SEROPREVALENCE OF TOXOPLASMA GONDII ANTIBODY IN VIETNAMESE VILLAGERS

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Abstract. Toxoplasmosis caused by Toxoplasma gondii is a protozoan infection found worldwide. It usually produces non-specific symptoms, but in pregnant women and immunocompromised individuals, it may cause severe and fatal illness. Many serological studies have been done in various parts of the world, but information is lacking for Vietnam. A seroprevalence study of T. gondii antibodies in Vietnamese villagers (n = 650) was performed using the Sabin-Feldman dye test. The average seroprevalence was 4.19% (95% CI = 1.78-4.62), including 6.36% (95% CI = 3.22-11.09), 4.73% (95% CI = 1.92-9.50) and 1.09% (95% CI = 0.23-3.15) from Nghe An, Lao Cai and Tien Giang provinces, respectively. This study confirmed the low prevalence of toxoplasmosis in Vietnam similar to other countries in the region. Further studies are necessary in order to provide a complete picture for the country.

INTRODUCTION

Toxoplasmosis caused by Toxoplasma gondii can occur in all warm-blooded animals, including humans. Domestic, cats and other felines can transmit the infection to humans. Human infections are often asymptomatic, but in some it can cause severe disease which is usually observed in congenitally infected children. It causes abortion and stillbirths, particularly in immunosuppressed individuals, including patients with acquired immune deficiency syndrome (AIDS). Natural infection is acquired by ingestion of food or water containing oocysts found in cat excreta, bradyzoites in raw or undercooked meat or by transplacental transmission. Sero-prevalence data indicate toxoplasmosis is one of the most common human infections throughout the world (Fig 1), however, the prevalence levels reported vary greatly for different countries (Sukthana, 2006). A high prevalence of infection in France has been related to a preference for eating raw or undercooked meat, whereas in Central America it has been due to large numbers of stray cats which favor the survival of oocysts. The prevalence of T. gondii in most countries of Southeast Asia is relatively low, ranging from 7 to 30%, (Sery et al, 1988; Lee et al, 2000; Sukthana et al, 2000; Fan et al, 2002; Nissapatorn et al, 2004), but is as high as 70% in Indonesia (Terazawa et al, 2003). A major concern is whether acquired toxoplasmosis is due largely to consuming infected meat or consuming food contaminated with oocysts from cat excreta.

In Vietnam, a study in a suburban agricultural area of Hanoi and in a mountainous valley near Lao PDR showed that 20% of im-
munocompetent individuals were found to have *T. gondii* antibodies (Sery et al., 1988). Since up-to-date serological data are an important indicator for implementing preventive measures, the seroprevalence of *T. gondii* antibodies in Vietnamese villagers was studied.

**MATERIALS AND METHODS**

After informed consent was obtained, six hundred fifty serum samples were collected from immunocompetent subjects from February to March, 2006 from three provinces in Vietnam: Nghe An, Lao Cai and Tien Giang provinces (Fig 2). Sera were stored at -20°C for testing IgG antibody to *Toxoplasma gondii* by the Sabin-Feldman dye test. Demographic data, including age, sex, occupation and living location were recorded.

For maintenance of *T. gondii*, ICR mice were inoculated intraperitoneally with *T. gondii* at a concentration of 30,000-50,000 cells/mm³ every 3-4 days to maintain the parasite. The mice were sacrificed and tachyzoites of *T. gondii* were removed by intraperitoneal washings using sterile normal saline.

IgG *Toxoplasma* antibodies were determined from each serum sample using the Sabin-Feldman dye test (Sabin and Feldman, 1948). This test, which has a high specificity and sensitivity, is regarded as the gold standard method for the serodiagnosis of toxoplasmosis (Reiter-Owona et al., 1999). It is based on complement-mediated cytolysis of antibody-coated live *T. gondii* tachyzoites, indicated by their inability to take up methylene blue. The accessory factor, including alserver solution and *T. gondii* sero-negative human serum, were added to promote a better reaction for each dilution of tested sera. After a one hour incubation, 30 µl of methylene blue were added to each well. This was then left for five minutes, and the result was read using a 40 x microscope. A positive result, was indicated by ≥55% unstained *T. gondii* organisms, while a negative result was indicated by stained *T. gondii* organisms. The method is semi-quantitative; thus, tested samples were reported as either positive with a titer or negative.
Table 1

Seropositive results for T. gondii from various provinces in Vietnam.

