APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 1981, p. 618-620
0099-2240/81/030618-03$02.00/0

Vol. 41, No. 3

Persistence of Human Rhinovirus Infectivity Under Diverse Environmental Conditions

KEVIN J. REAGAN, MARY L. McGEADY, AND RICHARD L. CROWELL*

Department of Microbiology and Immunology, Hahnemann Medical College, Philadelphia, Pennsylvania 19102

The persistence of human rhinovirus type 2 and type 14 infectivity was studied under various laboratory conditions designed to mimic those commonly found in the environment. The effects of temperature, ionic strength, protein content, and evaporation were compared. Both viruses were stable (<0.3-log decrease in titer) at 6 and 23°C for 24 h in the liquid state regardless of salt or protein additives; a titer decrease of <1.0 log was noted at 37°C. However, evaporation at 37°C reduced virus infectivity by 3.2 to 4.5 logs in buffered water, an effect which could be significantly lessened by the addition of bovine serum albumin in saline (2.0- to 2.6-log decrease in titer). These studies support and extend observations by others that the human rhinoviruses retain sufficient infectivity after drying on hard surfaces to permit their transmission to susceptible persons upon contact.

The human rhinoviruses include over 100 distinct immunotypes and are a common cause of mild upper respiratory disease in humans. A high incidence of infection exists, occurring in the United States approximately once per person per year (5). In spite of this prevalence, the mode of virion transmission is not well established. An airborne route has not been successfully reproduced in experimental studies approaching natural conditions (2, 13), even though inhalation of particle aerosols has artificially initiated infection (1). Hendley et al. (7, 10) have suggested that rhinovirus shed from infected individuals could be transferred to hands or inanimate objects which are then touched by susceptible persons, with the virus subsequently inoculated into conjunctival or nasal mucosa. It is readily apparent that for an indirect transmission such as this to be feasible, infectious virus must persist outside the host under a variety of environmental conditions. Hendley and co-workers (7) provided evidence that rhinovirus 39 could retain infectivity for several hours at room temperature after drying on hard surfaces. Similar studies have demonstrated survival of rhinovirus immunotypes on human skin, fabric, and a variety of nonporous surfaces (6, 11), yet an analysis of factors involved in rhinovirus survival has not been reported. In a detailed study of a related picornavirus, coxsackievirus B3, persistence of infectivity was influenced by temperature, pH, moisture, salt, and protein content (9).

The current investigation represents a continuation of studies to define factors affecting picornavirus survival on hard surfaces. The inactivation of two acid-labile rhinovirus immunotypes was compared quantitatively under controlled laboratory conditions which mimic those commonly found in the environment. The effects of temperature, ionic strength, protein content, and evaporation were examined.

MATERIALS AND METHODS

Cells and viruses. High-titered pools of human rhinovirus type 2 (HRV2; strain HGP) and type 14 (HRV14; strain 1059) were prepared in a line of HeLa cells susceptible to the rhinoviruses (HeLa-R) by methods described elsewhere (8) and resulted in stock suspensions containing at least 10^9 plaque-forming units per ml. Both viruses and cells were provided by K. Lonberg-Holm (Central Research Department, E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.). The identity of each viral serotype was confirmed by neutralization with reference antiserum in a plaque reduction assay. Cells were maintained either as suspension cultures in Eagle minimal essential medium (modified for spinner cells) with double-strength amino acids and vitamins, 10% horse serum (GIBCO Laboratories, Grand Island, N.Y.), and 100 U of penicillin and 100 μg of streptomycin per liter or as monolayer cultures in 2× basal Eagle medium in Hanks balanced salt solution 10% calf serum (GIBCO), and 0.6 mg of peptone, 100 U of penicillin, and 100 μg of streptomycin per ml.

Conditions of inactivation. Under the conditions of virus inactivation, stock HRV2 or HRV14 was first diluted 1:100 into one of three solutions buffered to pH 7.0 with 1 mM tris(hydroxymethyl)aminomethanehydrochloride (Tris). These solutions were: water, saline (0.85%), or saline with 0.25% crystalline bovine serum albumin (Schwarz/Mann, Orangeburg, N.Y.). All experiments were performed in linear polyethylene liquid scintillation vials (New England Nuclear Corp., Boston, Mass.) at 6, 23, or 37°C. The incubation of

samples under wet or drying conditions as well as the sampling protocols were essentially as described previously (9). Complete evaporation of the 200-μl inoculum was noted by 12 h at 37°C and by 22 h at 23°C. Relative humidity was determined for each experiment but not controlled. All samples were frozen at −70°C in Hanks balanced salt solution with 4.5% added calf serum until assayed.

