APPLIED MICROBIOLOGY, June 1970, p. 950-954

Vol. 19, No. 6
Printed in U.S.A.

Quantitative Studies on Fabrics as Disseminators of Viruses

IV. Virus Transmission by Dry Contact of Fabrics

ROBERT W. SIDWELL,[1] GLEN J. DIXON, LOUISE WESTBROOK, AND FLORENCE H. FORZIATI

Virus and Cell Biology Divisions, Southern Research Institute, Birmingham, Alabama 35205, and Consumer and Food Economics Research Service, U.S. Department of Agriculture, Washington, D.C. 20250

Received for publication 16 February 1970

Cotton and woolen fabrics and fabrics of synthetic fibers were exposed by direct contact (pipette) and by aerosolization to poliovirus and to vaccinia virus in separate experiments, allowed to dry for 16 hr at 25 C in 35% relative humidity, and randomly tumbled with sterile swatches of the same fabrics for 30 min. By use of a HEp-2 cell assay system, up to $10^{3.5}$ $CCID_{50}$ of poliovirus per ml and $10^{4.4}$ $CCID_{50}$ of vaccinia virus per ml were recovered from the originally sterile fabrics as early as 1 to 10 min after contact. Maximum transfer of both viruses was achieved with wool blanket material, although high titers of vaccinia virus were recovered from all fabrics tested. Poliovirus placed on the fabrics in an aerosol tended to be transferred to the sterile fabrics at a greater rate than when it was placed on the fabrics by direct contact. The method of exposure had essentially no effect on the rate of transfer of vaccinia virus.

Clothing and household textiles have been among the many possible fomites implicated in the transmission of viruses (1, 3-5, 13, 18). Recent experiments have shown that two viruses of public health importance, polio and vaccinia, are capable of persisting on various cotton and wool fabrics for sufficient periods of time for the materials to be potentially capable of their transmission (3, 13, 14). Such information on viral persistence, although of importance, is in itself inadequate if conclusions are to be drawn regarding the role of a fabric in virus dissemination. Still needed are studies to determine whether the persisting viruses can be released from such contaminated materials in sufficient amounts to be capable of dissemination to susceptible animal hosts. In the present report, experiments are described which demonstrate the transfer of poliovirus and vaccinia virus from a variety of dry fabrics, typical of those used in clothing or in household textiles, to sterile pieces of the same fabrics.

MATERIALS AND METHODS

Viruses. The Lederle chorioallantoic strain of vaccinia virus and the MEF-1 strain of poliovirus, obtained in cell culture suspensions from Parke, Davis & Co., Detroit, Mich., were used in this study. Virus stock was prepared in HEp-2 cells (9) and stored at −70 C until used. These were the same viruses used in our previously cited virus persistence studies (3, 13-15).

Fabrics. The following fabrics were used in this study: 1.28 cotton sheeting (no. 8/422, Testfabrics, Inc., New York, N.Y.), cotton terry cloth (no. S/420, Testfabrics, Inc.), washable wool (Wurlan finished) shirting (W. Fong, Western Utilization Research and Development Division, U.S. Department of Agriculture, Albany, Calif.), wool blanketing (Fieldcrest Mills, Spray, N.C.), dull nylon jersey (no. S/304A, Testfabrics, Inc.), and dacron 54/cotton 65/35 shirting (no. S/7406, Testfabrics, Inc.). All fabrics were bleached white and were untreated with mothproofing or antimicrobial finishes. All were laundered twice in anionic detergent (a "typical" product, containing sodium linear alkyl sulfonate, alkyl ethylene oxide condensate, sodium tripolyphosphate, sodium silicate, sodium sulfate, and miscellaneous, supplied by Procter and Gamble Co., Cincinnati, Ohio). The washing machine setting used was the one most appropriate for each fabric. After this preliminary laundering, all were cut with a mechanized die into swatches having a diameter of ca. 5 cm. Sterilization was accomplished with ethylene oxide (Steri-Vac Sterilizer, Minnesota Mining and Manufacturing Co., Minneapolis, Minn.; 16). After sterilization, the fabrics were held in closed petri dishes for at least 10 days at room temperature prior to exposure to virus.

Methods of virus exposure. The fabrics were exposed to virus by direct contact and by aerosol in separate experiments as described previously (17).

