PREVALENCE OF ANTIBODY TO CHLAMYDIA PNEUMONIAE IN RESIDENTS OF JAPAN, THE SOLOMON ISLANDS, AND NEPAL

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Abstract. Sera of 4,050 residents from Japan, 276 from the Solomon Islands, and 602 from Nepal were tested by an enzyme-linked immunosorbent assay to determine the prevalence of antibody to Chlamydia pneumoniae. The prevalence of IgG and IgA antibodies was significantly higher in the Solomon Islands (64.9% and 82.2%) and Nepal (73.1%, and 69.8%) than in Japan (53.6% and 41.1%). These prevalence rates increased throughout the teenage years in the Solomon Islands and Japan and leveled off with age, whereas in Nepal the prevalence rates gradually increased with age. The prevalence of a high (> 3.0) IgA antibody index, which is suggestive of acute infection, was significantly higher in the Solomon Islands (34.8%) than in Japan (3.2%) and Nepal (10.5%). The prevalence of IgG antibody ranged from 46.4% to 67.7%, and the prevalence of IgA antibody ranged from 33.7% to 61.8% in the four difference areas of Japan. These findings indicate considerable differences in the prevalence of antibodies to C. pneumoniae by age in these nations and between the regions of Japan tested.

INTRODUCTION

Chlamydia pneumoniae (C. pneumoniae) has recently attracted attention as a major cause of bacterial respiratory infections, including bronchitis, pharyngitis, and pneumonia.1,2 This organism is disseminated from person to person via respiratory secretions, and infection is likely to occur in small groups where comparatively close contact over longer periods of time can be expected.3,4 It was recently reported that the prevalence of antibody to C. pneumoniae is significantly higher in patients with coronary disease than in healthy controls and that C. pneumoniae is closely involved with arteriosclerosis.5−8

Conventional methods used to detect antibodies to C. pneumoniae in the serum include the micro-immunofluorescence (MIF) test and the microplate immunofluorescence antibody (MIFA) technique.5,6,9,10 However, use of the MIF method requires special skill, and there are wide differences in sensitivity among tests performed in different laboratories. A new technique for detection of antibodies to C. pneumoniae, an enzyme-linked immunosorbent assay (ELISA), has recently been developed.1,11−13 Numazaki and others14 reported that the sensitivity of the ELISA when compared with the MIF method was 90.4% for IgG and 84.6% for IgA and that the specificity was 89.9% for IgG and 86.7% for IgA in Japanese infants and children. Kishimoto and others14 also reported a good correlation between the ELISA and the MIF test in detecting antibodies to C. pneumoniae. The reported rates of agreement between the ELISA and Western blot methods were 80.0% for IgG and 87.5% for IgA. The procedures involved in the ELISA are easy to perform, there is less chance of contamination, which is more likely with the MIF test, and many samples can be measured simultaneously, making the ELISA convenient for epidemiologic studies.

Only a few large-scale epidemiologic studies of C. pneumoniae infection have been conducted in a wide age range of subjects in the United States.15 In the present study, the prevalence of IgG and IgA antibodies to C. pneumoniae was determined by an ELISA in the general population in Japan, the Solomon Islands, and Nepal.

MATERIALS AND METHODS

Patients. Serum samples were collected from 276 residents of The Solomon Islands in 1998 and from 602 residents of Nepal in 1996. The Solomon Islands consist of tropical islands located north of New Caledonia and Australia. Nepal is located in the mountains between China and India. Serum samples were collected from 4,050 residents of Japan between 1997 and 1999. They were obtained from four different areas and included 2,511 samples collected in K Town, 502 in H Village in Fukuoka prefecture, 558 in I Island in Nagasaki prefecture, and 479 in Y Island in Okinawa prefecture. Fukuoka prefecture is located in the northern part of Kyushu, one of the major islands of the Japanese archipelago. Both K Town and H Village are in Fukuoka prefecture, but the former is in a suburban area while the latter is situated in an isolated mountainous area. I Island is located approximately 25 km north of the main island of Kyushu. Y Island is in Okinawa prefecture, approximately 1,000 km southwest of Kyushu in the subtropical zone. The mean ages of the Solomon and Nepalese populations studied were 27.3 years (range = 0–57) and 44.7 years (range = 13–90), respectively. The mean age of the Japanese population was 52.2 years (range = 1–90): 54.7 years (range = 20–88) in K Town, 58.1 years (range = 23–90) in H Village, 46.2 years (range = 2–90) on I Island, and 40.2 years (range = 1–83) on Y Island. All serum samples were stored at -20°C before testing.

