

## Seroepidemiological Studies of *Chlamydia pneumoniae* Infections in 1–36 Months Old Children with Respiratory Tract Infections and Other Diseases in Poland

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### Abstract

Presence of specific IgM, IgG and IgA antibodies against *Chlamydia pneumoniae* was evaluated in children aged 1 week to 36 months to investigate the role of *C. pneumoniae* in respiratory infections and other diseases. Serum samples were obtained from 150 hospitalized children, including 123 children presenting the clinical symptoms of various respiratory tract infections, two children with acute diarrhoea, two children with meningitis, 14 children with urinary tract infection, and 9 children with non-infectious diseases. Levels of specific *C. pneumoniae* IgM, IgG and IgA serum antibodies were measured by enzyme-linked immunoassay (ELISA). *C. pneumoniae* IgM antibodies were detected in 16 (13.0 %) of 123 children with respiratory tract infections. Specific IgG antibodies were found in sera of 11 children under 12 months old. Among 27 children without symptoms of a respiratory tract disease, specific *C. pneumoniae* IgM were found in two (7.4%) children, including one child with meningitis and another child with urinary tract infection. Specific IgA antibodies were not found in any tested child. All cases of *C. pneumoniae* infections were identified within two calendar years out of eight that were analyzed, i.e. in 1997 and 2000. The incidence of *C. pneumoniae* infections varied seasonally, with most children infected in autumn. *C. pneumoniae* IgM antibodies were detected more often in girls (17.9%) than in boys (7.2%). *C. pneumoniae* infections occur among small children in central Poland. The results of this study indicate that *C. pneumoniae* may play a role in the etiology of respiratory tract infections in infants and young children.

**Key words:** children, infants, respiratory tract infections, *Chlamydia pneumoniae*, antibodies

### Introduction

*Chlamydia pneumoniae* is a human pathogen with a wide distribution all over the world. This pathogen is responsible for upper and lower respiratory tract infections such as pharyngitis, sinusitis, bronchitis and pneumonia and probably myocarditis, erythema nodosum and reactive arthritis. The list of diseases associated with *C. pneumoniae* infection is growing. It is postulated that the microorganism is involved in the development of chronic diseases such as asthma, sarcoidosis, atherosclerosis, Guillain-Barre syndrome, Reiter's syndrome, and Alzheimer disease (Dowel *et al.*, 2001; Grayston, 2000; Kuo *et al.*, 1995).

It has been recognized worldwide that 50% of adults have significant levels of specific serum antibodies against *C. pneumoniae* (Kuo *et al.*, 1995). Considerable data are available on *C. pneumoniae* respiratory tract infections in adults, but relatively little is known on the role of this pathogen in infants and young children. Thus the role of *C. pneumoniae* infections in pediatric patients is uncertain. Few reports on the association of *C. pneumoniae* with respiratory tract infections in children have been published so far. These studies have been performed in Gambia, Sudan, Thailand, the Philippines, Sweden, Finland, Germany, Switzerland, USA, and Chile (Forgie *et al.*, 1991; Heiskanen-Kosma *et al.*, 1999; Herrmann *et al.*, 1994; Likitnukul *et al.*, 2003; Lund-Olsen *et al.*, 1994; Saikku *et al.*, 1988; Tagle *et al.*, 2000), with divergent and sometimes equivocal results.

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The aim of our study was to investigate the presence of specific *C. pneumoniae* IgM, IgG and IgA antibodies in Polish children with respiratory tract and other diseases, aged 1–36 months.

## Experimental

### Materials and Methods

**Patients and specimens.** A randomly selected group of 150 children aged from 1 week to 36 months admitted to the Department of Pediatrics and Pediatric Nephrology, Medical University of Warsaw in 1995–2003, with clinical symptoms of respiratory tract infections or other diseases was studied. The study group included 23 newborns (age 1–4 weeks), 75 infants (age 5 weeks–12 months) and 52 children age 13–36 months. Sixty-seven girls and 83 boys were enrolled. Patients originated from the Mazowsze district – in central part of Poland. Diagnoses included recurrent respiratory tract infections in 37 patients, pneumonia in 28 patients, bronchitis in 28 patients, pharyngitis in 24 patients, laryngitis in two patients, otitis media in four patients, acute diarrhoea in two patients, purulent meningitis in two patients, and urinary tract infection in 14 patients. Nine children were diagnosed with a non-infectious disease, including dietary indiscretion, urinary tract malformation.

