ORIGINAL ARTICLE

Seroepidemiology of *Chlamydia pneumoniae* in Japan between 1991 and 2000

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Aim: To clarify the endemic and epidemic status of *Chlamydia pneumoniae* in Japan, the incidence of anti-*C pneumoniae* antibody was evaluated over a period of 10 years.

Method: Serum samples were collected from 4756 healthy individuals aged 6 months to 88 years (2488 male and 2268 female individuals) between 1991 and 2000. The antibody titre was determined by a microimmunofluorescence test.

Results: After stratification by age and sex in each year, distinct peaks with prevalences of 73.3% and 73.0% were noted in 1993 and 1999, respectively. The lowest prevalence rate was seen in 1996 (59.0%). The epidemic cycle has been estimated to be almost six years in this geographical area.

Conclusions: *Chlamydia pneumoniae* infection is highly endemic in Japan, as it is in Western countries, and there is a year to year variability. Long term studies in Japan are needed to clarify the epidemic occurrence of *C pneumoniae* infection.

Chlamydia pneumoniae has been established as an important cause of both lower and upper acute respiratory illnesses among children and adults.¹ Pneumonia and bronchitis are the most frequent of the recognised illnesses associated with *C pneumoniae*, whereas asymptomatic infection and unrecognised, mildly symptomatic illness are common results of *C pneumoniae* infection.¹ Recent studies have also suggested a possible association between *C pneumoniae* infection and atherosclerotic cardiovascular disease, both from seroepidemiological studies and demonstration of the organism in atheromas.¹

Chlamydia pneumoniae is notoriously difficult to grow in cell culture. The microimmunofluorescence (MIF) test is specific for *C pneumoniae* and is the standard method for chlamydia serology today.¹⁻² Epidemiological studies on the prevalence of antibody to *C pneumoniae*, carried out in various populations, have shown that over 50% of adults worldwide have antibodies to this organism. We have carried out epidemiological surveillance of *C pneumoniae* infections in Japan since 1991. Little is known about year to year variability in the incidence of *C pneumoniae* infections in Japan. The aim of our present study was to evaluate the prevalence of anti-*C pneumoniae* antibodies in healthy subjects during the past decade.

"*Chlamydia pneumoniae* is notoriously difficult to grow in cell culture"

MATERIALS AND METHODS

Study population

Okayama is located in western Japan, with a population of approximately 2 000 000. Kawasaki Medical School Hospital is the main hospital for the prefecture and includes all clinical specialities. Between April 1991 and December 2000, 4756 serum samples were collected from healthy individuals aged 6 months to 88 years (2488 males and 2268 females) who had lived in the same area of Okayama for more than 10 years (children under 10 years old had also lived in the same area since their birth). Serum samples were collected and stored at -70°C until measurement.

Antigen preparation

The *C pneumoniae* KK-pn15 strain, which was isolated in our laboratory from a patient with acute pharyngitis,³ was used as

the antigen and was propagated in HEP-2 cells. The elementary bodies of the KK-pn15 strain were purified by a method of continuous Urografin (Schering AG, Berlin/Bergkamen, Germany) gradient centrifugation (40-52%), as described previously.⁴ The elementary bodies were resuspended in a solution of 2% yolk sac in phosphate buffered saline (pH 7.2) containing 0.02% formalin.

Serological testing

A MIF test was used to measure antibodies to *C pneumoniae*.³ The presence of IgG and IgM antibodies against *C pneumoniae* was detected using commercial fluorescein isothiocyanate conjugated goat antihuman IgG and IgM (Medical and Biological Laboratories, Nagoya, Japan). Rheumatoid factors were absorbed with Gullsorb (Gull Laboratories, Salt Lake City, Utah, USA) before IgM titrations. The criterion for a positive serological test was a titre of $\geq 1/16$ for IgG.¹ An anti-*C pneumoniae* antibody of titre $\geq 1/16$ for IgM was considered to indicate a recent infection. Recent reports concerning serological criteria indicate that the definition of acute infection using a titre of $\geq 1/512$ IgG is controversial. Therefore, in our study, a titre of $\geq 1/512$ for IgG was defined as a high titre. Statistical analysis was done by means of the χ^2 test.