The age groupings of the tested individuals were as follows: Japan: < 10 years old (n = 143); 10–19 years old (n = 93); 20–29 years old (n = 180); 30–39 years old (n = 487); 40–49 years old (n = 702); 50–59 years old (n = 810); ≥ 60 years old (n = 1,635). Solomon Islands: < 10 years old (n = 28); 10–19 years old (n = 60); 20–29 years old (n = 60); 30–39 years old (n = 61); 40–49 years old (n = 28); 50–59 years old (n = 28); Nepal: 10–19 years old (n = 44); 20–29 years old (n = 109); 30–39 years old (n = 101); 40–49 years old (n = 100); 50–59 years old (n = 91); ≥ 60 years old (n = 157).

Serologic study. The presence of IgG and IgA antibodies to C. pneumoniae was determined by an ELISA (Hitzyme; Hitachi Kasei, Tokyo, Japan). Chlamydial outer membrane complex purified from the elementary bodies of the C. pneumo-
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moniae YK-41 strain was used as the test antigen. The antigen was solidified in a microplate and first tested with antibodies to C. pneumoniae in the serum sample, then with anti-human IgG antibody or anti-human IgA antibody. Subsequently, p-nitrophenyl phosphate was added as a substrate and the absorbance was determined at 405 nm. The antibody titer was indicated by an index obtained by dividing the corrected absorbance of the sample by the cut-off value (optical density = 0.200). An IgG or IgA antibody titer less than 1.1 was considered negative. A titer ≥ 3.0 was considered a high antibody index. Samples with a high IgG or high IgA antibody index by the ELISA were verified by a Western blot method.

**Statistical analysis.** The prevalence of IgG and IgA antibodies and that of high IgG and IgA antibody indexes to C. pneumoniae were analyzed by the Mantel-Haenszel test. The prevalence of IgG and IgA antibodies and that of high IgG and IgA antibody indexes to C. pneumoniae by age were analyzed by the Cochran-Armitage method. P values ≤ 0.05 were considered statistically significant.

**RESULTS**

Table 1 shows the prevalence of antibodies to C. pneumoniae in Japan, the Solomon Islands, and Nepal classified by sex. The prevalence of both IgG and IgA to C. pneumoniae in residents of the Solomon Islands (64.9% and 82.2%; P < 0.0001 and P < 0.0001) and Nepal (73.1% and 69.8%; P < 0.0001 and P < 0.0001) was significantly higher than that in residents of Japan (56.3% and 41.1%). The prevalence of IgA in Japan, the Solomon Islands, and Nepal classified by sex. The prevalence of a high IgG antibody index was significantly higher in the Solomon Islands (34.8%) than in Japan (3.2%)  (P < 0.0001) and Nepal 10.5%  (P < 0.001). The prevalence of high IgG and IgA antibody indexes in Japan (P < 0.001) and a high IgG antibody index in Nepal (P = 0.0278) were significantly higher in men than in women.

The prevalence of IgG antibody indexes was 13.3% (men = 18.5%, women = 7.8%) in H Village, 6.7% (men = 9.4%, women = 5.6%) in K Town, 3.0% (men = 3.5%, women = 6.2%) on I Island, and 7.3% (men = 8.9%, women = 5.7%) on Y Island. The prevalence of high IgA antibody indexes was 5.2% (men = 7.8%, women = 3.0%) in H Village, 2.4% (men = 3.5%, women = 2.9%) in K Town, 3.8% (men = 4.8%, women = 2.7%) on I Island, and 5.0% (men = 7.7%, women = 2.5%) on Y Island. The prevalence of a high IgG antibody index was higher in H Village than in the other three areas in Japan. The prevalence of a high IgA antibody index was higher in H Village than in the other three areas in Japan. The prevalence of a high IgG antibody index was higher in H Village than in the other three areas.

Figure 1 shows the prevalence of antibodies to C. pneumoniae by age in Japan, the Solomon Islands, and Nepal. The prevalence of both IgG and IgA increased greatly in individuals 10–19 years old on the Solomon Islands and in Japan (to 70% and 90% on the Solomon Islands and to 50% and 40% in Japan), but leveled off thereafter. The prevalence of IgA was especially high (> 80%) on the Solomon Islands for all residents more than 10 years old. The prevalence rates of IgG and IgA showed a tendency to gradually increase with age in Nepal (P < 0.0001). However, since sera of residents less than 10 years old were not obtained in this country, the prevalence in this age group is unknown. When compared with the other two countries, the prevalence was low in Japan in all groups more than 20 years old.

