

ORIGINAL ARTICLE

A population based seroepidemiological survey of *Chlamydia pneumoniae* infections in schoolchildren

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Aim: A serosurvey was carried out in schoolchildren from a northeastern area of Italy to define the burden of *Chlamydia pneumoniae* infection.**Methods:** A sample of 649 schoolchildren underwent a simplified version of the International Study of Asthma and Allergies in Childhood questionnaire and IgG and IgA antibodies were investigated using an enzyme immunoassay, followed by a microimmunofluorescence assay in reactive sera.**Results:** Of the children examined, 29% and 19.7% had IgG and IgA antibodies, respectively. The IgG prevalence increased with age. No other sociodemographical variable was related to *C pneumoniae* infection. An association was established between IgA prevalence and previous otitis media.**Conclusions:** A mesoendemic (intermediate between high and low endemic level) pattern of *C pneumoniae* infection is present in schoolchildren from this area and the prevalence rate is related to age. Moreover, this is the first epidemiological evidence of the role of *C pneumoniae* in otitis.

The species of the chlamydia genus, *Chlamydia pneumoniae*, which is clearly distinct from *C trachomatis* and *C psittaci*, was established as an important respiratory pathogen at the beginning of the 1980s.¹ Most infections caused by *C pneumoniae* are mild or asymptomatic. When a clinical infection occurs, a wide spectrum of respiratory disorders is reported, including upper respiratory tract infections, bronchitis, and pneumonia.^{2–3}

A particular feature of *C pneumoniae* is its ability to lead to longlasting or chronic infections. The persistence of *C pneumoniae* in the respiratory tract may play an important role in chronic diseases, such as chronic obstructive pulmonary disease and asthma.^{4–5} In recent years, two additional disorders have been associated with *C pneumoniae* infection; namely, otitis media and cardiovascular disease, although the importance of this last association remains to be clarified.^{6–7}

"A particular feature of *C pneumoniae* is its ability to lead to longlasting or chronic infections"

Seroepidemiology showed that almost all populations experience infection/re-infection during their life. The primary infection mostly occurs in children, in particular in schoolchildren.^{2–10} Therefore, this age group would be the most suitable for establishing the burden of *C pneumoniae* infection, identifying risk factors or protective variables, and defining the role of *C pneumoniae* in the main respiratory disorders during childhood. With these aims, we carried out this study on a sample of schoolchildren from an urban area of northeastern Italy.

METHODS

Population

The survey was carried out on stored sera collected during 1999 and 2000 from children attending 17 primary schools in Trieste (3665 children aged 5–12 years, representing 87% of that reference population) for a screening of coeliac disease: the survey was approved by the local ethics committee. For our present study, 665 children were selected by means of a cluster sampling procedure, using all the

classes, stratified according to the eight school years, as units of randomisation.

The parents of the selected children were asked to give their consent to the serological test for *C pneumoniae* to be carried out on the blood samples so far collected and to answer a simplified version of the standard ISAAC (International Study of Asthma and Allergies in Childhood) questionnaire. All but 16 (97.6% of eligible individuals) agreed. The questionnaire was administered by a trained interviewer; the items regarded personal factors (familiarity for allergy, breast feeding, crèche attendance, kindergarten attendance, number of brothers, external tobacco smoke) and previous diseases (diagnosis of sinusitis, otitis, or pneumonia in the previous 12 months; allergic rhinitis/conjunctivitis in the previous 12 months; hospital admission for otitis, pneumonia, or asthma; ever asthma, defined as asthma diagnosed by a physician during life).

Serology

A two step procedure was performed to evaluate seroprevalence: (1) an initial screening of IgG and IgA antibodies using an enzyme immunoassay (EIA) method; and (2) a microimmunofluorescence (MIF) test for IgG and IgA, performed on sera found to be reactive in the EIA test.

Screening of *C pneumoniae* specific antibodies was performed with a commercial EIA (Elegance *C pneumoniae* IgG/IgA enzyme linked immunosorbent assay; Bioclone Australia Pty Limited, Marrickville, Australia). The antigen coated on the solid phase is made of a highly purified *C pneumoniae* specific outer membrane protein. The optical density was measured at 405 nm and the results were expressed and assessed as index values. In our experience (data not shown), no false negative results occurred when the index value cutoff was established at 0.6. Accordingly, sera with an index value ≥ 0.6 in the EIA were submitted to the MIF confirmatory test. For the MIF assay, a commercial kit was used (chlamydia MIF; MRL Diagnostic, Cypress, California, USA), according to the manufacturer's instructions. IgG quantitative evaluation was done starting from a serum

Abbreviations: CI, confidence interval; EIA, enzyme immunoassay; MIF, microimmunofluorescence; OR, odds ratio

