Transmission of *Chlamydia pneumoniae*

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To investigate the transmission of *Chlamydia pneumoniae* via hands and environmental surfaces, its survival on various surfaces was studied. The organism remained viable on formica countertops for 30 h and in tissue paper for 12 h. Measurable quantities of chlamydiae were transferred from these environmental surfaces to hands. However, titers were low and survival time on hands was limited to 10–15 min. *C. pneumoniae* survived small particle aerosolization well and was infectious to mice by both direct inoculation and aerosolization. These observations suggest that several mechanisms of transmission of *C. pneumoniae* are possible, including the transfer of fomites from environmental surfaces with subsequent autoinoculation.

Chlamydia pneumoniae (TWAR) is a recently recognized *Chlamydia* strain that is emerging as an important cause of respiratory disease in humans. *C. pneumoniae* has been implicated as a cause of bronchitis, pneumonia, sinusitis, and pharyngitis [1]. Although the disease is presumed to be spread via the respiratory route from person to person, the actual mechanism of transmission is not known. Three modes of transmission of respiratory pathogens are theoretically possible: small particle aerosol (<10 μm mass median diameter); direct inoculation by droplets or large particles; and self-inoculation after touching contaminated surfaces. We sought to describe the survival of *C. pneumoniae* on environmental surfaces and skin and in small particle aerosol and to determine whether animals can be infected with *C. pneumoniae* by intranasal inoculation and by exposure to aerosolized organisms.

Materials and Methods

Study Design

Recovery of *C. pneumoniae* from three surfaces, formica, tissue paper, and skin, was examined over time. Recovery of *C. pneumoniae* from hands that had touched contaminated surfaces was also determined. Finally, the infectivity of aerosolized *C. pneumoniae* was measured. A second set of experiments was done to assess two modes of transmission (aerosol and direct inoculation) in mice.

Chlamydia Culture

A stock culture of *C. pneumoniae* (CWL-029; American Type Culture Collection, Rockville, MD) was grown in cycloheximide-treated HEp-2 cells using a modification of the methods described by Roblin et al. [2]. Sample (400 μL) was centrifuged at 1500 *g* for 1 h at room temperature onto 24-well Costar tissue culture plates (Costar, Cambridge, MA) containing confluent monolayers of HEp-2 cells overlying 12-mm coverslips. Plates were then incubated at 35°C for 1 h, after which the samples were removed and 1 mL of Iscove's modified Dulbecco's medium with 10% fetal calf serum, 100 μg/mL each gentamicin and vancomycin, and 0.5 μg/mL cycloheximide was added. Coverslips were methanol-fixed after 3 days of incubation at 37°C, and organisms were detected by immunofluorescent staining with specific anti–*C. pneumoniae* monoclonal antibody (Washington Research Foundation, Seattle). Tenfold dilutions were made of each sample, and the titer of *C. pneumoniae* was calculated by counting the inclusions present in the last dilution and expressing the value as inclusion-forming units. The titer of stock *C. pneumoniae* used in all experiments was 10^7 ifu/mL.

Recovery from Environmental Surfaces

The temperature of the room was kept between 23 and 25°C. Aliquots of sucrose-phosphate-glutamine (SPG) medium, recovered in each of the following experiments, were cultured and titered for *C. pneumoniae* using 10-fold dilutions. Experiments were repeated three times.

Countertop. Aliquots (100 μL) of *C. pneumoniae* stock were placed onto formica countertops. Samples were recovered by aspirating by pipette and washing the area with 100 μL of SPG. At 2 h, the initial specimens were dry, and samples were recovered by washing the area with 100 μL of SPG twice.

Tissue paper. *C. pneumoniae* stock (100 μL) was placed on 4 $\times$ 4 cm pieces of tissue paper. The tissue was rinsed with 400 μL of SPG at the appropriate time intervals.

Skin. Fingertips were dipped in 100 μL of *C. pneumoniae* stock and then rinsed with 400 μL of SPG at each interval. This experiment was repeated with three subjects.

