JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1980, p. 567-571
0095-1137/80/10-0567/05$02.00/0

Vol. 12, No. 4

Role of Infectious Secretions in the Transmission of Rhinovirus

F. PANCIC,* D. C. CARPENTIER, AND P. E. CAME
Sterling-Winthrop Research Institute, Rensselaer, New York 12144

In a series of studies aimed at investigating the role of environmental surfaces in the transmission of certain respiratory virus infections, it was shown that small amounts of nasal mucus containing rhinovirus (infectious mucus) can spread from fingertips to door knobs, faucet handles, or other environmental surfaces and remain infectious for many hours. These surfaces can serve as a reservoir of virus and may provide sufficient infectious material to contaminate hands. Recent studies have shown that once virus is on the fingers, it may be transferred to the nasal and conjunctival mucosa by means of autoinoculation. It has been estimated that as little as 1.0 plaque-forming unit can produce an infection in a susceptible human. In the present experiments, the amount of rhinovirus transmitted from fingers contaminated with infectious mucus to environmental surfaces and from there onto the fingers of a volunteer who touched the contaminated objects was quantitated, and the efficiency of transfer was studied. From 3 to 1,800 plaque-forming units of rhinovirus were recovered from the fingertips of volunteers (recipients) who handled either a door knob or a faucet that had previously been manipulated by another volunteer (donor) whose fingers were contaminated with infectious mucus. The average amount of rhinovirus recovered from the fingers of the recipients was approximately 13.5% of the amount recoverable from the fingers of the donor. In experiments in which there was direct hand-to-hand contact between donor and recipient, about 6.7% of the virus present on the fingertips of donors was recoverable from the recipients.

The role of environmental surfaces in the transmission of certain infectious diseases has been a controversial subject, and fundamental knowledge concerning the spread of many diseases is lacking. In recent years, however, studies have been reported which indicate that the common cold may be spread by contact with infectious secretions rather than by inhalation of small-particle aerosols, and a number of laboratories have shown that respiratory viruses can survive on environmental surfaces for appreciable periods of time (4, 7, 8; K. J. Reagan, M. L. McGeady, and R. L. Crowell, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, Q101, p. 210). Moreover, Gwaltney and co-workers (3) have demonstrated that rhinoviruses (RVs) can be transmitted from hand to hand, and once the virus is on the fingertips, the finger becomes a potential source of infection if it is brought in contact with the conjunctival or nasal mucosa. Referring to work previously published, Gwaltney and colleagues (3) point out that contamination of environmental surfaces may possibly provide an additional source of virus for self-inoculation. Until the first studies by Hendley and co-workers (7) were published in 1973, it was generally accepted that the transmission of RVs occurred chiefly by the airborne route, but more recently, Reed (8), D'Alessio et al. (1), and Hall et al. (4) reevaluated the route of transmission of RVs and other respiratory viruses. Hall et al. (4) have found that respiratory syncytial virus may spread from infant to infant in the hospital via infectious secretions on the hands of health care personnel. These workers also indicated that it seems feasible that environmental surfaces and self-inoculation play a role in the transmission of respiratory syncytial virus (5).

In an attempt to study the efficiency of transfer of virus from individual to individual via contaminated fingers to hard surface and back to the fingers of another individual, we conducted a series of experiments designed to mimic natural conditions where feasible. An RV recently isolated from an individual with a cold was added to a pool of "normal" human nasal mucus and was used to contaminate the pads of two or three fingers of a volunteer (the donor). The donor manipulated a door knob or a faucet handle, and subsequently, these contaminated objects were used by another volunteer (the recipient). At each step of the route, virus was

recovered by rinsing either the finger pads or the object and then was quantified. In other experiments, the donor did not handle an inanimate object, but rather touched the fingertips of a recipient directly. This report details the results of several experiments and provides a quantitative description of RV transmission via commonly encountered hard surfaces.

