Potential Role of Hands in the Spread of Respiratory Viral Infections: Studies with Human Parainfluenza Virus 3 and Rhinovirus 14

SHAMIM A. ANSARI, V. SUSAN SPRINGTHORPE, SYED A. SATTAR,* SYLVIE RIVARD,1 AND MAKSUDAR RAHMAN2

Department of Microbiology & Immunology, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5, and Division of Biometrics, Laboratory Center for Disease Control, Health and Welfare Canada, Ottawa, Ontario, Canada K1A 0L2

Received 18 March 1991/Accepted 28 June 1991

Hands often become contaminated with respiratory viruses, either directly or through contact with contaminated surfaces. Spread of such viruses could then occur by touching the nasal mucosa or the conjunctivae. In this quantitative study, we compared the survival of mucin-suspended human parainfluenza virus 3 (HPIV-3) and rhinovirus 14 (RV-14) and the transfer of the viruses to and from the fingers of adult volunteers. When each finger pad was contaminated with 10 μl of either HPIV-3 (1.3 × 103 to 5.5 × 105 PFU) or RV-14 (2.1 × 105 to 1.1 × 106 PFU), <1.0% of HPIV-3 and 37.8% of RV-14 remained viable after 1 h; after 3 h, nearly 16% of RV-14 could still be detected, whereas HPIV-3 became undetectable. Tests on the potential spread of viruses from contaminated hands or surfaces were conducted 20 min after contamination of the donor surface by pressing together donor and recipient surfaces for 5 s. Transfer of HPIV-3 from finger to finger or from metal disk could not be detected, but 1.5% of infectious HPIV-3 was transferred from disk to finger. Irrespective of the type of donor or recipient surface, 0.7 to 0.9% of RV-14 was transferred. The relatively rapid loss of HPIV-3 infectivity on hands suggests that their role in the direct spread of parainfluenza viruses is limited. However, the findings of this study further reinforce the view that hands can be vehicles for rhinovirus colds. These results also suggest a role for nonporous environmental surfaces in the contamination of hands with respiratory viruses.

Parainfluenza viruses are common human pathogens, and they are second only to the respiratory syncytial virus in causing lower-respiratory-tract infections in young children (8). Rhinoviruses are the most common cause of acute respiratory infections in humans (19). Apart from the disease caused in the general population (11, 16, 17, 33), these viruses frequently produce outbreaks in hospitals (10, 18, 32, 34, 36, 45, 48), schools (4, 39), day-care centers (12, 35), and other institutional settings (21, 30).

In spite of the relative importance of parainfluenza viruses as human pathogens, our understanding of the modes and vehicles for their spread is still very limited (8). These viruses have been found to survive for at least a few hours in air (37) and on environmental surfaces (6, 38). However, no studies have been conducted to properly elucidate the potential of human hands in the spread of these viruses.

The relative importance of air (13, 31), hands (22), and environmental surfaces (20, 40, 42) in the spread of rhinovirus colds has been studied, but, so far, quantitative studies have not been carried out to determine how long rhinoviruses can survive on human hands. Therefore, in this study we used the finger pad protocol (3) to compare the survival of parainfluenza viruses and rhinoviruses on human hands and to determine how efficiently these viruses can be transferred to and from contaminated hands.

MATERIALS AND METHODS

Viruses and cells. Strain 47885 of human parainfluenza virus 3 (HPIV-3) was obtained from the National Institute of Allergy and Infectious Diseases, Bethesda, Md. Human rhinovirus 14 (RV-14) was obtained from J. Gwaltney, Jr., University of Virginia, Charlottesville. These viruses were cultivated in MA-104 cells and the A-5 strain of HeLa cells, respectively. Methods for working with MA-104 (41) and A-5 HeLa (31) cells have been described in detail.

Plaque assays. For plaque assays, cell monolayers were prepared in 12-well plastic plates (Costar, Cambridge, Mass.). The overlay for HPIV-3 consisted of Earle minimal essential medium (GIBCO, Grand Island, N.Y.), 2% fetal bovine serum, 0.6% agarose (Sigma Chemical Co., St. Louis, Mo.), and 50 μg of gentamicin (Cidomycin; Roussel Canada, Montreal, Quebec) per ml. RV-14-infected monolayers were overlaid with M-199 medium (Flow Laboratories) supplemented with 0.22% NaHCO3, 100 μg of 5-bromodeoxyuridine (Calbiochem, San Diego, Calif.) per ml, 50 μg of DEAE-dextran (Sigma) per ml, 30 mM MgCl2 (Fisher Scientific), and 0.9% Oxoid agar no. 1 (Oxoid Ltd., Basingstoke, Hampshire, England). The plates with overlaid monolayers were incubated at either 37°C for 4 days (HPIV-3) or 33°C for 2 days (RV-14). The cultures were then fixed and stained (41) for counting plaques, and the titer was expressed as PFU.