<table>
<thead>
<tr>
<th>Province</th>
<th>Age (year)</th>
<th>Male No. positive / No. tested (%)</th>
<th>Female No. positive / No. tested (%)</th>
<th>Total No. positive / No. tested (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Nghe An</td>
<td>0.5-30</td>
<td>0/31 (0.0)</td>
<td>5/50 (10.0)</td>
<td>5/81 (6.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>31-60</td>
<td>3/23 (13.0)</td>
<td>2/46 (4.3)</td>
<td>5/69 (7.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥ 61</td>
<td>0/11 (0.0)</td>
<td>1/12 (8.3)</td>
<td>1/28 (4.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Average</td>
<td></td>
<td>6.36%</td>
<td>3.22-11.09</td>
</tr>
<tr>
<td>Lao Cai</td>
<td>0.5-30</td>
<td>2/66 (3.0)</td>
<td>2/38 (5.3)</td>
<td>4/104 (3.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>31-60</td>
<td>2/22 (9.1)</td>
<td>1/10 (1.0)</td>
<td>3/32 (9.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥ 61</td>
<td>0/6 (0.0)</td>
<td>0/6 (0.0)</td>
<td>0/12 (0.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Average</td>
<td></td>
<td>4.73%</td>
<td>1.92-9.50</td>
</tr>
<tr>
<td>Tien Giang</td>
<td>0.5 - 30</td>
<td>0/34 (0.0)</td>
<td>0/49 (0.0)</td>
<td>0/83 (0.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>31 - 60</td>
<td>0/57 (0.0)</td>
<td>1/92 (1.1)</td>
<td>1/149 (0.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥ 61</td>
<td>0/15 (0.0)</td>
<td>2/28 (7.1)</td>
<td>2/43 (4.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Average</td>
<td></td>
<td>1.09%</td>
<td>0.23-3.15</td>
</tr>
<tr>
<td>Unknown</td>
<td></td>
<td>-</td>
<td>-</td>
<td>3/54 (5.6)</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>24/650 (4.19)</td>
<td>1.78-4.62</td>
</tr>
</tbody>
</table>

Known positive serum samples and normal saline solution were included when perform the test as a positive and negative controls, respectively.

RESULTS

Twenty-four of 650 samples from immunocompetent individuals from various provinces of Vietnam were positive for T. gondii antibodies. The overall prevalence rate of T. gondii antibodies was 4.19%. The seroprevalence of T. gondii antibodies in Nghe An Province was 6.36% (95% CI = 3.22-11.09) with the titers varying from 1:4 to 1:16. In Lao Cai, it was 4.73% (95% CI = 1.92-9.50) with titers varying from 1:4 to 1:32 (without 1:16) while in Tien Giang Province, it was 1.09% (95% CI = 0.23-3.15) with titers varying from 1:4 to 1:32 (Table 1).

DISCUSSION

T. gondii infection occurs worldwide. High
prevalence rates are found in South America, Europe and Africa. In Asia, the figures vary from 6.9% to 70% (Lee et al, 2000; Terazawa et al, 2003). This study showed much lower T. gondii antibody prevalence levels compared to the study by Sery et al (1988) (4.1% vs 20%). This finding may be for two main reasons. First, our study was carried out 20 years later when hygiene conditions have been much improved and a lower rate of infection has ensued (Khe et al, 2002). Second, our study represents both northern and southern Vietnam, while the previous study was carried out only in the north (Fig 2). Moreover, the techniques used were different. The Sabin-Feldman Dye test, the gold standard method, was used in our study while the previous study used a microreaction of the complement fixation method. The sensitivity of the latter would account for false positive cases, thus giving a higher sero-prevalence result.

The sero-prevalence rate found in our study is also lower than that reported for other Asian countries (Fig 1). The sero-prevalence has been reported to be 6.9% in Korea (Lee et al, 2000), 12.4% in Thailand (Sukthana et al, 2000), and 23% in Taiwan (Fan et al, 2002). This study showed much lower rates than those found in Malaysia and Indonesia, where prevalence levels were 20-30% and 70%, respectively (Terazawa et al, 2003; Nissapatorn et al, 2004). In our study, the samples belonged to a low socioeconomic level, and eating meat is not a frequent practice among them. In addition, consumption of undercooked or raw meat is rarely found in this group. Fish is the most popular food item. These findings deserve more extensive studies, including larger study samples, including subjects belonging to at risk groups such as pregnant women, HIV/AIDS patients and organ transplants patients.

ACKNOWLEDGEMENTS

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