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Seroprevalence and associated risk factors of *Toxoplasma gondii* infection in the Korean, Manchu, Mongol and Han ethnic groups in eastern and northeastern China

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SUMMARY

A cross-sectional study was conducted from June 2013 to August 2015 to determine the seroprevalence and possible risk factors for human *Toxoplasma gondii* infection in Korean, Manchu, Mongol and Han ethnic groups in eastern and northeastern China. A total of 1842 serum samples, including Han ($n = 802$), Korean ($n = 520$), Manchu ($n = 303$) and Mongol ($n = 217$) groups, were analysed using enzyme-linked immunoassays to detect IgG and IgM *T. gondii* antibodies. The overall *T. gondii* IgG and IgM seroprevalences were 13·79% and 1·25%, respectively. Of these groups, Mongol ethnicity had the highest *T. gondii* seroprevalence (20·74%, 45/217), followed by Korean ethnicity (16·54%, 86/520), Manchu ethnicity (13·86%, 42/303) and Han ethnicity (11·35%, 98/802). Multiple analysis showed that the consumption of raw vegetables and fruits, the consumption of raw/undercooked meat and the source of drinking water were significantly associated with *T. gondii* infection in the Han group. Likewise, having a cat at home was identified as being associated with *T. gondii* infection in the Korean, Manchu and Mongol groups. Moreover, the consumption of raw/undercooked meat was identified as another predictor of *T. gondii* seropositivity in the Mongol group. The results of this survey indicate that *T. gondii* infection is prevalent in Korean, Manchu, Mongol and Han ethnic groups in the study region. Therefore, it is essential to implement integrated strategies with efficient management measures to prevent and control *T. gondii* infection in this region of China. Moreover, this is the first report of *T. gondii* infection in Korean, Manchu, and Mongol ethnic groups in eastern and northeastern China.

Key words: China, Korean ethnicity, Manchu ethnicity, Mongol ethnicity, seroprevalence, *Toxoplasma gondii*.

INTRODUCTION

Toxoplasma gondii, an opportunistic protozoan parasite, can infect nearly all warm-blooded animals worldwide, including humans [1–5]. It is estimated that nearly a third of the human population in the world has been infected by *T. gondii* [1]. The seroprevalence of *T. gondii* is evolving worldwide, subject to complex environmental, socioeconomic and health-related practices [6]. Humans acquire *T. gondii* infection primarily through the consumption of undercooked or raw meat containing tissue cysts from an infected intermediate host, unwashed vegetables and

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fruits, or drinking water contaminated by oocysts from the excrement of infected cats [4–7].

China is a multi-ethnic country with 56 ethnic groups. The Han nationality represents the majority of the Chinese nation, encompassing 92% of the national population; the remaining 8% are ethnic minorities. In the 55 ethnic minorities, Korean, Manchu, and Mongolian represent the largest populations. Each ethnic minority has its own lifestyle and eating habits. Human *T. gondii* seroprevalences have been reported in many regions and different research groups, including pregnant women [8–10], female sterilized patients [11], psychiatric patients [12, 13], children [14, 15], and cancer patients [16, 17]. Moreover, a previous study reported *T. gondii* infection in the Bai and Han ethnic groups in southwestern China [18]. However, limited information on *T. gondii* infection in the Korean, Manchu, and Mongol ethnic groups is available in China. Thus, the present study was conducted to determine the seroprevalence and possible risk factors for human *T. gondii* infection in the Korean, Manchu, Mongol and Han ethnic groups in eastern and northeastern China.

MATERIALS AND METHODS

Serum samples

This study was approved by the Ethics Committee of Jilin Agricultural University. A cross-sectional study was conducted and a total of 1842 blood samples were randomly collected from the Han ($n = 802$), Korean ($n = 520$), Manchu ($n = 303$) and Mongol ($n = 217$) groups in eastern and northeastern China from June 2013 to August 2015 (Fig. 1). 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. Volunteers/guardians provided informed consent on behalf of all child participants. The sera were collected with agreement from the volunteers. Approximately 5 ml of venous blood samples were drawn from participants. Blood samples were left overnight at room temperature to allow for clotting and centrifuged at 3000 rpm for 10 min. The sera were collected in Eppendorf tubes and stored at 4 °C for 24–72 h until being transported in an ice box to the College of Animal Science and Technology, Jilin Agricultural University, Changchun, Jilin Province, People's Republic of China, where they were kept at −20 °C until tested.