[1] Present address: Department of Virology, ICN Nucleic Acid Research Institute, Irvine, Calif. 92664.

Recovery of virus. Each swatch was macerated in a homogenizer with Eagle's (6) basal medium supplemented with 5.0% agamma calf serum and 0.5% chick embryo extract and adjusted to *p*H 7.2. The eluate resulting from the maceration process was diluted in a series of 10-fold dilutions and added to quintuplicate vinyl-plastic panel (12) cups containing HEp-2 cells. The presence of virus was determined by microscopically discernible virus-induced cytopathogenic effects seen in the cell monolayer. A 50% cell culture infectious dose per ml of eluate ($CCID_{50}$/ml) was calculated by using the Reed-Muench procedure (11).

Determination of virus transfer. Ten swatches of each fabric type were exposed to virus either by direct contact or by aerosol. After storage for 16 hr at 25 C in 35% relative humidity, 15 of the swatches were placed in a sterile battery jar (15 by 30 cm) with at least 15 sterile swatches of the same fabric. The sterile swatches were marked for recognition by a cut in the side of each. The battery jar was sealed, and the jar was turned manually in a random tumbling motion for 30 min. The tumbling speed was approximately the same in each experiment. Five of the originally sterile swatches were removed every 10 min and tested for virus content to determine the rate of virus transfer. When preliminary experiments showed that viral transfer was probably rapid, the sampling times were altered in an attempt to determine better the rate of transfer. Five originally contaminated swatches were tested at the beginning of the experiment to determine the initial titer of the contaminated swatches being tumbled with the sterile fabric. These five swatches were labeled "virus controls" in this study.

RESULTS

The results of a typical virus transfer experiment are shown in Table 1. In this experiment, wool blanketing was exposed to poliovirus by direct contact. A mean titer of $10^{4.4}$ $CCID_{50}$ of poliovirus per ml was recovered from the virus control swatches after storage for 16 hr at 25 C in 35% humidity. In this experiment, 25 sterile (test) swatches were added to the tumbling chamber with the 25 virus-contaminated swatches; five of the test swatches were removed 3, 6, 10, 20, and 30 min later and immediately assayed to determine the amount of virus received during the tumbling process. Approximately $10^{3.0}$ $CCID_{50}$ of virus per ml was recovered from each of the previously sterile swatches, with the maximum amounts demonstrated at the 20-min sampling period. Little variation in titer occurred between each sample within a particular group, the standard error usually being approximately $< 10^{0.2}$ $CCID_{50}$/ml.

The results of all of the poliovirus transfer experiments on all six fabrics are summarized in Table 2. The degree of transfer of this virus varied markedly according to the fabric type and method of exposure of the fabric to the virus. Those fabrics containing relatively small amounts of virus on the control swatches also had little or no demonstrable virus transferred to the sterile fabrics. This observation was particularly apparent with the cotton, the dacron/cotton, and the washable wool shirting fabrics, in which usually less than $10^{2.0}$ $CCID_{50}$ of virus per ml remained on

TABLE 1. *Transfer of poliovirus from wool blanketing exposed to virus by direct contact*

Swatch identification	Sample	Virus titer[a] ($CCID_{50}$/ml)	Mean virus titer ($CCID_{50}$/ml)
Virus control[b]	1	$10^{4.7}$	$10^{4.4}$
	2	$10^{4.0}$	
	3	$10^{4.2}$	
	4	$10^{4.2}$	
	5	$10^{4.6}$	
3-Min test[c]	6	$10^{2.7}$	$10^{2.8}$
	7	$10^{3.1}$	
	8	$10^{2.9}$	
	9	$10^{2.8}$	
	10	$10^{2.2}$	
6-Min test	11	$10^{2.8}$	$10^{2.8}$
	12	$10^{3.1}$	
	13	$10^{2.8}$	
	14	$10^{2.7}$	
	15	$10^{2.4}$	
10-Min test	16	$10^{3.6}$	$10^{3.3}$
	17	$10^{3.6}$	
	18	$10^{2.9}$	
	19	$10^{2.9}$	
	20	$10^{3.1}$	
20-Min test	21	$10^{3.4}$	$10^{3.5}$
	22	$10^{3.0}$	
	23	$10^{3.6}$	
	24	$10^{3.6}$	
	25	$10^{3.7}$	
30-Min test	26	$10^{2.9}$	$10^{3.0}$
	27	$10^{3.0}$	
	28	$10^{3.0}$	
	29	$10^{3.1}$	
	30	$10^{3.0}$	

[a] Concentration of virus in the eluate after maceration of each swatch. Each titer is the mean of quintuplicate tests on each swatch.

