

Quantitative Studies on Fabrics as Disseminators of Viruses

I. Persistence of Vaccinia Virus on Cotton and Wool Fabrics

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Received for publication 29 July 1965

ABSTRACT

SIDWELL, ROBERT W. (Southern Research Institute, Birmingham, Ala.), GLEN J. DIXON, AND ETHEL MCNEIL. Quantitative studies on fabrics as disseminators of viruses. I. Persistence of vaccinia virus on cotton and wool fabrics. *Appl. Microbiol.* 14:55-59. 1966.—The persistence of vaccinia virus on wool (blanket and gabardine) and cotton (sheeting, terry cloth, and knit jersey) fabrics was studied. The fabrics were exposed to the virus by three methods: direct contact, aerosol, and virus-containing dust having a high content of textile fibers. Fabrics exposed to virus by each method were held in 35 and 78% relative humidities at 25 C. Virus was recovered for up to 14 weeks from wool fabrics exposed to virus and held in the low humidity. In contrast, virus persisted for shorter periods of time on the cotton fabrics. No virus was detected on terry cloth as early as 3 days after exposure to virus. The virus appeared to be less stable in the high humidity, and the method of exposure of the fabrics to virus apparently had an effect upon the persistence of the agent. On all fabrics, viral persistence was of sufficient duration to be of epidemiological significance.

The dissemination of microorganisms by fabrics has been recognized for many years as a problem of great public health significance (6). To develop means for preventing or controlling such dissemination, a fuller knowledge of the basic factors behind the problem is necessary. Significant contributions to this knowledge have been made by many investigators in studies with bacteria, but, to date, little quantitative information exists concerning the persistence on and dissemination of viruses by fabrics. Wooding (M.S. Thesis, Univ. Connecticut, Storrs, p. 1-71, 1948) and Jungherr (4) reported experiments on the stability of infectious bronchitis, Newcastle disease, and fowlpox viruses on burlap bags. Wooding also studied the stability of Newcastle disease virus on leather and other types of surfaces. Naurybayev (8) studied the viability of foot-and-mouth disease virus on various surfaces and on cotton fabric, rubber, and leather, taking into consideration the effects of such factors as temperature, humidity, and season on the persistence of the agent. Virus-stability information is available on many of the better-known agents, but additional information is needed concerning the survival of viruses on fabrics of various fiber types and construction.

In the present report the persistence of vaccinia virus on a variety of wool and cotton fabrics was studied. The fabrics were exposed to the agent by direct contact, aerosol, and virus-containing dust and held in both low and high humidities at constant temperature.

MATERIALS AND METHODS

Virus. The Lederle chorioallantoic strain of vaccinia virus was used in these studies. This virus was received from Parke, Davis & Co., Detroit, Mich., in cell culture suspension. A stock virus was prepared by a single passage of the virus in HEp-2 cells (7).

Fabrics. The following fabrics were used in these studies: wool blanket (Fieldcrest Mills, Spray, N.C.), wool gabardine (J. P. Stevens Co., Inc., Milledgeville, Ga.), cotton sheeting (Pepperell Manufacturing Co., Abbeville, Ala.), cotton terry cloth (West Point Manufacturing Co., Fairfax, Ala.), and cotton knit jersey (Russell Mills, Inc., Alexander City, Ala.). All fabrics were bleached white and had not been treated with moth-proofing or antimicrobial agents. Each fabric was tested for inherent antimicrobial activity with *Staphylococcus aureus* and *Escherichia coli* in the agar plate test described in the AATCC Technical Manual (American Association of Textile Chemists and Colorists, Durham, N.C., vol. 39, p. B139-149, 1963). No activity could be demonstrated.

Each of the above fabrics was cut into standard swatches (diameter, 5.08 cm) with a mechanized die.

Humidity maintenance. A saturated solution of sodium chloride was used to produce a high relative humidity (ca. 78%), and dry potassium acetate was used to produce a low relative humidity (ca. 35%). Airtight, insulated cabinets held in a 25 C incubator room were employed for fabric storage to maintain the desired temperature and humidity. The fabric swatches were placed five to a dish in elevated-lid plastic petri dishes (Falcon Plastics Division of B-D Laboratories, Inc., Los Angeles, Calif.). The swatches were separated in the dish by sterile, glass-fiber screens.

Sterilization of materials. All fabrics and plastic materials were sterilized with ethylene oxide (Sterivac Sterilizer, Advanced Products Corp., Framingham, Mass.) at ambient temperature.