MATERIALS AND METHODS

Virus. A serologically untyped RV, strain HH, isolated at the University of Virginia School of Medicine from a patient with a naturally occurring cold, was obtained from J. M. Gwaltney, University of Virginia, Charlottesville. The nasal mucus obtained from this donor was tested and shown to be free of bacterial pathogens or other viruses. Six passages of the virus were performed in WI-38 monolayers in our laboratory. The virus pool prepared from the sixth passage contained 8.6×10^5 plaque-forming units (PFU)/ml.

Normal nasal mucus. Nasal mucus was forcibly expelled into a petri dish by a volunteer who was without symptoms of a cold. A small sample of this mucus was diluted 1:10 in tissue culture medium and used to inoculate monolayers of HeLa (Ohio) cells for detection of adventitious viruses. No cytopathic effect was observed after 5 days of incubation at 36°C.

Infectious mucus. Unless specified, the inocula used to contaminate donors' fingers consisted of a total volume of 0.05 ml of a 1:1 mixture of cell culture-prepared virus and normal nasal mucus. Experience and observation suggested that a volume of 0.05 ml (1 or 2 drops) was a reasonable estimate of the amount of mucus that could occasionally be encountered on the fingers or hand of an individual who had just attended his or her runny nose. Possibly a greater quantity might be expected on the hand of a young child suffering from a cold who did not use a tissue or handkerchief, and smaller quantities could be envisioned as contaminating the hands of an individual practicing better hygiene. The quantity of virus in the infectious mucus was adjusted to reflect estimates obtained from previous reports which indicated that nasal mucus from common cold sufferers at various stages of their illness contains a range of about 10 to 1×10^6 PFU or 50% tissue culture infective doses per ml of nasal discharge (1, 3, 8).

Plaque assay. Serial 10-fold dilutions of fluids were assayed in confluent monolayers of HeLa (Ohio) cells, 48 h old, by adding 1 ml of virus dilution, incubating at 33°C for 2 h, aspirating fluid, and adding 5 ml of an overlay containing 0.8% Oxoid no. 1 agar, 10% fetal bovine serum, and 0.6% $MgCl_2$ in medium 199. Assays were performed in triplicate for each dilution.

Cultures were incubated at 33°C for 6 days, and then the cells were fixed and stained by the addition of 10 ml of a crystal violet solution in 9% Formalin, incubated at room temperature for 2 h, and washed with tap water. Plaques were counted and expressed as PFU. Each plaque was considered to represent one virus particle in the original solution.

Environmental surfaces. For experimental convenience, sets of three door knobs and three faucets were mounted on a board. Faucets or brass door knobs were about 1 ft (30.48 cm) apart. Sets were placed on a stainless-steel bench and washed with water and a cloth, followed by applying 70% ethanol with a cloth.

Donors. Volunteers serving as donors were contaminated by spreading 0.05 ml of infectious mucus between the ball of the thumb and the index finger, which were gently rubbed together to facilitate distribution. Then donors made direct hand-to-hand contact with recipients or handled faucet handles or door knobs as described below.

Hand-to-environmental surface-to-hand transmission. These trials were designed to simulate the events that might be expected to occur when the contaminated hands of a cold sufferer (the donor) manipulate a commonly encountered environmental surface, resulting in its contamination. The contaminated object was handled by another volunteer (the recipient) whose fingertips were subsequently sampled to quantitate virus.

In two experiments, the donor contaminated a faucet handle by placing thumb and finger on either side of the handle and sliding them back and forth twice. Afterwards, the fingers of the donor, and the faucet handle, were immediately rinsed to determine virus titer.

In two other experiments, door knobs were used as the inanimate objects. The donor placed three fingers around the knob and twisted it twice as if opening or closing the door. The door knob was sampled by rinsing, as described for the faucet handle, as were the donor's and the recipient's fingers.

In two additional studies, also using door knobs, the size of the infectious mucus inoculum was increased to 0.1 ml to ascertain whether proportionate increases in virus recovery would be obtained at the various steps encountered in the transmission route.