[b] Swatches which received by pipette 0.4 ml of virus suspension. The titers shown were obtained after storage at 25 C in 35% relative humidity for 16 hr and immediately prior to adding identical control swatches to the tumbling chamber with the sterile swatches.

[c] Originally sterile swatches were tumbled for the indicated time intervals with virus-contaminated swatches and immediately assayed for virus titer.

TABLE 2. *Transfer of poliovirus to sterile fabrics during dry tumbling*

Fabric	Method of virus exposure	Maximum recovered virus[a] ($CCID_{50}$/ml)	Time to maximum virus transfer[b]	Control virus titer ($CCID_{50}$/ml)[c]
			min	
Cotton sheeting	Aerosol	$10^{0.6}$	10	$10^{2.2}$
	Direct contact	$10^{0.4}$	20	$10^{1.3}$
Cotton terry cloth	Aerosol	$10^{0.6}$	1[d]	$10^{1.5}$
	Direct contact	$10^{0.4}$	>30	$10^{0.6}$
Washable wool shirting	Aerosol	$10^{1.1}$	30	$10^{1.6}$
	Direct contact	$10^{0.4}$	>30	$10^{1.6}$
Wool blanketing	Aerosol	$10^{2.8}$	3	$10^{3.3}$
	Direct contact	$10^{3.5}$	20	$10^{4.4}$
Nylon jersey	Aerosol	$10^{1.2}$	1[d]	$10^{2.3}$
	Direct contact	$10^{1.5}$	30	$10^{3.9}$
Dacron/cotton shirting	Aerosol	$10^{0.4}$	1[d]	$10^{1.0}$
	Direct contact	$10^{0.4}$	1[d]	$10^{0.9}$

[a] Maximum mean virus titer demonstrated on sterile fabrics tumbled with virus-containing fabric of the same type.

[b] Sampling time interval, during which sterile and virus-containing fabrics were tumbled, when the maximum virus titer was recovered from the originally sterile fabric.

[c] Mean titer of virus demonstrated on five original virus-containing swatches 16 hr after virus exposure and immediately prior to initiation of tumbling.

[d] Earliest sampling time.

TABLE 3. *Transfer of vaccinia virus to sterile fabrics during dry tumbling*

Fabric	Method of virus exposure	Maximum recovered virus[a] ($CCID_{50}$/ml)	Time to maximum virus transfer[b]	Control virus titer ($CCID_{50}$/ml)[c]
			min	
Cotton sheeting	Aerosol	$10^{1.9}$	10[d]	$10^{4.0}$
	Direct contact	$10^{0.6}$	10	$10^{3.3}$
Cotton terry cloth	Aerosol	$10^{3.3}$	10	$10^{4.9}$
	Direct contact	$10^{4.2}$	20	$10^{5.3}$
Washable wool shirting	Aerosol	$10^{3.3}$	10	$10^{5.2}$
	Direct contact	$10^{1.8}$	10	$10^{4.1}$
Wool blanketing	Aerosol	$10^{4.2}$	20	$10^{5.3}$
	Direct contact	$10^{4.4}$	10	$10^{5.9}$
Nylon jersey	Aerosol	$10^{2.8}$	10	$10^{5.2}$
	Direct contact	$10^{2.8}$	10	$10^{5.1}$
Dacron/cotton shirting	Aerosol	$10^{3.0}$	10	$10^{5.2}$
	Direct contact	$10^{2.6}$	10	$10^{5.2}$

[a] Maximum mean virus titer demonstrated on sterile fabrics tumbled with virus-containing fabric of the same type.

[b] Sampling time interval, during which sterile and virus-containing fabrics were tumbled, when the maximum virus titer was recovered from the originally sterile fabric.

[c] Mean titer of virus demonstrated on five original virus-containing swatches 16 hours after virus exposure and immediately prior to initiation of tumbling.

[d] Earliest sampling time was 10 min in the vaccinia virus study.

the control swatches after the 16-hr storage period. Those fabrics receiving aerosolized virus tended to lose this virus at a greater rate to the sterile fabrics during the tumbling process, but the total amount of virus transferred was approximately the same as that transferred from fabrics exposed by direct contact. Maximum poliovirus transfer was demonstrated with the wool blanketing and nylon jersey materials.

