PREVALENCE OF ANTIBODY TO TOXOPLASMA GONDII AMONG INHABITANTS UNDER DIFFERENT GEOGRAPHICAL AND CLIMATIC CONDITIONS IN HYOGO PREFECTURE, JAPAN

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SUMMARY: Sera collected from 2,564 inhabitants of different areas in Hyogo Prefecture, Japan, were examined for antibody to Toxoplasma gondii by enzyme-linked immunosorbent assay. The overall prevalence rates of male (11.6%) and female populations (7.6%) were significantly different (P<0.001). The prevalence also depended on age of the population. Of 11 districts three, being far from the sea and occupied mainly by mountains with relatively high altitudes (95-195 m), showed a significantly lower average prevalence (5.0%) than the other districts with altitudes of 3-58 m, a part of which was adjacent to the sea (10.6%; P<0.001). The average prevalence obtained in two districts with annual rainfall of 1,862-2,062 mm (16.8%) was significantly higher than that obtained in six districts with annual rainfall of 1,026-1,204 mm (8.6%; P<0.001).

INTRODUCTION

Toxoplasma gondii is a protozoan parasite which infects humans at relatively high rates even in countries in the temperate zone. The infection is transmitted to humans mainly by accidental ingestion of the organism in two forms, the oocyst defecated by infected cats or the cyst contained in raw meat, except for con-
genital transmission (1). The reported prevalence among humans in the world differs from one geographical area to another, relating to climatic, hygienic and sociological factors in the survey area as well as occupations, manners and customs of the inhabitants. The difference in the Toxoplasma prevalence depending on the area may reflect the different transmission rates of this organism to humans depending on these environmental factors.

Several antibody surveys carried out in Japan (2-5) also have provided different prevalence rates among humans in different districts. Although the difference in the Toxoplasma prevalence depending on the district has been studied from the viewpoints of prevalence among cats and oocyst contamination in soil, geographical or climatic factors relating to the prevalence are not well known. A series of our study to know the present status of Toxoplasma infection in Japanese populations has consistently been performed with populations in Hyogo Prefecture, the middle-west part of the country (6-10). The purpose of this study was to compare the Toxoplasma antibody prevalence among inhabitants of different areas in this prefecture and to discuss possible environmental factors involved in transmission of Toxoplasma to humans.

MATERIALS AND METHODS

Sample population: A total of 2,564 sera surveyed were collected from general inhabitants of 11 administrative districts in Hyogo Prefecture at public medical examinations under the primary health care program organized by this prefecture. The locations of these districts covered the northern, middle and southern parts of the prefecture, the total area of which is about 8,400 km² (2) (Fig. 1). The station was not available in districts C, G or I, but we supposed that the environmental conditions of these districts were similar to those obtained at the nearest station. These environmental data and the population densities of the 11 districts are listed in Table I. The subjects for the survey included 1,070 males aged 16-62 years and 1,494 females aged 16-64 with mean ages of 34.6 and 32.7, respectively. The ages were grouped into 16-19, 20-29, 30-39, 40-49, and 50-64 years, and the mean ages in these districts were comparable.

Enzyme-linked immunosorbent assay (ELISA): Toxoplasma antigens used for ELISA tests were prepared from tachyzoites obtained from the peritoneal exudates of the mice infected with RH strain of Toxoplasma gondii. Tachyzoites were separated from leukocytes by filtration through polycarbonate membrane, sonicated and clarified as previously described (6). Antibodies to Toxoplasma in sera were measured by ELISA essentially as previously described (11). Briefly,
Fig. 1. Survey areas in Hyogo Prefecture, Japan. Serum samples were collected from inhabitants of 11 districts (A-K), and geographic and climatic data were obtained from eight meteorological stations (a, b, d, e, f, h, j and k).

The solid phase sensitized with Toxoplasma soluble antigen at 15 μg/ml was allowed to react with test sera at a 1:100 dilution and then with alkaline phosphatase-conjugated anti-human immunoglobulin G (gamma-chain specific: Tago Inc.) at a dilution of 1:1,000. The substrate was p-nitrophenyl phosphate at 1 mg/ml. Absorbance values obtained in duplicate were averaged and adjusted with the value for the positive control to minimize interplate variations. The borderlines differentiating positive from doubtful and doubtful from negative were determined by statistical analysis of ELISA values obtained with human sera that were diagnosed as positive and negative by the dye test (11).