Methods of virus exposure. Fabric swatches were exposed to virus in three ways: by direct contact, by aerosol, or by dust. For the direct contact method, 0.4 ml of a standardized virus suspension was pipetted onto the fabric. Exposure to aerosol was carried out in a specially designed, stainless-steel chamber. This chamber was cylindrical and measured 30 inches (76.2 cm) in length by 24 inches (59.96 cm) in diameter. The fabric swatches were attached to hooks on a grid placed 5 inches (12.7 cm) from the bottom of the chamber, and a suspension of the virus was aerosolized onto these swatches by use of a DeVilbiss atomizer under 13 psi of nitrogen gas pressure. This atomizer produced particles of which 95% were from 0.27 to 6.0 μ in diameter, at a density of 2.1×10^5 particles per liter, as determined by an aerosol photometer (11). The virus-containing fog was allowed to settle on the swatches for 1 hr prior to removal from the chamber. Preliminary studies in-

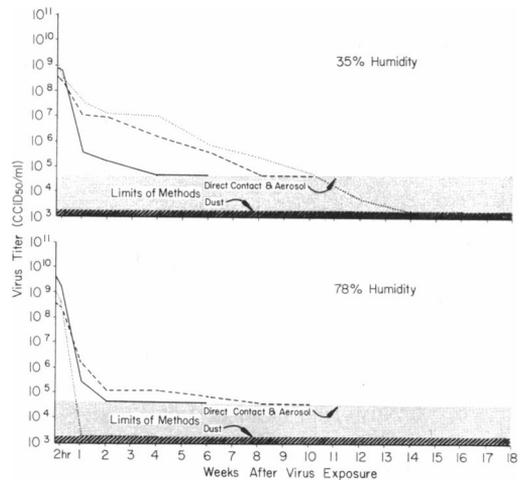


FIG. 2. Persistence of vaccinia virus on wool blanket material held at 25 C in two humidities. Data expressed as mean virus titers. Method of exposure to virus: solid line, direct contact; dashed line, aerosol; dotted line, virus-containing dust.

dicated that each of the swatches exposed to the virus aerosol received a uniform amount of the agent. To expose the swatches to virus-containing dust, a household dust was obtained which was made up predominantly of textile fibers. This material was suspended in a cell culture prepared virus suspension and then lyophilized. The fabrics were exposed to this virus by sealing them in a large battery jar with the virus-containing dust and giving the closed jar a random rotating, tumbling action for 15 min. Virus assays on randomly selected virus-contaminated swatches of all fabrics indicated that all swatches received essentially the same quantity of recoverable virus.

Recovery of virus from swatches. To demonstrate the presence, and to determine the titer, of virus on each fabric, each swatch was macerated with Eagle's (2) basal medium (BME) supplemented with 5.0% agamma calf serum and 0.5% chick embryo extract, and adjusted to pH 7.2. The maceration process was carried out in a Servall Omnimixer homogenizer (Ivan Sorvall, Inc., Norwalk, Conn.) run at maximal speed for 30 sec. The eluate was removed and centrifuged at low speed to remove suspended fibers. The virus titer in the supernatant fluid was determined by assay in HEP-2 cells grown in vinyl plastic panels, as described by Richtsel et al. (9).

Experimental design. Eighty sterile swatches of each fabric were exposed to 10^9 CCID₅₀/ml (cell culture 50% infectious dose) of virus by one of the three methods previously described. The fabrics were then placed in the humidity cabinets, and five swatches of each fabric were tested for the presence and titer of virus at the following time intervals: immediately after exposure to virus (zero-time), 2 hr, and 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 weeks after

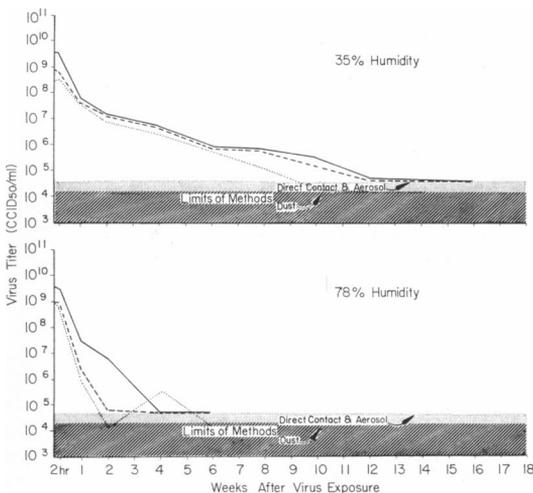


FIG. 1. Persistence of vaccinia virus on wool gabardine material held at 25 C in two humidities. Data expressed as mean virus titers. Method of exposure to virus: solid line, direct contact; dashed line, aerosol; dotted line, virus-containing dust.

virus exposure, or at least two time intervals after virus could no longer be recovered from the material.