Table 1 Age and sex composition of the sample compared with the reference population

Age	Sample					Population				
	Boys		Girls		Total	Boys		Girls		Total
	N	%	N	%		N	%	N	%	
≤6	37	53.6	32	46.4	69	726	52.6	653	47.4	1379
7	69	58.0	50	42.0	119	729	49.9	733	50.1	1462
8	53	47.7	58	52.3	111	759	52.7	681	47.3	1440
9	65	56.0	51	44.0	116	820	51.5	773	48.5	1593
10	68	57.6	50	42.4	118	787	51.7	735	48.3	1522
≥11	66	57.0	50	43.0	116	720	48.5	766	51.5	1486
Total	358	55.2	291	44.8	649	4541	51.1	4341	48.9	8882

dilution of 1/20, and the final titre was expressed as a reciprocal. IgA was assessed by MIF only in the starting dilution and expressed as a positive or negative result.

Statistical analysis

The data are presented as frequency, geometric mean of serum titres, and median. The differences in proportions were tested with the χ^2 test or Fisher's exact test, when requested by the lowest expected frequency. The linear trend was tested with Pearson's correlation test. The differences between means were evaluated using the Student's *t* test. The data were analysed using SPSS 10.0 (SPSS Inc, Chicago, Illinois, USA).

RESULTS

The sample resulting from the cluster sampling method was compared with the reference population for age and sex (table 1), and the frequency distribution for the two variables was similar in the two groups.

Table 2 shows the seroprevalence data, according to age and sex. Overall, 29% and 19.7% of schoolchildren had antibodies belonging to the IgG and IgA classes, respectively; all IgA positive (128) children were also IgG positive.

The seroprevalence rates did not differ significantly according to sex. The prevalence rates seemed to increase with age, but the test for linear trend was not significant, probably owing to the narrow age range. However, subdividing the sample by the median (9 years), the difference in IgG prevalence was significant (25.3% in the youngest *v* 32.4% in the others; odds ratio (OR), 1.41; 95% confidence interval, 1.005 to 1.992; *p* = 0.046).

The IgG and IgA prevalence rates and the IgG geometric mean titres were analysed according to some personal and social variables (data not shown).

Table 2 Prevalence of *Chlamydia pneumoniae* antibodies by sex and age

Variable (N)	IgG positive		p Value	IgA positive		p Value
	N	%		N	(%)	
Sex						
Boys (358)	102	28.5	NS	64	17.9	NS
Females (291)	86	29.6		64	22.0	
Age						
≤6 (69)	16	23.2	NS	10	14.5	NS
7 (119)	29	24.4		19	16.0	
8 (111)	30	27.0		25	22.5	
9 (116)	44	37.9		26	22.4	
10 (118)	36	30.5		22	18.6	
>11 (116)	33	28.4		26	22.4	
Total	188	29.0		128	19.7	

Neither breast feeding, nor a family history of allergy correlated with the *C pneumoniae* immune response. Day nursery attendance (children from 6 months to 2 years) did not seem to affect the prevalence; kindergarten attendance (children 3–5 years) was not investigated because almost all children had this exposure. The environmental factors explored—external tobacco smoke and number of siblings—did not correlate with *C pneumoniae* antibodies. With regard to external tobacco smoke, IgG and IgA reactivity was higher in exposed children, but the relation was not significant. Social conditions, evaluated by the parents' schooling level, were not significantly associated with *C pneumoniae* infection.

In table 3, the positivity of *C pneumoniae* antibodies is analysed in relation to certain respiratory disorders.

No association was found with rhinitis/conjunctivitis, sinusitis, pneumonia, or asthma (defined as history of hospitalisation for asthma or ever asthma). However, otitis seemed to be positively associated with *C pneumoniae*. The prevalence of IgG and the geometric mean of IgG titres were substantially higher in children with previous otitis, albeit in a non-significant way. Nevertheless, a trend towards significance seemed to appear when the prevalence of IgG was compared in the two groups, because the OR was 1.6 with a 95% CI of 0.9 to 2.9 (*p* = 0.07). In addition, IgA prevalence was significantly associated with this disease (OR, 1.81; 95% CI, 1.00 to 3.37; *p* = 0.05).

