Epidemiology of *Chlamydia pneumoniae* infection in a randomly selected population in a developed country

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SUMMARY

This cross-sectional study of 400 sera from a randomly selected adult population in Northern Ireland, using a microimmunofluorescence assay, demonstrated high overall seropositivity (70%) for IgG *Chlamydia pneumoniae* antibodies in developed populations. Seropositivity was shown to be unrelated to gender, age or smoking but there was an inverse trend between infection and educational level achieved as a measure of socio-economic status. IgG levels were also higher during the winter months suggesting seasonal variation of *Chlamydia pneumoniae* infection. The high prevalence of evidence of exposure to *Chlamydia pneumoniae* as described in this study may have implications for prevention of cardiovascular disease if further evidence conclusively determines that infection with this organism is a risk factor for cardiovascular disease.

INTRODUCTION

Chlamydia pneumoniae is a Gram-negative obligate intracellular bacterium first described in 1986 [1] as a respiratory pathogen. It is a common cause of bronchitis, sinusitis and pharyngitis and of community-acquired pneumonia [2] accounting for between 6 and 12% of pneumonias in young adults and children [3, 4]. Infection appears to be geographically widespread [2, 5] with individuals possibly being frequently re-infected [6] or harbouring infection for long periods [7]. Recent years have seen much interest in the association between infection with the organism and risk of cardiovascular disease [8–10]. However, little is known about the basic epidemiology of *C. pneumoniae* infection principally because few

studies have examined representative population samples. We present a study of the distribution and socio-demographic correlates of *C. pneumoniae* infection in a representative population sample of adults from Northern Ireland.

METHODS

Survey methods

Subjects were drawn from those recruited in Northern Ireland into the World Health Organisation co-ordinated MONItoring of trends and determinants in Cardiovascular disease Project's third population survey (MONICA 3). This survey employed a single stage random sampling procedure (sampling frame, the GP register held by Central Services Agency, Belfast) with stratification by age and sex. Subjects were screened by trained observers adhering to a

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standard protocol [11] between October 1991 and June 1992. Information collected by questionnaire included data on education and socio-economic status, past medical history, medication and lifestyle (smoking habit, alcohol consumption, diet and physical activity). Height, weight and resting blood pressure (Hawksley random zero sphygmo-manometer; diastolic phase V) were measured and a blood sample (semi-fasting) was taken, centrifuged and separated within 4 h, aliquoted and stored at −70 °C.

In this study, stored serum (which had been thawed on one previous occasion) from 400 subjects were analysed for the presence of anti-*C. pneumoniae* antibodies. These subjects were randomly selected from the survey population. When serum was not available for one of the selected subjects a replacement was randomly chosen from the remainder of the survey participants. Fifty-two replacements were made.

Laboratory methods

MRL Diagnostics *Chlamydia* MIF (microimmuno-fluorescence) assay [12] was used to detect the presence of IgG antibodies to antigens from *C. pneumoniae* (TW183), *C. psittaci* (strains 6BC and DD34) and *C. trachomatis* (sero-types D-K) at a serum dilution of 1 in 64. Slides were viewed at a magnification of 400 × on a Leitz fluorescence microscope within 24 h by two independent observers. Results were graded on a scale 0, +/−, 1+, 2+, 3+ and 4+ with 0 and +/− interpreted as negative. If the observers disagreed on a result the opinion of a third experienced observer was sought.

Subjects whose sera reacted with *C. trachomatis* or *C. psittaci* antigens were excluded from the analysis. This report was based on sera from 393 subjects (199 males and 194 females). The serum of 6 patients was excluded, 5 because of cross-reaction with *C. psittaci* or *C. trachomatis* respectively and 1 sample had been tested twice with inconsistent results.

Statistical/analytical methods

Occupational class of the head of the household, tenure of accommodation, highest educational level achieved and number of years spent at school were used as measures of socio-economic status. Occupational class was coded according to the Office of Population Censuses and Surveys Classification of Occupations [13] and was grouped into manual (IIIM,

Table 1. *Social class distribution of subjects studied*

Social class	Subjects studied (%)	MONICA 3 participants (%)	Northern Ireland population (%)
I	6·8	7·2	3·1
II	22·8	22·8	23·6
III NM	26·5	26·8	21·1
III M	24·1	24·7	25·7
IV	12·3	13·1	19·2
V	7·4	5·5	7·2

IV and V) and non-manual (I, II and IIINM) classes. Tenure was categorized into rented and owned and highest educational level achieved into elementary, primary or secondary school only; grammar school only; technical or nursing college and third level education. Number of years spent at school was categorized into 9 or less years, 10–11 years, 12–13 years and 14 or more years. Smoking habit was grouped into four categories: current smoker of 20 or more cigarettes a day, current smoker of less than 20 cigarettes a day, ex-smoker and never smoked. Alcohol intake was categorized into life-time abstinence, current abstinence, current alcohol intake less than 10 units per week and current intake of more than 10 units per week. Month of screening (and collection of blood sample) was grouped into winter (November, December, January) and not winter (all other months). Height, weight and body mass index were treated as continuous un-transformed variables. Both bi-variable (independent samples *t* test, Chi square, and Chi square for trend) and multivariable analyses (logistic regression) were performed using SPSS for Windows.

