Seroprevalence and associated risk factors of *Toxoplasma gondii* among Manchu pregnant women in northeastern China

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ABSTRACT

Toxoplasma gondii is an intracellular protozoan parasite infecting human and almost all warm-blooded animals. It may cause several severe symptoms if pregnant women infection with *T. gondii*, including misbirth. A cross-sectional study was conducted containing 313 Manchu pregnant women attending antenatal care from 2016 to 2017 in Jilin province, northeastern China, and were assessed by enzyme-link immunosorbent assay and the study utilized univariate analysis to identify the potential risk factors for *T. gondii* infection. Of the 313 investigated Manchu pregnant women, 51 (16.29%, 95% CI 12.2–20.4) were tested as *T. gondii*-seropositive, with 47 (15.02%) seropositive for *T. gondii* IgG antibodies and 8 (2.56%) IgM positive. The seroprevalence of *T. gondii* in different age groups varied from 13.50% (8.7–18.3) to 23.90% (13.9–41.9). Pregnant raising cat at home has a significantly higher seroprevalence of *T. gondii* than no cat at home. Pregnant consuming raw/undercooked meat has a significantly higher *T. gondii* seroprevalence than individuals did not consuming raw/undercooked meat. This is the first study of *T. gondii* infection seroprevalence in Manchu pregnant women. Risk factors analysis suggested that seroprevalence of *T. gondii* in investigated Manchu pregnant women was mainly related to consumption of raw/undercooked meat and raising cat at home. The findings will provide key and baseline data for prevention and control of toxoplasmosis among Manchu pregnant women and other people.

1. Introduction

Toxoplasma gondii is an intracellular protozoan parasite. The life cycle of *Toxoplasma* contains three infectious stage, including oocysts, pseudocysts or tachyzoits, and tissue cysts or bradyzoits [1,2]. Felines are the only definitive hosts for the parasite and virtually all warm-blooded animals are intermediate hosts such as humans [3,4]. *T. gondii* can be transmitted to humans through ingesting *T. gondii* tissue cysts or oocysts from contaminated food and water, or vertical spread to posterities through transplacental transmission [5].

Although a third of the people infects *T. gondii* around the world, most of them are asymptomatic [6]. However, immunocompromised individuals may cause severe illness [7]. Pregnant women who infect *T. gondii* are at risk of congenital transmission to the fetuses, which may result in severe congenital toxoplasmosis [7]. For example, fetuses infect *T. gondii* may cause blindness, mental retardation, foetal abnormalities and even death. Other bad consequences of pregnant women with infection increase the risk of abortion and infertility [8].

China is a multi-ethnic country with 55 minorities and Han nationality. The Manchu ethnic minority, with a population of 10,410,585 (year of 2010), lived in northeastern China mainly and has the habit of consuming pork, game and hot pot, which may represent a route of *T. gondii* transmission to humans. There are many investigations about *T. gondii* infection among pregnant women around the world [9–12], but no information was available about *T. gondii* infection among pregnant women who belong to the Manchu ethnic minority. The main objectives of this study were to assess the seroprevalence and to determine the risk factors associated with *T. gondii* infection among Manchu pregnant women in northeastern China, which will provide baseline data for prevent and control *T. gondii* infection among Manchu pregnant women.

2. Materials and methods

2.1. Study design and population

We conduct cross-sectional study and use systematic random sampling technique to obtain the samples. A total of 313 blood samples were collected among Manchu pregnant women in Jilin province, northeastern China, from 2016 to 2017. The individuals' occupations and names were not recorded to ensure confidentiality. The purpose and procedures of the study were explained to all participants, and written informed consent was obtained from them all.

2.2. Sample collection and serological tests

About 5 mL of venous blood was collected aseptically from each participant in Eppendorf tubes and kept at room temperature for 2 h. Then serum was separated from the whole blood by centrifugation at 3000 rpm for 10 min, which was labeled and frozen at −20 °C until use. Testing for *T. gondii* serology (IgG and IgM) was performed using commercial enzyme immunoassay kits (Demeditec Diagnostics GmbH, Germany). *Toxoplasma* IgM and IgG ELISA were done as per the protocol. Positive, negative and blank controls were included in every plate. Optical densities were measured by photometer at a wavelength of 450 nm. Values higher than the cut-off (10 IU/mL) were considered positive.

2.3. Date analysis

The data of questionnaire covered socio-demographic information such as age, resident area, source of drinking water and behavioral factors including cat at home, consumption of raw vegetables and fruits, consumption of raw/undercooked meat. The information of questionnaire and experimental results was entered on to an excel spreadsheet and transferred to SPSS v. 19.0 software package (SPSS Inc., USA). Univariate analysis and multivariate analysis were used to analyze the association between variables and *T. gondii* infection. probability (P) value < 0.05 was considered as statistically significant in the analysis and the odds ratios(OR) and its 95% confidence interval were calculated.