RESULTS

The results are expressed as mean titer of virus recovered from each group of five swatches tested at the various time intervals (Fig. 1-5). The virus titers of the individual swatches varied relatively little from the mean, regardless of the manner of exposure of the swatch to the virus; 95% confidence limits were usually less than $\pm 0.5 \log_{10}$ from the mean.

Each method of exposure of the swatches to virus resulted in approximately constant amounts (10^8 to 10^9 CCID₅₀/ml) of the agent being recoverable at zero-time. In most cases the persistence of the virus varied with the method of exposure. For example, in 35% humidity, virus in dust placed on cotton fabrics and on wool blanket material was recovered in higher titer than when placed on fabrics as an aerosol or by direct contact. In this same humidity, virus applied as an aerosol generally persisted longer than virus pipetted onto these same fabrics. In 78% humidity, the results were more varied regarding

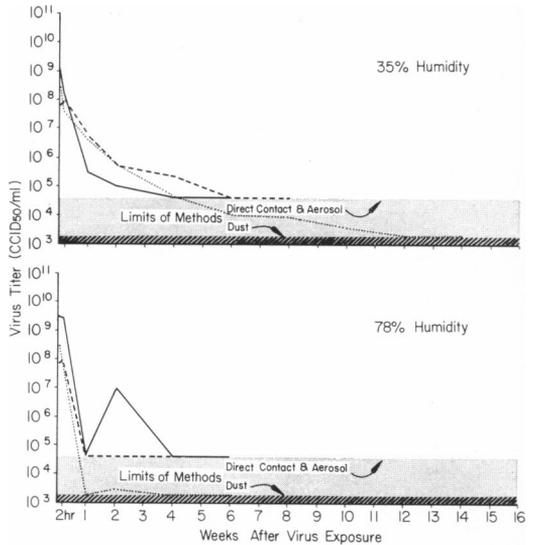


FIG. 4. Persistence of vaccinia virus on cotton knit material held at 25 C in two humidities. Data expressed as mean virus titers. Method of exposure to virus: solid line, direct contact; dashed line, aerosol; dotted line, virus-containing dust.

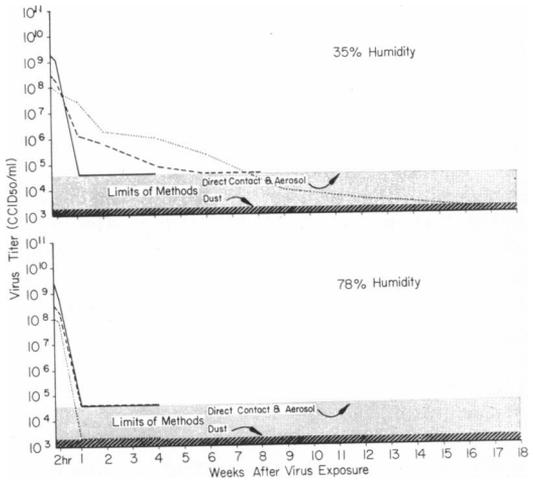


FIG. 5. Persistence of vaccinia virus on cotton terry cloth material held at 25 C in two humidities. Data expressed as mean virus titers. Method of exposure to virus: solid line, direct contact; dashed line, aerosol; dotted line, virus-containing dust.

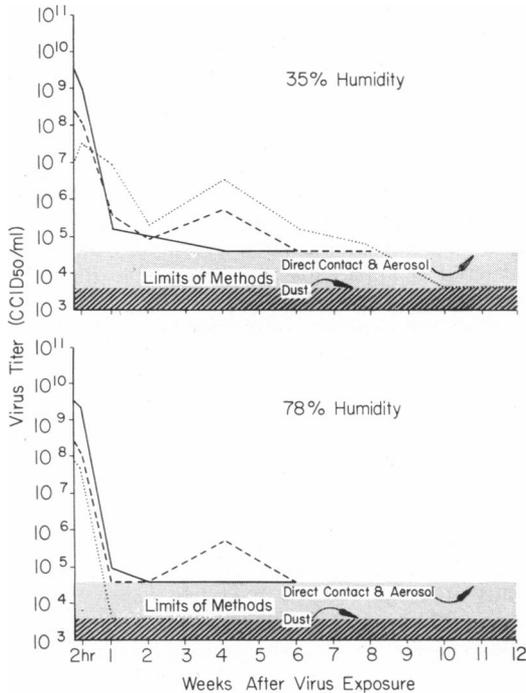


FIG. 3. Persistence of vaccinia virus on cotton sheeting material held at 25 C in two humidities. Data expressed as mean virus titers. Method of exposure to virus: solid line, direct contact; dashed line, aerosol; dotted line, virus-containing dust.

the effect of method of exposure to the virus. The virus generally could not be recovered by 2 to 4 weeks from wool fabrics and by 1 to 2 weeks from cotton fabrics held in the high humidity. It was observed that the virus was usually recoverable from the wool materials for longer periods of time than from cotton fabrics.

