

Rotavirus Survival on Human Hands and Transfer of Infectious Virus to Animate and Nonporous Inanimate Surfaces

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We tested the survival of the Wa strain of human rotavirus on the hands of volunteers and also studied infectious virus transfer between animate and inanimate (stainless steel disks) surfaces. The virus was diluted in a 10% suspension of feces, and 10 μ l (1×10^3 to 4×10^4 PFU) was placed on each of the four fingerpads of the left hand. One milliliter of 20% tryptose phosphate broth in Earle balanced salt solution was used for virus elution from each fingerpad, and the hands were disinfected with 70% ethanol before they were washed with an antiseptic soap and water. At 20, 60, and 260 min after inoculation, approximately 57, 43, and 7%, respectively, of the input infectious virus could be recovered. For virus transfer, the inoculum (2×10^4 to 8×10^4 PFU) was allowed to dry, and the donor surface was kept in contact with the recipient surface for 10 s at a pressure of approximately 1 kg/cm². At 20 and 60 min after virus inoculation, 16.1 and 1.8%, respectively, of the input infectious virus could be transferred from the contaminated hand to a clean disk; when a clean hand was pressed against a contaminated disk, virus transfer was 16.8 and 1.6%, respectively. Contact between a contaminated and a clean hand 20 and 60 min after virus inoculation resulted in the transfer of 6.6 and 2.8%, respectively, of the input infectious virus. These findings indicate the potential vehicular role for human hands in the spread of rotaviral infections.

Rotaviruses are among the major causes of acute diarrhea throughout the world (2, 6, 20). In developing countries, in particular, rotaviral infections contribute significantly to fatalities in children under 5 years of age (4, 5). In addition to community-based epidemics (9, 17, 19, 24), outbreaks of gastroenteritis due to these viruses are frequently observed in institutional settings such as hospitals (8, 18, 26, 27, 32, 35), nursing homes (15, 25), day-care centers (1, 7, 21, 29), and schools (16, 38).

Even though the exact vehicles of rotaviral spread during most such institutional outbreaks are not known, the available evidence strongly suggests that virus-contaminated hands may play an important role in this regard (22, 27, 29, 33, 35). Infectious rotavirus particles have been recovered from naturally contaminated and frequently handled surfaces in a day-care center (22), and it has also been shown (35) that these viruses can survive for several days on experimentally contaminated nonporous inanimate surfaces. Rotaviral antigens were detected in handwashings of persons who attended to patients with rotaviral gastroenteritis (33), and infectious rotavirus particles were recovered from the hands of a teacher in a day-care center (22). However, it is not known for how long rotaviruses can survive on human hands and how readily transfer of infectious virus can occur between hands and environmental surfaces. This study was initiated to address these questions.

MATERIALS AND METHODS

Cells. The MA-104 line of rhesus monkey kidney cells was used throughout this study. Cultivation, maintenance, and passage of these cells have been described in detail elsewhere (34). For virus plaque assays, cell monolayers were prepared in 12-well plastic plates (Costar, Cambridge,

Mass.) with Eagle minimal essential medium (EMEM; Flow Laboratories, Inc., Rockville, Md.) with 5% fetal calf serum and gentamicin (Cidomycin; Roussel, Montreal, Quebec, Canada) at a final concentration of 50 μ g/ml. The seeded plates were sealed in plastic bags (Philips, Toronto, Ontario, Canada) and incubated at 37°C for 48 h for monolayer formation.

Virus. The cell culture-adapted Wa strain of human rotavirus, kindly supplied by R. G. Wyatt (National Institutes of Health, Bethesda, Md.), was used in these experiments. The virus was grown in cell monolayers prepared in 75-cm² plastic flasks (Costar). The growth medium was discarded, and the remaining serum was washed out by rinsing the monolayers in three changes of Earle balanced salt solution (EBSS). They were then infected at a multiplicity of infection of 0.01, and the virus was allowed to adsorb for 1 h at 37°C. The maintenance medium consisted of EMEM with 5 μ g of trypsin per ml (Nutritional Biochemical Corp., Cleveland, Ohio). After 72 h at 37°C, the infected cultures were frozen (-80°C) and thawed (22°C) three times, and the contents were centrifuged at 1,000 \times g for 15 min. The supernatant was collected as the virus pool, diluted in the suspending medium (see below), and dispensed into vials for storage at -80°C.