Ten minutes after contamination of faucet handles or door knobs, they were manipulated by a recipient who was instructed to: (i) grasp the faucet handle firmly between thumb and index finger and slide them back and forth twice; or (ii) grasp a door knob firmly between thumb, index, and middle fingers and slide them around (180°) the knob twice. The fingers were rinsed and the fluids were titrated.

Assays for recoverable virus were made at the following steps in the transmission chain: (i) the infectious mucus mixture used to contaminate the fingers of the donors; (ii) a rinse of the donor's fingers immediately after the inoculum of infectious mucus was deposited on them to determine the efficiency of virus recovery from skin; (iii) a rinse of the donor's fingers immediately after manipulation of the object to determine how much virus was removed from the fingers while the object was being handled; (iv) a rinse of the contaminated object to determine the quantity of virus deposited on the object and to assess the efficiency of transmission from the donor's fingers to the object; (v) a rinse of the contaminated object after manipulation by the recipient, which was done to provide an estimate of how much virus remained on the object after it was handled by a recipient; (vi) titrations of the rinses from the recipient's fingers to estimate how many infectious virus particles were present and pre-

sumably available for self-inoculation by introduction into eye or nose.

Direct hand-to-hand transmission. This series of experiments was conducted to provide a quantitative estimate of the efficiency of transfer of RV from direct finger-to-finger contact. In the studies reported by Gwaltney and colleagues (3), the experimental design involved direct hand-to-hand transmission of RV colds. The fingers of donors were contaminated by depositing 0.05 ml of infectious mucus onto the pads of two fingers, which were then brought into contact with three fingers of the recipient by gently rubbing fingertip pads.

Virus was rinsed from the fingertips of donors or recipients on the following occasions: (i) The donor's fingertips were rinsed immediately after a 0.05-ml inoculum of infectious mucus was deposited on them; (ii) after the donor touched the recipient's fingers, the donor's fingertips were sampled to determine how much of the inoculum remained after touching; (iii) the recipient's fingertips were sampled to determine the amount of infectious virus available for self-inoculation.

RESULTS

Hand-to-environmental surface-to-hand transmission. The chain of events and the virus sampling procedure used in one of the described experiments are shown in Table 1. Experiments A and B show the results when a door knob served as the object. In experiment A, 915 PFU were deposited onto the donor's fingers. In experiment B, a slightly lower inoculum was used, 685 PFU. In both experiments, virus was recovered from the fingers of the recipients, 3 and 37 PFU, respectively. Also, in both trials, it can be seen that the amount of recoverable virus decreased as the number of manipulations increased and was consistent with expected losses due to <100% efficiency of transmission from one surface to another, dilution resulting from a surface distribution effect, adherence of virus to the surfaces or skin, and other factors.

Experiments C and D show the results obtained when faucet handles were used. The amounts of virus in the inocula were only 215 and 225 PFU in these trials. In both experiments, virus (3.7 and 7 PFU) was recovered from the recipient's fingers after the faucets were handled.

The results of experiments E and F, in which the virus inoculum was increased to 0.1 ml containing 6,300 and 18,000 PFU, show that the successive rinses from fingers and door knobs reflected a corresponding increase of virus contamination. Also, it can be seen that, at the termination of the experiments, there were 103 and 400 PFU remaining on the door knobs. In these two experiments, as well as in the four discussed above, a diminution of virus occurred as the number of manipulations increased, but in all six experiments, infectious virus was recoverable from the fingers of the recipient.