RESULTS

Response rates of the MONICA 3 survey

Of the 5000 names randomly selected from the CSA register, 565 were ineligible because they were deceased, resided outside the MONICA Project area (Belfast and surrounding districts) or because their age lay outside the specified range (25–64 years). A further 226 were untraceable after at least two visits to the last known address and reference to other sources. Two thousand and five of the 4209 eligible and traceable subjects participated giving a response rate of 47·6%. The random sampling procedure used to provide the subset of MONICA 3 subjects examined in this study delivered a sample with characteristics

Table 2. *Socio-economic status and infection*

Measure of socio-economic status	Number *C. pneumoniae* seronegative	Number (%) *C. pneumoniae* seropositive
Occupational class		
I	9	13 (59·1)
II	23	51 (68·9)
III NM	26	60 (69·8)
III M	19	59 (75·6)
IV	11	29 (72·5)
V	6	18 (75·0)
Tenure of accommodation		
Owned	94	226 (70·6)
Rented	19	40 (67·8)
Highest educational level achieved		
Primary, elementary or secondary school	59	172 (74·5)
Grammar school	12	29 (70·7)
Technical college, polytechnic or nursing college	25	35 (58·3)
University	18	36 (66·7)
Years spent at school		
9 or less	12	46 (79·3)
10 or 11	43	105 (70·9)
12 or 13	27	67 (71·3)
14 or more	36	57 (61·3)

(such as mean age, weight, total cholesterol etc.) closely resembling all participants in MONICA 3 and the social class distributions of subjects in the study were similar to that in the MONICA 3 survey and in Northern Ireland as a whole (Table 1).

Age, socio-economic status and seropositivity

Two hundred and seventy-five subjects (70%) possessed IgG antibodies to *C. pneumoniae*. There was a tendency towards increasing seropositivity with increasing age with 63·4, 69·3, 76·0 and 70·7% of subjects aged 25–34, 35–44, 45–54, 55–64 years, respectively, having IgG antibodies to *C. pneumoniae* but this trend did not reach statistical significance (χ^2 for trend 1·8, $P = 0·18$). More males than females were seropositive (72·9 *vs.* 67·0%) but statistical significance was not reached (χ^2 1·6, $P = 0·21$). The relationship between measures of socio-economic status and infection are shown in Table 2. Seropositivity increased with lower occupational class but statistical significance was not achieved (χ^2 for trend 1·76, $P = 0·18$). Seropositivity was unrelated to tenure of accommodation but there was a statistically significant trend towards decreasing prevalence of infection with higher educational level achieved (χ^2

for trend 4·09, $P = 0·04$). There was also an association between number of years spent at school and seropositivity (χ^2 for trend = 4·93, $P = 0·03$), which was almost sustained after adjustment for age using logistic regression: the odds ratio of seropositivity if school had been attended for more than 14 years relative to attendance for 9 years or less was 0·46, 95% confidence intervals 0·2 to 1·02, $P = 0·06$.

Lifestyle, anthropometry and seropositivity

There was no association between smoking habit (χ^2 for trend 0·07, $P = 0·79$) or consumption of alcohol (χ^2 for trend 0·1, $P = 0·75$) and seropositivity for *C. pneumoniae* (Table 3). Seropositives were slightly taller (168·1 cm *vs.* 167·3 cm) and considerably heavier (73·5 *vs.* 71·4) than seronegatives but these relationships did not achieve statistical significance. The weight difference was entirely confined to females with mean weight in seropositive females 2·4 kg higher than in seronegative females but the relationship was not statistically significant (95% confidence intervals of the difference in mean weight −0·1 to 5·7, $P = 0·16$). Seropositivity was not related to body mass index in all subjects or in either of the sexes. Adjustment for age had no effect on the relationships between

Table 3. *Lifestyle and seropositivity*

	Number seronegative	Number seropositive (%)
Smoking status		
Never smoked	51	115 (69·3)
Ex smoker	30	76 (71·7)
Current smoker (< 20 cigarettes/day)	17	42 (71·2)
Current smoker (20 or more cigarettes/day)	17	41 (70·7)
Alcohol intake		
Lifetime abstinence	16	32 (66·7)
Current abstinence	4	27 (87·1)
Current drinkers (< 10 units/week)	54	123 (69·5)
Current drinkers (10 or more units/week)	39	89 (69·5)

anthropometric measurements and seropositivity for *C. pneumoniae*.

