

## Asymptomatic Respiratory Tract Infection with *Chlamydia pneumoniae* TWAR

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***Chlamydia pneumoniae* is a newly recognized organism associated with respiratory tract infections. Asymptomatic infection with *C. pneumoniae*, although it has been suggested to occur, has not been previously documented. We describe two asymptomatic individuals infected with this organism; these infections demonstrate that *C. pneumoniae* is able to establish a subclinical infection.**

*Chlamydia pneumoniae* was first described as a respiratory tract pathogen by Grayston and colleagues in 1986 (7). Subsequently, much work has been done to characterize this organism and the diseases with which it is associated. Seroepidemiological surveys have shown *C. pneumoniae* to be a common infectious agent, with up to 50% of adults having serologic evidence of prior exposure (5). It has been most strongly associated with respiratory tract infections, in particular, pneumonia (5). Although asymptomatic infection has been suggested to occur, this has not been previously documented. As a result of a laboratory accident, we discovered two individuals with asymptomatic *C. pneumoniae* infections. We present and discuss these data.

(This study was presented in part at the 30th Interscience Conference on Antimicrobial Agents and Chemotherapy [8].)

In October 1989, a centrifuge malfunction in our laboratory caused the destruction of two 75-cm<sup>2</sup> flasks; each flask contained HeLa 229 cell monolayers inoculated with 10<sup>6</sup> inclusion-forming units of a clinical isolate of *C. pneumoniae*. This strain, T-2023, was isolated from a patient with an acute respiratory tract infection. Identification as *C. pneumoniae* was based on positive staining of inclusions by a species-specific fluorescein-conjugated monoclonal antibody (Washington Research Foundation, Seattle, Wash.) (7), and characteristic elementary body morphology (5) was noted on electron micrography. Two unmasked workers were present when the centrifuge was opened immediately after the accident.

In order to evaluate the consequences of this exposure, the following investigations were done. Nasopharyngeal swabs for *Chlamydia* culture were obtained from both individuals at 5 days and approximately 20 weeks postexposure. Specimens were placed in vials containing transport medium, vortexed, and frozen at -70°C until the specimens were cultured. Serial blood samples for chlamydial serology were taken at 5 days and approximately 5, 12 (case 1 only), and 20 weeks postexposure and centrifuged; and the sera were stored at -70°C until they were transported on dry ice to the University of San Francisco Chlamydia Laboratory for serological testing. *Chlamydia* cultures were prepared as described previously (3). Briefly, cultures were prepared in

duplicate in 96-well microtiter plates by inoculating fresh HeLa 229 cell monolayers pretreated with 30 µg of dextran-DEAE per ml with 0.1 ml of sample. The plates were centrifuged at 1,700 × g for 1 h, after which the supernatant was removed and 0.2 ml of overlay medium containing 0.5 µg of cycloheximide per ml was added. After 72 h of incubation, the monolayers were fixed with 95% ethanol and stained with either a genus-specific fluorescent antibody (Pathfinder Chlamydia Culture Confirmation System, Kallestad, Chaska, Minn.) or a species-specific fluorescent monoclonal antibody (Washington Research Foundation). Inclusions were identified as *C. pneumoniae* only if they stained positively with both antibodies. *Chlamydia* serologies were performed by the microimmunofluorescence (MIF) method with immunoglobulin G (IgG) and IgM conjugates (13). The antigen used was elementary bodies of strain TW-183, which makes this test specific for *C. pneumoniae* (4).

The culture and serological results are given in Table 1. The initial specimens from both cases 1 and 2 were positive on first passage. Negative controls were negative, and repeat cultures of the original specimens done at a later date were positive. Specimens taken at 20 weeks postexposure were culture positive for case 1 but were negative for case 2. Both individuals denied respiratory tract symptoms either before or after the exposure during 6 months of observation. Neither opted to take treatment when it became known that cultures of their specimens were positive. Serologically, case 2 had no evidence of prior exposure and failed to develop a detectable antibody response even by 20 weeks postexposure. Case 1 had evidence of prior exposure and at 5 weeks postexposure demonstrated a fourfold drop in IgG titer and an IgM titer of <1:8 (the lowest dilution of serum tested); thereafter, the IgG titer remained stable at 1:64 and the IgM titer fluctuated between <1:8 and 1:8.

We believe that both individuals became infected as a result of their exposure and that the route of infection was by droplet aerosolization. Even though these assumptions cannot be proven, it should be noted that these were probably laboratory-acquired infections and, as such, should serve to caution others working with this organism. Regardless of when or how these infections were acquired, they demonstrate that *C. pneumoniae* can cause asymptomatic infections. This has been demonstrated previously only in non-human primates (9).

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TABLE 1. Culture and serological results<sup>a</sup>

Case no.	Date of specimen collection (mo/day/yr)	Culture result <sup>b</sup>	Titer <sup>c</sup>	
			IgM	IgG
1	10/25/1989	+	8	64
	11/27/1989	ND	<8	16
	1/8/1990	ND	8	64
	3/6/1990	+	<8	64
2	10/25/1989	+	<8	<8
	11/27/1989	ND	<8	<8
	3/6/1990	-	<8	<8

<sup>a</sup> Cases were exposed on 20 October 1989.

<sup>b</sup> +, positive; ND, not done; -, negative.

<sup>c</sup> Titers are expressed as reciprocals.

Although these cases probably resulted from the accidental exposure, we suspect that asymptomatic infections occur with some frequency. The fact that subclinical infections are established by other species of *Chlamydia*, such as genital tract infections with *C. trachomatis*, is well described (11, 12). In a retrospective serological study of respiratory tract infections in military trainees caused by *C. pneumoniae* (10), the authors estimated that only about 10% of infections became clinically apparent. In another study (6) of pneumonias associated with this organism, the authors suggested that some of these infections may have represented a "re-activation of a quiescent TWAR infection in the respiratory tract." This would imply a subclinical and, possibly, a chronic carriage state. Case 1 in this study, who was culture positive on two occasions over a 5-month period, and another case of an individual described by Chirgwin and colleagues (2), who, subsequent to an acute infection, was culture positive on three occasions over a 12-month period, lend support to this hypothesis. The evidence that this organism can cause a subclinical infection raises several important issues, such as the prevalence of asymptomatic infections in the general population, whether such individuals represent a reservoir, and the implication for treatment when the organism is isolated.

The other interesting observation from our cases was their serologic response to infection with *C. pneumoniae*. Case 2 failed to develop detectable antibody at any time during the period of observation. The serology of case 1 is less clear. We interpret the initial titers as evidence of prior infection. The subsequent changes in both IgM and IgG titers may represent the serological response to acute reinfection (four-fold rise in titer) (5) or fluctuations of a low level of preexisting antibody. Whether the titer would have been higher if the MIF assay was performed with strain T2023 as the antigen is unknown. Isolation of this organism without detection of the characteristic serological response has been noted before. Berdal and associates (1) isolated *C. pneumoniae* from the nasopharynx of 5 of 30 symptomatic individuals with acute adenovirus infections. None of these five individuals had a serological profile suggestive of active infection with *C. pneumoniae*, even when the MIF was

repeated by using autologous isolates as the antigen. This observation led the authors to question the infectious role of this organism and the significance of its presence.

*C. pneumoniae* is a newly recognized pathogen about which much remains to be elucidated. The cases described here are the only ones we are aware of that document that *C. pneumoniae* can establish asymptomatic infections. Data are accumulating that question the pathogenic significance of this organism when it is isolated. This is an important issue that deserves further study.

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