Plaque assay. The samples to be assayed were diluted in EBSS. After the growth medium was removed, the monolayers in 12-well plates were washed at least three times with EBSS. Each well then received 0.1 ml of the inoculum; at least three wells were used for each sample dilution tested. Each control well was inoculated with 0.1 ml of the diluent. After 1 h of virus adsorption at 37°C in a CO₂ incubator, each monolayer was overlaid with 2.0 ml of EMEM (without any serum) containing 0.6% agarose (Sigma Chemical Co., St. Louis, Mo.) and 5 μ g of trypsin per ml. The plates were sealed in plastic bags and held at 37°C for 3 days. Before the

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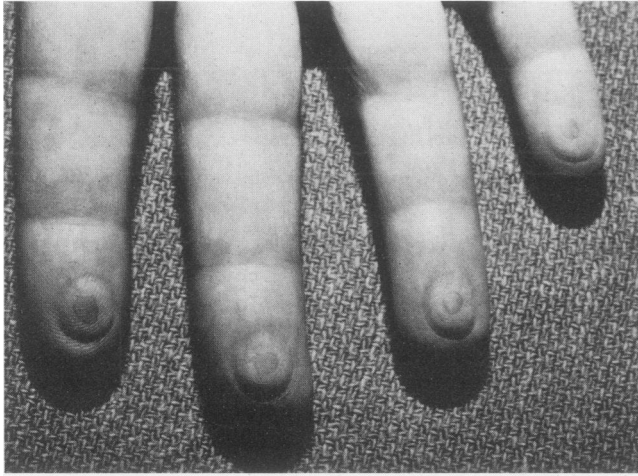


FIG. 1. Demarcated areas with dried inocula (10 μ l) on the fingerpads.

overlay medium was removed, the monolayers were fixed overnight by adding to each well 2.0 ml of 10% Formalin in normal saline. They were then stained with an aqueous solution of crystal violet to determine the number of plaque-forming units of the virus.

Virus suspension medium. A 10% suspension of infant feces was prepared in normal saline and centrifuged at 1,000 $\times g$ for 15 min to remove coarse particulate matter. Before its use, this suspension was tested for cytotoxicity to MA-104 cells and the presence of any endogenous viruses in the following manner. Each monolayer of MA-104 cells in 25-cm² culture flasks and in wells was inoculated with 0.1 ml of the undiluted fecal suspension or a 10-fold dilution of the suspension. After 1 h of adsorption at 37°C, each flask received 8 ml of EMEM with 5% fetal calf serum, and each monolayer in the wells was overlaid with 2 ml of EMEM with 0.6% agarose and 5 μ g of trypsin per ml. They were then held at 37°C for 5 days. No cytotoxic or cytopathic degeneration was observed in any of the flasks, and the plates remained free of detectable plaques.

Volunteers. Permission of the Ethics Committee of our School of Medicine was obtained for use of adult human volunteers, and three male and three female members of the departmental staff participated in this study. They ranged in age from 22 to 50 years.

Fingerpad contamination and virus elution. The pads of the index, middle, ring, and little fingers on the left hand of the volunteers were designated as F1, F2, F3, and F4, respectively. Before the fingerpads were contaminated, volunteers were asked to wash their hands in running tap water and then rinse them with 70% ethanol and allow them to air dry.

The fingerpads were pressed over the mouth (8-mm inside diameter) of an empty plastic vial (no. 72.694.006; Sarstedt Inc., Princeton, N.J.) to demarcate the area where the virus inoculum was to be deposited. Virus suspended in feces (10 μ l) was then placed in the center of the marked area on each fingerpad. The inocula were allowed to dry by keeping the contaminated hand in a laminar-flow hood. Figure 1 shows the fingerpads with the dried inocula. Once the inocula were dry, the volunteers were permitted to move about and perform any tasks as long as contact with the contaminated fingerpads could be avoided.