Statistical analysis: Significance of differences was evaluated by the Chi-square test with the Yates' correction factor (12).
Table I. Environmental data in 11 districts in Hyogo Prefecture

<table>
<thead>
<tr>
<th>District</th>
<th>Station[^a]</th>
<th>Altitude (m)</th>
<th>Temperature[^b] (°C)</th>
<th>Annual rainfall[^b] (mm)</th>
<th>Annual snowfall[^c] (cm)</th>
<th>Population density[^d] (per km²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>a</td>
<td>6</td>
<td>14.0</td>
<td>2,062</td>
<td>347</td>
<td>99</td>
</tr>
<tr>
<td>B</td>
<td>b</td>
<td>3</td>
<td>13.7</td>
<td>1,862</td>
<td>—</td>
<td>295</td>
</tr>
<tr>
<td>C</td>
<td>d</td>
<td>95</td>
<td>13.3</td>
<td>1,415</td>
<td>—</td>
<td>109</td>
</tr>
<tr>
<td>D</td>
<td>d</td>
<td>95</td>
<td>13.3</td>
<td>1,415</td>
<td>—</td>
<td>170</td>
</tr>
<tr>
<td>E</td>
<td>e</td>
<td>195</td>
<td>13.0</td>
<td>1,736</td>
<td>—</td>
<td>75</td>
</tr>
<tr>
<td>F</td>
<td>f</td>
<td>38</td>
<td>14.6</td>
<td>1,176</td>
<td>5</td>
<td>1,659</td>
</tr>
<tr>
<td>G</td>
<td>f</td>
<td>38</td>
<td>14.6</td>
<td>1,176</td>
<td>5</td>
<td>1,812</td>
</tr>
<tr>
<td>H</td>
<td>h</td>
<td>58</td>
<td>15.5</td>
<td>1,204</td>
<td>3</td>
<td>2,656</td>
</tr>
<tr>
<td>I</td>
<td>h</td>
<td>58</td>
<td>15.5</td>
<td>1,204</td>
<td>3</td>
<td>10,444</td>
</tr>
<tr>
<td>J</td>
<td>j</td>
<td>5</td>
<td>15.3</td>
<td>1,026</td>
<td>—</td>
<td>277</td>
</tr>
<tr>
<td>K</td>
<td>k</td>
<td>33</td>
<td>15.7</td>
<td>1,113</td>
<td>6</td>
<td>284</td>
</tr>
</tbody>
</table>

[^a] Meteorological stations where geographic and climatic data were obtained (see Fig. 1).  
[^b] Average of data obtained from 1979 through 1987.  
[^c] Average of data obtained from 1961 through 1990.  
[^e] Data not available.

RESULTS

Age and Sex Dependency of Prevalence

Figure 2 shows age-dependent prevalence curves of males and females. Males showed higher seropositive rates than females in all but one age group of 40-49 years. The overall prevalence was 11.6% (124/1,070) in males and 7.6% (114/1,494) in females, which were significantly different (P<0.001; Table II).

Difference in Prevalence among 11 Districts

The prevalence rates in the 11 survey districts widely ranged from 4.2% to 18.7% with significant differences observed in some pairs (Table II). When each population was divided into males and females, the maximum rate was observed in males in district A (21.3%) and the minimum rate in females in district D (1.9%). The positive rates were higher in males than in females in all districts, among which the difference was significant in district D (P<0.05).
Relation between Prevalence and Environmental Factors

Based on the above results, the prevalence rates were analyzed among districts grouped according to environmental factors. Significant differences were observed in two comparisons made in terms of altitude and annual rainfall. Mountain areas corresponding to districts C, D, and E, which had relatively high altitudes (95-195 m), showed lower prevalence rates (5.0% in average) than seaside areas corresponding to the other districts (10.6% in average; P<0.001), which had relatively low altitudes (3-58 m). The northern part of the prefecture corresponding to the districts A and B which had more annual rainfall (1,862-2,062 mm), showed higher prevalence rates (16.8% in average) than the southern part corresponding to districts F-K (8.6% in average; P<0.001), which had less annual rainfall (1,026-1,204 mm).
Table II. Incidence of *Toxoplasma* antibodies in males and females in 11 districts in Hyogo Prefecture

<table>
<thead>
<tr>
<th>District</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>21.3% (32/150)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.1% (24/149)</td>
<td>18.7%&lt;sup&gt;b&lt;/sup&gt; (56/299)</td>
</tr>
<tr>
<td>B</td>
<td>14.1% (14/99)</td>
<td>13.4% (13/97)</td>
<td>13.8%&lt;sup&gt;c&lt;/sup&gt; (27/196)</td>
</tr>
<tr>
<td>C</td>
<td>8.6% (6/70)</td>
<td>3.3% (4/123)</td>
<td>5.2%&lt;sup&gt;e&lt;/sup&gt; (10/193)</td>
</tr>
<tr>
<td>D</td>
<td>7.2% (1/125)</td>
<td>1.9% (3/158)</td>
<td>4.2%&lt;sup&gt;f&lt;/sup&gt; (12/283)</td>
</tr>
<tr>
<td>E</td>
<td>7.1% (3/42)</td>
<td>5.9% (6/102)</td>
<td>6.3%&lt;sup&gt;e&lt;/sup&gt; (9/144)</td>
</tr>
<tr>
<td>F</td>
<td>6.6% (7/106)</td>
<td>3.8% (5/133)</td>
<td>5.0%&lt;sup&gt;e&lt;/sup&gt; (12/239)</td>
</tr>
<tr>
<td>G</td>
<td>9.1% (8/88)</td>
<td>8.6% (14/162)</td>
<td>8.8%&lt;sup&gt;d&lt;/sup&gt; (22/250)</td>
</tr>
<tr>
<td>H</td>
<td>9.3% (14/150)</td>
<td>6.6% (11/167)</td>
<td>7.9%&lt;sup&gt;e&lt;/sup&gt; (25/317)</td>
</tr>
<tr>
<td>I</td>
<td>20.0% (10/50)</td>
<td>12.6% (18/143)</td>
<td>14.5%&lt;sup&gt;c&lt;/sup&gt; (28/193)</td>
</tr>
<tr>
<td>J</td>
<td>11.6% (15/129)</td>
<td>7.2% (11/153)</td>
<td>9.2%&lt;sup&gt;d&lt;/sup&gt; (26/282)</td>
</tr>
<tr>
<td>K</td>
<td>9.8% (6/61)</td>
<td>4.7% (5/107)</td>
<td>6.6%&lt;sup&gt;e&lt;/sup&gt; (11/168)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>11.6% (124/1070)</strong></td>
<td><strong>7.6% (114/1494)</strong></td>
<td><strong>9.3% (238/2564)</strong></td>
</tr>
</tbody>
</table>

