Seroprevalence of IgG antibodies against *Chlamydia pneumoniae* in Chinese, Malays and Asian Indians in Singapore

Woon Puay Koh,a Mark B Taylor,b Kenneth Hughes,a SK Chew,c CW Fong,c MC Phoon,b KL Kangb and Vincent TK Chownb

**Background** *Chlamydia pneumoniae*, a bacterium that causes respiratory infections, is probably under-diagnosed. There is also interest in its possible role in the aetiology of coronary heart disease. This is the first population-based seroprevalence survey of *C. pneumoniae* infection in Singapore.

**Methods** A random sample of 1068 people aged 18–69 years was selected from the participants of the Singapore National Health Survey conducted in 1998. Sera and data on certain clinical measurements and conditions had been collected. IgG antibodies for *C. pneumoniae* were detected using an indirect microimmunofluorescence test and positivity graded. Seropositivity was defined as IgG titre ≥1:16.

**Results** There were no statistically significant differences in the prevalence rates of seropositivity to *C. pneumoniae* for age group 18–69 years among the three ethnic groups, i.e. Chinese (males 76.7%, females 68.3%), Malays (males 75.4%, females 59.1%), and Asian Indians (males 74.6%, females 59.4%). The seropositivity rate for people aged 18–69 years in Singapore was 75.0% for males and 65.5% for females (difference of 9.5%, *P* < 0.001). In both genders combined, seropositivity increased from 46.5% in the age group 18–29 to reach a plateau of 78.9% in the age group 40–49, which remained stable to 60–69 years. There was no association of seropositivity with smoking, diabetes mellitus, hypertension or body mass index after adjustment for age and gender.

**Conclusion** The high prevalence rates in our study population and the higher rate in males compared to females are consistent with studies from other parts of the world. No significant difference in prevalence rates was observed among Chinese, Malays and Indians. The pattern of rising and levelling off of seropositivity with age suggests that *C. pneumoniae* infection occurs early in life, and in older ages the high level of seropositivity is probably maintained by re-infections or chronic infections. *Chlamydia pneumoniae* infection was not found to be associated with the cardiovascular risk factors examined.

**Keywords** *Chlamydia pneumoniae*, Singapore, seroepidemiological studies, ethnicity, smoking, hypertension, diabetes mellitus, body mass index

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*Chlamydia pneumoniae* is an intracellular bacterium that was first isolated in 1965 from the eye of a child in Taiwan,1 and subsequently established as a unique chlamydial species distinct from other chlamydial species in 1987.2 It has been implicated in community-acquired respiratory tract infections in all ages, except under 5 years, in temperate zone countries.3 It has been estimated that *C. pneumoniae* causes about 10% of all community-acquired pneumonia in adults and 5% of bronchitis and sinusitis.4 This organism is also associated with chronicity and re-infectivity, and with chronic respiratory conditions such as chronic bronchitis,5 fibrosing alveolitis6 and chronic asthma.7
There is some evidence that *C. pneumoniae* may play a role in the aetiology of coronary heart disease,

and clinical trials are being conducted to assess the efficacy of anti-chlamydial antibiotics in the secondary prevention of coronary artery disease.

Singapore is a modern city with a population of 4.0 million living in an area of 682.7 km², with an ethnic distribution of 77% Chinese, 14% Malays, 8% Asian Indians and 1% of other races. In Singapore, as in other parts of the world, Asian Indians are more susceptible to coronary heart disease than other ethnic groups,

which is not fully explained by established risk factors. No epidemiological data are available on *C. pneumoniae* infection in Singapore, and the organism may well be under-recognized as a pathogen for community-acquired pneumonia.

This study was therefore conducted as the first population-based survey of *C. pneumoniae* infection in Singapore, and measured the seroprevalence and ethnic differences, and also its relationship with established coronary risk factors.

**Methodology**

**Study population**

The study population was assembled from the subjects who participated in the National Health Survey conducted in Singapore in 1998. The National Health Survey population was randomly assembled through a two-phase sampling process from the 2.16 million Chinese, Malay and Indian residents aged 18–69 years in Singapore. In the first phase, a sample of household addresses was selected after stratification by house-type. In the second phase, 7500 people within the specified age group 18–69 years from these addresses were randomly selected after stratification by age and ethnic group. In all, 4723 people eventually responded and participated in the survey. After stratification by ethnic group to ensure approximately equal numbers in the three ethnic groups, a random sample of 1068 people was selected from the 4723 people for our study.

