Prevalence of antibodies to *Chlamydia pneumoniae* in an Israeli population without clinical evidence of respiratory infection

M Ben-Yaakov, G Eshel, L Zakonski, Z Lazarovich, I Boldur

**Aims:** To estimate the occurrence of recent, past, and "persistent" infections with *Chlamydia pneumoniae*—as indicated by serology—in an Israeli population without clinical evidence of respiratory infection.

**Methods:** Serum samples from 402 subjects (172 children and 230 adults), without known respiratory symptoms, were collected. Antibodies to *C pneumoniae* (IgG, IgA, and IgM) were evaluated using the microimmunofluorescence (MIF) assay. Antibody prevalence and indication of recent, past, and persistent infections were calculated and their distribution determined according to age, sex, and season.

**Results:** Antibodies to *C pneumoniae* were detected in 53 children (31%) and 171 adults (74%). Recent infection was indicated in only one of 50 children under 5 years of age, in nine of 122 older children, and in 19 of 230 adults. IgM antibodies were detected in nine children, but only in three adults. Past infection was indicated in six of 96 young children (aged 1–10 years), in 28 of 76 teenagers, and in 128 of 230 adults. Persistent infection was indicated in three young children, in six teenagers, and in 24 adults, with a significantly higher frequency (p = 0.012) in men (18 of 117) than in women (six of 113). No seasonal differences could be detected.

**Conclusions:** Infection with *C pneumoniae* was detected serologically in children and adults without clinical signs of respiratory disease. These results should serve as a basis for studies on the role of *C pneumoniae* infections and their sequelae in Israel and contribute to the general understanding of asymptomatic infection with *C pneumoniae*.

*Chlamydia pneumoniae* has recently been recognised as a common and important intracellular bacterium, implicated in upper and lower respiratory tract infections in humans worldwide.

*Chlamydia pneumoniae* can cause severe clinical disease, but often symptoms are only mild or even subclinical. A seemingly healthy population will include individuals with serological indications of past infection, persistent (chronic) infection, and recent (acute) infection with *C pneumoniae*. Mixed infections with *C pneumoniae* are common and should be taken into account when planning antimicrobial treatment for community acquired pneumonia. It is not possible to distinguish patients with atypical pneumonia from those with pneumonia of undetermined aetiology by clinical features.

Because *C pneumoniae* often persists in the body after acute infection, it may be involved in chronic respiratory diseases, such as chronic obstructive pulmonary disease and asthma.

The microimmunofluorescence (MIF) test is the serological testing method of choice for the diagnosis of acute *C pneumoniae* infection. The specificity of the test can be attributed to the use of purified elementary bodies of *C pneumoniae* that can detect species specific antibodies.

Because of the lack of standardisation of the test, the interpretation of published data is difficult. Interlaboratory and regional variations exist. Although there is extensive variation in the numerical titre values, the overall percentage agreement with the reference standard titres from the university of Washington is 80%.

The prevalence and titre of antibodies to *C pneumoniae* in children and adults in our region, without clinical evidence of respiratory infection, has not yet been reported. It is therefore important to evaluate these criteria as a basis for further considerations on the role of *C pneumoniae* in infection and thereby contribute to the general understanding of asymptomatic respiratory infection with *C pneumoniae*.

**MATERIALS AND METHODS**

Our study included 172 children of both sexes, age range 1–19 years, visiting the emergency room and the paediatric surgery...
orthopaedic clinic of the Assaf Harofeh Medical Centre, from October 1996 to March 1997. Exclusion criteria were positive physical findings relating to past respiratory infection or any symptoms of respiratory tract infection or complaints during the previous three months.

Our study also included 230 healthy adults of both sexes, visiting outpatient clinics from January to August 1997 for annual check ups, or healthy hospital personnel without respiratory symptoms three months before the study. Blood samples were obtained for necessary routine laboratory tests, and aliquots were used for our study according to the guidelines for human experimentation (Helsinki committee). A single blood specimen was drawn from each individual. Sera were stored at −20°C until tested.

Antibodies to *C. pneumoniae* were detected by the microimmunofluorescence assay using self prepared slides with purified dotted formalinised *C. pneumoniae* elementary bodies, 10⁷ particles/ml (AR39; Washington Research Foundation, Seattle, USA) in 0.5% yolk sac, as an antigen. The slides were fixed with acetone. IgG, IgA, and IgM antibodies were measured using fluorescein isothiocyanate conjugated goat anti-human immunoglobulins (Jackson Immuno Research, West Grove, USA) with Evans blue (0.05%) as a counterstain. A single blood specimen was drawn from each individual. Sera were stored at −20°C until tested.

For each conjugate the optimal dilution was determined by titration with a high titred serum. Positive and negative sera were included in each run and reproducibility between runs was checked by the titration of control serum samples were included in each run and aliquots were used for our study according to the guidelines for human experimentation (Helsinki committee). A single blood specimen was drawn from each individual. Sera were stored at −20°C until tested.

Table 1 summarises the *C. pneumoniae* antibody prevalence in adults, according to age and sex. Recent infection was indicated in one of 50 children under the age of 5 years. Frequency increased with age to four of 46 at the age of 5–10 years and to five of 76 in teenagers. Nine of 10 children with an indication of recent infection had IgM antibodies. Past infection was indicated in six of 96 children under the age of 10 years, with a significant rise to 28 of 76 in teenagers (p = 1.2 × 10⁻⁴).