Infectivity assay. To quantitate remaining infectivity, serial 10-fold dilutions in Hanks balanced salt solution plus 4.5% calf serum were prepared from thawed samples. Duplicate 200-μl portions were placed on 90 to 95% confluent HeLa-R monolayers in 60-mm tissue culture dishes (Falcon Plastics, Oxnard, Calif.). A 30-min incubation period at 34.5°C was allowed for virus attachment followed by an overlay consisting of 2× basal Eagle medium in Hanks balanced salt solution, 0.6 mg of peptone per ml, 4.5% calf serum, 0.02 M *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES; pH 7.5), 100 U of penicillin and 100 μg of streptomycin per ml, and 0.5% agar (Difco Laboratories, Detroit, Mich.). Cultures were incubated for 3 days at 34.5°C in 5% CO_2 in air and then fixed with Formalin before the monolayers were stained with crystal violet. The addition to the agar overlay of $MgCl_2$ or diethylaminoethyl dextran alone or in combination was not found to enhance plaque size, number, or clarity. Virus control preparations were included in each set of titrations.

RESULTS

Tables 1 and 2 summarize the results for the survival of HRV2 and HRV14 under various incubation conditions. Data are expressed as the $\log_{10}$ decrease in plaque-forming units at a given time interval in comparison with the amount present at zero time (approximately 10^7 plaque-forming units/ml). Overall, a high degree of stability was seen for both immunotypes.

Under nonevaporating conditions, HRV2 and HRV14 demonstrated as little as a 0.3-log decrease in titer at lower temperatures and up to a 1.0-log decrease at 37°C, regardless of salt or protein additives. Greater losses of infectivity occurred among samples subjected to evaporation. At higher temperatures, where fluid vol-

TABLE 1. *Survival of HRV2 at pH 7 under diverse conditions of incubation*

Temp (°C)	Diluent	Log decrease in virus titer							
		Time (h) wet				Time (h) drying			
		1	3	6	24	1	3	6	24
6	Tris (10^{-3} M)	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.0
	Tris-saline	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Tris-saline-BSA[a]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
23	Tris (10^{-3} M)	0.2	0.0	0.0	0.2	0.0	0.0	0.0	0.2
	Tris-saline	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.5
	Tris-saline-BSA	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.5
37	Tris (10^{-3} M)	0.0	0.0	0.1	0.5	0.0	0.1	0.2	3.2
	Tris-saline	0.0	0.0	0.1	1.0	0.0	0.1	0.2	2.9
	Tris-saline-BSA	0.3	0.2	0.2	0.7	0.1	0.1	0.2	2.0

[a] 0.25% bovine serum albumin.

TABLE 2. *Survival of HRV14 at pH 7 under diverse conditions of incubation*

Temp (°C)	Diluent	Log decrease in virus titer							
		Time (h) wet				Time (h) drying			
		1	3	6	24	1	3	6	24
6	Tris (10^{-3} M)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Tris-saline	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
	Tris-saline-BSA[a]	0.1	0.1	0.1	0.2	0.1	0.1	0.0	0.1
23	Tris (10^{-3} M)	0.0	0.0	0.0	0.2	0.0	0.0	0.0	1.5
	Tris-saline	0.0	0.0	0.3	0.3	0.1	0.0	0.2	1.7
	Tris-saline-BSA	0.1	0.0	0.0	0.2	0.0	0.0	0.0	1.3
37	Tris (10^{-3} M)	0.1	0.1	0.3	0.6	0.0	0.0	0.1	4.5
	Tris-saline	0.0	0.1	0.1	0.4	0.0	0.1	0.0	3.7
	Tris-saline-BSA	0.1	0.1	0.1	0.3	0.0	0.1	0.1	2.6

[a] 0.25% bovine serum albumin.

umes were completely eliminated during the 24-h incubation period, titer decreases of 0.2 to 1.7 logs (23°C) and 2.0 to 4.5 logs (37°C) were noted. A protective effect of bovine serum albumin in saline was apparent among samples drying at 37°C (2.0- to 2.6-log decreases in Tris-saline-bovine serum albumin in comparison with 3.2- to 4.5-log decreases in buffered water).