Virus suspending medium. Normal saline containing 5 mg of bovine mucin (product no. M-4503; Sigma) per ml was used as the suspending medium for both viruses to simulate human nasopharyngeal secretions (14). The virus under test was diluted 1:10 in the suspending medium and used for the experimental contamination of animate and inanimate surfaces.

Disks. Clean and sterile stainless-steel disks (1-cm diameter) were used as representative nonporous inanimate sur-
faces. These disks were punched out of no. 4 polished stainless-steel sheets (0.75-mm thickness) purchased locally. Prior to the experimental contamination with the virus suspension, the disks were cleaned by sonication for 10 min in a 7× cleaning solution (Linbro; Flow Laboratories) followed by thorough rinsing in running distilled water. They were then soaked in 95% ethanol for 1 h, air dried, and autoclave sterilized in a screw-capped glass vial. After preparation, disks were placed and moved only with sterile forceps. Survival of HPIV-3 on disks was in accordance with the procedure described before (43).

**Volunteers.** Three adult males and one adult female participated as volunteers in this study. Permission to place the viruses on their hands was obtained from our university’s ethics committee. Volunteers were thoroughly briefed on the experimental protocol and the infectious agents to be used before their consent was obtained.

Just before each experiment, the volunteers were required to wash their hands with warm tap water, rinse them with 70% ethanol, and allow them to air dry in order to minimize the influence of accumulated materials such as emollients on virus survival (2).

Immediately after each experiment, the contaminated finger pads were decontaminated by wiping them with agents which had been shown previously to be effective against the test virus; 70% ethanol was used for HPIV-3 (44), and 5% acetic acid was used for RV-14. The volunteers were then required to wash their hands thoroughly with a germicidal liquid soap (Dial Corp., Scottsdale, Ariz.) and warm tap water.

**Virus survival experiments on hands.** The method developed to test human rotavirus survival on hands (3) was used in this study. Ten microliters of the test virus suspension in mucin was placed on the pad of the middle finger of the right hand, using a positive-displacement pipette (Gilson Medical Instruments, Villiers-le-Bel, France), and immediately eluted with 1.0 ml of Earle balanced salt solution to represent the amount of infectious virus placed on each finger pad (0-min control). The pads of the index, middle, ring, and small fingers of the left hand were then contaminated with 10 μl of the virus suspension. The deposited virus was eluted from one of these fingers, in random order, at specified sampling intervals. Unless otherwise stated, each experiment was repeated at least three times on each one of the four volunteers and all assays were performed in triplicate.

**Virus transfer experiments.** Three models of virus transfer were investigated: finger to disk, disk to finger, and finger to finger (3). To study virus transfer, each virus donor surface was inoculated with 10 μl of the virus suspension under test, using a positive-displacement pipette. The inoculum was allowed to become visibly dry, which occurred by 20 min under ambient conditions. Therefore, 20 min postinoculation was selected as a standard time for virus transfer. To determine the amount of infectious virus remaining at the end of the 20-min drying period, virus from a representative surface in each experiment was eluted as described earlier (3) and plaque assayed.

During virus transfer, the donor and recipient surfaces were pressed together for 5 s at a pressure of approximately 1 kg/cm²; this was deemed equivalent to the pressure applied in a handshake or to a doorknob when opening a door. Virus was eluted from both surfaces, and eluates were plaque assayed. A recording hygrothermograph (Cole-Parmer Instrument Co., Chicago, Ill.) was used to monitor the air temperature and relative humidity (RH) of the atmosphere continuously. Each experiment was repeated at least three times, and all assays were performed in triplicate.

**Statistical analysis.** A computerized statistical program, Statistical Analysis System, was used to analyze the data. The differences in percent virus survival among volunteers were analyzed by using analysis of variance on transformed data. If a significant difference among the variables was found, the least significant difference test was used for subsequent pairwise comparisons.

**RESULTS**

The amount of input HPIV-3 on each finger pad ranged from 1.3 × 10⁵ to 5.5 × 10⁵ PFU, and, initially, survival of this virus on fingers was tested over a period of 3 h. Repeated trials showed that virus infectivity became virtually undetectable 1 h after its deposition on the finger pads. In view of this, HPIV-3 survival on the hands of the four volunteers is shown over a period of 60 min only (Fig. 1). Virus infectivity dropped very rapidly within the first 10 min; only 5.3% of the input infectious virus remained detectable at the end of that period, and after 1 h we could recover <1.0% of the input PFU. Statistical analyses of data indicate that there are no observed differences between fingers or experimental replicates (P = 0.6425), but there were slight differences between volunteers.