**Procedures**

Information had been collected by questionnaire, measurements and tests. A smoker was someone who smoked at least one cigarette a day, and an ex-smoker was a former smoker who had given up the habit completely. A hypertensive was diagnosed as either a person on medication or who had a systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥90 mmHg. Diabetes mellitus was diagnosed as a person on medication or who had a 2-hour plasma glucose concentration ≥11.1 mmol/l after an oral glucose tolerance test. Weight and height measurements were taken for each subject and body mass index (BMI) calculated.

**Antibody assay**

A sample of venous blood had been collected from each subject and the sera stored at −20°C. IgG antibodies against *C. pneumoniae* were determined by a microimmunofluorescence (MIF) test kit (MRL Diagnostics, Cypress, CA, USA), which has performed reasonably well in comparative studies. The kit uses *C. pneumoniae* elementary bodies (strain TW183) as antigen, with the inclusion of *C. trachomatis* (eight serotypes, D–K) and *C. psittaci* (strains 6BC, DD34) antigens as controls. These antigens were treated to remove interfering genus-reactive lipopolysaccharide and suspended in 3% yolk sac matrix. All the three antigens and a yolk sac control were mounted onto wells on slides. Some 25 µl each of the positive control, negative control and serum samples (diluted 1:16 in PBS) were applied to the slide wells and incubated in a humid chamber at 37°C for 30 minutes. After washing with PBS to remove unbound serum antibodies, each slide was overlaid with fluorescein-labelled goat anti-human antibody to IgG and incubated. After repeated washing, drying and mounting, the slides were examined by two experienced technicians within 24 hours using a Microphot-FXA fluorescence microscope (Nikon, Japan) at 400× magnification, and graded as 0, 1+, 2+, 3+ or 4+. Serum samples were considered positive if they reacted only with *C. pneumoniae* antigen, or if they reacted with the other chlamydial species but showed the highest grade of reaction with *C. pneumoniae* antigen. People with IgG titres ≥1:16 were classified as seropositive.

In another study using the same test kit and method performed in the same laboratory and by the same operator, identical results were obtained for a repeat test with ten randomly selected samples (one non-specific, one cross-reactive and eight positive results). Statistical analyses

**Ethnicity**

Table 1 shows *C. pneumoniae* seropositivity rates by gender, ethnic group and two age groups. Although the prevalence rates were highest in Chinese for both genders, the differences between ethnic groups were not statistically significant. For the three ethnic groups combined in the overall 18–69 age group, 72.5% of Chinese, 67.2% of Malays and 66.9% of Indians were seropositive, but none of the differences were statistically significant; between Chinese and Malays (P = 0.141), Chinese and Indians (P = 0.122), and Malays and Indians (P = 0.936).

**Gender**

Table 1 also shows that the prevalence of seropositivity was higher in males than females for each age/ethnic group. In the overall 18–69 age group, the seropositivity rate in males was higher than in females in all three ethnic groups, the differences being Chinese 8.4% (P = 0.098), Malays 16.3% (P = 0.001) and Indians 15.2% (P = 0.002). For the three ethnic groups combined in the overall 18–69 age group, 75.6% of males and 62.3% of females were seropositive, with the difference of 13.3% being statistically significant (P < 0.001).

In order to estimate the overall prevalence of seropositivity to *C. pneumoniae* in Singapore, the rates by age and ethnic group were directly standardized to the age and ethnic group distribution of the 1998 mid-year population of Singapore. This gave seroprevalence rates of 75.0% for males and 65.5% for females. The difference of 9.5% was very highly statistically significant (P < 0.001).
The distribution of IgG seropositivity to *C. pneumoniae* in the different age groups from 18 to 69 years is shown in Table 2 and Figure 1. As the youngest subjects were 18-years-old, information was not available on seroprevalence in children and teenagers. However, by ages 18–29 years, as high as 58.4% of males and 35.0% of females were seropositive, with the gender difference of 23.4% being statistically significant (*P* = 0.001).

Hence, nearly 50% of the population had been infected by the third decade of life. Seropositivity continued to increase with age in both genders (χ² test for trend, *P* < 0.001). In males, the rate of increase in seropositivity was greatest at ages 30–49, but then levelled off in older age groups. In females, the greatest increase was at ages 18–39, with seropositivity continuing to increase thereafter, but at a much lower rate.

