Anti-Toxoplasma Antibody Prevalence, Primary Infection Rate, and Risk Factors in a Study of Toxoplasmosis in 4,466 Pregnant Women in Japan

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Toxoplasmosis is a zoonosis caused by infection with Toxoplasma gondii and is prevalent worldwide under various climatic conditions. It is usually asymptomatic, but infection in pregnant women can pose serious health problems for the fetus. However, epidemiological information regarding toxoplasmosis in Japanese pregnant women is limited. This study aimed to determine the prevalence of anti-Toxoplasma antibodies, the primary infection rate, and the risk factors for toxoplasmosis in Japanese pregnant women. We measured anti-Toxoplasma antibody titers in 4,466 pregnant women over a period of 7.5 years and simultaneously conducted interviews to identify the risk factors for toxoplasmosis. The overall prevalence of anti-Toxoplasma antibodies was 10.3%, and it was significantly higher in women aged above 35 years. The rate of primary Toxoplasma infection during pregnancy was estimated to be 0.25%. A possibility of infection in the later stages of pregnancy was identified for those women who were not infected in the early stages. A history of raw meat intake was identified to be a risk factor related to toxoplasmosis. Therefore, to lower the risk of toxoplasmosis, pregnant women should refrain from eating raw and undercooked meat and maintain personal hygiene.

Toxoplasmosis is a zoonosis caused by infection with Toxoplasma gondii, which is a polyenous pathogenic protozoan parasite belonging to the Apicomplexa phylum. It can.parasitize humans, in addition to a wide range of other vertebrate species. Toxoplasmosis is prevalent worldwide and occurs under various climatic conditions. Considering that cultural habits and behaviors, such as personal hygiene, food-handling practices, and cooking preferences, vary among countries, the seroprevalence of T. gondii infection and the associated risk factors also vary among countries. The seroprevalence of T. gondii ranges from 6.1% to 74.5% among different regions of the world (1, 2, 9, 10, 12, 15, 18, 20, 23, 24, 26–29). Various risk factors for toxoplasmosis have been reported, including childbearing age (3, 10, 26); previous pregnancy (3); consumption of raw meat (1, 6, 10, 11, 13, 16–18, 21, 23, 31); physical contact with cats (3, 7, 11, 13, 16, 18, 20) and contaminated soil (16, 17, 27); consumption of unwashed raw vegetables or fruits (18, 20), unboiled water (10), and unpasteurized milk (29); low educational standards (3, 18, 20); and residence in rural areas (18).

In general, toxoplasmosis is asymptomatic in immunocompetent hosts and induces a self-limiting disease. However, infection in pregnant women can pose serious health problems for the fetus. If T. gondii infects a pregnant woman with no previous exposure to the parasite, it can migrate through the placenta into the fetus and subsequently cause congenital toxoplasmosis (4, 25, 31). Many of the clinical symptoms may present themselves in various forms in the fetus; these include hydrocephalus, retinochoroiditis, thrombocytopenia, mental retardation, epilepsy, and even fetal death (4, 11, 18, 19, 22, 30).

Conversely, a lot of pregnant women have a history of infection. It is particularly important to precisely determine whether infection occurred in the early stage of pregnancy or before pregnancy because identification of the time of primary infection is crucial for the clinical management of pregnant women infected with T. gondii. However, even if Toxoplasma antibodies are not detected in the early stages of pregnancy, we should consider the possibility of infection during the later stages because all individuals are surrounded by some risk factors for toxoplasmosis. To the best of our knowledge, there is limited epidemiological information concerning toxoplasmosis in Japanese pregnant women.

Therefore, this study was conducted to investigate the prevalence of anti-Toxoplasma antibodies and the primary infection rate of Toxoplasma in Japanese pregnant women. We measured anti-Toxoplasma antibodies in both early and late pregnancy and calculated the rate of anti-Toxoplasma antibody seroconversion during pregnancy. This study also aimed to identify the possible risk factors associated with toxoplasmosis in Japan.

