

Survival of *Chlamydia pneumoniae* following contact with various surfaces

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Objective: In this study, the survival and recovery of *Chlamydia pneumoniae* (Cp) strains TW-183, AR-39, AR-388 and CWL-029 were measured after inoculation on glass, stainless steel, Formica[®] laminate, paper, fabric and human skin.

Methods: Inoculum in throat washes from healthy volunteers was applied to each surface. Samples were taken immediately after inoculum application and at specified intervals thereafter to determine infectivity.

Results: Infectious Cp was recovered from glass for up to 4 h, from paper and fabric for up to 3 h, from Formica[®] laminate for up to 2 h, from stainless steel for up to 60 min and from human skin for up to 30 min. Drying of the inoculated area had no significant effect on the recovery of infectious Cp. Further experiments demonstrated that infectious Cp could be transferred to hands by touching these contaminated surfaces and could be recovered from these hands for up to 3 min. Addition of albumin, surfactant or phosphatidylcholine had no significant effect on the survival of Cp.

Conclusions: These results suggest that contact with contaminated surfaces may be a potential mode of transmission of Cp.

Key words: *Chlamydia*, *Chlamydia pneumoniae*, survival, transmission, tissue culture

Laboratory evidence of *Chlamydia pneumoniae* (Cp) infection has been demonstrated in approximately 10% of community-acquired pneumonia cases [1-3]. However, the organism has also been associated with bronchitis [4,5], asthma [6], reactive arthritis [7] and a number of other diseases [8-10]. Recent studies provide evidence that Cp infection may play a causal role in coronary artery disease [11-13], although many Cp infections are mild or asymptomatic and may therefore remain undiagnosed [14-16].

Specific antibodies against Cp have been found in 50 to 95% of the adult populations in many different countries of the world [3,17-19]. The prevalence of Cp-related antibodies in The Netherlands is approxi-

mately 80% in subjects between 20 and 80 years of age, and no significant difference is found between men and women. These data suggest that most people are infected and reinfected with Cp throughout life [3,19]. Current evidence suggests that Cp infections spread slowly and the case-to-case interval is long. Epidemics have been described in Norway, Sweden, Finland and the US [5,20-22].

Although Cp infection is presumed to be spread from person to person, the exact route of transmission has not been established. The survival of Cp, albeit brief, in small-particle aerosols has been demonstrated [23]. This study was carried out to determine whether Cp remains infectious in respiratory secretions on various surfaces and whether hand contact with these contaminated surfaces results in transfer of infectious Cp.

MATERIALS AND METHODS

Cell culture

Buffalo green monkey cells (BGM; PAMM, Veldhoven, The Netherlands) were grown as monolayers in plastic culture flasks at 35°C with 5% carbon dioxide (CO₂) in a fully humidified cabinet (Costar Europe Ltd, The

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Netherlands). The BGM cells were cultured in Eagle Minimal Essential Medium (EMEM; Life Technologies Ltd, Renfrewshire, Scotland), which contained 10% fetal calf serum (Life Technologies Ltd, Renfrewshire, Scotland), 1% non-essential amino acids (ICN Bio-medicals Inc., Costa Mesa, CA), 10 mg/L gentamicin (Pharmachemie, Haarlem, The Netherlands), 25 mg/L vancomycin (Eli Lilly, Indianapolis, IN) and 4 mg/L amphotericin B (Bristol Myers-Squibb, Epernon, France) [24].

***Chlamydia pneumoniae* strains**

Stock cultures of TW-183, AR-39, AR-388 (Washington Research Foundation, Seattle, WA) and CWL-029 (American Type Culture Collection, Rockville, MD) were used in this study. BGM cells were seeded into 75-cm² tissue-culture flasks (Costar Europe Ltd, The Netherlands) and incubated at 35°C with 5% CO₂ in a fully humidified cabinet. Cell monolayers were examined on the day of inoculation for confluency. After inoculation with Cp, tissue-culture flasks were centrifuged at 900 g at 25°C for 60 min and subsequently incubated with fresh medium containing 0.6 mg/L cycloheximide (Sigma Chemical Company, St Louis, MO).