Season and seropositivity

IgG antibodies to *C. pneumoniae* were more common in blood samples taken during winter months (November, December, January) than in samples taken during the rest of the year: 75·9 *vs.* 66·7% seropositive respectively. This relationship almost achieved conventional statistical significance (χ^2 3·66, $P = 0.06$).

DISCUSSION

C. pneumoniae infection has seldom been investigated in representative population samples [14, 15]. Subjects investigated in this study were selected from among the participants in the MONICA 3 survey who were randomly chosen from a population register. A further random selection process was used to ensure that the study group were representative of the MONICA 3 population. Unfortunately the parent study had a lower response rate than was desirable which may have introduced a degree of selection bias. Responders tended to be older than non-responders and compared to the 1991 Northern Ireland Census higher social classes were over-represented and lower social classes under-represented in both MONICA 3 and this study. This may lead to some difficulty in extrapolating the results of this study to the Northern Ireland population as a whole.

Although differences in study design confound comparisons between *C. pneumoniae* sero-prevalence surveys, the high prevalence of IgG antibodies to the organism observed in adulthood in this study is a fairly consistent finding throughout the developed world [14–17]. Our failure to observe an increase in IgG seropositivity with age within the limits of this study is also consistent with other studies [14, 15]. Anti-*C. pneumoniae* IgG antibody levels tend to fall with time following acute infection, but this may be a protracted process [6] suggesting persistent or chronic infection. Repeated infection in adulthood also occurs and is accompanied by a rise in IgG [6]. Therefore, interpretation of a raised IgG titre to *C. pneumoniae* antigen in a cross-sectional study, such as this, is difficult. Raised IgG indicates previous exposure to the organism but this may be in the form of a recent primary infection, a repeated infection or chronic infection. There is clear evidence that exposure to *C. pneumoniae* increases substantially during childhood and adolescence [6] but the lack of observation of association between IgG seropositivity and age in this and other studies suggests that exposure to the organism may remain fairly constant throughout adult life. There is, however, some evidence that, in adulthood, chronic infection (as indicated by raised IgA antibodies) [18] increases with age [15].

We, in common with Mendall and colleagues [14] and at variance with other stuides [15, 19], did not find any association between seropositivity and smoking. However, evidence for association with raised *C. pneumoniae* specific IgA is more convincing than for raised IgG suggesting that smoking is a risk factor for chronicity or persistence of *C. pneumoniae* infection rather than for exposure to the organism. We may have observed an association with smoking had we measured IgA antibodies.

Several seroprevalence studies have demonstrated an excess of seropositivity in males [15, 16, 20], which has been attributed to exposure to the infection outside the home although sex-specific differences in

subject recruitment may be a more likely explanation of the association. In tune with the findings of other investigators [14] we did not observe an association between male sex and seropositivity. It is intriguing to speculate that *C. pneumoniae* infection might contribute to the sex-specific difference in incidence of cardiovascular disease but further investigation in representative population samples is required to confirm a higher prevalence of infection in males.

The present study did not uncover any relationship between infection and anthropometry. Although, it was not expected to find any association with weight it was felt that *C. pneumoniae* infection might be associated with decreased height in a similar manner to *Helicobacter pylori* [21, 22]. Perhaps, unlike *H. pylori* which is a chronic infection acquired predominantly in childhood, infection with *C. pneumoniae* occurs too late in life to affect growth, or is too transient an infection to have an effect on growth should it occur earlier.

The relationship between *C. pneumoniae* infection and socio-economic status has been infrequently examined with no clear pattern emerging [14, 15]. We did not observe an association between IgG seropositivity and occupational class but evidence of infection was more common in subjects with fewer years of schooling or who had achieved lower educational levels. This is in keeping with the inverse relationship between IgG seropositivity and educational achievement observed in a random sample of Jerusalem residents [15]. Interestingly no such relationship was observed for IgA levels in the Jewish study perhaps indicating that, although there may be a social gradient in exposure to *C. pneumoniae* infection, persistence of infection may be governed by factors unrelated to socio-economic status.

Subjects who had blood taken during winter months were more likely to be seropositive than subjects who had blood taken during other seasons. This indicates that infection with *C. pneumoniae* may exhibit seasonality with a higher risk of acquisition of infection during winter. Alternatively, as we only had data relating to one winter period and as previous serological studies have failed to show seasonality [15, 16], we may merely have observed a single epidemic of *C. pneumoniae* infection occurring during the winter months.

In conclusion, we have demonstrated high prevalence of IgG antibodies to *C. pneumoniae* in a western European population. Seropositivity was not related to gender or smoking habit and did not increase with age. However, infection was more common in those who had spent fewer years in school or who had achieved lower educational levels, and in subjects who had blood taken during winter months. The continuing accrual of evidence implicating *C. pneumoniae* in the pathogenesis of cardiovascular disease underlines the need for further investigation of the epidemiology of the infection in order that appropriate intervention strategies can be devised.

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