Table 3 *Chlamydia pneumoniae* seroprevalence and respiratory disorders

Variable (N)	IgG positive			IgA positive	
	N	%	GM	N	(%)
Allergic rhinitis/conjunctivitis					
Yes (53)	19	35.8	5.93	14	26.4
No (596)	169	28.4	4.01	114	19.1
Sinusitis					
Yes (18)	5	27.8	4.09	0	—
No (631)	183	29.0	4.14	128	20.3
Otitis					
Yes (54)	20	37.0	6.23	16*	29.6
No (595)	168	28.2	3.97	112*	18.8
Pneumonia					
Yes (7)	1	14.3	2.08	1	14.3
No (642)	187	29.1	4.19	127	19.8
Hospitalisation for asthma					
Yes (12)	1	8.3	1.45	1	8.3
No (637)	187	29.4	4.99	127	19.9
Ever asthma					
Yes (47)	8	17.0	2.36	5	10.6
No (602)	180	29.9	4.35	123	20.4

*Odds ratio, 1.81; 95% confidence interval, 1.00 to 3.37; *p* = 0.05. GM, geometric mean of IgG titres.

DISCUSSION

This is one of the largest population based, cross sectional surveys performed to date on the prevalence of *C pneumoniae* in children. About 7.5% of the reference population living in an urban area of northeastern Italy was screened for *C pneumoniae* antibodies. We believe that the sampling method, coupled with the very low refusal rate among eligible subjects, led to the enrolment of an unbiased group of children. Overall, 29% of the studied subjects tested positive for *C pneumoniae* IgG antibodies. Therefore, the pattern of *C pneumoniae* infection in this area may be defined as mesoendemic, intermediate between the low prevalence rates recorded in North America and northern Europe, but substantially lower than those reported in series from Taiwan, Japan, Spain and in general from tropical areas.^{2 8 11–17} In addition, our data agree with two previous reports from Italy.^{18 19}

“An IgG antibody prevalence rate of about 23% in the first age class is consistent with an early exposure to *Chlamydia pneumoniae* infection in the community”

The reasons for these wide geographical differences are poorly understood. It has been claimed that seroprevalence rates are related to population density²⁰; however, crowding and hygienic conditions could be more relevant. In this respect, our study revealed no clear exposure (or protective factor) that could be related to *C pneumoniae* antibody prevalence, except for age. An increase in seroprevalence was evident when comparing the groups under and over the median, thus underlining the importance of school settings in favouring *C pneumoniae* transmission. Nevertheless, an IgG antibody prevalence rate of about 23% in the first age class is consistent with an early exposure to *C pneumoniae* infection in the community.²¹ We found that the seroprevalence was not related to crèche attendance, family crowding, social status, or external tobacco smoke. Collectively, our data show that several social and environmental conditions are not related to the burden of *C pneumoniae* infection in this area, and that only strong differences in exposure could translate into substantial differences in seroprevalence.

The role of *C pneumoniae* in child pneumonia has been well established in prospective studies carried out in clinical settings, although variation in rates, ranging from a few cases to 10%, were reported.^{22–25} In this population based study, the rarity of this disease made the association impossible to assess.

The role of *C pneumoniae* in asthma is still debatable, at least in some groups of patients. According to our study, primary infections occurring in children may not be important for asthma onset, although in adults *C pneumoniae* infections can act as a trigger for an already existing disorder.^{26–28}

Take home messages

- We carried out a seroprevalence survey in school-children from northeastern Italy to define the burden of *Chlamydia pneumoniae* infection
- A mesoendemic (intermediate between high and low endemic level) pattern of *C pneumoniae* infection was found and the prevalence rate was related to age
- Our study provided the first epidemiological evidence that *C pneumoniae* is involved in otitis media

The role of *C pneumoniae* infection in otitis media (acute otitis media or secretory otitis media) has been defined in recent years. The presence of *C pneumoniae* in fluid from the middle ear was repeatedly confirmed, in spite of a single large series with negative results (D Davis *et al.* Failure to detect *Chlamydia pneumoniae* in middle ear aspirates from patients with otitis media with effusion [abstract]. Proceedings of the 97th general meeting of the American Society for Microbiology, Miami Beach, Florida, USA, 4–8 May 1997:C-351).^{29 30} The involvement of *C pneumoniae* in middle ear flogosis has been confirmed by our survey. IgG prevalence and a high IgG titre, expressed as a geometric mean, showed a trend in significance for the outcome, namely otitis media occurring in the past year. Moreover, we found a significant relation between IgA seroprevalence and otitis. IgA reactivity, alone or coupled with high IgG titres, was frequently considered to be an indirect marker of chronic or persistent *C pneumoniae* infection, because of the short half life of this class of immunoglobulin. In our survey, the distinction between acute otitis and chronic/recurrent otitis could not be made: this issue needs to be studied further. Nevertheless, *C pneumoniae* seems to play a role in otitis media. Therefore, this aetiology must be considered in the clinical setting whenever evidence of the most common pathogens is lacking; furthermore, *C pneumoniae* should be suspected as a possible co-pathogen when treatment with a β lactamic proves unsuccessful.

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