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Toxoplasma Gondii Infection among Pregnant Women in Guangdong Province, Subtropical Southern China

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Abstract

Little is known about the prevalence of infection by *T. gondii* in pregnant women in subtropical Southern China. The seroprevalence of *T. gondii* infection and associated risk factors were investigated in Guangdong province between April 2009 and August 2011. 5006 pregnant women participated in this study. Participants answered survey questions and blood samples were collected from these pregnant women. IgM and IgG antibodies were detected with Enzyme-Linked Immunoadsorbent Assay (ELISA). The odds ratio (OR) and 95% confidence interval (95% CI) between anti-IgM, IgG antibodies and relative risk factors were calculated using an association analysis based on unconditional logistic regression. Totally, 0.46% and 7.01% of the pregnant women were found to be positive for *T. gondii* IgM and IgG antibodies, respectively. Risk factors for *Toxoplasma gondii* infection in women were: contact with cats [odds ratio (OR) 4.99], consumption of raw or under-cooked wild and domestic animals (rats, rabbits and cats) or pork products (OR 4.08) and two administrative regions of Shaoguan and Zhanjiang with habits of eating non-cooked meat (OR 3.93 and 2.58). No significant relations were observed between anti-Toxoplasma IgM and IgG antibodies and unwashed raw vegetables or fruits, educational level and different age group. This is the first report of seroprevalence and risk factors for *T. gondii* infection among pregnant women in subtropical southern China. This study provides a basis for the design of successful preventive measures against *T.gondii* infection in pregnant women.

Keywords: *Toxoplasma gondii;* Pregnant women; Seroprevalence; Risk factors; ELISA; Subtropical southern

Introduction

Toxoplasmosis, a zoonotic disease of humans and most animals with a worldwide distribution, is caused by the opportunistic intercellular parasite *T. gondii* [1,2]. The main routes of transmission in humans are ingestion of tissue cysts in undercooked or oocyst contaminated food and through placental transmission to the fetus [3]. When the infection occurs in pregnant women, it can cause severe disease symptomatology including toxoplasmic encephalitis, blindness, fetal abnormalities, abortion and even stillbirth [4,5]. Previous reports showed that 39% of fetuses showed subclinical or clinical infection when the pregnant women acquired the infection for the first time [6]. Incidence of congenital toxoplasmosis ranges from 0.01-1.1% live births [4].

The seroprevalence of exposure to *T. gondii* is influenced by many factors such as climate and environmental conditions, food habit and culture [1]. The reported infection rate in pregnant women varies between countries as well as different areas within the same country [7]. Higher infection rates have been reported in the countries of Central and South America, Africa and Asia [3, 8, 9] compared to Northern Europe and the United States [9-11]. For Asian countries, the prevalence in India, Malaysia and Nepal are higher (41.8-51.4%) than that in Korea (0.8%), Vietnam (11.2%) and China (10.6) [8, 12]. Many researches have shown that pregnant women with positive IgG but negative IgM are latently infected, and only with a positive IgM titer were they considered to have an active infection [8, 9, 12]. Within China, because of the different environmental and food habits, there is considerable variation in infection rates among pregnant women in the different areas and provinces [13]. Specifically, there is very limited epidemiological information on *T. gondii* infection among pregnant women in Guangdong province. Guangdong faces the South China Sea to the south and is the most populous province in China, with a popu-

		IgM		Total
		+	-	
IgG	+	20	331	351(7.01%)
	-	3	4652	4655
	Total	23(0.46%)	4983	5006

χ^2>100.00, p<0.001

Table 1: The IgM and the IgG of *T.gondii* infection in pregnant women.

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lation of approximately 110 million people, including approximately 30 million migrants. The purpose of this study was to determine the prevalence of *T. gondii* infection and evaluate associated risk factors of T.gondii infection among pregnant women in Guangdong province, subtropical southern China.

Methods

Study population

This study was conducted in Guangdong province (20°13′-25°31′ N, 109°39′-117°19′E), which extends about 179,800 km2 in southern China. The climate of the Guangdong is subtropical; the average annual temperature and precipitation are 23.3′ and 1300-1500mm respectively (http://www.gd.gov.cn/gdgk/sqgm/zrdl).