MATERIALS AND METHODS

The present study included a total of 4,466 pregnant women aged between 16 and 46 years (median age, 27.4 years) who received antenatal care in a private hospital located in Miyazaki Prefecture in Kyushu, Japan. Miyazaki Prefecture is located in the southeast region of Japan (approximately 31°N, 130°E), and it covers an area of 7,734 km². This research was conducted over a period of 7.5 years between July 1997 and December 2004. The subjects comprised 2,761 primiparous women, 1,468 women with a history of one pregnancy, and 237 women with a history of two pregnancies.
After obtaining informed consent from the participants, we measured anti-\emph{Toxoplasma} antibodies in maternal blood. Pregnant women were tested for the presence of anti-\emph{Toxoplasma} antibodies using an assay kit for \emph{Toxoplasma} latex agglutination (LA) microtiters, called Toxotest-MT Eiken (provided by Eiken Chemical Co., Ltd., Japan), according to the manufacturer’s instructions. In this kit, LA titers of 1:32 or more are regarded to be a positive result. A $\chi^2$ test was used to verify the rate of anti-\emph{Toxoplasma} antibody prevalence from the viewpoint of maternal age.

Moreover, to determine the primary infection rate during pregnancy, we calculated the anti-\emph{Toxoplasma} antibody seroconversion rate in 2,696 subjects. Their antibodies were measured in both early and late pregnancy. The mean interval between antibody measurements (early and late pregnancy) was 16.2 weeks. Therefore, we estimated the average gestational period to be 40 weeks, and the rate of \emph{Toxoplasma} infection during pregnancy was estimated to be 0.25%.

### Risk factors for toxoplasmosis
A total of 4,035 women completed the interview on the risk factors for toxoplasmosis. The history of raw meat intake was considered a risk factor (95% confidence interval [CI], 0.543 to 0.936; $P = 0.02$). Conversely, cat ownership was not significantly related to the infectious risk of \emph{Toxoplasma} (95% CI, 0.680 to 0.100; $P = 0.05$). When the prevalence of anti-\emph{Toxoplasma} antibodies in the South Kyushu region, which consists of three prefectures (Miyazaki, Kagoshima, and Kumamoto), was compared with that in the other areas of Japan, the rate was significantly higher in the former than in the latter (95% CI, 1.161 to 2.769; $P = 0.01$).

Therefore, owing to these significant trends, we also performed multivariate analysis for the history of raw meat intake and residence in the South Kyushu region. The results revealed that residence in the South Kyushu region was not a risk factor (95% CI, 0.523 to 1.070; $P = 0.11$), whereas history of raw meat intake was identified to be an independent risk factor (95% CI, 0.359 to 0.785; $P = 0.0015$) for toxoplasmosis.

### DISCUSSION
In this study, the prevalence of anti-\emph{Toxoplasma} antibodies in Japanese pregnant women was 10.3%. With regard to age, the prevalence was significantly higher in women above 35 years of age than in women below 35 years. In general, the prevalence of anti-\emph{Toxoplasma} antibody was almost 1 in every 5 pregnant women above 35 years of age. This suggests that the probability of coming into contact with sources of infection increases with age. As the number of high-risk pregnancies, including late childbearing, has continued to increase in recent years (8), the probability of contacting potential sources of infection may also increase. Older pregnant women should be followed carefully through antenatal care.

### RESULTS
**Prevalence of anti-\emph{Toxoplasma} antibodies.** Among 4,466 pregnant women, 459 tested positive for anti-\emph{Toxoplasma} antibodies; therefore, the overall anti-\emph{Toxoplasma} antibody prevalence was 10.3% in this study. In generational terms, the prevalence of anti-\emph{Toxoplasma} antibodies was as follows: late teenage years, 5/126 (4.0%); early 20s, 89/1,057 (8.4%); late 20s, 172/1,886 (9.1%); early 30s, 135/1,100 (12.3%); late 30s, 55/268 (20.5%); and 40s, 3/29 (10.3%) (Fig. 1). The rate of \emph{Toxoplasma} infection was significantly higher among women above 35 years of age (58/297) than among women below 35 years of age (401/4,169) ($P < 0.001$).

