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 present 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 micro-immunofluorescence 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 antihuman immunoglobulins (Jackson Immuno Research, West Grove, USA) with evans blue (0.05%) as a counterstain. For each conjugate the optimal dilution was determined by titration with a high titred serum. Positive and negative control serum samples were included in each run and reproducibility between runs was checked by the titration of positive control serum. All the tests were performed blinded by the same very experienced staff member (BYM).

Sera were diluted from 1/16 to endpoint for IgG determination. IgA and IgM were tested at a dilution of 1/20, after IgG neutralisation by Gullsorb (Gull Lab, Utah, USA)10–16 and positive sera were further diluted to endpoint.

The antibody titre for each serum was recorded and for each study group antibody prevalence and indication of recent, past, or persistent infections were calculated.

A titre of >= 1/20 in the IgM fraction and/or >= 1/512 in the IgG fraction was considered indicative of recent infection. A chlamydia antibody titre of 1/16 to 1/256 in the IgG fraction was considered indicative of past infection. Because there is as yet no standardisation of serological criteria for persistent infection,14 we considered antibody titres of >= 1/20 in the IgA fraction, together with IgG titres of 1/64 to 1/256, to be indicative of persistent infection.

Age, sex, and seasonal distribution were noted.

The significance of the data was determined by Fisher's exact test or by the \( \chi^2 \) test for numbers larger than five in all cells. A p value < 0.05 was considered significant.

RESULTS

Table 1 summarises the \( C. pneumoniae \) antibody prevalence 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 x 10−6). Persistent infection, as indicated by the presence of IgA antibodies at a titre of >= 1/20, together with IgG 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. \( C. pneumoniae \) antibody prevalence at the age of 19–30 years was 71%, significantly higher than in teenagers, with a 51% prevalence (p < 0.05).

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 adults (56%) than in teenagers (37%) (p = 7 × 10−6). 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.

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Table 1  \( Chlamydia pneumoniae \) (CP) infection and antibody prevalence in children according to age and sex

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Boys</th>
<th>Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–5</td>
<td>1–5</td>
<td>5–10</td>
</tr>
<tr>
<td>1.2–4.9</td>
<td>5.3–9.8</td>
<td>10.3–16.0</td>
</tr>
<tr>
<td>Mean years</td>
<td>2.5</td>
<td>6.7</td>
</tr>
<tr>
<td>No. tested</td>
<td>17</td>
<td>25</td>
</tr>
<tr>
<td>No. with CP antibodies</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Recent infection (IgM Ab)</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>Past infection</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>“Persistent” infection</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Recent infection categorised as IgM titre >= 1/20 or IgG titre >= 1/512 with or without IgA titre >= 1/20. Past infection categorised as IgG titre 1/16–1/256. Persistent infection categorised as IgA titre 1/64–1/256.

*p< 0.05 compared with children under the age of 10 years (Fisher’s exact test); **p< 0.05 compared with children under the age of 10 years (χ² test).

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Table 2  \( Chlamydia pneumoniae \) (CP) infection and antibody prevalence in adults according to age and sex

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>19–30</td>
<td>31–50</td>
<td>51–65</td>
</tr>
<tr>
<td>51–65</td>
<td>31–50</td>
<td>51–65</td>
</tr>
<tr>
<td>No. tested</td>
<td>46</td>
<td>41</td>
</tr>
<tr>
<td>No. with CP antibodies</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Recent infection (IgM Ab)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Past infection</td>
<td>20</td>
<td>23</td>
</tr>
<tr>
<td>“Persistent” infection</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>IgG: mean geometric titre</td>
<td>1/160</td>
<td>1/152</td>
</tr>
</tbody>
</table>

Recent infection categorised as IgM titre >= 1/20 or IgG titre >= 1/512 with or without IgA titre >= 1/20. Past infection categorised as IgG titre 1/16–1/256. Persistent infection categorised as IgA titre 1/64–1/256.
Prevalence of antibodies to Chlamydia pneumoniae

In young children, an IgA titre of $\geq 1/20$, 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 $\geq 1/40$, 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 problematical 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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