Table 2 shows the prevalence of a high antibody index (≥ 3.0) to C. pneumoniae in Japan, the Solomon Islands, and Nepal classified by sex. The prevalence of a high IgG antibody index in Nepal (24.9%) was significantly higher than that in Japan (7.1%) (P < 0.0001) and the Solomon Islands (9.8%). The prevalence of a high IgA antibody index was significantly higher in the Solomon Islands (34.8%) than in Japan (3.2%) (P < 0.0001) and Nepal (10.5%) (P < 0.001). The prevalence of high IgG and IgA antibody indexes in Japan (P < 0.001) and a high IgG antibody index in Nepal (P = 0.0278) were significantly higher in men than in women.

**Table 1**

Prevalence rates of IgG and IgA antibodies to Chlamydia pneumoniae in residents of Japan, the Solomon Islands, and Nepal, classified by sex and country.*

<table>
<thead>
<tr>
<th>Country</th>
<th>Antibody</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. tested</td>
<td>No. positive (%)</td>
<td>No. tested</td>
<td>No. positive (%)</td>
</tr>
<tr>
<td>Japan</td>
<td>1,438</td>
<td>834  (58.0)*</td>
<td>2,612</td>
<td>1,337  (51.2)b</td>
</tr>
<tr>
<td></td>
<td>1,438</td>
<td>676  (47.0)c</td>
<td>2,612</td>
<td>998    (37.9)d</td>
</tr>
<tr>
<td>Solomon Islands</td>
<td>146</td>
<td>99   (67.8)</td>
<td>130</td>
<td>80     (61.5)</td>
</tr>
<tr>
<td></td>
<td>146</td>
<td>122  (83.6)</td>
<td>130</td>
<td>105    (80.8)</td>
</tr>
<tr>
<td>Nepal</td>
<td>297</td>
<td>228  (76.8)</td>
<td>305</td>
<td>212    (69.5)</td>
</tr>
<tr>
<td></td>
<td>297</td>
<td>221  (74.4)</td>
<td>305</td>
<td>199    (65.2)b</td>
</tr>
</tbody>
</table>

* P < 0.0001 for a vs b, c vs d, i vs k and m, j vs l and n, and l vs n.

P = 0.0262 for e vs f. P = 0.0006 for g vs h.
reaching 43.3% or more in residents ≥ 60 years old. The prevalence of a high IgA antibody index in Japan was 5.4% in individuals 10–19 years old. This decreased to 2% in those 20–50 years old, and increased to 5.4% in those ≥ 60 years old. The prevalence of a high IgA antibody index on the Solomon Islands was 35% or higher in all age groups and 45% in residents ≥ 20 years old. In Nepal, the prevalence of a high IgA antibody index was 2.3% in individuals 10–19 years old, and increased to 17.2% in those ≥ 60 years old.

All samples assessed as having a high IgG or IgA antibody index by the ELISA were also positive by Western blot method.

**DISCUSSION**

All serum samples used in the present study were simultaneously tested for IgG and IgA antibodies to *C. pneumoniae*. Simultaneous detection is important because of differences in the times of appearance and half-lives of IgG and IgA antibodies among patients infected with *C. pneumoniae*, which are important in assessing acute and persistent infections. The ELISA is a simple method that enables the detection of antibodies to this organism in a large number of samples without being too time-consuming. This method is useful in clinical practice and epidemiologic research. The present study is the first report to demonstrate *C. pneumoniae* infection detected by ELISA in a large population.

The prevalence of antibodies to *C. pneumoniae* has been reported to vary depending on the region investigated and the age of the population tested. Wang and Grayston reported an IgG prevalence of 45% (determined by the MIF method) in seven countries, including Japan. However, Kishimoto reported an IgG prevalence of approximately 60% (determined by the MFA method) in healthy Japanese adults. According to a report that compared IgG prevalence determined by the MIF method in 12 areas of the United States

### Table 2

<table>
<thead>
<tr>
<th>Country</th>
<th>Antibody</th>
<th>Male No. tested</th>
<th>Male No. positive (%)</th>
<th>Female No. tested</th>
<th>Female No. positive (%)</th>
<th>Total No. tested</th>
<th>Total No. positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan</td>
<td>IgG</td>
<td>1438</td>
<td>142 (9.9)*</td>
<td>2612</td>
<td>146 (5.6)*</td>
<td>4050</td>
<td>288 (7.1)*</td>
</tr>
<tr>
<td></td>
<td>IgA</td>
<td>1438</td>
<td>73 (5.1)*</td>
<td>2612</td>
<td>58 (2.2)*</td>
<td>4050</td>
<td>131 (3.2)*</td>
</tr>
<tr>
<td>Solomon Islands</td>
<td>IgG</td>
<td>146</td>
<td>18 (12.3)</td>
<td>2612</td>
<td>9 (6.9)</td>
<td>276</td>
<td>27 (9.8)</td>
</tr>
<tr>
<td></td>
<td>IgA</td>
<td>146</td>
<td>43 (29.5)</td>
<td>2612</td>
<td>53 (40.8)</td>
<td>276</td>
<td>96 (34.8)*</td>
</tr>
<tr>
<td>Nepal</td>
<td>IgG</td>
<td>297</td>
<td>86 (29.0)*</td>
<td>305</td>
<td>64 (21.0)*</td>
<td>602</td>
<td>150 (24.9)*</td>
</tr>
<tr>
<td></td>
<td>IgA</td>
<td>297</td>
<td>36 (12.1)</td>
<td>305</td>
<td>27 (8.9)</td>
<td>602</td>
<td>63 (10.5)*</td>
</tr>
</tbody>
</table>