Chest roentgenogram was performed in all children with respiratory infections. A diagnosis of pneumonia was based on radiological findings including hyperinflation, prominent bronchovascular markings or diffuse interstitial and patchy alveolar infiltrates.

A diagnosis of bronchitis was based on clinical findings including wheezing, cough, dyspnea or tachypnea and normal chest radiograph. Children with more than six incidents of upper or lower respiratory tract infections within a year were categorized as having recurrent respiratory tract infections.

Blood samples were taken from each patient prior to administration of antibiotic treatment.

Laboratory tests included: leukocyte count and differential white blood cell count, CRP and serological tests.

**Serology.** Anti-chlamydial IgM, IgG and IgA antibodies were determined by enzyme-linked immunoassay (ELISA), with a major outer membrane protein as an antigen. For IgM antibodies, *Chlamydia pneumoniae* IgM ELISA (Viracell, Granada, Spain) test was used. This test has 91% sensitivity and 98% specificity (Numazaki *et al.*, 1996; Gutierrez *et al.*, 2002). For IgG and IgA, ELEGANCE *Chlamydia pneumoniae* IgG & IgA ELISA (Bioclone, Sydney, Australia) tests were used. The ELEGANCE *Chlamydia pneumoniae* ELISA test has 71.7% sensitivity and 95.8% specificity for IgG and 73.9% sensitivity and 92.9% specificity for IgA (Kishimoto, 1990; Kishimoto, 1996; Ekman, 1993). Presence of IgM antibodies was regarded as an indication of acute infection. The presence of *C. pneumoniae* IgG antibodies in children aged up to 12 months was assumed to be maternal origin.

## Results

Among 150 tested children, *C. pneumoniae* IgM and IgG antibodies were detected in sera of 18 (12%) and 12 (8%), respectively.

In children with symptoms of a respiratory tract infection, specific serum *C. pneumoniae* IgM antibodies were detected in 16 (13.0%) of 123 patients. Specific IgG antibodies were found only in sera of 12 children younger than 12 months. Specific IgG antibodies were not detected in children with specific *C. pneumoniae* IgM. Specific IgA antibodies were not found in any of 150 tested children.

Acute *C. pneumoniae* infection was detected in patients with various respiratory tract diseases (Table I). Most commonly, it was found in children with bronchitis – 32.1% (9/28), including 77.7% (7/9) of children with wheezing. *C. pneumoniae* infections were also detected in 10.8% (4/37) of children with recurrent respiratory tract infections, 8.3% (2/24) of children with pharyngitis, and in one child (out of two) with laryngitis. *C. pneumoniae* infection was detected neither in patients with otitis media (0/4) nor in patients with pneumonia (0/26). Among 27 children with a non-respiratory illness, specific serum *C. pneumoniae* IgM antibodies were found in two (7.4%) patients, including one child with meningitis and another one with urinary tract infection. Specific IgM and IgG antibodies were not detected in children with non-infectious diseases and in children with acute diarrhoea (Table I).

Serological evidence of *C. pneumoniae* infection was found in 6.3% of infants, 13.5% of children aged 1–2 years and in 46.7% of those aged 2–3 years. Specific *C. pneumoniae* antibodies were found more commonly in girls (12/67; 17.9%) than in boys (6/83; 7.2%).

Only three children with *C. pneumoniae* infection presented fever, including a 10-month-old infant with pharyngitis, a 16-month-old child with bronchitis complicated by seizures and a 9-month-old infant with purulent streptococcal meningitis. In children with acute *C. pneumoniae* infection, average leukocyte count in peripheral blood was  $12\ 100/\text{mm}^3$  with range 7 000–27 000 (normal values 5 000–17 500/ $\text{mm}^3$ ). Leukocytosis of  $27\ 000/\text{mm}^3$  was found in a 22-month-old girl with acute bronchitis. Differential white blood cell count showed predominant lymphocytes from 51 to 80% in 8 children in this group, CRP level was slightly elevated and averaged 0.6 with range 0.01–0.8 mg/dL (normal level <0.01 mg/dL). Among the remaining children with symptoms of a respiratory tract infection and no specific *C. pneumoniae* IgM antibodies, 23 children presented fever, leukocyte count ranged from 5 000 to 24 000 and averaged  $11\ 600/\text{mm}^3$ , again