RESULTS

Figure 1 shows the age related incidence of anti-*C pneumoniae* antibodies during the entire period. The antibody detection rate was 57%, broken down by age as follows: 6 months to 1 year old, 4%; 2-4 years old, 5%; 5-9 years old, 40%; 10-14 years old, 56%; 15-19 years old, 59%; 20-29 years old, 57%; 30-39 years old, 63%; 40-49 years old, 67%; 50-59 years old, 67%; 60-69 years old, 70%; and ≥ 70 years old, 71%. In subjects over 15 years old, anti-*C pneumoniae* antibodies were present in 1331 (67.5%) of 1971 males and 1078 (60.6%) of 1777 females. There were 14 cases (0.3%) of recent *C pneumoniae* infection among these healthy individuals, as determined by IgM.

As shown in fig 1, the prevalence of antibody to *C pneumoniae* varies in the population in relation to age and sex. Therefore, to estimate year to year differences we

Abbreviations: MIF, microimmunofluorescence

Figure 1 Population prevalence of anti-*Chlamydia pneumoniae* IgG antibody by age and sex in 4756 individuals (closed circles, male; open circles, female individuals).

Table 1 Prevalence of *Chlamydia pneumoniae* antibody in different year groups

Year	No. of subjects	No. (%) positive		
		IgG $\geq 1/16$	IgG $\geq 1/512$	IgM $\geq 1/16$
1991	300	180 (60.0)	9 (3.0)	0
1992	300	189 (63.0)	10 (3.3)	2 (0.7)
1993	300	220 (73.3)	14 (4.6)	2 (0.7)
1994	300	192 (64.0)	11 (3.6)	1 (0.3)
1995	300	191 (63.6)	10 (3.3)	0
1996	300	177 (59.0)	8 (2.6)	0
1997	300	187 (62.3)	10 (3.3)	1 (0.3)
1998	300	181 (60.3)	11 (3.6)	1 (0.3)
1999	300	219 (73.0)	23 (7.6)	2 (0.7)
2000	300	193 (64.3)	15 (5.0)	1 (0.3)

randomly selected sera that were matched for stratified age (15–29, 30–49, 50–69, and ≥ 70 years old) and sex in each year and compared them. Table 1 shows the prevalence of anti-*C pneumoniae* antibody in the different year groups between 1991 and 2000. The antibody positivity rate was between 59.0% and 73.3%. In 1993 and 1999, there were distinct peaks in the positivity rate: 73.3% and 73.0%, respectively. In contrast, in 1996, the prevalence was 59.0%, which was the lowest during the observation period. There were significant differences in the prevalence between the highest years (1993 and 1999) and other years (1993, $p = 0.00058$; 1999, $p = 0.00092$). Table 1 also shows the prevalence of high titres of IgG ($\geq 1/512$) and IgM $\geq 1/16$. The prevalence of high titre sera was between 2.6% and 7.6%. In 1999, there was also a peak in the prevalence rate of high titres and this was significantly higher than in other years ($p = 0.00075$).

We also analysed the season and seropositivity. No evidence of seasonality was noted, in that parallel seasons in different years did not show similar patterns of seroprevalence.

DISCUSSION

The seroepidemiology of *C pneumoniae* infection in Japan was studied by analysing the prevalence of anti-*C pneumoniae* antibody over a 10 year period. Our data and other epidemiological data support a major role for *C pneumoniae* infections in causing changes in the anti-*C pneumoniae* antibody positivity rate.^{5,6} In our study, year to year variability was detected in the incidence of *C pneumoniae* infections. In our area, we encountered outbreaks of *C pneumoniae* infection in some schools and families in 1993 as confirmed by the isolation of cell cultures, the polymerase chain reaction, and serology. The high prevalence in 1993 was thus related to the occurrence of *C pneumoniae* infection in our area. Therefore, the higher antibody prevalence in 1999 may reflect the occurrence of an outbreak of *C pneumoniae* infection in the same year, as described

by Karvonen *et al.*⁵ The high prevalence of extremely high IgG titres in 1999 may also point to the possibility that an epidemic of *C pneumoniae* occurred in our area.

