ORIGINAL ARTICLE

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

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

J Clin Pathol 2002;**55**:355–358

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*.

See end of article for authors' affiliations

Correspondence to:
Dr M Ben-Yaakov,
Department of
Microbiology, Assaf
Harofeh Medical Center,
Zerifin, 70300 Israel;
iboldur@asaf.health.gov.il

Accepted for publication
29 October 2001

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,^{1–3} but often symptoms are only mild or even subclinical.⁴ 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 common⁵ 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.⁵

Because *C pneumoniae* often persists in the body after acute infection, it may be involved in chronic respiratory diseases, such as chronic obstructive pulmonary disease⁶ and asthma.⁷ An association of *C pneumoniae* with chronic non-respiratory diseases such as atherosclerosis and coronary heart disease has been suggested by several investigators, based on seroepidemiology and the demonstration of the agent in vessel walls, although negative studies have also been reported.^{8–11}

The isolation of the organism is difficult because of poor growth in culture.¹ The detection of *C pneumoniae* by molecular biology based amplification methods has recently been introduced as a diagnostic aid,^{12–13} but most reports are still based on serology. Antibody prevalence studies have shown that almost everybody is infected with *C pneumoniae* at some time during their lifetime and that reinfection is common.^{1,2} Sero-prevalence studies from different parts of the world suggest a low antibody prevalence during childhood, which increases during the teenage years, and relatively high values in middle age and old age.^{1,14,15}

“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%.¹⁶

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

Abbreviations: MIF, microimmunofluorescence; PCR, polymerase chain reaction

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

	Boys			Girls		
Age group (years)	1–5	5–10	10–18	1–5	5–10	10–18
Actual range (years)	1.2–4.9	5.3–9.8	10.3–16.0	1.3–4.8	5.6–9.9	10.1–17.5
Mean (years)	2.5	6.7	12.4	2.6	7.9	13.6
No. tested	17	25	34	33	21	42
No. with						
CP antibodies	2	3	19*	3	6	20**
Recent infection (IgM Ab)	1 (1)	1 (1)	2 (2)	0 (0)	3 (2)	3 (3)
Past infection	1	2	14*	1	2	14*
"Persistent" infection	0	0	3	2	1	3

Recent infection categorised as IgM titre $\geq 1/20$ and/or IgG titre $\geq 1/512$ with or without IgA titre $\geq 1/20$. Past infection categorised as IgG titre $1/16$ – $1/256$. Persistent infection categorised as IgA titre $\geq 1/20$ with IgG 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 (χ^2 test).

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, 10^9 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 Gullisorb (Gull Lab, Utah, USA)^{17,18} 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 $\geq 1/20$ in the IgM fraction and/or $\geq 1/512$ in the IgG fraction was considered indicative of recent infection.¹ A *Chlamydia* antibody titre of 1/16 to 1/256 in the IgG fraction was considered indicative of past infection.¹

Because there is as yet no standardisation of serological criteria for persistent infection,^{6,19} we considered antibody titres of $\geq 1/20$ 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 χ^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 ($p = 1.2 \times 10^{-6}$).

Persistent infection, as indicated by the presence of IgA antibodies at a titre of $\geq 1/20$, 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. *Chlamydia 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 $\geq 1/512$, whereas only three had IgM antibodies. The indication of past infection was significantly higher in adults (56%) than in teenagers (37%) ($p = 7 \times 10^{-3}$). 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,

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

	Men			Women		
Age group (years)	19–30	31–50	51–65	19–30	31–50	51–65
No. tested	46	41	30	39	44	30
No. with						
CP antibodies	32	32	26	28	32	21
Recent infection (IgM Ab)	4 (0)	4 (1)	2 (1)	2 (0)	3 (1)	4 (0)
Past infection	20	23	19	24	27	15
"Persistent" infection	8	5	5	2	2	2
IgG: mean geometric titre	1/160	1/152	1/126	1/110	1/108	1/146

Recent infection categorised as IgM titre $\geq 1/20$ and/or IgG titre $\geq 1/512$ with or without IgA titre $\geq 1/20$. Past infection categorised as IgG titre $1/16$ – $1/256$. Persistent infection categorised as IgA titre $\geq 1/20$ with IgG titre $1/64$ – $1/256$.