Five thousand and six pregnant women who reported for antenatal care in Sun Yet-sen memorial hospital for the first or second time from April, 2009 to August, 2011 gave verbal consent for the detection of *Toxoplasma gondii*. An individual questionnaire was completed for each woman. The pregnant women were from different districts across Guangdong province (Table 1) and ranged in age from 16-46 years old (Table 2). Inclusion criteria for the study subjects were: age, home town, residency in rural or urban area, type of housing (apartment or house), knowledge of toxoplasmosis, consumption of unwashed raw vegetables or fruit, meat consumption (beef, goat, lamb, pork, poultry and/or rabbit) and consumption of raw or undercooked meat.

Ethical aspects

This study was approved by both the ethics committee of Sun Yat-

Districts	Pregnant women tested (n)	Relative distribution (%)	Ig M Seropositive (n)	IgM Seroprevalence (%)	P-value	95% CI	Ig G Seropositive (n)	IgG Seroprevalence (%)	P-value	95% CI
Guangzhou	3713	74.17	15	0.40	0.356	3.08-6.18	231	6.22	0.388	48.21-78.72
Shenzhen	249	4.98	1	0.40	0.335	3.02-6.05	16	6.42	0.395	46.03-72.43
Huizhou	157	3.14	1	0.64	0.067	4.46-8.95	11	7.01	0.253	49.76-80.29
Zhuhai	226	4.52	1	0.44	0.393	3.13-6.31	17	7.52	0.362	51.36-81.81
Foshan	181	3.62	1	0.55	0.112	3.75-8.39	13	7.18	0.177	49.78-79.08
Shaoguan	219	4.37	2	0.91	0.00091**	6.39-15.52	30	14.16	0.00102**	83.27-91.58
Zhanjiang	258	5.15	2	0.78	0.025*	5.43-12.86	33	12.79	0.0213*	80.53-90.22
Total	5006	100.00	23	0.46	0.168	3.26-8.66	351	7.01	0.139	49.06-80.01

*: Significantly higher than in other districts (P<0.05);
**: Significantly higher than in all the other districts (P<0.01)

Table2: Seroprevalence of T. gondii infection among pregnant women in different districts in Guangdong province.

Age group (years)	Pregnant women tested (n)	Relative distribution (%)	Ig M Seropositive (n)	IgM Seroprevalence (%)	P-value	95% CI	Ig G Seropositive (n)	Ig G Seroprevalence (%)	P-value	95% CI
16-20	95	1.90	0	0.00	-	-	3	3.12	0.023*	20.01-31.59
21-25	396	7.91	1	0.25	0.053	1.72-4.16	15	3.79	0.039*	21.38-32.79
26-30	1926	38.47	9	0.47	0.392	3.13-8.22	136	7.06	0.576	49.85-81.21
31-35	1652	33.00	8	0.48	0.351	3.41-8.58	131	7.93	0.257	52.30-81.31
36-40	751	15.00	4	0.53	0.176	3.65-8.99	57	7.59	0.295	51.33-80.36
40-46	186	3.72	1	0.54	0.139	3.77-9.37	9	4.84	0.062	27.55-38.09
Total	5006	100.00	23	0.55	0.373	3.21-7.35	351	7.01	0.418	42.58-79.73

*: Significantly higher than in other districts (P<0.05);

Table 3: Seroprevalence of T. gondii infection in pregnant women at different age groups.