<sup>a</sup>Percent positivity (number of positive sera/number of tested sera).

<sup>b</sup>Significantly different from d,e,f (P < 0.001).

<sup>c</sup>Significantly different from e(P < 0.05) and f (P < 0.001).

<sup>d</sup>Significantly different from f(P < 0.05).

**DISCUSSION**

Difference in the *Toxoplasma* antibody prevalence between men and women has been reported in several countries. Females were more frequently infected in Norway (13), Poland (14), Finland (15) and Iran (16), but males were more frequently infected in Panama (17) and China (18). In Japan, where several populations have been surveyed (2,4,5), males and females did not show any significant difference in the antibody prevalence. However, our surveys in Hyogo Prefecture have consistently indicated that the prevalence rate was significantly higher in males than in females in populations of outpatients (6) and farmers (7). The present study also confirmed the difference in the prevalence between males and females in Hyogo populations.

The areas surveyed in this study were not divided on the basis of geographical conditions, but were administrative districts in which a large number of sera
were available at medical examinations. In such a survey system, common geographical factors were necessarily shared at least by adjacent districts, so that difference in prevalence depending on these factors, even if present, may possibly come to be insignificant. However, the 11 districts in this study showed significant difference in the antibody prevalence rate.

Altitude has been considered as a factor involved in the *Toxoplasma* prevalence. Studies in Mexico (19), Guatemala and Costa Rica (20), Panama (17) and Brazil (21) have indicated the highest prevalence at the lowest altitude. When the survey area had the relatively narrow limits of altitude, prevalence may not correlate with elevation like a survey in El Salvador (22) with a maximum altitude of 1,200 m. In this study, a significant difference was observed between inhabitants of mountain areas and those of the other areas, in spite of limited altitudes. However, because low prevalence rates were also observed in lower areas, such as district F, the difference in the prevalence is not likely to be explained by altitude only. It is unknown which epidemiological factors involved in transmission of this parasite to humans are directly related to the altitude.

A significant difference in antibody prevalence was observed between inhabitants of districts with different amounts of rainfall. Although Remington et al. (22) concluded no correlation between *Toxoplasma* infection and rainfall in their survey in El Salvador, high prevalence associated with heavier rainfall observed in the districts A and B may be explained by the fact that toxoplasma oocysts can survive longer in more humid soil. Kobayashi (2) described that sporulated oocysts survived at 7°C for 8 months in water, for 4 months at 66% relative humidity and for 2 months at 32% relative humidity. However, the heavier rainfall in the northern part of Hyogo Prefecture is attributable in part to snowfall, the effect of which on the transmission of *Toxoplasma* is not known.

The antibody prevalence among inhabitants did not correlate with that prevalence among stray cats collected at most areas in Hyogo Prefecture (M. Goto, personal communication). For instance, seropositivity among the cats collected in districts A and B, where positive rates were high in humans, ranged from 0 to 22.2%, whereas that in cats collected in district D where the rate in humans was lowest ranged from 19.0% to 31.6%. Although oocysts are undoubtedly a major agent of transmission of *Toxoplasma*, a previous serological survey in Japan (23) also showed no significant correlation between prevalence rates in humans and cats.

The prevalence of *Toxoplasma* infection among humans is affected by several factors. Some districts surveyed in this study included both urban and rural
environments, in which the inhabitants were considerably different in the occupation, living style and customs. Moreover, the place of residence is sometimes different from the place of birth or growing up. It is highly probable that these factors may complicate the interpretation of the survey data concerning the difference in Toxoplasma antibody prevalence in humans. However, the significant differences between Toxoplasma prevalence among humans and environmental factors such as altitude and annual rainfall obtained in this study indicate that some epidemiological factors involved in the environment are related to the transmission of Toxoplasma to humans in nature.

REFERENCES