Table I  
Prevalence of *C. pneumoniae* serum antibodies in 1–36 months old children with various diseases

Disease	No tested	No seropositives <sup>1</sup>		No (%) <i>C. pneumoniae</i> infections <sup>***</sup>
		IgM	IgG <sup>2</sup>	
Recurrent RTI *	37	4	0	4 (10.8)
Bronchitis	28	9	0	9 (32.1)
Pneumonia	28	0	6	0
Pharyngitis	24	2	5	2 (8.3)
Otitis media	4	0	1	0
Laryngitis	2	1	0	1
Meningitis	2	1	0	1
UTI **	14	1	0	1 (7.1)
Acute diarrhoea	2	0	0	0
Non-infectious diseases	9	0	0	0

\* RTI – respiratory tract infections, \*\* UTI – urinary tract infections, \*\*\* The presence of specific *C. pneumoniae* IgM antibodies was considered an acute infection, # Specific *C. pneumoniae* IgA antibodies were not detected in any tested serum, <sup>1</sup> Specific *C. pneumoniae* IgG antibodies were only detected in children under 12 months of age and were assumed to be maternal origin.

with differential white blood cell count showing predominant lymphocytes from 51 to 75% in 33 children, and CRP averaged 0.5 mg/dL.

Serological testing revealed that all cases of *C. pneumoniae* infections occurred within only two out of eight analyzed years (Figure 1). In 1997, specific *C. pneumoniae* antibodies were detected in 4 of 16 tested children (25.0%), and in 2000, 14 (25.0%) of 56 examined children were seropositive. In contrast, negative results were obtained in serum samples collected in other years (1995, 1996, 1998, 1999, 2001 and 2002) in all 78 tested children. In addition, it appears that the incidence of *C. pneumoniae* infections varies seasonally. Cases were detected mostly in November (1997 – 2/4, 2000 – 9/14), October (2000 – 2/14) and December

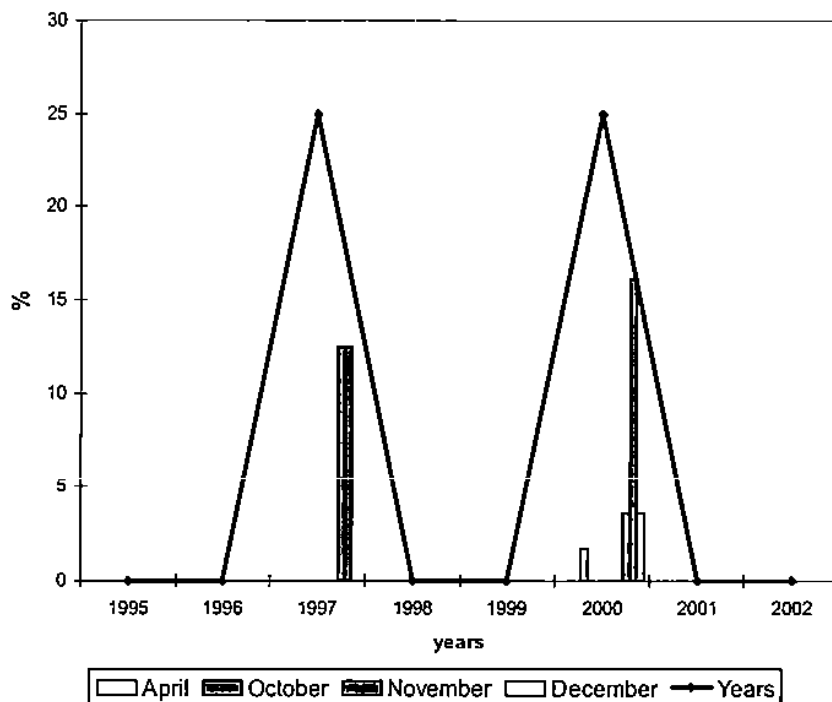


Fig. 1. *C. pneumoniae* infections by years and months

\* Specific *C. pneumoniae* antibodies were not detected in any child in January, February, March, May, June, July, August and September

(1997– 2/4, 2000 – 2/14). Among children tested in April, there was one child with *C. pneumoniae* infection. Specific *C. pneumoniae* antibodies were not found in serum samples collected in January, February, March, May, June, July, August and September.