Persistent infection, as indicated by the presence of IgA antibodies at a titre of ≥1/20, together with IgG titres of 1/64 to 1/256, was present in only three girls under the age of 10 years, but was detected in six of 76 teenagers.

Table 2 summarises the results for *C. pneumoniae* infection and antibody prevalence in adults, according to age and sex. Among adults (56%) than in teenagers (37%) (p = 0.012). The frequency of recent infection in adults (8%) was similar to the frequency in teenagers (7%), but 16 of 19 adults had an IgG titre of ≥1/512, whereas only three had IgM antibodies. The indication of past infection was significantly higher in men (56%) than in teenagers (37%) (p = 7 × 10⁻⁴). Persistent infection at the age of 19–30 years was 12%, which was not significantly different to that seen in teenagers (p = 0.6). The prevalence of persistent infection was significantly higher in men (18 of 117) than in women (6 of 113) (p = 0.012).

No significant difference according to season was recorded for *C. pneumoniae* recent infections. Five of 51 children showed an indication of recent infection from October to December.
Prevalence of antibodies to Chlamydia pneumoniae

compared with five of 121 from January to March (p = 0.27). An indication of recent infection was detected in 11 of 168 adults during January to April compared with eight of 62 during May to August (p = 0.2).

DISCUSSION
A group of children and adults, without clinical evidence of respiratory infection, was evaluated for the presence of C pneumoniae antibodies. The recommended serological test for the diagnosis of C pneumoniae infection—the MIF assay—was used.1

A progressive increase in antibody prevalence, from early childhood up to the age of young adulthood (19–30 years), was documented. At an older age, no further significant increase was detected. These findings correlate with data on populations, without clinical evidence of respiratory infection, from various parts of the world,1 15 21 and do not significantly differ from our previous results concerning symptomatic patients. At present, there is no validated serological marker for persistent infection.

The frequency of past infection was significantly higher in young adults (age 19–30), compared with teenagers, but became relatively stable with increasing age. Acute primary infections in adults—based on the presence of IgM antibodies (≥ 1/16 titre)—were frequent (16 of 230). These infections were not primary or on a clinical basis. The possibility that they are in the incubation period cannot be excluded because no second (convalescent) blood sample was available.

"At present, there is no validated serological marker for persistent infection"

Recent infection was indicated in nine of 122 children older then 5 years. Nine of 10 cases of recent infection in children were primary infections, with IgM antibodies (≥ 1/16 titre). Although we cannot be sure that these children represent a healthy childhood population, it has to be re-emphasised that they had no signs of acute respiratory disease or other known manifestations of acute C pneumoniae infections, at anamnesis or on a clinical basis. The possibility that they are in the incubation period cannot be excluded because no second (convalescent) blood sample was available.

In our study, acute infection using a single serum sample was detected in 8.3% of the adults. This value was lower than the value of 18.4% reported by Hyman et al.,21 but higher than the value of 3.9% reported recently by Miyashita et al.,22 probably as a result of geographical variations in the distribution of C pneumoniae. The value of 3.9% was established in children and did not show signs of known C pneumoniae associated acute diseases.

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Recently, the lack of correlation between positive cultures and/or PCR positivity and serology using the MIF test has been reported not only in children,21 but also in an adult population.24 At present, there is no validated serological marker for persistent infection; an increased IgA titre may be a good candidate because IgA antibodies have a short half life of only five to seven days.

Variable cut off values for IgA positivities are used in different publications.1 15 We considered IgA values of ≥ 1/20, with IgG values of 1/64 to 1/256, as indicative of persistent infection.

In young children, an IgA titre of ≥ 1/20, with an IgG titre of 1/64 to 1/256, was seldom detected, but in teenagers 8% already had indications of persistent infection. In adults, the prevalence of persistent infection was not significantly different to that seen in teenagers. If we were to calculate the results according to higher cut off values—IgA titres ≥ 1/40, with IgG titres of 1/128—only four adults (two men and two women) would have been included in this category. Prevalence was significantly higher in men (18 of 117) than in women (six of 113), p = 0.0012.

We feel that the importance of our study goes beyond establishing a background for our further studies by contributing to the worldwide trials to standardise this method.

Serological testing of C pneumoniae remains problematic because of difficulties in obtaining paired serum samples, discrepancies in the antibody values of a healthy population from different geographical locations, and the lack of standardised tests and reagents. It is therefore of crucial importance to establish a background for the particular population which a laboratory intends to diagnose.

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Take home messages
• Infection with Chlamydia pneumoniae was detected serologically in children and adults without clinical signs of respiratory disease
• Evidence of persistent infection was rarely seen in young children, but 8% of teenagers already had indications of persistent infection, and the prevalence of persistent infection was similar in adults
• Prevalence was significantly higher in men than in women
• No significant differences according to season were recorded
• These results should serve as a basis for studies on the role of C pneumoniae infections and their sequelae in Israel and contribute to the general understanding of asymptomatic infection with C pneumoniae
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