After 3 days, the flasks were aspirated and the monolayers suspended in 5 mL sucrose-phosphate-glutamate buffer (SPG) using a cell scraper (Costar Europe Ltd). Tenfold dilutions were made of each stock solution, and the titer of Cp was calculated by counting the inclusions present in the last dilution. The titer was expressed as inclusion-forming units (IFU)/mL. All stock solutions were stored at -80 °C prior to use. The final concentration of each sample was 5 × 10⁸ IFU/mL.

Table 1 *Chlamydia pneumoniae* antigen recovery from various surfaces

Surface	<i>Chlamydia pneumoniae</i> antigen recovered after:					
	15 min	30 min	60 min	120 min	180 min	240 min
Glass	44±5	48±13	35±9	46±13	34±8	28±5
Stainless steel	42±2	42±4	50±4	41±6	33±5	37±3
Formica ^R						
laminate	60±4	59±10	72±2	61±7	60±4	57±4
Paper	61±5	66±7	60±7	41±10	31±7	52±11
Fabric	81±3	65±2	65±7	60±6	54±7	57±6
Human skin	22±3	19±3	20±3	19±3	17±3	n.t.

n.t. = not tested.

The data are presented as mean %±SE antigen recovered by Magic^R Lite *Chlamydia* immunoassay, which reports antigen concentrations as relative light units (RLU)/sec. All data are expressed as % compared with RLU in the stock solution used for inoculation (average of three separate experiments with four different strains of *C. pneumoniae*).

***Chlamydia pneumoniae* culture**

BGM cells were seeded onto 24-well tissue-culture plates (Costar Europe Ltd) and incubated at 35°C with 5% CO₂ in a fully humidified cabinet. All cell monolayers were examined on the day of inoculation for confluency. After inoculation, the 24-well plates were centrifuged at 900 g at 25°C for 60 min and subsequently incubated with fresh medium containing 0.6 mg/L cycloheximide. After 3 days, the 24-well plates were aspirated and fixed with methanol (Merck, Darmstadt, Germany).

The fixed monolayers were rinsed once with phosphate-buffered saline and stained by the fluorescent-antibody technique, using *Chlamydia* genus-specific mouse monoclonal antibody (kindly provided by J.M. Ossewaarde, National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands) as described elsewhere [2]. Rabbit anti-mouse immunoglobulin (Ig) labeled with fluorescein isothiocyanate (Dako A/S, Glostrup, Denmark) was used as a conjugate. Evans blue at 0.05% (Sigma Chemical Company, St Louis, MO) was used as a counterstain.

Magic^R Lite *Chlamydia* immunoassay

The Magic^R Lite *Chlamydia* immunoassay (Ciba Corning Diagnostics Corp., Medfield, MA) was used for the measurement of antigen recovery at each interval. The test is based on a genus-specific chemiluminometric antigen-detection assay, and uses constant amounts of acridinium ester-labeled monoclonal anti-*Chlamydial* antibody and polyclonal anti-*Chlamydial* antibody covalently coupled to paramagnetic particles [25]. The test records antigen concentrations as relative light units (RLU)/sec. All data are expressed as % compared with RLU found in the stock solution used for inoculation.

Throat washes from healthy volunteers

These consisted of 10-mL phosphate-buffered saline collected as throat washes from healthy volunteers. Ten throat washes were pooled and stored at -20°C prior to use.

Recovery and survival of *Chlamydia pneumoniae* from various surfaces

The surfaces used were Formica^R laminate, glass, stainless steel, fabric, paper and human skin (fingertips). A 5 µL inoculum (2.0 × 10⁷ IFU/mL) in pooled throat washes from healthy volunteers was applied to each surface. Formica^R laminate, glass, stainless steel and human skin were sampled with a wet swab that was subsequently suspended in 1 mL SPG. Fabric and paper were rinsed with 1 mL SPG, and a 100 µL sample was

used to inoculate cell monolayers to determine the infectivity (as described above). The Magic^R Lite *Chlamydia* immunoassay was used to calculate the recovery of Cp antigen, using a 200 μ L sample for the detection of Cp antigen as described by the manufacturer. Samples were taken immediately after inoculum application and at specified intervals thereafter. Temperature and humidity were monitored and maintained at 22 to 28°C and 40 to 60% relative humidity.