In preliminary experiments, undiluted tryptose phosphate broth (Difco Laboratories, Detroit, Mich.), EBSS, and 20%

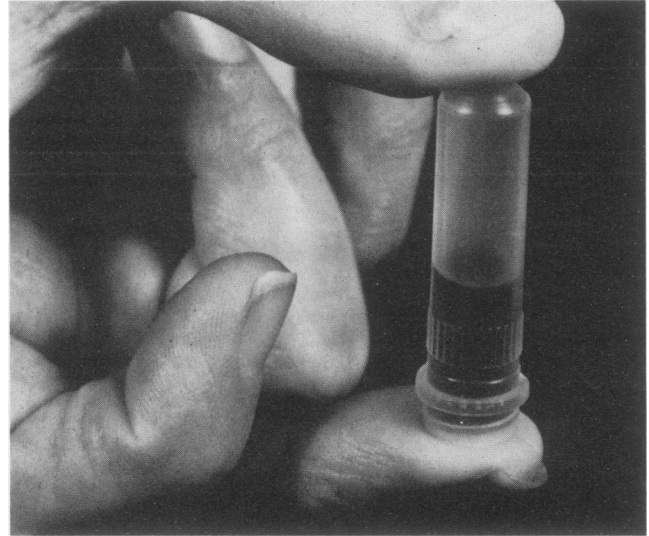


FIG. 2. Positioning of the vial (with 1 ml of eluent) on the fingerpad for virus recovery.

tryptose phosphate broth in EBSS, all adjusted to a pH of 7.2, were tested as virus eluents from fingerpads; 20% tryptose phosphate broth in EBSS was found to give a consistently high recovery of the virus. It was therefore used as the eluent in all subsequent experiments.

The contaminated area was placed over the mouth of a plastic vial containing 1.0 ml of the eluent, and the vial was inverted (Fig. 2) to soak the dried virus inoculum for 5 s. This was followed by 20 repeated inversions of the vial while it was still in place, another 5 seconds of soaking, and a further 20 inversions. Finally, the vial, with the contaminated finger still over it, was turned upright, and the fingerpad was scraped against the inside rim of the vial to recover as much as possible of the liquid. The eluates were then plaque assayed. The contaminated fingers were thoroughly rinsed in 70% ethanol before they were washed with antiseptic soap and water.

Disks. Clean and sterile stainless steel disks (1-cm diameter) were used in the virus transfer experiments as representatives of nonporous inanimate surfaces. The methods for the decontamination, cleaning, and sterilization of these disks were described in detail earlier (35). Each clean disk was contaminated with 10 μ l of the virus suspension, and the inoculum was allowed to dry for either 20 or 60 min under ambient conditions. For virus recovery, each disk was placed in a vial containing 1.0 ml of 20% tryptose phosphate broth in EBSS and sonicated for 10 min in a Bransonic bath (O.H. Johns Scientific, Toronto, Ontario, Canada).

Procedure for testing virus survival. Virus survival was tested by placing 10 μ l (1×10^3 to 2×10^4 PFU) of the virus suspension on each fingerpad and eluting the inocula from at least two fingerpads at each sampling period (Fig. 3).

Procedures for testing virus transfer. We studied three models of virus transmission: (i) a contaminated hand to a clean inanimate surface, (ii) a contaminated inanimate surface to a clean hand, and (iii) a contaminated hand to a clean hand. A 10- μ l inoculum (2×10^4 to 8×10^4 PFU) was used, and contact was made between the donor and recipient surfaces for 10 s with a pressure of approximately 1 kg/cm². The extent of virus transfer was determined by virus recov-