### Discussion

Among children younger than 3 years old with respiratory tract diseases, acute *C. pneumoniae* infections were detected in 13.0% of them. The youngest patient with *C. pneumoniae* infection detected serologically was one-month-old. The frequency of *C. pneumoniae* etiology in respiratory tract infections in pediatric populations varies from 0% up to more than 18% (Likitnukul *et al.*, 2003; Lund-Olsen *et al.*, 1994; Saikku *et al.*, 1988; Tagle *et al.*, 2000; Hammerschlag *et al.*, 2003; Schmidt *et al.*, 2002; Baer *et al.*, 2003; Chirgwin *et al.*, 1991; Block *et al.*, 1995). These differences are probably related to the population studied and the presence or absence of local outbreaks in the respective communities during the investigation period as well as to applied specific diagnostic procedures (Hammerschlag *et al.*, 2003).

It is well known that *C. pneumoniae* infections spread as long-lasting epidemics that occur at irregular intervals of several years' duration (Grayston, 2000; Kuo *et al.*, 1995). There is a paucity of data on respiratory tract infections caused by *C. pneumoniae* in children in Poland. Similarly, there are no data on *C. pneumoniae* epidemics in Poland. Our results may suggest that such epidemics occurred in 1997 and 2000. Additionally, a seasonal trend for *C. pneumoniae* cases throughout a year was observed, with a peak of incidence in autumn. These results are in accordance with studies by T. Heiskanen-Kosma *et al.* who observed incidence peak of *C. pneumoniae* infections in October (Heiskanen-Kosma *et al.*, 1998).

Specific *C. pneumoniae* antibodies have been detected most frequently among children aged 2–3 years. Serological evidence of *C. pneumoniae* infection was found in 46.7% children in this age group. Majority of cases were associated with recurrent respiratory tract infections. *C. pneumoniae* antibodies were also detected in children without symptoms of respiratory tract diseases: a 9-month-old girl with meningitis and in a 3-month-old boy with a urinary tract infection. Detection of specific *C. pneumoniae* antibodies, in particular IgM class, in children without respiratory tract infections suggest the occurrence of asymptomatic chlamydial infections in early childhood. In Finland, it was shown that 4.0 to 6.0% of 2–4 years old healthy children were seropositive. It confirms probability of the occurrence of asymptomatic *C. pneumoniae* infections in this age group (Tuuminen *et al.*, 2000).

The significance of asymptomatic *C. pneumoniae* infections and their sequelae requires further investigations. In adult populations, *C. pneumoniae* antibodies are present with significantly higher frequency in men than in women. This is, however, at variance with our results in a group of children aged 1–36 months. *C. pneumoniae* antibodies were detected more often in girls than in boys.

These results are in accordance with a study by Lin *et al.* who found higher prevalence of *C. pneumoniae* antibodies in females than in males among subjects aged 6 months to 20 years (Lin *et al.*, 2004).

Most of the seroepidemiological studies on *C. pneumoniae* infections in children were performed with the MIF method. According to the recent recommendations of Centers for Disease Control and Prevention (USA) and the Laboratory Centre for Disease Control (Canada) (Dowell *et al.*, 2001) this technique is considered a reference method, although there are studies showing low sensitivity of this method in children (Kutlin *et al.*, 1998).

Simultaneous application of MIF and culture in the examination of children shows that MIF is not a sensitive technique for detecting *C. pneumoniae* antibodies in children under 5 years old. Since positive culture results were achieved in seronegative children. According to some studies only 20% to 30% of children with culture-documented *C. pneumoniae* infection had antibodies detectable by MIF (Schmidt *et al.*, 2002; Hyman *et al.*, 1995; Emre *et al.*, 1994). Kutlin *et al.* found that over 80% of culture-positive and MIF-negative children had antibodies to *C. pneumoniae* detected by immunoblotting (Kutlin *et al.*, 1998).

The lack of standardized methods, including serology and PCR, makes it difficult to perform a specific microbiologic diagnosis. Although there is currently no validated diagnostic marker for *C. pneumoniae* infections, and the value of the EIA method in diagnosis *C. pneumoniae* infections is an open issue, detection of *C. pneumoniae* IgM antibodies in several children below 3 years of age may suggest that *C. pneumoniae* infections occur in early childhood. Moreover, frequent detection of *C. pneumoniae* antibodies in children with respiratory tract infections suggests that the bacterium may be an etiological agent of respiratory diseases in infants and young children.

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