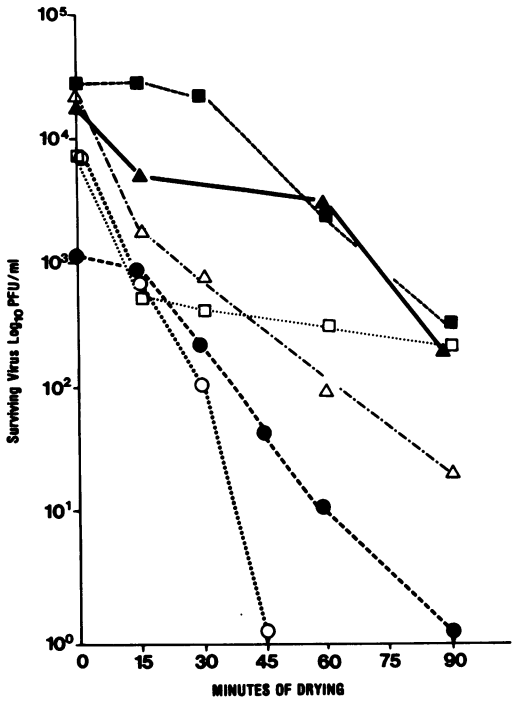

FIG. 1. Survival of rotavirus, poliovirus, and phage f2 on impermeable surfaces. The points represent the means of at least two trials. Symbols: ○, rotavirus SA-11 in water; ●, rotavirus SA-11 in stool solution; △, phage f2 in water; ▲, phage f2 in stool solution; □, poliovirus in water; ■, poliovirus in stool solution.

min. Samples were inoculated onto monolayers of MA-104 cells, incubated for 24 h, and assayed by fluorescence (13).

Briefly, cultures of MA-104 cells in chambered slides (Lab-Tek Products) were inoculated with 0.1- to 0.4-ml portions of the specimens, centrifuged at 3,000 × *g* for 60 min, overlaid with MEM containing 0.5 μg of trypsin (type IX; Sigma Chemical Co.) per ml, and incubated for 24 h at 37°C. After fixation in cold methanol, the infected cells were stained with a guinea pig antiserum to human rotavirus (antiserum V-710-501-558; National Institute of Allergy and Infectious Diseases) and a goat anti-guinea pig fluorescein isothiocyanate-labeled serum (Miles Laboratories, Inc.). Positive (rotavirus SA-11) and negative (phosphate-buffered saline) control samples were included for each set of assays.

RESULTS

Survival studies. Cotton swabs have been recommended for detecting viruses on environmental surfaces (11), although newer methods of detecting viruses in the environment have employed positively charged filter material (14). Preliminary tests under a variety of conditions in our laboratory indicated that cotton swabs wetted with phosphate-buffered saline or MEM gave results comparable to those obtained by eluting cotton swabs or filter pads. For convenience, therefore, all experiments were conducted with cotton swabs without elution.

Figure 1 shows that rotavirus suspended in water and misted onto counters was sensitive to drying and could not be detected after 45 min of exposure and drying. Poliovirus and coliphage f2 could be detected for at least 90 min after application. Bacteriophage f2 was detectable after 24 h of drying (data not shown). It also is evident that the viruses and bacteriophage f2 survived longer in the presence of fecal material than in water. The efficiency of recovery for each virus was calculated from the titer of the virus solution before it was sprayed onto the counter and the total amount of virus recovered from a known surface area at zero time. The levels of recovery were 16.8 ± 6.0, 42.3 ± 1.9, and 10.6 ± 5.7% (mean ± standard error) for rotavirus, poliovirus, and phage f2, respectively.

A fluorescence assay (FA) was used to detect human isolates. To test the sensitivity of this system, the FA was compared with the plaque assay by using rotavirus SA-11 suspended in a 10% stool preparation as a model of human rotavirus contamination. Samples were collected as described above at 15-min intervals. Table 1 shows that virus was detectable after 60 min of drying with both assays and that 1 $\log_{10}$ PFU of rotavirus SA-11 per ml was detected by the FA.

Environmental isolates. A total of 25 samples were collected from various objects and surfaces on 3 days at the same DCC (Table 2). Four (16%) of these samples were positive for rotavirus by the FA. No obvious fecal contamination was present on any of the surfaces. Contaminated objects adjacent to the diapering area included a refrigerator door handle, a diaper pail lid, and a sink. The diaper-changing counter, which is routinely cleaned, was negative on three occasions. One swab taken from a hand of a teacher

TABLE 1. Comparison of plaque assay and FA for detection of rotavirus SA-11 on environmental surfaces[a]

Time of sample (min)	Titer (PFU/ml)	FA result
0	1.2×10^3	+++
15	1.9×10^2	++
30	2.3×10^2	+
45	3.5×10^1	+
60	1.0×10^1	+
90	0	−

[a] Rotavirus SA-11 suspended in a 10% stool solution was sprayed onto a counter top. The surface was sampled with a cotton swab.

TABLE 2. Human rotavirus isolation from environmental surfaces in DCC

Sample location	FA for rotavirus
Bathroom wall	0/1[a]
Toilet seat	0/1
Sink	1/3
Sink tap handle	0/1
Diaper pail lid	1/1
Diaper-changing counter	0/3
Playpen	0/1
Floor	0/3
Walker	0/2
Toy	0/3
Table	0/1
Hands (teacher)	1/1
Hall floor	0/1
Stairs	0/1
Refrigerator door handle	1/1
Kitchen counter	0/1

[a] Number positive/number tested.

who frequently diapered children was also positive.