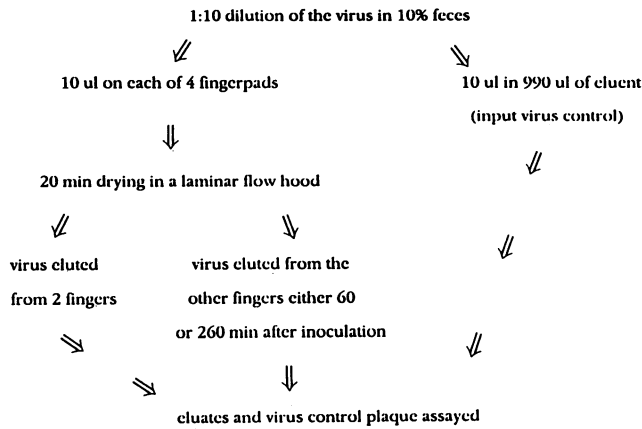


FIG. 3. Procedure for testing rotavirus survival on hands.

ery from recipient surfaces. The flow chart for the three types of transfer experiments is given in Fig. 4.

Experimental design. In the virus survival experiments, four fingers of one hand of each individual were inoculated with the virus suspension. Virus was eluted from two fingers

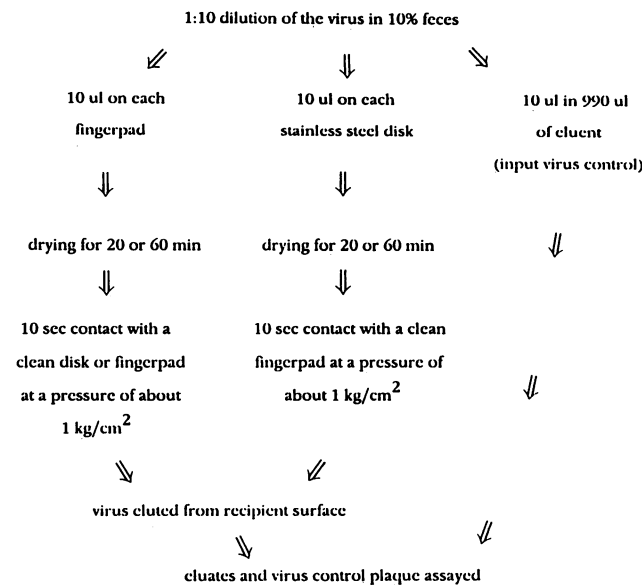


FIG. 4. Procedure for studying rotavirus transfer from an experimentally contaminated surface to a clean surface.

at 20 min after deposition and from the other two fingers at 260 min after deposition of the virus suspension. Virus survival at 60 min after deposition was determined in a subsequent trial with three fingers of each volunteer.

In the virus transfer experiments, three fingers of one hand of each individual were used, and the experiment was repeated at least three times on each volunteer. Five individuals participated when the virus transfer was tested 20 min after inoculation, and the results for virus transfer at 60 min postinoculation are based on experiments with six volunteers.

Nested analyses of variance were conducted on the data from various experiments to ascertain the amount of variation attributable to the various factors considered in these experiments.

RESULTS

Virus survival on hands. Virus survival on hands was determined 20, 60, and 260 min after deposition of the virus suspension on fingerpads (Table 1). At 20, 60, and 260 min after virus inoculation, 57, 42.6, and 7.1%, respectively, of the input infectious virus could be recovered from the contaminated fingers.

In examining the variance components in each trial, 90, 95.4, and 65.8% of the total variation at 20, 60, and 260 min, respectively, was attributable to random error and subject variation was responsible for 10, 4.6, and 33.9%, respectively, of the total. These analyses also showed that there was little or no finger-to-finger variation.

Virus transfer. Results from the virus transfer experiments are given in Table 2. The amounts of infectious virus transferred from contaminated hands to clean disks and from contaminated disks to clean hands were very similar. At 20 min after virus inoculation 16.1 to 16.8% of the input infectious virus could be transferred to and from an animate and inanimate surface, and at 60 min postinoculation such transfer amounted to 1.6 to 1.8%. After 20 and 60 min of inoculation, 6.6 and 2.8%, respectively, of the input infectious virus could be transferred from a contaminated hand to a clean hand.