Recovery from Skin after Transfer from Environmental Surfaces

Again, 100 μL of *C. pneumoniae* was placed on countertops, tissue, and hands. Samples were then taken from different hands after touching the contaminated surfaces and were cultured as described above.

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Aerosolization

C. pneumoniae stock (2 mL) was placed in a 35b Devilbiss ultrasonic nebulizer (particle size, 0.5–3.0 μm). Aerosolized material was directed by sealed tubing to a chamber (20 × 20 × 15 cm). Aerosol was condensed in a sterile Petri dish on ice (10 μL volume) and cultured.

Animal Studies

Inoculation. Twenty-eight 4-week-old Swiss mice were inoculated intranasally with 100 μL of *C. pneumoniae* stock. Animals were observed daily for signs of illness. Four animals each were sacrificed on days 1, 2, 4, 7, 10, 16, and 30. Lungs were removed, and a homogenate was prepared using SPG. The suspension was filtered through a 5-μm filter and assayed for infectious organisms by titration on HEp-2 monolayers. The ID_{50} for Swiss mice was established by inoculating eight groups of 3 mice each with 100 μL of *C. pneumoniae* stock in serial 10-fold dilutions (10^{-1} to 10^{-7}). Mice were sacrificed and lungs cultured on day 7.

Aerosolization. Six 4-week-old Swiss mice were placed in the chamber, and 6 mL of *C. pneumoniae* stock was aerosolized over 30 min. Mice were sacrificed at days 3 and 11, and cultures were done as outlined above.

Results

Environmental survival. *C. pneumoniae* was recovered from countertops for up to 30 h, from tissue paper for up to 12 h, and from skin up to 15 min (figure 1A). The titer of viable chlamydiae fell most rapidly on skin. No organism could be recovered from any individual after 15 min.

C. pneumoniae was recovered from hands contacting the contaminated surfaces as shown in figure 1B. Chlamydiae were recoverable from hands after touching tissue only immediately afterwards. The organism was recoverable immediately after hand-to-hand transfer; however, the titer fell rapidly, with a titer of 1 ifu/mL at 5 min and no recoverable organisms at 10 min. Chlamydiae persisted somewhat longer after counter-to-hand transfer, but again only at low titers of 5 ifu/mL at 10 min and 1 ifu/mL at 15 min. Chlamydiae survived aerosolization well, although there was a 10-fold drop in titer from 1×10^7 to 1.4×10^6 ifu/mL.

Animal studies. Of mice that received *C. pneumoniae* intranasally, animals sacrificed on day 1 had no organisms recovered from lungs. Organisms were recovered from all mice on days 2, 4, 7, 10, and 16, with highest titers present on day 10. Two of 4 animals remained culture-positive at day 30 (figure 2). The ID_{50} was calculated to be $10^{-4.6}$/mL of stock. Of the mice exposed to the *C. pneumoniae* aerosol, 0 of 3 had positive cultures at day 3 and 1 of 3 was positive at day 11 (titer, 60 ifu/mL). No animal appeared ill throughout the study period.