Since the virus inoculum was visibly wet after 10 min on the finger pad, the relatively rapid loss in HPIV-3 infectivity on the skin was unlikely to be due to the drying of the inoculum alone. To verify this, further experiments were conducted to determine the extent of HPIV-3 inactivation in the first 10 min after its deposition on finger pads. The results, shown in Table 1, confirm the earlier observations.

The poor survival of HPIV-3 on hands prompted us to investigate its survival on disks. Under ambient conditions (50% ± 5% RH and 22 ± 2°C), HPIV-3 survived much better on disks than on fingers (Fig. 1) and the difference was statistically significant at the α = 0.05 level. Twenty minutes after inoculation, 37.8% ± 5.3% of the original virus inoculum remained infectious on the disks. In spite of its poor survival on hands, we attempted to determine whether any infectious HPIV-3 could be transferred from contaminated hands to clean hands or disks after 20 min of drying. Repeated experiments did not show any detectable virus transfer from finger to finger or finger to disk. However, nearly 1.5% ± 0.98% of infectious virus could be transferred...
from contaminated disks to clean hands (Table 2); this represents a minimum of 204 PFU.

The amount of RV-14 placed on each finger pad ranged from \(2.1 \times 10^5\) to \(1.1 \times 10^7\) PFU. As shown in Fig. 2, there was a gradual loss in virus infectivity over a period of 60 min. However, nearly 16% of the input virus remained viable on the finger pads even after 3 h; this represents a minimum of 3,000 PFU. The rate of loss of RV-14 infectivity on fingers was clearly much slower than that for HPIV-3. As was the case for HPIV-3, there were slight person-to-person differences observed, but no differences were noted between fingers or experimental replicates.

The extent of RV-14 transfer between experimentally contaminated and clean surfaces was also determined under ambient conditions (50% ± 5% RH and 22 ± 2°C). Nearly 69% of the input infectious virus survived the 20 min of drying on the disks. Results given in Table 2 show that, irrespective of the type of donor or recipient surface, the amounts of infectious virus transferred were similar. After 20 min of deposition of the virus on finger pads or disks, infectious virus transferred ranged between 0.7 and 0.9%; this represents a minimum of 190 PFU.

**DISCUSSION**

In general terms, the potential of a vehicle to spread a given infectious agent is directly related to the capacity of the agent to survive in or on that vehicle. Hands have long been implicated in the spread of infectious diseases and are often suggested to be the most important vehicle (1, 5, 27, 29). In spite of this, their role in the spread of respiratory diseases remains unclear, and there is relatively little information on the capacity of respiratory pathogens to survive on hands. Most of the evidence in this regard comes from work with rhinoviruses (22, 28, 40) and respiratory syncytial virus (24).

If hands are to be important vehicles in the spread of respiratory pathogens, they must transfer to the susceptible host a quantity of infectious agent at least equal to the minimal infective dose. The minimal infective dose for rhinoviruses through the nasal route has been found to be <1 50% tissue culture infective dose (9); humans can also be readily infected by rhinoviruses through the conjunctival route (7). Limited testing with parainfluenza viruses shows their minimal infective dose for humans by the intranasal route to be nearly 80% tissue culture infective doses (46). Fingers may be the most important part of the hand in the spread of pathogenic microorganisms. They frequently come in contact with infectious fluids or surfaces and fomites contaminated directly or by large-particle aerosols. Such acquisition and carriage of pathogens by the fingers may result in inoculation of self or others, particularly during caring for the sick, the young, and the elderly.

The finger pad protocol used here was developed in our laboratory (3) as a simple, reproducible, and quantitative method to study the survival of viruses and bacteria on human hands, their transfer between hands and inanimate objects, and their antisepsis (2). As has been discussed before (47), this protocol can be controlled better than the methods that use the whole hand. The results of this study show that this protocol could be readily used for the study of respiratory viruses as well. Stainless-steel disks were used in this study because virus survival on such disks may be representative of hard environmental surfaces. Furthermore, as was shown with rotavirus (43), virus survival on stainless-steel disks may be almost the same as on glass and plastics.

The nature of the medium used for suspending the test virus can influence its survival (15). The most appropriate

**TABLE 1. Survival of HPIV-3 on finger pads within 10 min of deposition**

<table>
<thead>
<tr>
<th>Expt no.</th>
<th>1 min</th>
<th>4 min</th>
<th>7 min</th>
<th>10 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>82.65</td>
<td>57.80</td>
<td>29.48</td>
<td>07.54</td>
</tr>
<tr>
<td>2</td>
<td>84.12</td>
<td>25.09</td>
<td>16.32</td>
<td>11.17</td>
</tr>
<tr>
<td>3</td>
<td>65.93</td>
<td>24.72</td>
<td>23.54</td>
<td>10.00</td>
</tr>
<tr>
<td>4</td>
<td>94.90</td>
<td>63.20</td>
<td>20.50</td>
<td>02.00</td>
</tr>
<tr>
<td>5</td>
<td>86.21</td>
<td>45.34</td>
<td>40.43</td>
<td>10.40</td>
</tr>
</tbody>
</table>

Overall mean (SD) 82.76 (10.54) 43.23 (17.94) 26.05 (9.06) 08.22 (3.73)

*a All virus plaque assays were performed in triplicate.

