

Seroepidemiology of *Toxoplasma gondii* infection in Bai and Han ethnic groups in southwestern China

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SUMMARY

Eating raw pork and/or liver is a custom of the Bai ethnic group in China. Most people living in Dali Bai Autonomous Prefecture, Yunnan Province, southwestern China are of Bai ethnicity. Little is known of the seroprevalence of *Toxoplasma gondii* in Bai and Han ethnic populations in this region. In the present survey, a total of 555 and 595 blood samples were obtained from Bai and Han ethnic groups in Dali urban and rural areas, respectively. Enzyme-linked immunosorbent assay was performed to examine *T. gondii* IgG antibodies. Total positive rate of anti-*T. gondii* IgG in Bai and Han groups in this region was 21·6% (248/1150). The total seroprevalence of *T. gondii* was significantly higher in the Bai ethnic group (32·3%, 179/555) than in the Han ethnic group (11·6%, 69/595) ($P < 0\cdot01$). The results of statistical analysis indicated that there was no significant difference between cat feeding/non-cat feeding groups in the Bai ethnic group, the most important risk factor was consumption of raw pork and/or liver for the Bai group, but feeding a cat may be the main route of *T. gondii* infection for the Han group. Therefore, it is essential to implement integrated strategies to prevent and control *T. gondii* infection in this unique region of the world.

Key words: Bai ethnicity, seroepidemiology, *Toxoplasma gondii*, toxoplasmosis, Yunnan Province.

INTRODUCTION

Toxoplasma gondii can infect a wide range of hosts including wild mammals, birds, livestock, poultry and human beings, leading to zoonotic toxoplasmosis.

Most *T. gondii* infections in immunocompetent individuals are asymptomatic; however, blindness and mental retardation can occur in congenitally infected children [1–3], and the occurrence of toxoplasmosis in immunocompromised hosts may cause severe diseases or even death.

The prevalence of human toxoplasmosis ranges from 4% in Korea to 92% in Brazil [4], and a mean prevalence of 7·9% has been estimated in China [5]. The different *T. gondii* prevalences may be due to different factors including sample size, age, occupation,

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serological techniques, and living customs and habits. Earlier studies indicated that the seroprevalence of *T. gondii* in slaughter pigs in Yunnan Province in China was 22.28% [6], and the genotype was Toxo DB#9 [7], representing a widespread lineage from pigs, cats, and human patients in China. Dali Bai Autonomous Prefecture is located in the west of Yunnan Province, where 26 ethnic groups live, with the main ethnic groups being of Bai and Han ethnicity. The Bai ethnic group has the habit of consuming raw or undercooked animal meat and/or organs, which may represent a route of *T. gondii* transmission to humans, and thus stimulated the aims of the present study, which were to investigate *T. gondii* seroepidemiology and analyse the epidemiological correlation between *T. gondii* infection and risk factors in Bai and Han populations.

MATERIALS AND METHODS

Serum samples

A total of 555 and 595 blood samples were collected from Bai and Han groups in Dali urban and rural areas, respectively, by medically trained staff. The individuals' occupation and name were not available because of confidentiality. The information including age, sex, ethnicity, education, consumption of raw pork and/or liver and cat feeding information were obtained through questionnaires and recorded during the sample collection (March–December 2013). Blood was collected by venepuncture in plain tubes and centrifuged to remove blood cells. All sera were stored at -20°C after deactivation of complement at 56°C for 30 min before use. This study was approved by the Ethics Committee of Dali University.

Serological tests

All the serum samples were tested for *T. gondii* IgG antibodies using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Haitai Co. Ltd, China) according to the manufacturer's instructions. Positive and negative serum controls were included in every plate. The absorbance was measured at 450 nm using an ELISA reader (Bio-Tek ELISA \times 808, USA). All samples were run in triplicate.

Statistical analysis

Between the two ethnic groups, the χ^2 test was used to analyse *T. gondii* prevalence relating to cat feeding (or

not), level of education and various degrees of consumption of raw pork and/or liver by the subjects using SPSS software (release 16.0 standard version, SPSS Inc., USA). For all data, the variation in *T. gondii* seroprevalence (y) of humans of different ethnic groups (x_1), sex (x_2), cat-feeding behaviour (x_3), education (x_4), raw pork and/or liver consumption (x_5) and age group (x_6) was analysed by χ^2 test. Using multivariable regression analysis, each of these variables was included in the Binary Logit Model as an independent variable. The differences were considered as statistically significant between or among levels within factors and interactions when the probability (P) value was <0.05 . Odds ratios (OR) with 95% confidence intervals (CI) were calculated by multivariate analysis using logistic regression.