In five of the experiments, the amount of virus recovered from the fingers of the recipient amounted to an average of approximately 7.9% of the virus recoverable from the donor's fingers, whereas in the sixth experiment, in which a high inoculum of 18,000 PFU was used, 22% of the

TABLE 1. *Quantitation of RV during the transmission sequence from the donor's fingers to objects[a] and back onto the hands of recipients*

Transmission step	Virus titer (PFU)					
	Expt A	Expt B	Expt C	Expt D	Expt E	Expt F
(i) The 0.05-ml (0.1 ml in expt E and F) inoculum directly from the pipette	915	685	215	225	6,300	18,000
(ii) Rinsings from donor's fingers immediately after inoculation but before manipulation of the object	207	177	63	130	2,300	8,100
(iii) Rinsings from the donor's fingers immediately after manipulation of the object	73	53	37	43	1,650	2,700
(iv) Rinsings from the contaminated objects before handling by the recipients	13	37	27	37	1,200	3,000
(v) Rinsings from the contaminated objects immediately after manipulation by the recipients	7	3.3	1	13	103	400
(vi) Rinsings of the recipients' fingers	3	37	3.7	7	70	1,800
(vii) Efficiency of transfer (%) from donor's fingers (step ii) to surface to recipients' fingers (step vi)[b]	1.4	20.9	5.8	5.3	3.0	22.0

[a] Door knobs were used in experiments A, B, E, and F, and faucet handles were used in experiments C and D.

[b] Average = 13.2%.

virus inoculum was recovered.

Direct hand-to-hand transmission. Table 2 shows the results of eight separate trials of direct hand-to-hand transmission. It can be seen that the average of approximately 5.9% of the virus recoverable from the hands of the donors could be rinsed from the fingers of the recipients. It can also be seen that the amount of virus found on the fingers of recipients was related to the diminished amount of virus remaining on the fingertips of donors after contact with the recipients.

DISCUSSION

In most of these trials, an inoculum of 0.05 ml of RV in human nasal mucus was selected to represent the volume of infectious mucus that might be found at least occasionally on the fingers of a child or adult cold sufferer who has wiped, blown, or picked his or her nose. Calculations from studies in volunteers (1, 3, 8) show that a volume of 0.05 ml of mucus from a cold sufferer may contain 2 to 50,000 PFU of infectious virus. Using several concentrations of virus expected to be within the range found in natural colds, a series of four experiments was conducted in which human nasal mucus containing 215 to 915 PFU was deposited onto the fingers of a volunteer to mimic conditions that undoubtedly occur with great regularity in nature during the common cold season. Two additional experiments used inocula of 6,300 and 18,000 PFU; these larger inocula were used to mimic the occasional situation where more generous amounts of nasal secretions might be encountered, e.g., a small child using fingers directly to remove mucus from the nose. A volunteer (the donor) then used his/her contaminated hand to manipulate door knobs or faucet handles. Subsequently, the contaminated objects were handled by a volunteer (the recipient) and, afterwards, virus was recovered from the recipient's fingers in concentrations sufficient to induce infection, although no attempt was made to infect volunteers in this study. In earlier studies, Douglas (2) and Hendley et al. (6) reported that small quantities of RV can efficiently initiate infection in volunteers under experimental conditions.

Survival of RV on skin and surfaces has been reported by other workers. Recovery of virus from the backs of volunteers' hands, stainless-steel spoons, plastic ballpoint pens, and plastic table tops was reported by Reed (8). During a 3-h period, a slight decrease in titer was noted on the skin but little decrease was seen on the inanimate surfaces. After 24 h, virus was still recoverable from various objects, although at lower titers. Survival of RV types 2 and 14 under varying laboratory conditions was also reported by Reagan et al. (Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, Q101, p. 210), and results indicated that human RVs retain sufficient infectivity after drying on hard surfaces to permit potential transmission on contact. Hendley et al. have demonstrated the survival of RV type 39 in Hanks balanced salt solution and in nasal mucus for periods up to 24 h (7).