### Smoking status, diabetes mellitus, hypertension, and body mass index

Table 3 shows the percentages of *C. pneumoniae* seropositivity by smoking status, diabetes mellitus, hypertension and BMI.

### Table 1 Seroprevalence of *Chlamydia pneumoniae* IgG by gender, age and ethnic group

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age group</th>
<th>Ethnic group</th>
<th>Sample (N)</th>
<th>Positive (n)</th>
<th>Positive % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>18–44</td>
<td>Chinese</td>
<td>105</td>
<td>73</td>
<td>69.5 (60.7–78.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Malays</td>
<td>103</td>
<td>71</td>
<td>68.9 (60.0–77.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Indians</td>
<td>103</td>
<td>72</td>
<td>69.9 (61.0–78.8)</td>
</tr>
<tr>
<td></td>
<td>45–69</td>
<td>Chinese</td>
<td>75</td>
<td>65</td>
<td>86.7 (76.8–93.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Malays</td>
<td>72</td>
<td>61</td>
<td>84.7 (74.3–92.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Indians</td>
<td>74</td>
<td>60</td>
<td>81.1 (70.3–91.3)</td>
</tr>
<tr>
<td></td>
<td>18–69</td>
<td>Chinese</td>
<td>178</td>
<td>138</td>
<td>76.7 (70.5–82.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Malays</td>
<td>175</td>
<td>132</td>
<td>75.4 (69.1–81.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Indians</td>
<td>177</td>
<td>132</td>
<td>74.6 (68.2–81.0)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>All</td>
<td>532</td>
<td>402</td>
<td>75.6 (71.9–79.2)</td>
</tr>
</tbody>
</table>

### Table 2 Seroprevalence of *Chlamydia pneumoniae* IgG by gender and age group

<table>
<thead>
<tr>
<th>Age group</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample (N)</td>
<td>Positive (n) (%)</td>
<td>Sample (N)</td>
</tr>
<tr>
<td>18–29</td>
<td>113 66 (58.4)</td>
<td>117 41 (35.0)</td>
</tr>
<tr>
<td>30–39</td>
<td>140 99 (70.7)</td>
<td>133 84 (63.2)</td>
</tr>
<tr>
<td>40–49</td>
<td>105 91 (88.3)</td>
<td>106 74 (69.8)</td>
</tr>
<tr>
<td>50–59</td>
<td>109 89 (81.7)</td>
<td>103 76 (73.8)</td>
</tr>
<tr>
<td>60–69</td>
<td>67 57 (85.1)</td>
<td>77 59 (76.6)</td>
</tr>
<tr>
<td>Total</td>
<td>532 402 (75.6)</td>
<td>536 334 (62.3)</td>
</tr>
</tbody>
</table>

### Age

The distribution of IgG seropositivity to *C. pneumoniae* in the different age groups from 18 to 69 years is shown in Table 2 and Figure 1. As the youngest subjects were 18-years-old, information was not available on seroprevalence in children and teenagers. However, by ages 18–29 years, as high as 58.4% of males and 35.0% of females were seropositive, with the gender difference of 23.4% being statistically significant (*P* = 0.001). Hence, nearly 50% of the population had been infected by the third decade of life.

Seropositivity continued to increase with age in both genders (χ² test for trend, *P* < 0.001). In males, the rate of increase in seropositivity was greatest at ages 30–49, but then levelled off in older age groups. In females, the greatest increase was at ages 18–39, with seropositivity continuing to increase thereafter, but at a much lower rate.

### Figure 1 Prevalence of IgG antibodies to *Chlamydia pneumoniae* by age group
hazard ratios (relative risks) were calculated after adjustment for age and gender, as they were confounding variables for these factors.

Although the proportion of seropositivity was lowest in non-smokers compared to daily and ex-smokers, there was no increased risk of seropositivity with smoking habit after adjustment for age and gender. When the analysis was confined only to males, as the number of female smokers was small, smoking was still not a risk factor for seropositivity. There was also no association of \textit{C. pneumoniae} seropositivity with diabetes mellitus, hypertension, or BMI after adjustment for age and gender.

