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High prevalence of antibodies to *Chlamydia pneumoniae*; determinants of IgG and IgA seropositivity among Jerusalem residents

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SUMMARY

The prevalence of antibodies to *Chlamydia pneumoniae* was examined in a stratified random sample of 581 Jerusalem adult residents between August 1987 and March 1989. IgG and IgA titres were measured by microimmunofluorescence, and associations with smoking and socio-demographic variables were assessed. IgG antibodies were found in 84·5% (95% confidence interval (CI): 80·4–87·9) of men and 68·7% (95% CI: 61·6–75·0) of women ($P < 0·0001$ for sex difference), indicating a very high rate of exposure in this population. IgA antibodies, postulated to represent persistent infection, were present in 45·1% (95% CI: 40·1–50·2) of men and 23% (95% CI: 17·4–29·7) of women ($P < 0.0001$ for sex difference). Factors associated with IgG seropositivity included family size, education and social class. On the other hand, age (in men) and smoking were associated with IgA seropositivity. These findings support the hypothesis that low socioeconomic status and household crowding may be predictive of exposure to or infection with this organism (IgG seropositivity), whereas they do not explain persistence of the infection putatively expressed as IgA seropositivity.

INTRODUCTION

Chlamydia pneumoniae is a recently recognized respiratory tract pathogen found to be responsible for outbreaks of both upper and lower respiratory infection among children and adults. [1–3]. Although the association of this organism with respiratory infection was described in 1985 [4], analysis of stored sera indicates a high prevalence of antibodies to this organism among residents of Finland as early as 1958 [5]. Serological studies from Seattle [6, 7] have suggested a general pattern for *C. pneumoniae* seroprevalence in Western countries: low prevalence in childhood up to 5 years of age with rapid acquisition at school age. After age 20 about 50% of adults have antibodies, men more frequently than women.

Besides the respiratory infection caused by this organism, interest has been focussed on a possible association with cardiac disease, including peri-, myo- and endocarditis [8–10]. Further research has suggested an association between *C.*

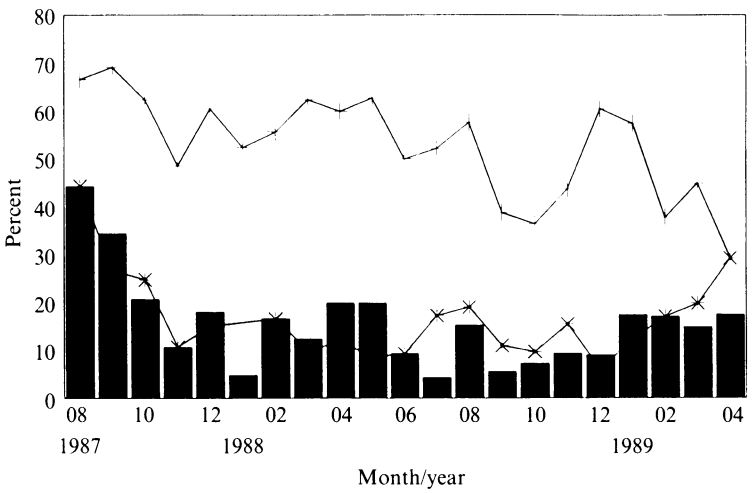

Fig. 1. *Chlamydia pneumoniae* seropositivity by month, Jerusalem residents (Both sexes). Titres: Solid bars IgG $\geqslant$ 512**, crosses IgG $\geqslant$ 128*, asterisks IgA $\geqslant$ 80. *P = 0·006, **P = 0·08 by Mantel-Haenszel for linear association.

pneumoniae infection and coronary artery disease [11–15]. The seroprevalence of this infection was heretofore unknown in Israel. We therefore undertook a study of the seroepidemiology of *C. pneumoniae* among a representative sample of the population of Jerusalem.

MATERIALS AND METHODS

Study subjects (410 men and 207 women) were Jewish residents of Jerusalem aged 25–64 who were randomly selected from the Israel Population Registry and consented to enter the study. The overall response rate was 83%. The sample was stratified by age and sex and sampled to provide heavier weights to older age groups and to men [16]. Women within 6 months of termination of pregnancy (live birth or other) as well as homebound or institutionalized individuals were excluded. The subjects were invited for an interview, physical examination and blood drawing between August 1987 and March 1989. The interview included a questionnaire concerning education, family size, degree of religious observance, country of origin and occupation as well as personal habits including smoking. For this study, age was categorized by decades; smoking status was studied in terms of smokers versus non-smokers or categorized by level of smoking, i.e. 0, 1–19, and 20+ cigarettes per day, as well as by former or current or never smoked; and education was stratified according to completion of primary, secondary or post-secondary education. Social class was determined according to an Israeli modification of the British Registrar General's method [17].