DISCUSSION

Before the present study, no information was available on the capacity of rotaviruses to survive on human hands. This report shows that rotaviruses can retain their infectivity for several hours on the skin, and that transfer of infectious rotavirus can occur readily between animate surfaces as well as between animate and nonporous inanimate surfaces. Virus survival on hands and transfer of infectious virus to

TABLE 1. Percent rotavirus survival on experimentally contaminated fingerpads^a

Volunteer	20 min		60 min		260 min	
	n	Mean % survival (SD)	n	Mean % survival (SD)	n	Mean % survival (SD)
1	6	85.3 (47.33)	3	50.7 (18.98)	6	8.5 (5.16)
2	6	62.7 (14.89)	3	37.2 (12.50)	6	1.8 (2.02)
3	6	58.8 (51.57)	3	41.3 (5.04)	6	4.4 (3.13)
4	6	52.9 (34.33)	2	30.3 (10.33)	6	14.3 (7.35)
5	12	51.1 (23.32)	3	40.3 (5.42)	12	10.2 (9.72)
6 ^b	6	37.3 (21.02)	3	51.9 (14.96)	6	0.02 (0.04)

^a Overall mean percent survival (standard deviation) was 57.0 (36.61), 42.6 (12.68), and 7.1% (7.98%) at 20, 60, and 260 min, respectively.

^b One volunteer was different for the 60-min sampling time.

TABLE 2. Transfer of infectious rotavirus from contaminated to clean surfaces in three models of virus transmission

Time (min) between virus contamination and transfer	No. of volunteers ^a	Mean % infectious virus transfer (SD)		
		Hand to surface	Surface to hand	Hand to hand
20	5	16.1 (5.43)	16.8 (5.17)	6.6 (2.61)
60	6	1.8 (1.50)	1.6 (1.18)	2.8 (2.11)

^a Three fingers were used with each volunteer.

and from hands was previously demonstrated for rhinovirus (11, 12, 28, 30) and respiratory syncytial virus (13, 14).

A variety of porous and nonporous inanimate surfaces can act as rotavirus donors or recipients in institutional settings. However, earlier studies have shown that rotaviruses are more readily recovered from experimentally contaminated nonporous inanimate surfaces and that the extent of virus survival is virtually the same on plastic, glass, and stainless steel (35). In view of this, disks of stainless steel were chosen here as representative nonporous inanimate surfaces.

For the survival-inactivation experiments, the first sampling time (20 min) was selected because this was the minimum period required for the inocula to become visibly dry. The last sampling time, after a further 4 h (260 min), was chosen because we believe this to be the maximum period expected between handwashings for institutional staff and those in their care. Furthermore, during this period caregivers would be expected to make direct or indirect contact with several susceptible individuals in institutional settings; this could include self-inoculation. It was also difficult to schedule and conduct experiments involving volunteers for more than 4½ h. A 1-h sampling time was included to assess the quantity of the initial virus inoculum which would be available for transfer to a clean surface after 1 h.

It is difficult to make a comparison between human rotavirus inactivation on the skin surface and that previously observed under defined conditions of temperature and relative humidity for the same virus dried onto inanimate surfaces (35). Although the experiments here were conducted under ambient conditions (approximately 22 ± 2°C and 50% relative humidity), the exact temperature and relative humidity microclimate of the hand were unknown. It is also possible that chemicals present on or secreted by the skin may be detrimental to virus survival.

A 10% suspension of feces was used to suspend the virus in these experiments because the large amounts of particulate matter and the thicker consistency made more concentrated stool samples difficult to work with. The 10-µl inoculum used in this study was large enough to be measured with reasonable accuracy, and yet it was considered small enough to represent a realistic amount of contamination that could be acquired during contact with fecally contaminated material.

The washing and ethanol rinsing of hands before the deposition of the virus on fingerpads were considered necessary to minimize the influence of accumulated materials, such as emollients, on virus survival and to eliminate the possibility of any background levels of virus on the hands. In spite of this, survival of the virus was consistently poorer on the hands of certain volunteers. This may be due to slight differences in the biochemistry, physiology, and topography of the skin from one individual to another. Whereas this requires further investigation, we observed that, in one case where the quantity of survival virus was very variable, lack

of virus survival after 260 min often coincided with excessive perspiration on the hand.