Characteristic	Pregnant women tested (n)	Relative distribution (%)	Ig M Seropositive (n)	Ig M Seroprevalence (%)	P-value	95% CI	Ig G Seropositive (n)	Ig G Seroprevalence (%)	P-value	95% CI
Degree of meat cooking										
Well done	1113	22.23	3	0.27	P=0.0029**	1.78-4.13	46	4.13	P=0.0041**	26.66-39.09
Raw or undercook	3893	77.77	20	0.51		3.23-7.82	305	7.83		51.78-81.52
Contact with the cat										
Yes	2719	54.31	18	0.66	P=0.00073**	4.23-8.66	289	10.63	P=0.00030**	79.91-88.36
No	2287	45.69	5	0.22		1.51-3.61	62	2.71		15.03-21.38
Unwashed raw vegetables or fruits										
Yes	817	16.32	4	0.49	P=0.267	3.18-7.80	61	7.47	P=0.598	50.13-81.05
No	4189	83.68	19	0.45		3.13-7.72	290	6.92		48.53-79.71
Education level										
Educational	589	11.76	3	0.51	P=0.203	2.93-8.02	52	8.83	P=0.097	52.75-82.67
No Educational	4417	88.24	20	0.45		3.20-7.62	299	6.77		47.16-78.05

**: Significantly higher than in all the other districts (P<0.01)

Table 4: Risk factors associated with T. gondii infection in pregnant women in Guangdong province.

	IgG Seroprevalence (%)	OR	95% CI	IgG Seroprevalence (%)	OR	95% CI
Districts						
Total	0.46	1.0		7.01	1.0	
Shaoguan	0.91	3.93(P<0.001)	2.28-8.03	14.16	3.89(P<0.001)	2.19-7.33
Zhanjiang	0.78	2.58 (P<0.05)	1.41-5.11	12.79	2.29 (P<0.05)	1.25-4.78
Degree of meat cooking						
Well done	0.27	1.0		4.13	1.0	
Raw or undercook	0.51	4.08 (P<0.001)	2.76-7.11	7.83	3.36 (P<0.05)	2.11-5.67
Contact with cats						
No	0.22	1.0		2.71	1.0	
Yes	0.66	4.99 (P<0.001)	2.39-9.98	10.63	6.57(P<0.001)	3.88-13.33

Table 5: Identification of risk factors for *T.gondii* infection in pregnant women by multiple logistic regression analysis.

sen University and by the medical boards of Sun Yet-sen memorial hospital. The purpose and procedures of this investigation were explained to all participants, and informed consent was obtained from all the women studied.

Serological analysis for T.gondii antibodies

Blood samples were taken from the women under aseptic conditions; the sera were separated and stored at -80 oC until further testing. Serum samples were tested for IgM and IgG against T.gondii using enzyme-linked immunoadsorbent assay (*Toxoplasma gondii* IgM and IgG μ-capture Elisa assay; Hamburg, German). Serological tests were performed according to the manufacture's instruction at the biological laboratory of Sun Yet-sen memorial hospital.

Statistical analysis

All data were analyzed with SPSS 13.0 Data Editor (SPSS Inc, Chicago, IL, USA). Bivariate and multivariate analyses were used to assess all the different districts, age differences and risk factors associated with *T. gondii* infection. If the P value < 0.3 in the bivariated analysis, the variable was included into the multivariate analysis. 95% confidence intervals (CIs) and odds ratios (ORs) were calculated by multivariate analysis using a multiple, unconditional logistic regression model. The differences were considered to be statistically significant when the p value obtained was less than 0.05.

Results

T. gondii infection in pregnant women in different districts in Guangdong province

Total twenty-three (0.46%) and 351 (7.01%) of 5006 pregnant women were found to be positive for *T. gondii* IgM and IgG antibodies, respectively (Table 1). Estimated seroprevalence of toxoplasma IgM and IgG among the districts ranged from 0.4% to 0.91 and 6.22%-14.16% respectively. Two districts, Shaoguan (0.91%, p=0.00091 for IgM and 14.16%, P=0.00102 for IgG) and Zhanjiang (0.78%, p=0.025 for IgM and 12.79%, p=0.0213 for IgG) have significantly higher infection rates compared to other districts (Table 2).

T. gondii infection in pregnant women in different age groups

The pregnant women tested were divided into 5 age groups: under 20 years, 21-25 years, 26-30 years, 31-35 years, 36-40 years and over 40 years. The distribution and the positive rates are shown in Table 3. Although the active infection positive rate varied in different age groups, the IgM positive rate ranged from 0% (<20 years) to 0.54% (>40 years) and IgG positive rate ranged from 3.12% (<20 years) to 7.93% (31-35 years). No statistically significant differences were observed among all age groups (Table 3, P>0.05).