Chlamydia pneumoniae is spread through person to person transmission by droplets, and outbreaks of infection have been reported in families, schools, military barracks, and nursing homes. Countrywide epidemics of *C pneumoniae* have also been recorded in Scandinavian countries.⁶ Epidemics, obviously caused by *C pneumoniae* infections, have occurred in the Scandinavian countries in 1976/7, 1981/2, and 1987/8 and in Finland in 1977 and 1987/8.^{5–7} The epidemic cycle, from the peak of high incidence to low incidence, has been estimated to be approximately four to six years in Scandinavia and approximately 10 years in eastern Finland.⁵ In our area, we detected two waves of largely subclinical infections that occurred at approximately six year intervals. Epidemiological surveillance of *C pneumoniae* infection must be continued to determine whether this interval reflects the epidemic cycle.

“It is intriguing to speculate that *Chlamydia pneumoniae* infection might contribute to the sex specific difference in the incidence of cardiovascular disease”

Surveys in various parts of the world have demonstrated that antibodies to *C pneumoniae* occur in over 50% of adult populations. This finding supports the assumption that antigenic boosting occurs throughout life.^{1,2} There have been only two seroepidemiological studies of *C pneumoniae* infection among the Japanese population, using the MIF test.^{8,9} Our seroepidemiological studies demonstrated that a very small proportion of children under 5 years of age show serological evidence of past infection with *C pneumoniae*. The prevalence then increases dramatically from ages 5 through to 14 years, and by age 20 years approximately 55% of those studied have detectable amounts of antibody to the organism. The seroprevalence continues to increase among older age groups, but at a slower rate, and reaches approximately 70% in the elderly. These observations are consistent with former reports.^{8,9} However, the antibody prevalence appears to be higher in the lower age groups in Japan than in Western countries.^{1,2}

Pronounced sex differences in the prevalence of antibodies to *C pneumoniae*, with an excess of seropositivity among men, have been observed in many countries.^{1,2} However, we found very little sex differences in our study; seropositivity was 59.9% in male and 54.3% in female individuals. However, the prevalence of anti-*C pneumoniae* antibodies in elderly individuals (≥ 60 years old) was significantly higher in men than in women (75.5% *v* 64.8%; $p = 0.00029$). It is intriguing to speculate that *C pneumoniae* infection might contribute to the sex specific difference in the incidence of cardiovascular disease, but further investigation in representative population samples is required to confirm a higher prevalence of infection in men. Recently, O'Neill *et al* reported that subjects who had blood taken during the winter months were more likely to be seropositive than subjects who had blood taken during other seasons.¹⁰ They suggested that infection with *C pneumoniae* may exhibit seasonality, with a higher risk of acquisition of infection during winter. In our study, however, we detected no evidence of seasonality in the past decade. This may be because only one winter period was described in O'Neill's study.¹⁰

In conclusion, we have shown that *C pneumoniae* infection is as highly endemic in Japan as it is in Western countries, and have detected year to year variability. Long term studies in Japan are needed to clarify the epidemic occurrence or periodicity of *C pneumoniae* infection.

Take home messages

- *Chlamydia pneumoniae* infection is highly endemic in Japan and has a year to year variability
- Although there was very little difference between the prevalence in men and women as a whole, the prevalence of anti-*C pneumoniae* antibodies was significantly higher in men than in women in elderly individuals (≥ 60 years old)
- The epidemic cycle appeared to be approximately six years, but further studies are needed to clarify this
- There was no evidence of seasonality in the past 10 years

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