Chlamydia serology was performed on 386 men and 195 women. Sera for the measurement of chlamydial antibodies were stored for 2–5 years at −20 °C until shipment of coded specimens on dry ice to Finland where testing was performed. Antibodies to *C. pneumoniae* using the TWAR (AR39 Washington Research

Table 1. *Sex-specific prevalence of antibodies to* Chlamydia pneumoniae *among Jerusalem residents 1987–9*

Antibody type	Titre	Prevalence in men (%) ($N = 386$)	Prevalence in women (%) ($N = 195$)	
C. pneumoniae IgG	⩽ 16	15·5 (17·1)	31·3 (29·7)	χ^2, 2 D.F. = 30·8, $P < 0·0001$
	32–64	23·6 (24·2)	30·8 (32·3)	
	⩾ 128	60·9 (58·6)	37·9 (37·9)	
C. pneumoniae IgA	< 20	54·9 (55·7)	76·9 (77·2)	χ^2, 2 D.F. = 30.1, $P < 0·0001$
	20–40	26·2 (25·8)	17·4 (14·9)	
	⩾ 80	18·9 (14·9)	5·6 (6·1)	

Percentages in parentheses are age-weighted to world standard population for ages 35–64. Weights are 12/31 (age 35–44); 11/31 (age 45–54); 8/31 (age 55–64).
The χ^2 tests are for differences in unadjusted rates between men and women.

Foundation) antigen were measured in a simplified microimmunofluorescence test as previously described [11]. IgA titres were measured after neutralization of IgG with Gullsorb (Gull Laboratories, Salt Lake City, Utah) treatment as previously described [18]. Pooled antigens of *C. trachomatis* (B–E–D, C–H–I–J, G–F–K, Washington Research Foundation, Seattle) and *C. psittaci* (6BC, OA, Slovak Academy of Sciences, Bratislava) were used as controls.

Titres for *C. pneumoniae* IgG were considered 'low' if they were measured at ⩽ 16, 'medium' if they were between 32 and 64, and 'high' if they were ⩾ 128. Corresponding IgA cut-offs were ⩽ 10, 20–40 and ⩾ 80.

The data were analysed using SPSS version 4·1 for VAX/VMS (SPSS Inc, Chicago, Illinois). Differences in proportions were tested using χ^2 tests for contingency tables. Linear associations for ordinal variables were tested by the Mantel Haenszel procedure. Multiple regression was used to assess the association of smoking with log transformed IgG and IgA titres controlling for age and other factors. The sex-specific prevalence proportions of IgG and IgA antibodies in the Jerusalem population were weighted according to the world standard population for ages 35–64 to facilitate comparison with other studies. The weights used were 12/31 (for age 35–44), 11/31 (for ages 45–64) and 8/31 (for ages 55–64), as in previous studies [19].

RESULTS

Antibody prevalence

Antibodies to *C. pneumoniae* were highly prevalent in this population: 79% (95% confidence interval (CI): 75·4–82·2) were seropositive with IgG titres greater or equal to 32 and 53% (95% CI: 48·9–57·8) of the sample had elevated IgG titres of ⩾ 128. There was significant variation over the duration of the study (Fig. 1), with a borderline significant decrease in log IgG titres over the study period ($P = 0·08$ for linear trend). No evidence of seasonality was observed, in that parallel seasons in different years did not show similar patterns of seroprevalence.

The prevalence of positive IgA titres was lower than IgG seropositivity (Table

Table 2. *Chlamydia antibodies and sociodemographic variables among Jerusalem residents 1987–9*