3. Results and discussion

Of the 313 investigated Manchu pregnant women, 51 (16.29%, 95% CI 12.2–20.4) were tested as *T. gondii*-seropositive, with 47 (15.02%) seropositive for *T. gondii* IgG antibodies. *Toxoplasma* IgG Avidity ELISA was not done in this present study. This would have indicated if the infection was recent or past [13]. A total of 8 (2.56%) IgM positive were found in this study which indicates an urgent state or an infection that has occurred within the last year. Of them, 1.28% Manchu pregnant women were positive for both IgG and IgM (Table 1). According to univariate analysis, pregnant women raising cat at home (28.33%, 95% CI 16.6–40.1) has a significantly higher seroprevalence of *T. gondii* than no cat at home (13.44%, 95% CI 9.2–17.7, P = 0.006). Pregnant consuming raw/undercooked meat (24.60%, 95% CI 17.0–32.2) has a significantly higher *T. gondii* seroprevalence than individuals do not

Table 1
Combined IgG and IgM anti-*T. gondii* antibodies seroprevalence in Manchu pregnant women in Jilin Province, northeastern China.

Seroreation	Positive	%(95%CI)
Positive for IgG only	43	13.74 (9.9–17.6)
Positive for IgM only	4	1.28 (0.0–2.5)
Positive for IgG and IgM	4	1.28 (0.0–2.5)
Negative for IgG and IgM	262	83.71 (79.6–87.8)
Positive for either IgG or IgM	51	16.29 (12.2–20.4)

consuming raw/undercooked meat (10.70%, 95% CI 6.2–15.2, P = 0.001). The highest seroprevalence of *T. gondii* was found in pregnant aged of > 35 years (27.90%, 95% CI 13.9–41.9), followed by less than 25 years (17.14%, 95% CI 8.1–26.2) and 25–35 years (13.50%, 95% CI 8.7–18.3). Seroprevalence of *T. gondii* in pregnant lived in urban areas was 16.95% (95% CI 11.4–22.5), and rural areas was 15.44% (95% CI 9.3–21.6) (Table 2). Optimized by Fisher's scoring technique, forward stepwise logistic regression analysis was conducted to evaluate the impacts of multiple variables on *T. gondii*. In the final model, two variables had effects on the infectious disease, described by the equation: $y = 0.9113\,x3 + 0.9863\,x5 + 0.4372$. Kept cat at home and Consumption of raw/undercooked meat had positive effects on the risk of *T. gondii*, for which the ORs were 2.49 (95%CI 1.26–4.92) and 2.68 (95%CI 1.44–5.00), respectively.

In the present study, we surveyed the seroprevalence of toxoplasmosis among Manchu pregnant women in Jilin province. The overall *T. gondii* seroprevalence of them were 16.29%, which was higher than the rest of pregnant women in mainland China, ranged from 2.4% to 5.0% and the data obtained from Bayesian hierarchical models [14]. It was also higher than the previous studies conducted in Mexico (6.20%) [9] by EIA and ELFA and in Zambia (5.87%) [15], Egypt (8.30%) [16] by ELISA, but lower than those reported in Iran (33.3%) [17] (52.2%) [18], Ethiopia (85.3%) [11], Turkey (31%) [19], Lebanon (82.6%) [7], Brazil (51%) [20], Côte d'Ivoire (67.92%) [21], Burkina Faso (31.1%) [12], Sri Lanka (29.9%) [22] by ELISA and in Ethiopia (88.6%) [23] (85.4%) [24] by latex agglutination test, in Saudi Arabia (28.5%) [25] by chemiluminescent microparticle immunoassays. Such seroprevalence variations may be due to climate conditions, sanitation, socioeconomic, host susceptibility, geographical location and the custom of local residents, for example, kept cats at home. It is also effect the seropositivity that using different detection methods.

Previous study reported higher seroprevalence of *T. gondii* in humans from rural areas [22], meanwhile, as we know, soil contact which is more common in rural area, also found to be a risk factor in other sero-epidemiological study [26], but in the present study the higher seroprevalence was found in humans from urban areas followed by rural areas, but the difference was not statistically significant. This may be due to the number of pet cats has soared in urban families. Moreover, the present study also found that elder had a higher *T. gondii* seroprevalence, which further confirmed the results of the previous studies [27–29].

Cat is the only definitive host of *T. gondii* and it can excrete oocysts into environment [6], which may become a potential resource of toxoplasmosis. Domestic cats are the popular pets for the majority of households. In this study, raising cats at home was considered as a main risk factor for pregnant women to acquire toxoplasmosis. The findings showing that the raising cats at home group had a 2.55 times higher risk of being seropositive compared to not raising cats at home group (OR = 2.55, 95% CI 1.31–4.97, P = 0.006). Human toxoplasmosis can be acquired by consumption of undercooked or/and raw meat containing tissue cysts [6,8,30]. In the present study, the results also showed that consumption of raw/undercooked meat had positive effect on the risk of *T. gondii*, which accordance with the findings of previous studies [2,3,28]. China has 56 ethnic groups, and Manchu is one of the minority nationalities. In northeastern China, Manchus have similar diets and culture to the Hans who like to eat barbecue (including raw/undercooked meat), which was one of the most important resources for transmitting toxoplasmosis. The findings showed that pregnant women who consume raw/undercooked meat has a 2.73-fold at risk of *T. gondii* infection compare to women who not consume raw/undercooked meat groups (OR = 2.73, 95% CI 1.47–5.05, P = 0.001). The present finding also suggested that habits of eating raw/undercooked meat should be avoided in future.

Vertical transmission is the primary way for fetuses acquiring toxoplasmosis [8]. *T. gondii* infection in pregnant women not only can