The vaccinia virus experiments are summarized in Table 3. Higher titers of virus were demonstrated on the control fabrics after the 16-hr storage, and more virus was apparently transferred to the test swatches than was observed in the poliovirus experiments. In the majority of the experiments, maximum virus transfer was seen at the 10-min testing time, the earliest used in the vaccinia virus study. Method of exposure of the control fabrics to the virus had essentially no more effect on the rate of transfer than occurred in the poliovirus experiments, but the total amount of virus transferred was usually higher (up to $10^{1.0}$ $CCID_{50}$/ml) on the fabrics originally exposed to the aerosolized virus. Maximum virus transfer occurred on the wool blanketing material; the least virus transfer was seen with the cotton sheeting.

DISCUSSION

Poliovirus and vaccinia virus can persist for significant periods of time on certain fabrics (3, 13–15). This observation has been extended to a degree in the present study, since the dry persisting viruses were obviously transferred from contaminated to sterile fabrics during dry random contact. It is yet to be determined whether an animal, and, specifically, a human being, would become clinically infected by the quantity of virus that was transferred to the sterile fabrics used in the present study, since quantitative data are lacking regarding the amount of these viruses required to cause a clinical human infection. From a practical viewpoint, the large quantities of virus placed on the fabrics initially in these experiments should also be considered. In domestic uses, such initial high-virus inocula would not usually be expected, although Downie et al. (5) have reported smallpox virus to be recovered in significant quantities from the bedclothes of infected patients. Also to be considered is the fact that the viruses used were only representative of the many which are of public health importance. Because of the wide differences between poliovirus and vaccinia virus, however, we may cautiously assume that perhaps most of the important human viruses would behave similarly under the conditions used in this study. Additional studies with other viruses are needed to confirm this assumption. Herpesvirus and parainfluenza virus have been recovered in relatively high titer from wool gabardine material after drying (16), a finding similar to that seen with poliovirus and vaccinia virus, but it is not yet known whether these other viruses will transfer to other fabrics during dry contact.

Certain factors were found to enhance noticeably the degree of virus transfer. Fabric type was particularly important. As we have reported previously, both poliovirus and vaccinia virus tend to survive more readily on woolen fabrics than on cotton materials. Wool blanketing apparently provided the most suitable environment, probably as a result of the physical construction of the wool itself, since the wool fiber has overlapping scales and a higher moisture content than the cotton fabrics, although such a construction would not explain the ease of virus transfer observed with this fabric. The two fabrics containing synthetic fibers also allowed a greater degree of viral persistence and transfer than was seen with the cotton materials. The method of exposure of the fabrics to the viruses was of importance, with the aerosolized viruses being transferred more readily than those placed on the fabrics by direct contact. Such a variation may have been caused by an alteration in construction of the virus aerosol particle which would prevent the particle from adhering as strongly to the fabrics as the viruses placed on the fabric in aqueous suspension with a pipette. Such alterations would be expected, since dehydration would remove much of the bound water in the macromolecule (19), and other physical and chemical stresses are known which will influence the viability of aerosolized microorganisms (2, 20).

A surprising observation was the relatively short period of time needed to achieve a maximum virus transfer in the majority of these experiments. Even in those experiments in which the time to maximum virus transfer was as long as 20 min, a significant quantity of virus was demonstrated on the sterile material at the earliest times tested. Such rapid transfer would suggest that the virus particles were adhering very loosely to the fabric and would probably be disseminated from contaminated clothing and household textiles with little difficulty under normal conditions of use.

The mechanisms by which viruses are disseminated in human populations depend to a considerable extent on the type of virus. These mechanisms are still not completely understood, particularly with regard to the two viruses specifically employed in this study. It is clear that close association is needed in poliovirus transmission (10), particularly in view of the rapid inactivation rate of the virus upon drying (2, 7, 8). The virus

will survive for significant periods of time, however, on certain fabrics (2), and in the present study it was shown to be capable of transferral from fabrics after drying. Smallpox infection has definitely been attributed to contaminated textile articles in certain instances (1, 4, 5). The present studies with the closely related vaccinia virus provide further evidence that clothing is a potential disseminator of this disease agent.