Effect of additives on survival of *Chlamydia pneumoniae* on various surfaces

Human serum albumin at 50 mg/L (The Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands), surfactant at 34 mg/L (kindly provided by J.F. van Iwaarden, Laboratory of Veterinary Biochemistry, University of Utrecht, The Netherlands) and phosphatidylcholine at 1.2 mg/L (Sigma Chemical Company, St. Louis, MO) was added to the inoculum prior to contamination of the various test surfaces.

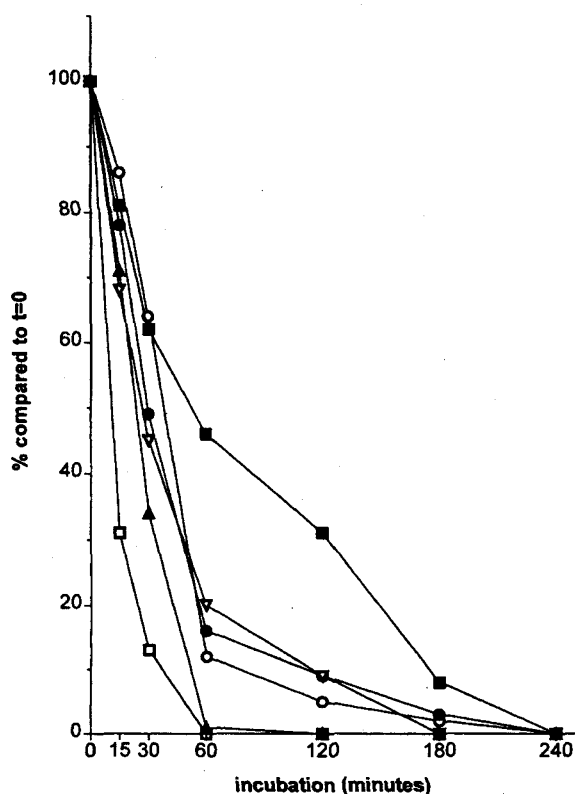


Figure 1 Mean % Cp survival and antigen recovery following contact with glass (■), Formica^R laminate (▽), paper (●), fabric (○), stainless steel (▲) and human skin (□). The data represent the average of four separate experiments with four different *C. pneumoniae* strains.

Recovery of infectious *Chlamydia pneumoniae* from human skin after transfer from environmental surfaces

A 5 μ L inoculum (2.0×10^7 IFU/mL) was administered to a glass surface. Samples were taken from hands after having touched the contaminated surface and were cultured as described above.

RESULTS

Antigen recovery

The average rate of recovery of Cp antigen after Cp contamination (Table 1) from glass, stainless steel, Formica^R laminate and paper at 15 min was 44%, 42%, 60% and 61%, respectively, compared with 81% and 22% from fabric and human skin, respectively. The antigen recovery was significantly lower from human skin compared with the other surfaces tested ($p < 0.05$). The Cp-antigen recovery rate remained constant over time on all surfaces; drying of the inoculated area had no significant effect on Cp-antigen recovery, and no significant differences were found among the four Cp isolates.

Survival of *Chlamydia pneumoniae* following contact with various surfaces

Infectious Cp was recoverable from Formica^R laminate and human skin for up to 120 and 30 min, respectively (Figure 1). Drying of the inoculated area had no significant effect on Cp survival. After 15 min, the recovery of infectious Cp from Formica^R laminate was 1.8×10^4 IFU whereas, on human skin, the survival rate was only 2.9×10^3 IFU (68% and 31% compared with t_0 , respectively). In contrast, recovery of Cp antigen from these surfaces was stable over the time period sampled, as determined by the Magic^R Lite *Chlamydia* assay (Table 1).

Infectious Cp was recoverable from glass, paper, fabric and stainless steel for up to 240, 180, 180 and 120 min, respectively. After 15 min, recovery of infectious Cp was 1.7×10^4 , 2.1×10^4 , 3.2×10^4 and 1.4×10^4 IFU on the respective surfaces (81%, 78%, 86% and 71%, respectively, compared with t_0). Cp survival on stainless steel was of significantly shorter duration compared with glass and Formica^R laminate ($p < 0.05$; data not shown).