In the present experiments, at each step along the chain of transmission from donor's fingers to recipient's fingers, rinse samples were taken and the amount of infectious virus was quantitated, which permitted the efficiency of transfer from the inoculum to the fingers of the recipient to be determined. The diminution of virus along the transmission route was consistent with anticipated losses due to adherence of virus to surfaces, less than 100% efficiency of transfer from skin to hard surface or hard surface to skin, and a dilution effect due to distribution of virus by smearing. However, in five experiments in which inoculum size varied from as low as 215 PFU to as high as 6,300 PFU, an average of about 7.9%

TABLE 2. *Quantitation of RV directly from the fingers of donors to recipients*

Recovery step	Virus titer (PFU)							
	Expt 1	Expt 2	Expt 3	Expt 4	Expt 5	Expt 6	Expt 7	Expt 8
(i) Recovery from the fingers of donors before contact with recipients	710	1,930	4,000	300	1,900	70	297	80
(ii) Recovery from the fingers of donors after contact with recipients	107	600	3,600	267	930	40	120	77
(iii) Recovery from the fingers of recipients	43	147	57	27	73	7.3	10.3	2
(iv) Efficiency of transfer (%) from donors (step i) to recipients (step iii)[a]	6.1	7.6	1.4	9.1	3.8	10.4	3.5	2.5

[a] Average = 5.9%.

was recoverable from the recipient's fingers. The only exception occurred in an experiment in which the inoculum contained 18,000 PFU in 0.1 ml, and recovery of the virus was increased to 22%.

In the experiments that assessed the efficiency of direct hand-to-hand transmission, the recovery of RV from the recipient's hand (about 5.9% of that recoverable from the donor's hands) was only slightly less than that found on the recipients in the trials involving hand-to-hard surface-to-hand transmission (13.2%), and the significance of this apparent difference is unknown.

Another conclusion that can be derived from the data presented in Tables 1 and 2 is that a contaminated donor's hand may be capable of contaminating multiple surfaces or hands and, similarly, a contaminated surface may provide a source of infectious virus for more than one recipient.

In these experiments, no attempts were made to rigorously control temperature, humidity, etc., but a number of conditions may be envisioned which could influence virus survival and transmissibility; for example, low humidity and high room temperature might decrease survival time of viruses on surfaces. Likewise, high humidity and cooler conditions might prolong survival. It seems probable that mucus containing RVs deposited on a milk or soda bottle or other refrigerated items would retain its infectious potential for a long time.

In the present study, the ease and frequency of recovery from fingers of sufficient infectious virus to induce disease in humans compel one to conclude that similar circumstances must occur in the home, school, or work place. It is known that RV can be recovered from both the hands of persons suffering from colds and from selected objects in their homes (1, 3, 7, 8). It also seems reasonable that disinfection of contaminated objects or hand cleansing or both would interrupt the transmission of some infectious diseases.

LITERATURE CITED

1. **D'Alessio, D. J., J. A. Peterson, C. R. Dick, and E. C. Dick.** 1976. Transmission of experimental rhinovirus colds in married couples. J. Infect. Dis. **133**:28-36.
2. **Douglas, R. G., Jr.** 1970. Pathogenesis of rhinovirus common cold in human volunteers. Ann. Otol. Rhinol. Laryngol. **79**: 563-571.
3. **Gwaltney, J. M., Jr., P. B. Moskalski, and J. O. Hendley.** 1978. Hand to hand transmission of rhinovirus colds. Ann. Intern. Med. **88**:463-467.
4. **Hall, C. B., R. G. Douglas, Jr., and J. M. Geiman.** 1980. Possible transmission by fomites of respiratory syncytial virus. J. Infect. Dis. **141**:98-102.
5. **Hall, C. B., J. M. Geiman, R. G. Douglas, Jr., and M. P. Meagher.** 1978. Control of nosocomial respiratory syncytial viral infections. Pediatrics **62**:728-732.
6. **Hendley, J. O., W. P. Edmondson, Jr., and J. M. Gwaltney, Jr.** 1972. Relation between naturally acquired immunity and infectivity of two rhinoviruses in volunteers. J. Infect. Dis. **125**:243-248.
7. **Hendley, J. O., R. P. Wenzel, and J. M. Gwaltney, Jr.** 1973. Transmission of rhinovirus colds by self-inoculation. N. Engl. J. Med. **288**:1361-1364.
8. **Reed, S. E.** 1975. An investigation of the possible transmission of rhinovirus colds through indirect contact. J. Hyg. **75**:249-258.