RESULTS

The age and sex distribution of *T. gondii* seropositivity in Bai and Han ethnic groups are summarized in Table 1. In total, 248 (21.6%) of the 1150 serum samples were anti-*Toxoplasma* IgG positive. The overall seroprevalence of *T. gondii* was higher in the Bai group (32.3%, 179/555) than in the Han group (11.6%, 69/595) ($P < 0.01$). Within the Bai group, seroprevalence was significantly higher in males (37.8%) than in females (27.4%), but this difference was not significant between males (10.2%) and females (12.4%) within the Han group ($P = 0.414$). The infection risk was higher in males than in females in the Bai group ($P = 0.008$, OR 5.346, 95% CI 3.25–8.8). These results confirmed the highly significant difference in seroprevalence of both males and females between Bai and Han ethnic groups ($P < 0.01$).

As shown in Table 1, the *T. gondii* seroprevalence in the <18 years age group was lower than other age groups in both Bai and Han populations. In the Han group, the seropositivity of *T. gondii* was 0% in the 1–18 years age group, 6.8% in the 18–40 years age group, 16.8% in the 41–65 years age group, and 15.9% in the >65 years age group. For the Bai group, the seroprevalence was also 0% in <18 years age group, but seroprevalence was significantly higher in the 18–40 years age group (34.1%, $P < 0.001$), 41–65 years group (33.1%, $P < 0.001$), and >65 years group (29.8%, $P = 0.013$) in the Bai group than in the Han group.

The results of characteristics of study subjects and risk factors associated with the seropositivity of *T. gondii* in Bai and Han groups are detailed in

Table 1. Age and sex distribution of *Toxoplasma gondii* seroprevalence in Bai and Han ethnic groups, Yunnan Province, southwestern China

Nationality	Age (years)	Male			Female			Total		
		No. examined	No. positive	Prevalence, %	No. examined	No. positive	Prevalence, %	No. examined	No. positive	Prevalence, %
Han ethnicity	<18	17	0	0	11	0	0	28	0	0
	18–40	71	3	4.2	179	14	7.8	250	17	6.8
	41–65	65	10	15.4	120	21	17.5	185	31	16.8
	>65	72	10	13.9	60	11	18.3	132	21	15.9
	Subtotal	225	23	10.2*	370	46	12.4†	595	69	11.6‡
Bai ethnicity	<18	7	0	0	4	0	0	11	0	0
	18–40	102	44	43.1	109	28	25.7	211	72	34.1
	41–65	116	44	37.9	123	35	28.5	239	79	33.1
	>65	34	10	29.4	60	18	30	94	28	29.8
	Subtotal	259	98	37.8*	296	81	27.4†	555	179	32.3‡
Total		484	121	25	666	127	19.1	1150	248	21.6

OR, Odds ratio; CI, confidence interval.

P was estimated using the χ^2 test.

* *P* < 0.001, OR 5.346, 95% CI 3.25–8.8.

† *P* < 0.001, OR 2.654, 95% CI 1.777–3.962.

‡ *P* < 0.001, OR 3.629, 95% CI 2.668–4.936.

Table 2. The subjects were categorized into four education groups: primary school, junior high school, senior middle school and college. There was no significant difference in *T. gondii* seroprevalence within the Bai group of different education levels ($P=0.144$), but the infection risk decreased with increasing level of education in the Han group, and the difference was significant ($P=0.01$).

Consumption of raw pork and/or liver was divided into the following categories: never, occasionally (<6 times a year), not often (>6 to <12 times a year), often (≥ 12 times a year). In contrast to the 'never' group, our statistical analysis showed that the increasing frequency of consumption of raw pork and/or liver is related to high *T. gondii* seroprevalence in both Bai and Han ethnic groups ($P<0.001$). The ratio of people who ate raw pork and/or liver in the Bai group was far greater than in the Han group, but there was no significant difference in the 'often' group between the two ethnic populations ($P=0.257$), and the seropositivity was 59% and 50% in Bai and Han ethnic groups, respectively.

There was no significant difference in *T. gondii* seroprevalence between cat feeding (33.3%) or non-cat feeding (32.1%) groups in the Bai groups. By contrast, cat feeding (or not) was significantly associated with *T. gondii* infection in the Han group ($P<0.001$), and the seroprevalence was significantly higher in the cat-feeding group (34.3%, 35/102) than in the non-cat-feeding group (6.9%, 34/493).

The effects of ethnicity, sex, cat feeding, education level, consumption of raw pork and age group associated with *T. gondii* seropositivity in the present study were analysed using univariate analyses. Optimized by Fisher's scoring technique, forward stepwise logistic regression analysis was conducted to evaluate the risks of multiple variables relating to *T. gondii* infection. In the final model, two variables had effects on the infectious disease, described by the equation $y=4.12-1.52x_3-x_5$. Not feeding a cat and consuming less raw meat had negative effects on the risk of *T. gondii* infection, for which the ORs were 0.22 (95% CI 0.134–0.355) and 0.37 (95% CI 0.315–0.428), respectively.