No significant differences in survival and antigen recovery were found among the four Cp strains tested, nor did the addition of albumin, surfactant or phosphatidylcholine have significant effects on the survival and recovery of Cp on any of the surfaces tested (data not shown).

Recovery of *Chlamydia pneumoniae* from human skin after transfer from environmental surfaces

Cp was recoverable from hands that had touched the contaminated glass, although recovery of infectious Cp was restricted to the first 3 min following contact with the contaminated area. The average recovery of infectious Cp from fingertips after 1 and 3 min was 580 (6%) and 96 (1%) IFU, respectively. No infectious Cp or Cp antigen was recovered if hands were washed with soap and water prior to sampling.

DISCUSSION

Chlamydia pneumoniae is considered an important pathogen associated with approximately 10% of pneumonia cases and other respiratory infections [1-3]. Many Cp infections are mild or asymptomatic and may therefore remain undiagnosed [14-16]. However, the recent association of Cp with coronary artery disease has prompted a reevaluation of the pathogenesis of Cp infection in patients [11-13]. The high prevalence of Cp-specific antibodies among Dutch adults indicates the possibility of reinfection. However, little is known of the mode of transmission of this organism. Survival of Cp in small-particle aerosols is possible, especially at relatively high humidities, but the duration is relatively short [23].

One study of the survival of Cp following contact with environmental surfaces has been described by other investigators [26] in contrast to the many survival studies with respiratory viruses. The feasibility of the spread of rhinoviruses and respiratory syncytial virus (RSV) has been well documented [27,28]. Investigators have demonstrated that rhinovirus transmission is mostly by hand contact with contaminated surfaces and occasionally after large-particle exposure, but not following small-particle exposure [27]. Transmission of RSV infection is also possible through indirect contact with contaminated surfaces [29].

In the present study of Cp survival and transmission, a theoretical chain of several steps was examined, starting with Cp-antigen recovery from various surfaces, which remained constant over time on all surfaces. Drying of the inoculated area had no significant effect on antigen recovery. A significantly lower recovery was observed from human skin compared with the other surfaces tested. One possible explanation for this phenomenon may be an increased adherence of the microorganism to the surface of human skin.

The survival of Cp on various surfaces lasted for long periods of time and was transferable to hands. Drying of the inoculated area had no significant effect on the rate of survival, which was apparently poorer on

human skin and stainless steel than on the other surfaces tested. One reason for the significantly reduced survival on human skin may be the presence of lactic acid, which causes the pH of skin to be acid. Also, the presence of skin surface lipids and excretory products of the resident microbiological flora may limit the survival of Cp on human skin [30]. Addition of albumin, surfactant or phosphatidylcholine, however, had no effect on the survival time of Cp. The reason for the short survival on stainless steel is unexplained. An antimicrobial action of ferrous ions may be one explanation for the accelerated inactivation of Cp.

Infectious Cp was recoverable from hands, but only during the first 3 min following contact with surfaces contaminated with Cp. No significant differences were found among the four Cp strains used in this study.

Falsey and Walsh have compared the survival of Cp after contact with human skin, tissue and Formica^R laminate [26], and the results of the present study are comparable to their findings. However, discrepant results are found with Formica^R laminate: Falsey and Walsh reported a survival period of up to 30 h, which is significantly longer than the 3-h survival in the present study. Such a significant difference may be the result of substantial differences in methodology. In the present study, a 5 µL Cp-stock suspension was applied to each surface tested whereas Falsey and Walsh used 100 µL. Also, in our study, the Cp-stock suspensions were diluted in pooled throat washes from healthy volunteers prior to sampling whereas SPG was used by Falsey and Walsh. In our opinion, the use of small sample volumes that were diluted in human pooled throat washes is closer to reality.

In conclusion, the results of the present study indicate that Cp is able to survive sufficiently long in the environment to allow transfer of infectious Cp to hands in contact with contaminated surfaces. As with many respiratory viruses, this may be the most efficient route of transmission of these microorganisms.

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