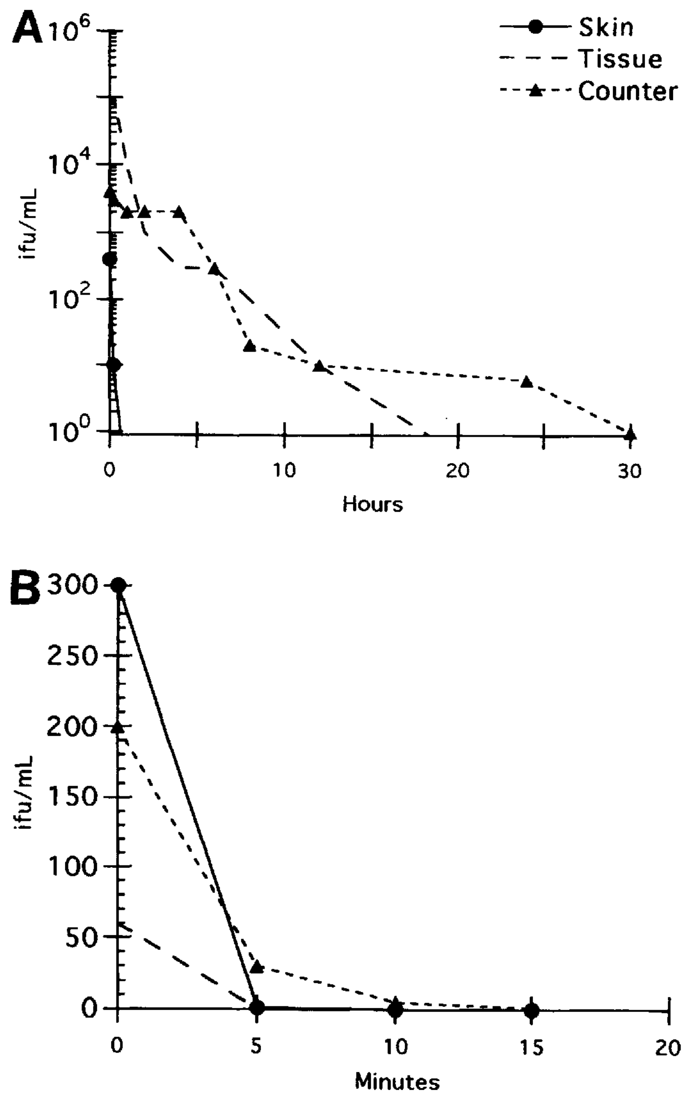

Figure 1. **A,** Titer of *C. pneumoniae* (ifu/mL) and duration of recovery over 30 h from skin, tissue, and counter. Titer is expressed in log 10 scale. **B,** Titer and recovery on hands after contact with contaminated skin, tissue, and counter over 20 min. Titer is expressed in linear scale (representative examples of 3 experiments).

Discussion

Although *C. pneumoniae* has been recognized as a significant pathogen of the human respiratory tract, little information is available on the mode of transmission. *Chlamydia psittaci* is known to be transmitted by small particle aerosol, and the organism has been demonstrated to survive for months in dry bird feces. *Chlamydia trachomatis* is spread by direct contact with infected genital secretions [3]. *C. pneumoniae* is presumed to be spread from human to human by aerosol droplets, and there is no known animal reservoir. A single episode of transmission of *C. pneumoniae* by aerosol inhalation during a laboratory accident has been docu-

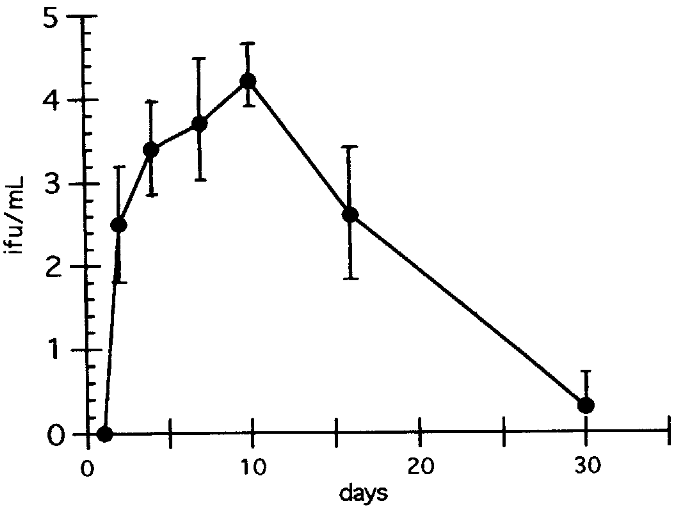

Figure 2. Mean titer of *C. pneumoniae* recovered from mouse lungs over 30 days (log 10 scale) ± SD.

mented; however, definitive studies of transmission are lacking [4].