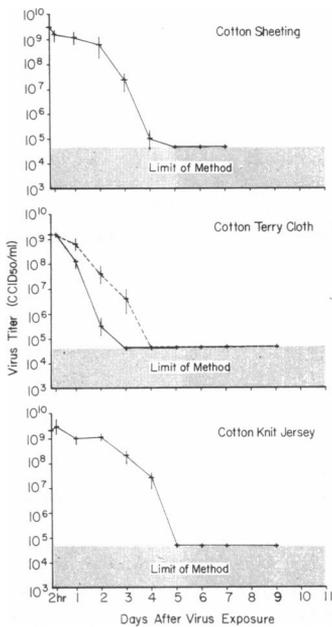


FIG. 6. Persistence of vaccinia virus pipetted on cotton sheeting, terry cloth, and knit jersey materials held at 25 C in 35 or 78% humidities. Data expressed as mean virus titers $\pm 95\%$ confidence limits at each time tested. Solid line, 78% humidity; dashed line, 35% humidity.

Because of the apparent short persistence (1 week or less) of virus on cotton fabrics held in 78% humidity, these experiments were repeated by exposing the swatches to virus by direct contact and testing them daily for 10 days after exposure. The results of these experiments are shown in Fig. 6. On sheeting and knit jersey, virus was detected until day 5 after virus exposure. No virus was recovered from terry cloth swatches held in 35% humidity 4 days after virus exposure, nor from swatches held in 78% humidity 3 days after virus exposure.

It was thought that perhaps residual ethylene oxide from the sterilization procedure might be exerting a virucidal effect during these studies. Therefore, an experiment was carried out to determine the persistence of vaccinia virus on cotton sheeting material which had been sterilized by autoclaving rather than by ethylene oxide. The virus persisted for the same period of time on this material as it did in the above studies with ethylene oxide-sterilized cotton sheeting.

It was postulated that the virus might survive a longer period of time if it were in a more natural state, such as in infected mouse lung tissue. A stock vaccinia virus was prepared from the lungs of intranasally inoculated Swiss mice. This mouse

lung virus preparation had a titer of $10^{9.2}$ CCID₅₀/ml in HEp-2 cells. Wool gabardine, cotton sheeting, and cotton terry cloth materials were exposed by direct contact to this virus preparation and tested for virus at the selected time intervals. In this study, virus titers decreased in a manner analogous with that observed with swatches exposed to virus prepared in cell culture.

The cell culture methods employed for determining the titer of the recoverable virus were relatively sensitive, rapid, and reproducible. An experiment was carried out comparing the relative sensitivity of the cell culture method with a method involving intracerebral inoculation of 8- to 10-g and 18- to 21-g Swiss mice; the cell culture technique was approximately 100 times more sensitive than the *in vivo* procedure.

DISCUSSION

In a review by Langmuir (5), it was pointed out that microorganisms can be spread by contact, droplet, droplet nuclei (residues resulting from evaporation of moisture from droplets), and by dust. In the present investigation three of these methods were used to study the survival and persistence of vaccinia virus on cotton and woolen fabrics. Vaccinia virus can be stored in glycerol at 4 C for long periods of time, and is viable for years when stored at -10 C or lower (1). The virus loses little of its infectivity when lyophilized (3), but is readily inactivated by ultraviolet light (12).

The present studies indicate that vaccinia virus is relatively stable in a dry atmosphere, but when held in a high humidity it appears to lose its viability quite rapidly. Virus prepared from lung material is apparently no more stable than the virus prepared in cell culture.

Since the virus was recoverable from wool materials for longer periods than from cotton fabrics, the fiber type or construction of the cloth may have an effect upon the stability of the agent. Wool fibers consist mainly of keratin and have a cuticle of overlapping scales. Cotton fibers are flattened, twisted cellulose tubes with a small amount of pectins and waxes in the outer wall. The natural moisture content of wool is somewhat higher than that of cotton. There is also the possibility that the virus is held less tightly to the woolen fabrics than to the cotton materials, thus allowing the virus to be recoverable in higher titer. However, since the virus titer decreased, in most cases, at a steady rate with increasing time, the implication is that the agent lost its viability upon storage.

These results would support the thesis that fabrics can play an important role in the dissemi-

nation of pox viruses. The role of fabrics as fomites has been implied by Taylor and Knowelden (10), who reported the occurrence of secondary cases of smallpox among laundry sorters in England.

ACKNOWLEDGMENTS

The authors express their appreciation to Louise Westbrook, Karen Chapman, Mary Jones, and Mary Summerville for their technical assistance in these investigations.

This investigation was supported by USDA contract no. 12-14-100-7189 (63).

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