**Discussion**

**Comparison with other studies**

There is no international standardization for the MIF test although it has been shown to measure species-specific chlamydial antibodies reliably when used by an experienced reader. \(^1^6\) Differences in the antigenic strains and methods used in the various kits, coupled with the high operator dependence for interpretation of MIF results, make comparisons of seropositivity rates from different studies very difficult. Consequently, consensus recommendations on standardized testing for \textit{C. pneumoniae} by MIF have been published recently, which state that an IgG titre of $\geq 1:16$ be interpreted as presumed past infection. \(^1^7\) In our study, we have also adopted this criterion as the definition of seropositivity. Some studies have also used the same criterion, \(^1^8\)–\(^2^0\) while others have set it as $\geq 1:32$. \(^2^1\)–\(^2^3\) Nevertheless, studies all over the world have reported estimates of seropositivity ranging from 60 to 90% in most adult populations, indicating that \textit{C. pneumoniae} infection is common and globally distributed. Our study estimated a population sero-prevalence of 75.0% for men and 65.5% for women, which is comparable to other countries in Asia such as China, \(^1^8\) Japan, \(^2^1\) Korea, \(^2^3\) Taiwan, \(^1^9\) and Thailand, \(^2^4\) where seroprevalence reaches 50% by the third decade of life, especially in male adults, and continues to increase with age.

**Age**

In most countries, the seropositivity rate is low in infancy, begins to increase during school-age years, and reaches the level in the adult population by 15–20 years. \(^2^0\), \(^2^5\), \(^2^6\) Our data showing relatively high seropositivity by the ages of 18–29 years is therefore consistent with these previous findings, and may imply that exposure is common in adolescents and young adults, with or without pneumonia. Our study also demonstrates that the high rate of seropositivity is maintained in the elderly population at fairly constant levels at about 45 years of age and above in both genders.

Raised IgG indicates past exposure to \textit{C. pneumoniae} either as a recent primary infection, a re-infection or a chronic infection. The IgG antibodies from the primary acute infection tend to decrease with time, and usually disappear in 3 to 5 years. \(^4\) In \textit{C. trachomatis} infection, antibody prevalence diminishes sharply after the age of 40 to 50, \(^2^7\) in contrast to the persistence of \textit{C. pneumoniae} antibody into old age in many cross-sectional sero-epidemiological studies. It has been suggested that the maintenance of antibody at a stable level in older age is due to re-infection that occurs throughout life. \(^4\), \(^2^7\) This phenomenon also suggests that exposure to this organism and/or its chronic persistence remains fairly constant throughout the rest of adult life after reaching a peak in the second or third decade. \(^2^8\)

Consistent with this suggestion, a longitudinal study has shown that after initial infections, adults continue to be at risk from re-infections. \(^2^9\) Several authors have therefore advocated that the observation of a persistently high seropositivity into advanced ages is best explained by frequent re-infections and persistent chronic infections in old age. \(^1^9\), \(^2^3\), \(^2^4\) although it remains possible that the multiple re-infections may have occurred at younger ages or that the rate of primary infection is maintained at a constant level in the elderly. Some studies have reported a steady increase with age, \(^3^0\) or a saddle-shaped curve due to a second peak in the elderly due to a surge of re-infections in this group. \(^1^9\) As we do not have data from subjects $\geq 70$ years, we are unable to see if this surge of re-infections occurs in our population as well.

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**Table 3** Descriptive data and hazard ratios for \textit{Chlamydia pneumoniae} seropositivity

<table>
<thead>
<tr>
<th>Factors</th>
<th>Sample (N)</th>
<th>No. Positive (%)</th>
<th>Hazards ratio (95% CI)(^a)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Smoking status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smoker</td>
<td>812</td>
<td>537 (66.1)</td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>72</td>
<td>58 (80.6)</td>
<td>1.039 (0.845–1.278)</td>
<td>0.718</td>
</tr>
<tr>
<td>Daily smoker</td>
<td>184</td>
<td>141 (76.6)</td>
<td>0.976 (0.729–1.306)</td>
<td>0.869</td>
</tr>
<tr>
<td><strong>Diabetes mellitus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>922</td>
<td>618 (67.0)</td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>146</td>
<td>118 (80.8)</td>
<td>1.020 (0.825–1.262)</td>
<td>0.853</td>
</tr>
<tr>
<td><strong>Hypertension</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>791</td>
<td>515 (65.1)</td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>277</td>
<td>221 (79.8)</td>
<td>1.033 (0.864–1.235)</td>
<td>0.721</td>
</tr>
<tr>
<td><strong>Body mass index</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underweight ((&lt;18.5))</td>
<td>74</td>
<td>44 (59.5)</td>
<td>0.992 (0.724–1.359)</td>
<td>0.959</td>
</tr>
<tr>
<td>Normal weight (18.5–24.9)</td>
<td>569</td>
<td>381 (67.0)</td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>Overweight ((\geq25))</td>
<td>425</td>
<td>311 (73.2)</td>
<td>1.057 (0.908–1.230)</td>
<td>0.477</td>
</tr>
</tbody>
</table>