	Titres in men						Titres in women					
	IgG			IgA			IgG			IgA		
Variable	Number	⩽ 16	32–64	⩾ 128	< 20	20–40	⩾ 80	Number	⩽ 16	32–64	⩾ 128	< 20	20–40	⩾ 80
		60	91	235	212	100	73		61	60	74	150	34	11
Age (%)														
25–34	28	17·9	21·4	60·7	75·0	14·3	10·7	8	50·0	25·0	25·0	75·0	25·0	0
35–44	71	21·1	25·4	53·5	64·8	22·5	12·7	30	26·7	36·7	36·7	76·7	16·7	6·7
45–54	102	15·7	23·5	60·8	49·0	27·5	23·5	55	32·7	29·1	38·2	78·2	16·4	5·5
55–64	185	13·0	23·2	63·8	51·4	28·6	20·0	102	30·4	30·4	39·2	76·5	17·0	5·9
		$MH\chi^2 = 2·9$, $P = 0·14$			$MH\chi^2 = 5·87$, P 0·02				$MH\chi^2 = 0·38$, $P = 0·54$			$MH\chi^2 = 0·01$, $P = 0·89$		
Smoking (%)														
Non-smokers	247	15·8	25·5	58·7	58·7	22·7	18·6	163	33·1	29·4	37·4	79·8	15·3	4·9
< 20 cigarettes/day	58	20·7	19·0	60·3	62·1	25·9	12·1	21	19·0	42·9	38·1	66·7	28·6	4·8
20+ cigarettes/day	81	11·1	21·0	67·9	38·3	37·0	24·7	10	30·0	30·0	40·0	60·0	20·0	20·0
		$MH\chi^2 = 1·62$, $P = 0·12$			$MH\chi^2 = 5·09$, $P = 0·02$				$MH\chi^2 = 0·33$, $P = 0·56$			$MH\chi^2 = 4·21$, $P = 0·04$		
Education (%)														
Primary school	116	7·8	28·4	63·8	50·0	25·9	24·1	66	25·8	33·3	40·9	74·2	21·2	4·5
Incomplete High School	103	16·5	20·4	63·1	60·2	25·7	14·7	41	29·3	24·4	46·3	80·5	7·3	12·2
High School Matriculation	71	12·7	22·5	64·8	57·7	17·8	16·9	53	30·2	34·0	35·8	77·4	17·0	5·7
University Degree	96	26·0	21·9	52·9	53·1	26·7	18·8	35	45·7	28·6	25·7	77·1	22·9	0
		$MH\chi^2 = 6·91$, $P = 0·01$			$MH\chi^2 = 0·46$, $P = 0·5$				$MH\chi^2 = 3·67$, $P = 0·06$			$MH\chi^2 = 0·37$, $P = 0·54$		
Family size (%)														
1–3 Siblings	124	20·2	24·2	55·6	56·5	25·8	17·7	65	40·0	32·3	27·7	83·1	7·7	9·2
4–5 Siblings	80	21·3	21·3	57·5	55·0	25·0	20·0	52	32·7	30·8	36·5	75·0	21·2	3·8
6+ Siblings	181	9·9	24·3	65·7	54·1	26·5	19·3	78	23·1	29·5	47·4	73·1	23·1	3·8
		$MH\chi^2 = 5·87$, $P = 0·02$			$MH\chi^2 = 0·17$, $P = 0·64$				$MH\chi^2 = 6·94$, $P = 0·008$			$MH\chi^2 = 0·21$, $P = 0·63$		

$MH\chi^2$, Mantel Haenszel Chi square for linear association.

1). There was a significant correlation between IgG and IgA titres (Pearson $r = 0{\cdot}61$, $P < 0{\cdot}0001$ in men and $r = 0{\cdot}51$, $P < 0{\cdot}0001$ in women). Despite this correlation, predictors of elevated IgA and IgG serology differed as described below.

Relationships between chlamydial antibodies and socio-demographic variables and smoking:

Sex: Table 1 shows the sex-specific prevalence of antibodies to *C. pneumoniae*. Antibodies to *C. pneumoniae*, whether IgG or IgA, were significantly more prevalent among men than women. Among the seropositive, men also had substantially higher titres than women (Table 1).

Age (Table 2): The presence of IgA antibodies among males was associated with increasing age but there was no such association among women. No age effect was seen in the prevalence of IgG antibodies in either sex.

Smoking (Table 2): There was a positive relationship between current smoking and the presence of elevated IgA titres to *C. pneumoniae* among men, particularly with heavy smoking. No difference was observed between never smokers and past smokers. After controlling for age, smoking 20 or more cigarettes per day remained highly associated ($P = 0{\cdot}002$) with elevated log IgA titres. Among women, a trend was observed between smoking and elevated IgA titres ($P = 0{\cdot}04$). IgG antibody levels were not associated with smoking in either sex.

Education (Table 2): In men, the prevalence of high titre IgG antibodies was inversely related to educational level ($P < 0{\cdot}01$) with highest titres evident among those with only a primary school education. In women a similar trend was seen but was of borderline significance ($P = 0{\cdot}06$). No association between IgA titre and educational level was observed.

Family size (Table 2): In both sexes, those with larger sibships had a higher prevalence of elevated IgG, but not IgA titres.

Place of origin (data not shown): Among both men and women, those born in Asia were more likely to be IgG seropositive, although the findings were not statistically significant. No association between IgA serology and place of origin was seen.

Social class (occupational status) (data not shown): Among women, high titres of IgG antibody were significantly more common among women in the lowest socioeconomic group ($\chi^2 = 7{\cdot}64$, $P = 0{\cdot}015$), but no such association was observed for men. There was no association between IgA serology and social class.