DISCUSSION

In China, *T. gondii* infection has not been given high priority owing to the limited knowledge of the route of transmission to humans, e.g. the infection risk of the well known food named 'Heige' (fresh pork or liver)

in many restaurants in Yunnan Province. Therefore, the local cuisine including 'Heige' in Dali Bai Autonomous Prefecture, Yunnan Province may represent a potential risk of *T. gondii* infection, especially for the Bai group which has the habit of eating raw pork and/or liver. Significantly, this potential risk has led to 21.6% anti-*T. gondii* IgG positivity in Dali Bai Autonomous Prefecture, which is higher than that in northeast and the south of China (12.3%) [8], and it is also much higher than the national average (7.9%) [5]. Although a previous study reported that the *T. gondii* seroprevalence was 25.4%, 25.3%, 17.1% and 16.7% in Miao, Buyi, Mongol and Zhuang ethnic groups, respectively [5], our study, focusing on the Bai ethnic group, showed the highest seroprevalence of 32.3%.

Some previous reports indicated that the seropositivity of *T. gondii* was related to age [9, 10]. Consistent with these studies, the present study showed highly significant differences in *T. gondii* seropositivity in the different age groups (18–45, 45–65, >65 years) in the two ethnic populations. For subjects aged <18 years, there were a limited number of samples in this study, thus the data were inconclusive.

As the definitive hosts for *T. gondii*, cats play a key role in transmitting *T. gondii* due to their ability to pass oocysts in their faeces [11, 12]. In China, it has been shown that excretion of *T. gondii* oocysts in the environment by cats leads to the increased possibility of human infection [12]. The present study showed 33.3% (22/66) and 34.3% (35/102) seropositivity of *T. gondii* in Bai and Han ethnic groups, respectively, who feed cats. Although there was no significant difference in *T. gondii* seropositivity between the Bai groups that feed/do not feed a cat, a significant difference was found in *T. gondii* seropositivity between Han groups that feed cats and those that do not.

Previous studies have indicated that level of education was associated with *T. gondii* infection [13–15]. Similarly, our study showed that *T. gondii* seroprevalence was significantly different in Han ethnic populations of different educational levels, showing that the proportion of subjects who have college education had lower *T. gondii* prevalence than others, although there was no significant difference in the Bai ethnic group for different education levels.

CONCLUSION

The present study revealed for the first time that human infection with *T. gondii* is common in Bai

Table 2. Characteristics of study subjects and risk factors associated with the seropositivity of *Toxoplasma gondii* in Bai and Han ethnic groups, Yunnan Province, southwestern China

Characteristic or risk factor	Bai ethnicity						Han ethnicity					
	No. examined	No. positive	Prevalence, %	<i>P</i> *	OR	95% CI	No. examined	No. positive	Prevalence, %	<i>P</i> *	OR	95% CI
Education												
Primary school	142	50	35.2	0.144	Ref.	—	116	23	19.8	0.01	Ref.	—
Junior high school	200	66	33.0		0.906	0.576–1.426	243	28	11.5		0.527	0.288–0.962
Senior middle school	179	58	32.4		0.882	0.554–1.405	188	15	8.0		0.351	0.175–0.704
College	34	5	14.7		0.317	0.116–0.871	48	3	6.3		0.27	0.077–0.094
Consumption of raw pork and/or liver												
Never	35	3	8.6	0.000	Ref.	—	412	29	7.0	0.000	Ref.	—
Occasionally	132	14	10.6		1.266	0.343–4.675	83	7	8.4		1.216	0.514–2.878
Not often	176	37	21.0		2.839	0.824–9.789	52	9	17.3		2.764	1.228–6.224
Often	212	125	59.0		15.326	4.549–51.635	48	24	50.0		13.207	6.69–26.073
Feeding cat												
No	489	157	32.1	0.841	Ref.	—	493	34	6.9	0.000	Ref.	—
Yes	66	22	33.3		1.057	0.613–1.825	102	35	34.3		7.052	4.122–12.066

OR, Odds ratio; CI, confidence interval; Ref., used as the reference group.

* Estimated using the χ^2 test.

and Han ethnic groups in Dali Bai Autonomous Prefecture, Yunnan Province, southwestern China. The overall *T. gondii* seroprevalence in the Bai ethnic group was 32.3%. Eating raw pork and/or liver was the most important risk related to *T. gondii* infection in the Bai ethnic group. Cat feeding and raw meat consumption had positive effects on the risk of *T. gondii* in both Bai and Han ethnic groups in this area. Improved food habits and hygiene practices should be promoted to reduce the prevalence of *T. gondii* infection in humans in this unique region of the world.

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DECLARATION OF INTEREST

None.

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