*P* < 0.0001 for a vs b, c vs d, g vs j, h vs i and k, and i vs k.

*P* = 0.0278 for e vs f.
and in other countries, this prevalence was higher in tropical countries, such as Panama (62%) and Taiwan (75%), than in more northern, developed countries, such as Nova Scotia, Canada (41%), Finland (44%), and Denmark (45%). The IgG prevalence (MIF method) was reported to be low in children less than five years old, increasing by the age of 15 to the same level found in adults. An IgG antibody titer of 512 or higher in a single serum sample tested by the MIF method is regarded as an acute infection (initial or recurrent). Ekman and others reported an MIF IgA titer > 512, which is diagnostic of acute *C. pneumoniae* infection. An IgG or IgA antibody titer of 512 or higher determined by the MIF method corresponds to an antibody index of 3.0 or more by an ELISA. Therefore, a high antibody index determined by the ELISA in the present study may indicate acute infection.

In the present study, the prevalence of IgG and IgA specific for *C. pneumoniae* in Japan increased in those individuals 10–19 years old, and the prevalence of high IgG and IgA antibody indexes, which suggests initial infection, was also highest in this age group. In Japan between 1997 and 2000, 19 (5.9%) of 323 adults and 21 (19.6%) of 107 children seroconverted from IgG negative to IgG positive (unpublished data, Kikuchik, Kyushu University Hospital, Department of General Medicine). These results suggest that initial *C. pneumoniae* infections occurred in children less than 10 years of age and new infections rarely occurred in adults.

The prevalence of IgA in residents ≥ 10 years old in the Solomon Islands was higher than that observed in Japan. In addition, the prevalence of a high IgA antibody index was also greater in the Solomon Islands than in Japan and Nepal. These results suggest that there may have been an epidemic of *C. pneumoniae* in the Solomon Islands shortly before the time of serum collection in 1998. Because the half-life of IgA is generally short, it is used as an index of current or recent infection. *Chlamydia pneumoniae* is also an important epidemic pathogen, causing recurrent infection. Because the Solomon Islands are a region that is highly endemic for malaria, respiratory diseases, and viral infections, recurrent *C. pneumoniae* infections might also occur frequently. High prevalence rates of IgA have been observed in chronic pulmonary diseases, such as in the acute exacerbation of bronchial asthma, suggesting a relationship with persistent respiratory disease. Wang and Grayston reported the prevalence of IgG determined by the MIF method to be 15% in the Solomon Islands in a small number of serum samples collected in 1966. These discrepancies can be explained by the results of the present study.

In the present study, the prevalence of IgG and that of a high IgG antibody index were greater in Nepal compared with those observed in Japan and the Solomon Islands. Moreover, the prevalence of IgG and a high IgG antibody index increased with age, especially in Nepal, and were notably higher than those observed in Japan and the Solomon Islands among residents ≥ 40 years old. These results suggest that there may have been an epidemic of *C. pneumoniae* shortly before the time of serum collection. Epidemics of *C. pneumoniae* are likely to occur in crowded areas. In addition, the increase in the prevalence of the high IgG antibody index with age suggests recurrent or persistent infection in the elderly.

Our study showed that infection with *C. pneumoniae* was more prevalent in men than in women, as was reported in...
studies from Japan, the United States, and Sweden. In men, susceptibility to infection with *C. pneumoniae* may be due to smoking or having an increased opportunity for contact with others. In general, women seem to have a greater capacity to eliminate microorganisms, such as hepatitis B virus, hepatitis C virus, and *Strongyloides stercoralis*. Infection with *C. pneumoniae* also may be more persistent in men than in women.

By measuring both IgG and IgA antibodies to *C. pneumoniae* by an ELISA, we found that the age at the peak and epidemic periods of *C. pneumoniae* infection are different in Japan, the Solomon Islands, and Nepal. This investigation showed a considerable difference in the prevalence of antibodies to *C. pneumoniae* in these three countries, as well as in different areas in Japan.

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