For a proper distinction between the survival and inactivation of virus on hands and the efficiency of virus elution from hands it would be necessary to conduct such survival and transfer experiments with the aid of a suitable physical tracer incorporated into infectious rotavirus particles. Although we have previously used ¹⁴C-labeled rotavirus preparations to determine the efficiency of virus elution from nonporous inanimate surfaces (35), we are not aware of any such tracer that could be readily incorporated into infectious virus and which would at the same time be considered safe for use on human skin. Consequently, the survival of the contaminating virus and the retention of that virus by the experimentally contaminated surface are confounded in the data. For this reason, and to be able to compare the results properly, it was considered necessary to relate all of the data to the levels of input virus. Moreover, we have separately determined that the amount of virus recovered from the hands immediately after inoculation was approximately 82% of the input virus. It can therefore be stated that the figures we have quoted in the results section for both survival and transfer would be minimum values only.

Cases of rotaviral diarrhea have been found to excrete up to 10¹¹ virus particles per g of feces (7), and it has been demonstrated that at least 1 of every 4 × 10⁴ of these particles is infectious for cell cultures (40). In this study the level of infectious virus in the 10-µl inoculum was therefore within the range one might encounter in field situations. It has also been shown that the minimum infective dose for animal (10) as well as human (39) rotaviruses is 1 cell culture infective unit. Even though less than 10% of the input infectious virus remained detectable on hands after 260 min, this remaining fraction still represented between 1.3 × 10² and 1.7 × 10³ PFU.

In the transfer experiments only one level of pressure was tested, and the contact between the donor and recipient surface was made without any friction or rubbing motion. We estimate that the pressure used here is generally what is applied in a number of routine functions such as turning doorknobs and shaking hands. Further testing would be required to determine how different levels of pressure and friction would influence virus transfer.

In all three models of virus transmission, rotavirus transfer after 20 min was quite high compared with that at 60 min. This suggests that although the inocula were visibly dry after 20 min, drying was incomplete at this time. This is in agreement with previous observations with rhinovirus (30), where virus transfer was greatest when the inoculum was still damp. At 20 min the lowest percentage of transfer was observed in hand-to-hand transmission. If chemicals on the skin are detrimental to rotavirus survival, then the virus transferred from hand to hand may be exposed to a second chemical challenge which would reduce the infectious virus recovered. It is also interesting to note that at 1 h nearly twice as much virus is transferred in the hand-to-hand model compared with that transferred between hands and inanimate surfaces. The reasons for this are unknown but could be related to the presence of some moisture on both donor and recipient surfaces. The amount of infectious virus that could be transferred even after 60 min of drying was at least 3.0 × 10² PFU, which is considerably higher than the estimated minimal infective dose for rotaviruses.

In suspension tests, rotaviruses proved to be relatively resistant to inactivation by many commonly used chemical disinfectants (36, 37). A number of surface disinfectants and

topical antiseptics were also found to be ineffective against these viruses in carrier tests (23). We are now in the process of assessing the efficacy of topical antiseptics in rotavirus inactivation on human hands.

The role of hands in infectious disease transmission is widely accepted. However, experimental transmission of viruses by contaminated hands has only been reported for rhinoviruses (12). Hands frequently come into contact with environmental surfaces, where they can acquire or deposit infectious agents. The results of this study strongly suggest that hands may play a potential vehicular role in rotavirus transmission. Since rotaviruses may remain infectious on inanimate surfaces for prolonged periods (35), the roles of fomites and hands in their transmission are complementary and may be synergistic. In institutions housing infants and young children the numbers of hand-hand and hand-object contacts are inevitably high. Children frequently suck their fingers and put toys and other objects into their mouths; in one study, these activities were noted as often as every 2 to 3 min (3). Such frequency of contacts may help to explain the high incidence of rotavirus infection and disease in susceptible populations of infants and young children.

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