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 20} and do not significantly differ from our previous results concerning symptomatic patients.¹⁴ In our study, children under the age of 5 years seldom showed serological indications of infection with *C pneumoniae* (past, persistent, or acute). This result is in accordance with other reports.²⁰ Recent prospective studies comparing polymerase chain reaction (PCR) detection and serology claim that serology is useful in the diagnosis of *C pneumoniae* infection only in children older than 5 years.^{21 22}

Recent infection was indicated in nine of 122 children older than 5 years. Nine of 10 cases of recent infection in children were primary infections, with IgM antibodies ($\geq 1/16$ 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.

“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—were rare (three of 230) compared that seen in children (nine of 172) ($p < 0.05$). Possible acute infections in adults, indicated by high IgG values ($\geq 1/512$ titre), were frequent (16 of 230). These infections were not primary infections, but seemed to be reinfections.¹ Adults with a serological indication of recent infection were objectively healthy and did not show signs of known *C pneumoniae* associated acute diseases.¹

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.*,²³ but higher than the value of 3.9% reported recently by Miyashita *et al.*,²⁴ probably as a result of geographical variations in the distribution of *C pneumoniae*.

Recently, the lack of correlation between positive cultures and/or PCR positivities and serology using the MIF test has been reported not only in children,^{21 22} 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.^{6 19} We considered IgA values of $\geq 1/20$, with IgG values of 1/64 to 1/256, as indicative of persistent infection.

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*

In young children, an IgA titre of $\geq 1/20$, with an IgG titre of 1/64 to 1/265, 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 1/128⁶—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.

ACKNOWLEDGEMENT

This study was supported by grant number 3970 from the Chief Scientist's Office of the Ministry of Health, Israel.

.....

Authors' affiliations

M Ben-Yaakov, Z Lazarovich, I Boldur, Department of Microbiology, Assaf Harofeh Medical Center, Zerifin, 70300 Israel

G Eshel, L Zaksonski, Division of Pediatrics, Assaf Harofeh Medical Center, affiliated to the Sackler Faculty of Medicine, Tel Aviv University, 69978 Israel

I Boldur, Department of Life Sciences, Bar-Ilan University, Ramat Gan, 52900 Israel

REFERENCES

- 1 Kuo CC, Jackson LA, Campbell LA, *et al.* Chlamydia pneumoniae (TWAR). *Clin Microbiol Rev* 1995;**8**:451–61.
- 2 Peeling RW, Brunham RC. Chlamydiae as pathogens: new species and new issues. *Emerg Infect Dis* 1996;**2**:307–19.
- 3 Lieberman D, Shvartzman P, Lieberman D, *et al.* Etiology of respiratory tract infection in adults in a general practice setting. *Eur J Clin Microbiol Infect Dis* 1998;**17**:685–9.
- 4 Blasi F. Clinical features of Chlamydia pneumoniae acute respiratory infection. *Clinical Microbiology and Infection* 1996;**1**(suppl 1):S14–18.
- 5 Lieberman D, Ben-Yaakov M, Lazarovich Z, *et al.* Chlamydia pneumoniae community-acquired pneumonia: a review of 62 hospitalized adult patients. *Infection* 1996;**24**:109–14.
- 6 Von Hertzen L, Alakarppa H, Koskinen R, *et al.* Chlamydia pneumoniae infection in patients with chronic obstructive pulmonary disease. *Epidemiol Infect* 1997;**118**:155–64.
- 7 Hahn DL, Peeling RW, Dillon E, *et al.* Serologic markers for Chlamydia pneumoniae is asthma. *Ann Allergy Asthma Immunol* 2000;**84**:227–33.
- 8 Saikku P. Chlamydia pneumoniae and cardiovascular diseases. *Clinical Microbiology and Infection* 1996;**1**(suppl 1):S19–22.
- 9 Gupta S. Chronic infection in the etiology of atherosclerosis—focus on Chlamydia pneumoniae. *Atherosclerosis* 1999;**143**:1–6.
- 10 Siscovick DS, Schwartz M, Caps M, *et al.* Chlamydia pneumoniae and atherosclerotic risk in populations: the role of seroepidemiology. *J Infect Dis* 2000;**181**(suppl 3):S417–20.