Risk factors associated with *T. gondii* infection in pregnant women

Risk factors related to eating preferences and hygienic habits are given in (Table 4). In pair wise comparisons, no statistically significant differences were found between the groups with and without a formal education, between the groups who consumed unwashed raw vegetables or fruits and those who washed them. However, significant differences (p<0.01) were detected between the group not consuming raw or under-cooked meat and those eating raw or under-cooked meat (3.23-7.82, p=0.0029 for IgM and 51.78-81.52, p=0.0041 for IgG); all possible risk factors were explored, degree of meat cooking, between the groups having no contact with cats (1.51-3.61 for IgM and 79.91-88.36 for IgG) and contact or owning cats (4.23-10.66;for IgM and 15.03-21.38,).

The analysis of odds ratios (ORs) and 95% confidence intervals (95% CIs) between anti-IgM antibodies and relative risk factors are given in Table 5. Risk factors for T.gondii infection in decreasing order were: contact cats (OR 4.99 for IgM and OR 6.57 for IgG), consumption of raw or under-cooked meat (OR 4.08 for IgM and OR 7.83 for IgG) and geographic locations (Shaoguan, OR 3.93 and Zhangjiang, OR 2.58).

Discussion

In China, policy of healthy pregnancy had strengthened to execute for women of childbearing age to improve the quality of reproduction for several years. Most pregnant women now voluntarily go to the hospital to obtain prenatal care. If a woman becomes actively infected with T.gondii during pregnacy there is a high probability that there will be deleterious effects on the development of the fetus including abortion and stillbirth [14-17]. Almost all of the public hospitals in China, screen for the IgM and IgG of *T. gondii* at early pregnancy. The emergence of IgM and IgG positive against *T. gondii* showed is a strong indicator of active infection and immediate measures should be taken to assure r treatment.

The 0.46% and 7.01% of *T. gondii* IgM and IgG psoitivity in pregnant women from Guangdong province, respectively, is much lower than Tirana and vlore 1.3% of IgM and 48.6% of IgG positive in Albania [18], 4.1% of IgM and 29.4% of IgG positive in Kosovo [19], 1.22% of IgM and 49.2% of IgG positive in brazil [20], 3,26% of IgM and 53.03% of IgG positivein southern brazil [21], 2.8% of IgM and 45.8% of IgG positive in Cali, Colombia, South America [3]. Our result is much higher than the reported 0% of IgM and 10.6% for IgG positive from other cities in China, for example a 0% active infection in pregnant women in Changchun [8], but is much lower than 6.6% of IgM in Wuhan [22] and 27.2 % of IgM positive in Weifang [23].

The potential risk for the pregnant women to become infected with *T. gondii* in our investigation showed that contact with cats, and consumption of undercooked or raw meat to be the most significant risk factors in Guangdong province consistent with previous reports [7, 8, 9, 10, 24]. Evidence that companion animals such as cats are hosts of *T. gondii* had been previously confirmed [1, 2, 6, 8, 10, 12, 18, 20, 24, 25, 26]. Thus, pregnant women in contact with these infected pets will naturally be at greater risk of acquiring active infection. The study confirmed that consumption of undercooked or raw meat and contact with the cats were the greater risk factors for the pregnant women studied. The subjects from Shaoguan and Zhanjiang districts had increased seroprevalence because people living in those areas have habits of both consumption of undercooked or raw meat of cats and regular contact with cats.

In summary, the present investigation revealed that 0.46% of IgM and 7.01% of pregnant women in subtropical southern China were seropositive for IgM and IgG against *T. gondii*.

It is interesting to note that contact with cats and consumption of uncooked or under-cooked meat were a significant risk factors for pregnant women. This is the first report of seroprevalence and risk factors for *T. gondii* infection among pregnant women in subtropical southern China. This study provides a basis for the design of successful preventive measures against *T.gondii* infection in pregnant women.

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