DISCUSSION

The prevalence of *C. pneumoniae* antibodies varies across populations. This study demonstrates that exposure to *C. pneumoniae* is very common in the Israeli context. In fact, the prevalance of high titres of IgG antibodies ($\geqslant 128$) to this organism was higher in this Jerusalem sample than in Scandinavian countries [2, 11] and Northwestern USA [20]. One possible explanation for the unequal prevalence observed between populations is population density [21] which has been proposed as an explanation for the high seroprevalence observed in Asia [22]; however, the prevalence documented in this urban Jerusalem sample is even

higher than that reported from a Japanese urban sample, where presumably the population density is considerably higher. On the other hand we have shown an association with social class and sibship size supporting increased transmission in crowded households.

Epidemics of *C. pneumoniae* have been documented in Scandinavia especially in the military [23, 24]. Serological samples from earlier or later years may help to clarify whether the high seroprevalence observed in this study particularly at the onset of the study period reflects the usual level of exposure to this organism in this population. A fourfold rise in titre or a level of > 512 of IgG indicate recent infection [1]. In our study we did not have paired samples, however we found very high IgG titres (i.e. > 512) in 12·7% (95% CI: 10·2–15·8) of our study sample (see Fig. 1). The higher prevalence of extremely high IgG titres (> 512) at the beginning of the study points to the possibility that an epidemic of *C. pneumoniae* occurred in the Jerusalem area a short time prior to the initiation of the study. A borderline significant decrease ($P = 0{\cdot}08$) in log IgG titre was noted over the study period, a finding which was not altered after adjustment for smoking, age, family size or educational level.

Marked sex differences in the prevalence of antibodies to *C. pneumoniae* with an excess of seropositivity among men were observed in our sample, as previously reported in European and American populations [20, 25], but not in Japan [22]. Higher titres of IgG and IgA antibodies were observed in men of all ethnic subgroups (i.e. birthplace Asia, Africa, Europe or Israel) of our population. Although the levels of IgG titre correlated well with IgA seropositivity, the factors associated with IgA seropositivity appear to differ from the predictors of an IgG response. It is generally accepted that IgG antibodies reflect past infection or exposure to the organism [1], while IgA levels may reflect persistence or chronicity of the infection [2, 12].

The factors which are associated with elevated IgG titres in the Jewish population of Jerusalem appear to be those which are based on social environment, such as country of origin, education, family size and a measure of social class. These factors may also be interpreted as reflecting living conditions such as crowding, which would positively influence the opportunity to acquire a respiratory infection both in early childhood and at later ages. The possibility of other routes of infection has not been excluded. Intrafamilial spread of disease has been shown to be important in other chlamydial infections. For example, Barenfanger [26] found that family members of infants with repetitively positive eye scrapings for trachoma were consistently more likely to be infected than siblings of uninfected infants. On the other hand, recent work by Aldous and colleagues [7] among mainly white, middle class Seattle families with school-aged children, has shown that acute *C. pneumoniae* infections involve more often single rather than multiple family members. In the Jerusalem population, even in families characterized by small sibships, IgG seropositivity was common, reaching 80% in men and 60% in women and pointing to substantial past exposure in all social class groupings among residents of the city.

Hahn and Golujatnikov [27] found an association between elevated (titre ⩾ 128) IgG serology and current smoking, and argued that the reported association between atherosclerosis and *C. pneumoniae* may be confounded by

smoking. We did not find an association between IgG serology and smoking; however, factors associated with elevated IgA titres included age and smoking. Saikku and colleagues [12] have proposed that IgA titres reflect ongoing active or chronic infection with *C. pneumoniae* rather than past exposure and infection as expressed by IgG seropositivity. This hypothesis is based on observations of the IgA response to other viral or bacterial infections. In fact, two groups have shown that in the case of *C. trachomatis* infection, the probability of positive culture is increased among those with positive IgA serology [28, 29]. In addition, IgA levels can be used to measure response to treatment in *C. trachomatis* infection, since they fall after successful treatment while IgG titres remain elevated [30].

The fact that both smoking and age were associated with IgA seropositivity but not with exposure *per se* suggests that these factors may play a role in persistence of infection. The consequences of this persistence may, in fact, include an increased risk of coronary events. In the Helsinki Heart study [12] as well, there was an association between smoking and IgA seropositivity. This latter finding, confirmed in our survey, suggests perhaps that individuals with damaged airways or impaired immune response as a result of smoking, despite being equivalently at risk for exposure to *C. pneumoniae*, are more likely to develop chronic or persistent infection.

In conclusion, we have shown that antibodies to *C. pneumoniae* are frequently found in Jerusalem adults, more so in men than in women, and that the presence of persistent infection (as expressed by elevated IgA titres) is highly prevalent and is associated with age and smoking. Infection and transmission, as reflected by IgG seropositivity are apparently related to family crowding and socioeconomic status. Further epidemiological studies should help to clarify the relationship between smoking and persistent *C. pneumoniae* infection. At the same time more research is required to elucidate whether there are extra-pulmonary sequelae of this infection, such as an elevated risk of coronary heart disease.

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