DISCUSSION

Rotavirus is thought to be spread by person-to-person transmission via the fecal-oral route in children in DCC. However, the high levels of virus shed in stools suggest that rotavirus may also be spread via a contaminated environment, as demonstrated in animal studies (7). In a limited sample we found that 16% of apparently clean surfaces in a DCC were contaminated with rotavirus. Zavate et al. (15) have reported that 6% of samples collected from the surfaces of various objects in a child care facility were contaminated with enteroviruses. Our previous study of bacteria on environmental surfaces in DCC demonstrated that 13 to 17% of the samples collected from toys, baby walkers, and teachers hands were contaminated. Thus, diarrhea-causing microorganisms are commonly present on environmental surfaces in DCC. Furthermore, secondary transmission of clinical illness via contaminated objects has been documented for rhinovirus (3).

Poliovirus, coxsackievirus, and echoviruses have been reported (6) to survive for 2 to 12 days on painted wood, glass, and cotton fabric. The simian rotavirus SA-11 used in our study was more sensitive to drying than either poliovirus or bacteriophage f2. The reason for this greater sensitivity of rotavirus SA-11 is unclear; however, a recent report (10) has suggested that moderate levels of humidity and elevated temperatures adversely affect the survival of human rotaviruses on impermeable surfaces; these were the conditions used in our study.

The sensitivity of the swab method for recovery of human rotaviruses was not determined. Thus, even though as few as 10 PFU of culture-adapted rotavirus SA-11 was detected in comparative tests, our swab method may not be sensitive enough to detect low numbers of viable human rotaviruses. Such low numbers of virus may be important in DCC environments if the minimal infectious dose of rotavirus is as low (less than 10 PFU) as has been demonstrated with poliovirus and echovirus (8, 9). Furthermore, the presence of fecal material increased the survival of viruses on surfaces, and the presence of fecal material is known to protect viruses from the action of disinfectants (4).

The importance of handwashing (1) and the disinfection of objects that may be placed in the mouths of small children is emphasized by our results, as are our previous finding (G. F. Lemp, M.S. thesis, University of Texas School of Public Health, Houston, 1981) that childhood diarrhea is associated with DCC teachers who both prepare food and change diapers. It is evident from the recovery of virus from a refrigerator door handle that fecal contamination can be spread to food preparation areas.

Handwashing and regular disinfection of toys and environmental surfaces are necessary to reduce person-to-person transmission of enteropathogens in DCC.

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LITERATURE CITED

1. **Black, R. E., A. C. Dykes, K. E. Anderson, J. G. Wells, S. P. Sinclair, G. W. Gary, M. H. Hatch, and E. J. Gangarosa.** 1981. Handwashing to prevent diarrhea in day care centers. Am. J. Epidemiol. **133**:445–451.
2. **Goyal, S. M., and C. P. Gerba.** 1979. Comparative adsorption of human enterovirus, similan rotavirus, and selected bacteriophages to soil. Appl. Environ. Microbiol. **38**:241–247.
3. **Gwaltney, J. M., and J. O. Hendley.** 1982. Transmission of experimental rhinovirus infection by contaminated surfaces. Am. J. Epidemiol. **116**:828–833.
4. **Hejkal, T. W., F. W. Wellings, P. LaRock, and A. L. Lewis.** 1979. Survival of poliovirus within organic solids during chlorination. Appl. Environ. Microbiol. **38**:114–118.
5. **Holmes, I. A.** 1979. Viral gastroenteritis. Prog. Med. Virol. **25**:1–36.
6. **Kiseleva, L. F.** 1968. Survival of enteric viruses in water and food stuffs and on various surfaces. Hyg. Sanit. (Jerusalem) **33**:439–440.
7. **Leece, J. G., M. W. King, and W. B. Dorsey.** 1978. Weaning regimen producing piglet diarrhea (rotavirus) and its relevance to acute infantile diarrhea. Science **199**:776–778.
8. **Melnick, J. L., and V. Rennick.** 1980. Infectivity titers of enterovirus as found in human stools. J. Med. Virol. **5**:205–220.

9. **Minor, T. E., C. I. Allen, A. A. Tsiatis, D. B. Nelson, and D. J. Alessio.** 1981. Human infective dose determinations for oral poliovirus type 1 vaccine in infants. J. Clin. Microbiol. **13**:388–389.
10. **Moe, K., and J. A. Shirky.** 1982. The effects of relative humidity and temperature on the survival of human rotavirus in feces. Arch. Virol. **72**:179–186.
11. **Phillips, C. A., and G. A. Grim.** 1965. Comparison of the elution and adsorption of picornaviruses by cotton and calcium alginate wool swabs. Appl. Microbiol. **13**:457–459.
12. **Pickering, L. K., and W. E. Woodward.** 1982. Diarrhea in day care centers. Pediatr. Infect. Dis. **1**:47–52.
13. **Smith, E. M., and C. P. Gerba.** 1982. Development of a method for detection of human rotavirus in water and sewage. Appl. Environ. Microbiol. **43**:1440–1450.
14. **Sobsey, M. D., and J. S. Glass.** 1980. Poliovirus concentration from tap water with electropositive adsorbent filters. Appl. Environ. Microbiol. **40**:201–210.
15. **Zavate, O., F. Cotro, A. Ivan, S. Tiron, and G. Avram.** 1980. Investigation on the circulation of enteroviruses in a children community. Rev. Roum. Med. Virol. **31**:289–293.