**Seroconversion rate during pregnancy.** Of the 2,969 subjects in whom anti-\emph{Toxoplasma} antibodies were detected both in early and in late pregnancy, 3 subjects (0.1%) exhibited seroconversion during pregnancy. The backgrounds of these three pregnant women are presented in Table 1; they all lived in the South Kyushu region (two in Miyazaki Prefecture and one in Kagoshima Prefecture) and had a history of raw meat intake.

This result indicated that pregnant women who are not infected by \emph{Toxoplasma} in the early stages of pregnancy can be infected during the later stages. The mean interval between the two antibody measurements (early and late pregnancy) was 16.2 weeks. Therefore, we estimated the average gestational period to be 40 weeks, and the rate of \emph{Toxoplasma} infection during pregnancy was estimated to be 0.25%.

**TABLE 1 Characteristics of three pregnant women who demonstrated seroconversion during pregnancy**

<table>
<thead>
<tr>
<th>Case</th>
<th>Maternal age (yr)</th>
<th>Early stage of pregnancy</th>
<th>Late stage of pregnancy</th>
<th>History of raw meat intake</th>
<th>History of owning a cat</th>
<th>Residence in the South Kyushu region</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>16 (1:16)</td>
<td>31 (1:128)</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>36</td>
<td>16 (&lt;1:16)</td>
<td>32 (1:32)</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>19 (&lt;1:16)</td>
<td>32 (1:64)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

$^a$ LA, latex agglutination.
The rate of primary infection with *Toxoplasma* during pregnancy was estimated to be 0.25%. Although the seroconversion rate was low, it did not rule out the possibility that pregnant women not infected in the early stages of pregnancy could be infected during the later stages. Therefore, continuous monitoring for toxoplasmosis and its risk factors should be performed during the entire duration of pregnancy. Prospective monitoring of changes in anti-*Toxoplasma* antibody titers should be a useful approach for diagnosing primary infection during pregnancy.

Consumption of raw meat was a risk factor for *Toxoplasma* infection. This finding is consistent with those of previous reports (1, 6, 10, 11, 13, 16–18, 21, 23, 31). Pregnant women should refrain from eating raw or undercooked meat. Toxoplasmosis can largely be prevented by cooking meat at a safe temperature. Although the prevalence of anti-*Toxoplasma* antibodies in the South Kyushu region was higher than that in other areas, eating habits but not residence in the South Kyushu region were revealed to be an independent risk factor for toxoplasmosis. In the South Kyushu region, the consumption of raw meat (e.g., horse meat) is prevalent; therefore, women in this region should be advised regarding their eating habits during pregnancy.

In this survey, a history of owning a cat tended to increase the risk for toxoplasmosis, but this tendency was not significant. However, this finding does not mean that cats cannot be a source of toxoplasmosis. Those who have lived with a cat for long periods may have been infected with *Toxoplasma* before pregnancy. For women who own or work with cats, the desire to give birth in the future is a sufficient reason to undergo *Toxoplasma* antibody testing to detect previous *Toxoplasma* infection. If no anti-*Toxoplasma* antibodies are detected, further informed precautions should be taken to prevent toxoplasmosis during pregnancy, and prospective mothers who own cats should avoid coming into contact with cat litter boxes.

The limitation of this study is that we did not detect the genome of *T. gondii* from raw meat. However, we believe that health education for women of childbearing age is extremely important to prevent maternal toxoplasmosis (5, 14, 16, 19). If women conceive, they should be educated regarding their eating habits and the value of good hygiene at the first visit for antenatal care. In particular, they should avoid eating raw and undercooked meat. Therefore, counseling pregnant women about the risk factors for toxoplasmosis can reduce the risk of congenital toxoplasmosis.

REFERENCES