Losses of infectivity among evaporating samples could not be adequately explained by an aggregation of intact particles or their absorption to vessel walls. After conditions of greatest inactivation (Tris-water, 37°C), a treatment of incubation vessels with aggregate-dissociating methods (sodium dodecyl sulfate, sonication, or increased ionic strength) could not recover any increased infectivity (data not shown). In addition, whereas low-ionic conditions have been shown to cause rapid aggregation of several picornaviruses, including poliovirus T1, and small-plaque variants of coxsackievirus B3 (4, 12), a similar event did not occur here. Both HRV2 and HRV14 appeared stable in Tris-water for up to 24 h in wet samples and at least 6 h in evaporating samples.

DISCUSSION

An assessment of the significance of these findings in relation to the pathogenesis of rhinovirus infection can be inferred. Virus shed from infected individuals contaminates a variety of environmental surfaces. This study supports the contention that contaminated surfaces might serve as fomites for susceptible individuals. At lower temperatures (6 and 23°C), especially when suspended in fluid, both HRV2 and HRV14 were quite stable over a 24-h incubation period. When subjected to evaporation virus was less stable, but a protective effect of salt and protein existed, resulting in the persistence of considerable infectivity. Considering that most virus shed from infected individuals is surrounded by significant amounts of mucous and that the infectious dose of rhinovirus for humans is low (3), it is reasonable to suspect that viruses remaining might easily serve as an infectious dose for others. Studies have shown the ability of rhinovirus to be transferred to hands by rubbing contaminated areas (7). Subsequent nose or eye rubbing could result in self-inoculation.

In addition to quantitating rhinovirus inactivation, this study provides a rationale for the testing of disinfectants with potential virucidal activity. Nonenveloped viruses such as rhinovirus or coxsackievirus B3 (9), which retain infectivity after drying as a film in the presence of saline and a noninhibitory protein such as bovine serum albumin, should prove an adequate test for the efficacy of the disinfectant.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of Agneta Nordstrom.

This research was supported by Lehn and Fink Products, Montvale, N.J., and by Public Health Service grant AI-03771 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. **Cate, T. R., R. B. Couch, W. F. Fleet, W. R. Griffith, P. J. Gerone, and V. Knight.** 1965. Production of tracheobronchitis in volunteers with rhinovirus in a small particle aerosol. Am. J. Epidemiol. **81**:95–105.
2. **D'Allesio, D. J., C. R. Dick, and E. C. Dick.** 1972. The transmission studies in volunteers infected with rhinovirus type 55, p. 115. *In* J. L. Melnick (ed.), International virology, vol. 2: Proceedings of the Second International Congress for Virology. S. Karger, Basel.
3. **Douglas, R. G., Jr., W. F. Fleet, T. R. Cate, and R. B. Couch.** 1968. Antibody to rhinovirus in human sera. I. Standardization of a neutralization test. Proc. Soc. Exp. Biol. Med. **127**:497–502.
4. **Floyd, R., and D. G. Sharp.** 1978. Viral aggregation: effects of salts on the aggregation of poliovirus and rhinovirus at low pH. Appl. Environ. Microbiol. **35**: 1079–1094.
5. **Gwaltney, J. M.** 1975. Medical reviews: rhinoviruses. Yale J. Biol. Med. **48**:17–45.
6. **Hendley, J. O., L. A. Mika, and J. M. Gwaltney.** 1978. Evaluation of virucidal compounds for inactivation of rhinovirus on hands. Antimicrob. Agents Chemother. **14**:690–694.
7. **Hendley, J. O., R. P. Wenzel, and J. M. Gwaltney.** 1973. Transmission of rhinovirus colds by self inoculation. N. Engl. J. Med. **288**:1361–1364.
8. **Korant, B. D., K. Lonberg-Holm, J. Noble, and J. T. Stasny.** 1972. Naturally occurring and artificially produced component of three rhinoviruses. Virology **48**: 71–86.
9. **McGeady, M. L., J-S. Siak, and R. L. Crowell.** 1979. Survival of coxsackievirus B3 under diverse environmental conditions. Appl. Environ. Microbiol. **37**:972–977.
10. **Moskalski, P. B., and J. W. Hendley.** 1978. Hand to hand transmission of rhinovirus colds. Ann. Int. Med. **88**:463.
11. **Reed, S. E.** 1975. An investigation of the possible transmission of rhinovirus colds through indirect contact. J. Hyg. **75**:249–258.
12. **Totsuka, A., K. Ohtaki, and I. Tagaya.** 1978. Aggregation of enterovirus small plaque variants and poliovirus under low ionic strength conditions. J. Gen. Virol. **38**:519–533.
13. **Tyrrell, D. A. J.** 1965. Common colds and related disease. The Williams & Wilkins Co., Baltimore.