Seroepidemiologic studies to date suggest that *C. pneumoniae* infections are common, with 50% of adults in the general population having IgG antibody titers ⩾1:16 [5]. Both asymptomatic and chronic *C. pneumoniae* infections have been documented by culture [5, 6]. Spread of infection, however, appears to be slow even in closed populations, suggesting either that the organism is not highly transmissible or that the incubation period may be long. In a study of Finnish military recruits experiencing an epidemic of TWAR pneumonia, the mean case interval was estimated at 30 days [7]. A recent large serologic study of families in the Seattle area demonstrated that 12 households had 2 or 3 members affected, whereas in 29 households only 1 family member was infected [8]. Using serology alone to define the epidemiology of TWAR infections may, however, underestimate the true incidence of infection and rate of spread, since symptomatic seronegative infection has been described [9].

The small particle aerosol (<10 μm mass diameter) generated by talking, coughing, or sneezing can spread for distances of >1.8 m. Organisms transmitted by this mechanism, such as influenza, varicella zoster, and measles viruses, tend to cause explosive outbreaks. Transmission of organisms by small particle aerosol depends on both the size of the particles generated and the ability of the organism to survive desiccation. Large droplet transmission (particles of 20–50 μm) requires close person-to-person contact. Spread by fomites with subsequent autoinoculation requires that an organism remain viable in the environment and on skin for sufficient time to allow transmission. Spread of infections by the latter two modes tends to be slower [10].

The data reported here indicate that *C. pneumoniae* can remain viable on environmental surfaces for long periods of time and is transferable to hands, although survival time on skin appears to be brief. The reason for this shortened survival on skin compared with that on inert material is unexplained. Skin temperature or intrinsic antimicrobial activities of keratinized epithelia, such as pH or surface lipids to which chlamydiae are sensitive, may be factors that decrease survival [11]. Rhinovirus, which has been demonstrated to spread primarily via fomites and self-inoculation, survives on skin for >1 h [12]. Several studies have provided evidence that respiratory syncytial virus (RSV) is spread via fomites. RSV in small particle aerosol (10 μm) has been shown to be unstable at relative humidities of 20%–30%, as are found in air during winter [13]. In addition, Hall et al. [14] showed that RSV could be recovered from hands after touching contaminated counters for an average of 20 min. Our results demonstrate prolonged environmental survival, with 60–300 ifu of *C. pneumoniae* immediately recoverable on hands in contact with each contaminated surface. The titer fell rapidly with time in all cases; however, sufficient time for autoinoculation is theoretically possible.

C. pneumoniae survived well in aerosolized particles of <5 μm. Survival of bacteria during aerosolization has been shown to be favored by low ambient temperatures and high relative humidity. No attempt was made during these experiments to simulate natural conditions of low humidity. Despite optimal conditions, the organism was recoverable from only 1 of 6 mice exposed to the aerosol. Since the ID_{50} was $10^{-4.6}$ and a large number of organisms was aerosolized (6×10^7), one would have expected more mice to become infected if this was a primary mode of spread. Direct inoculation was clearly a more efficient method of transmission in this mouse model, with high titers of organisms recoverable for long periods of time. In volunteer studies with influenza virus, a pathogen known to be primarily spread by aerosol, the ID_{50} was found to be up to 300 times higher when inoculation was via nasal drops than when it was by inhalation [15]. However, the mouse is not a natural host for *C. pneumoniae*, and direct comparison with human infection is not possible.

The slow patterns of spread of TWAR infection suggest either fomites or large droplets as the primary mode of transmission; however, a number of factors that may influence the epidemiology, such as the titer of infectious *C. pneumoniae* in respiratory secretions, the minimal infectious dose, and the precise incubation period for symptomatic disease, are unknown. Unfortunately, problems of chronic infection and the possible association of *C. pneumoniae* infection with atherosclerotic heart disease make volunteer exposure studies impossible. Although further work to more clearly define transmission of this agent is needed to develop effective control strategies, our results suggest that close contact is required for the transmission of *C. pneumoniae* and that environmental contamination and spread by fomites is possible. In addition, the very rapid loss of viability on skin may explain the slow spread during outbreaks.

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