ACKNOWLEDGMENT

This study was carried out under contract no. 12-14-100-9533(62) with the Agricultural Research Service, U.S. Department of Agriculture, administered by the Consumer and Food Economics Research Division, Washington, D.C.

LITERATURE CITED

1. Cramb, R. 1951. Smallpox outbreak in Brighton, 1950-51. Pub. Health (London) 64:123-128.
2. De Jong, J. C., and K. C. Winkler. 1968. The inactivation of poliovirus in aerosols. J. Hyg. 66:557-565.
3. Dixon, G. J., R. W. Sidwell, and E. McNeil. 1966. Quantitative studies on fabrics as disseminators of viruses. II. Persistence of poliomyelitis virus on cotton and wool fabrics. Appl. Microbiol. 14:183-188.
4. Downie, A. W. 1965. Poxvirus group, p. 944-948. *In* F. L. Horsfall, Jr., and I. Tamm (ed.), Viral and rickettsial infections of man. J. B. Lippincott Co., Philadelphia.
5. Downie, A. W., M. Meiklejohn, L. St. Vincent, A. R. Rao, B. V. Sundara Babu, and C. H. Kempe. 1965. The recovery of smallpox virus from patients and their environment in a smallpox hospital. Bull. W.H.O. 33:615-622.
6. Eagle, H. 1955. The minimum vitamin requirements of the L and HeLa cells in tissue culture, the production of specific vitamin deficiencies and their cure. J. Exp. Med. 102:595-606.
7. Hemmes, J. H., K. C. Winkler, and S. M. Kool. 1960. Virus survival as a seasonal factor in influenza and poliomyelitis. Nature (London) 188:430-431.
8. Howe, H. A. and J. L. Wilson. 1959. Poliomyelitis, p. 447. *In* T. M. Rivers and F. L. Horsfall, Jr. (ed)., Viral and rickettsial infections of man. J. B. Lippincott Co., Philadelphia.
9. Moore, A. E., L. Sabachewsky, and H. W. Toolan. 1955. Cultural characteristics of four permanent lines of human cancer cells. Cancer Res. 15:598-602.
10. Paffenberger, R. S., Jr., V. O. Wilson, D. Bodian, and J. Watt. 1954. The spread of poliomyelitis; an analysis of contact during epidemic periods. Amer. J. Hyg. 60:63-82.
11. Reed, L. J., and A. Muench. 1938. A simple method of estimating fifty percent end points. Amer. J. Hyg. 27:493-498.
12. Rightsel, W. A., P. Schultz, D. Muething, and I. W. McLean, Jr. 1956. Use of vinyl plastic containers in tissue culture for virus assays. J. Immunol. 76:464.
13. Sidwell, R. W., G. J. Dixon, and E. McNeil. 1966. Quantitative studies on fabrics as disseminators of viruses. I. Persistence of vaccinia virus on cotton and wool fabrics. Appl. Microbiol. 14:55-59.
14. Sidwell, R. W., G. J. Dixon, and E. McNeil. 1967. Quantitative studies on fabrics as disseminators of viruses. III. Persistence of vaccinia virus on fabrics impregnated with a virucidal agent. Appl. Microbiol. 15:921-927.
15. Sidwell, R. W., and G. J. Dixon. 1969. Role of virucides in controlling virus dissemination by fabrics. J. Amer. Oil Chem. Soc. 46:532-536.
16. Sidwell, R. W., G. J. Dixon, L. Westbrook, and E. A. Dulmadge. 1969. Procedure for the evaluation of the virucidal effectiveness of an ethylene oxide gas sterilizer. Appl. Microbiol. 17:790-796.
17. Sidwell, R. W., L. Westbrook, G. J. Dixon, and W. F. Happich. 1970. Potentially infectious agents associated with shearling bedpads. I. Effect of laundering with detergent-disinfectant combinations on polio and vaccinia viruses. Appl. Microbiol. 19:53-59.
18. Taylor, J., and J. Knowelden. 1957. Principles of epidemiology, p. 108. J. & A. Churchill Ltd., London.
19. Webb, S. J. 1959. Factors affecting the viability of airborne bacteria. I. Bacteria aerosolized from distilled water. Can. J. Microbiol. 5:649-669.
20. Zentner, R. J. 1966. Physical and chemical stresses of aerosolization. Bacteriol. Rev. 30:551-557.