- 11 **Danesh I**, Whincup P, Walker M, *et al*. Chlamydia pneumoniae IgG titre and coronary heart disease: prospective study and meta-analysis. *BMJ* 2000;**321**:208–13.
- 12 **Boman J**, Gaydos CA, Quinn TC. Molecular diagnosis of Chlamydia pneumoniae infection. *J Clin Microbiol* 1999;**37**:3791–9.
- 13 **Tijhe JH**, Dorigo-Zetsma JW, Roosendaal R, *et al*. Chlamydia pneumoniae and Mycoplasma pneumoniae in children with acute respiratory infection in general practices in the Netherlands. *Scand J Infect Dis* 2000;**32**:13–17.
- 14 **Ben-Yaakov M**, Lazarovich Z, Beer S, *et al*. Prevalence of Chlamydia pneumoniae antibodies in patients with acute respiratory infection in Israel. *J Clin Pathol* 1994;**47**:232–5.
- 15 **Ferrari M**, Poli A, Olivieri M, *et al*. Seroprevalence of Chlamydia pneumoniae antibodies in a young adult population sample living in Verona. European Community Respiratory Health Survey (ECRHS). Verona. *Infection* 2000;**28**:38–41.
- 16 **Peeling RW**, Wang SP, Grayston JT, *et al*. Chlamydia pneumoniae serology: interlaboratory variation in microimmunofluorescence assay results. *J Infect Dis* 2000;**181**(suppl 3):S426–9.
- 17 **Verkooyen RP**, Hazenberg MA, Van Haaren GH, *et al*. Age related interference with Chlamydia pneumoniae microimmunofluorescence serology due to circulating rheumatoid factor. *J Clin Microbiol* 1992;**30**:1287–90.
- 18 **Jauhainen T**, Tuomi T, Leinonen M, *et al*. Interference of immunoglobulin G (IgG) antibody in IgA antibody determinations for Chlamydia pneumoniae by microimmunofluorescence test. *J Clin Microbiol* 1994;**32**:839–40.
- 19 **Schumacher A**, Lerkerq AB, Seljeflot I, *et al*. Chlamydia pneumoniae serology: importance of methodology in patients with coronary heart disease and healthy individuals. *J Clin Microbiol* 2001;**39**:1859–64.
- 20 **Mono R**, Dibitonto G, Marcuccio L, *et al*. Antibodies to Chlamydia pneumoniae in healthy children from Bari, South Italy. *Microbiologica* 1998;**21**:281–4.
- 21 **Normann E**, Gnarp J, Gnarp H, *et al*. Chlamydia pneumoniae in children attending day-care centers in Gavle, Sweden. *Pediatr Infect Dis J* 1998;**17**:474–8.
- 22 **Kutlin A**, Roblin PM, Hammerschlag MR. Antibody response to Chlamydia pneumoniae infection in children with respiratory illness. *J Infect Dis* 1998;**177**:720–4.
- 23 **Hyman CL**, Roblin PM, Gaydos CA, *et al*. Prevalence of asymptomatic nasopharyngeal carriage of Chlamydia pneumoniae in subjectively healthy adults: assessment by polymerase chain reaction-enzyme immunoassay and culture. *Clin Infect Dis* 1995;**20**:1174–8.
- 24 **Miyashita N**, Niki Y, Nakajima M, *et al*. Prevalence of asymptomatic infection with Chlamydia pneumoniae in subjectively healthy adults. *Chest* 2001;**119**:1416–19.

Reference linking to full text of more than 200 journals

Toll free links

You can access the FULL TEXT of articles cited in the *Journal of Clinical Pathology* online if the citation is to one of the more than 200 journals hosted by HighWire (<http://highwire.stanford.edu>) without a subscription to that journal. There are also direct links from references to the Medline abstract for other titles.

www.jclinpath.com