\(^a\) Cox proportional hazards regression, adjusted for age and gender.
Gender
While many studies have shown a significantly higher seroprevalence in men compared to women,31–33 other studies have failed to show this.18,20,21 Our data showed a higher prevalence in males than females, and this was present in all three ethnic groups, though the difference was least in Chinese. A study in China showed that there was no difference in seroprevalence between males and females.18 A higher rate in males than females has been attributed to a greater proportion of men working outside the home than women.22 To date, studies showing a higher prevalence in adult men compared to women have not been able to offer any other explanation.33,34 In particular, this gender difference has not been explained by the higher prevalence of smoking in men.34

Ethnicity
Few studies have examined C. pneumoniae infection in different ethnic groups living in the same geographical location. One such study in the UK showed that C. pneumoniae seropositivity was most prevalent among Afro-Caribbeans, then Asians, and then Caucasians.35 This suggests that different ethnic groups may have different risks of susceptibility to C. pneumoniae infection. In our study, we did not find any difference among the three ethnic groups. This may well be due to the fact that these three ethnic groups live together in close proximity in a country of relatively high population density. In support of this hypothesis, high seropositivity has been associated with increased population density in several studies.18,23,36

There is a possible association between C. pneumoniae infection and coronary heart disease,4,8 although it is likely that a very imperfect correlation exists between the presence of IgG antibodies and the pathological effect of the organism on the disease process. Despite the increased susceptibility of Indians to coronary heart disease compared to Chinese and Malays in Singapore,9,10 Indians do not have a higher seroprevalence. However, the similar seroprevalence in the different ethnic groups does not exclude a role for C. pneumoniae in the higher rate of coronary disease among Indians. This may indicate similar exposure rates, but questions remain concerning potential genetic differences in response to infection, differences in unmeasured cofactors, and other contributory risk factors.

Smoking
Chlamydia pneumoniae has demonstrated a marked ciliostatic effect in vitro, completely aborting ciliary motion within 48 hours. This can contribute to both initiation and progression of respiratory infections by C. pneumoniae.37 While both present and past smoking has been associated with an increased risk for C. pneumoniae,34,38 our study, together with other studies,22,28,31 failed to show such an association. Differences may be partly attributed to the classification of smoking, as it may be only heavy smoking that is associated with increased C. pneumoniae infection.33,39

Risk factors for coronary heart disease
Established risk factors for coronary heart disease include hypertension, diabetes mellitus and obesity.40 Recent studies have suggested an association between C. pneumoniae seropositivity and coronary heart disease, but some investigators have cautioned that C. pneumoniae may not act independently but as a confounder or an effect modifier on the risk factors of atherosclerosis41,42

Hypertension and obesity have been found to be associated with an increased susceptibility to C. pneumoniae infection.43,44 Our study did not find an association between C. pneumoniae seropositivity and hypertension or obesity (as measured by BMI), as well as diabetes. This difference between our study and other studies may arise from factors such as definition and classification of risk factors as well as the titre or class of antibodies examined.

Conclusions
Chlamydia pneumoniae seroprevalence is high among all three ethnic groups in Singapore, and is higher in males than females. Given that C. pneumoniae is currently under-diagnosed and under-recognized in Singapore, our data have practical implications for the awareness and diagnosis of infections associated with this pathogen, so that prompt treatment with appropriate antibiotics can be achieved. The data also warrant further investigations in our patients with coronary heart disease to clarify the relationship between C. pneumoniae infection and atherosclerosis.

Acknowledgements
This project was supported by research grants from the National Medical Research Council and Biomedical Research Council, Singapore. We would also like to acknowledge B Ishak for technical assistance.

KEY MESSAGES
• There is a high seroprevalence rate of IgG antibodies against Chlamydia pneumoniae in the adult population aged 18–69 years in Singapore, with no significant difference in the rates among Chinese, Malays and Indians.
• The seroprevalence rates are significantly higher in males compared to females, and they rise and level off with age.
• There is no association between C. pneumoniae infection and smoking, hypertension, diabetes mellitus or overweight status in our population.
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