**TABLE 2. Virus transfer from donor surface to recipient surface after 20 min of drying**

<table>
<thead>
<tr>
<th>Transfer model</th>
<th>Virus</th>
<th>PFU transferred (SD)</th>
<th>% PFU transferred (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hand to disk</td>
<td>RV-14</td>
<td>148 (36)</td>
<td>0.92 (0.3)</td>
</tr>
<tr>
<td></td>
<td>HPIV-3</td>
<td>Undetectable</td>
<td></td>
</tr>
<tr>
<td>Disk to hand</td>
<td>RV-14</td>
<td>131 (29)</td>
<td>0.67 (0.1)</td>
</tr>
<tr>
<td></td>
<td>HPIV-3</td>
<td>239 (40)</td>
<td>1.48 (1.0)</td>
</tr>
<tr>
<td>Hand to hand</td>
<td>RV-14</td>
<td>170 (70)</td>
<td>0.71 (0.2)</td>
</tr>
<tr>
<td></td>
<td>HPIV-3</td>
<td>Undetectable</td>
<td></td>
</tr>
</tbody>
</table>

*a Each experiment was conducted three times, and all virus plaque assays were performed in triplicate.

**FIG. 2. Comparison of survival of HPIV-3 and RV-14 on the finger pads of adult volunteers. The amount of input HPIV-3 on each finger pad ranged from 1.3 \(\times 10^6\) to 5.5 \(\times 10^7\), and that of RV-14 ranged from 2.1 \(\times 10^4\) to 1.1 \(\times 10^7\).**
suspension for cell culture media. This remote distance ensures that the virus is not only harmless to virus infectivity but also highly efficient in virus elution. Elution of the virus inoculum immediately from the experimentally contaminated surfaces without the tracer. The reduction in the infectivity titer of HPIV-3 was apparent well before the inoculum became dry on the contaminated surfaces. This suggests that hands may be a much more important vehicle for the spread of rhinoviruses than for that of parainfluenza viruses. If spread of parainfluenza viruses through hands is to occur, it may only be within the first few minutes after their contamination with the infectious material.

The transfer of HPIV-3 and RV-14 from disks to clean fingers supports a role for fomites in the contamination of hands with both viruses. The numbers of PFU transferred for HPIV-3 and RV-14, respectively, were about 200 and 170. This represents just over 3 minimal infective doses for HPIV-3 (46). With a rapid loss of HPIV-3 infectivity on the skin, the chances of directly infecting susceptible hosts through hands are limited. Since no finger-to-finger or finger-to-object transfer of the virus could be demonstrated when the inoculum was allowed to dry for 20 min, indirect passage of the virus from hands is even less likely to result in disease spread. In contrast to this, the small minimal infective dose for rhinoviruses and their ability to remain viable on human skin for longer periods suggest that hands may play an important role in their spread.

The survival of aerosolized parainfluenza viruses is favored when the RH level is about 20% (37). The RH levels were not reported in the study by Brady et al. (6) in which parainfluenza virus survival on environmental surfaces was determined. Parkinson et al. (38) tested parainfluenza virus survival on plastic petri plates indoors where the RH was quite low (8 to 33%). In the present study, the RH was approximately 50%, and about 12% of the inoculated HPIV-3 on disks remained infectious after 1 h. Whether higher or lower levels of RH or a febrile state could influence virus survival on skin remains to be tested.

Proper and regular handwashing as well as disinfection of hard environmental surfaces may play a role in minimizing the spread of these viruses, particularly in institutional settings. Our earlier studies (44) have shown that HPIV-3-contaminated, hard, nonporous surfaces are relatively easy to disinfect with a variety of commercial and noncommercial formulations. However, no information is available on the in vivo efficacy of antiseptics in dealing with HPIV-3-contaminated hands. Rhinoviruses have also been found to be fairly susceptible to chemical disinfectants in studies on inanimate surfaces and on hands (23, 26). We have also shown (44a) that a medicated liquid soap could reduce the infectivity titer of RV-14 >99.0% on experimentally contaminated finger pads in a contact time of 10 s.

ACKNOWLEDGMENTS

We thank W. Tostowaryk for his help in the design of this study. We are thankful to all of the volunteers who participated in this study.

Financial support for S.A.A. has been provided by a scholarship from the Ministry of Education, Government of Pakistan. Complimentary supplies of Cidomycin were received from Roussel Canada.

REFERENCES