Socio-demographic and behavioural data collection

A questionnaire was used to collect data from study participants during blood sample collection which assessed socio-demographic and behavioural data. Socio-demographic data, including age, gender, birthplace and residence, were obtained from all participants, and behavioural data, including the presence of cats and dogs at home, the consumption of raw/undercooked meat, the consumption of unwashed vegetables and fruits, the source of drinking water, and exposure to soil were also obtained. These variables were selected based on the literature.

Serological tests

Sera were analysed for the presence of IgG and IgM antibodies against *T. gondii* using commercially available enzyme immunoassay kits (Demeditec Diagnostics GmbH, Germany) according to the manufacturer's instructions. Positive and negative serum controls were included in every plate. To avoid bias, the serology test was performed in a double-blind manner. Samples from different ethnic groups were randomly mixed, and the person performing the test was unaware of the source of the samples. Optical densities were measured by a photometer at a wavelength of 450 nm. Values higher than the cut-off (10 IU/ml) were considered positive. Values ±20% of the cut-off were considered to be equivocal and were re-tested.

Statistical analysis

Results were analysed with the SPSS v. 19.0 software package (SPSS Inc., USA). To compare the frequencies among groups, the Mantel–Haenszel test and, when indicated, Fisher's exact test were used. Bivariate and multiple analyses were used to assess the association between the characteristics of the subjects and *T. gondii* infection. Variables were included in the multiple analysis with $P \leqslant 0{\cdot}25$ in the bivariate analysis [6, 8]. The adjusted odds ratios (aORs) and 95% confidence interval (CIs) were calculated by multiple analysis using multiple unconditional logistic regression. $P < 0{\cdot}05$ was considered statistically significant.

RESULTS

The overall prevalence of anti-*T. gondii* IgG antibodies in the examined participants was 13·79%

Fig. 1. Geographical distribution of the sampled regions in eastern and northeastern China.

(254/1842, 95% CI 12·22–15·36). Of these, Mongol ethnicity had the highest *T. gondii* seroprevalence (19·36%, 42/217, 95% CI 14·10–24·61), followed by Korean ethnicity (15·77%, 82/520, 95% CI: 12·64–18·90), Manchu ethnicity (12·87%, 39/303, 95% CI 9·10–16·42) and Han ethnicity (11·35%, 91/802, 95% CI 9·15–13·54). Han ethnicity had a significantly lower seroprevalence than other ethnicities ($P < 0{\cdot}001$). Moreover, anti-*T. gondii* IgM antibodies were found in 23 (1·25%) participants, and six participants were positive for both IgG and IgM antibodies. Detailed information is summarized in Table 1.

Risk factor analysis

For the Han group, bivariate analysis showed a number of socio-demographic characteristics and behavioural characteristics with a P value $\leqslant 0{\cdot}25$, including area of residence, the consumption of raw vegetables and fruits, the consumption of raw/undercooked meat, exposure to soil, and source of drinking water (Table 2). Accordingly, multiple analysis of these socio-demographic and behavioural characteristics showed that the consumption of raw vegetables and fruits (aOR 1·654, 95% CI 1·083–2·528), the consumption of raw/undercooked meat (aOR 2·092, 95% CI 1·364–3·210) and the source of drinking water (aOR 1·607, 95% CI 1·045–2·472) were significantly associated with *T. gondii* infection in the Han group (Table 3). Likewise, having a cat at home was identified to be associated with *T. gondii* infection in the Korean (aOR 2·913, 95% CI 1·786–4·752), Manchu (aOR 2·400, 95% CI 1·171–4·918) and Mongol (aOR 2·188, 95% CI 1·055–4·535) groups (Table 3). Moreover, the consumption of raw/undercooked meat was identified as another predictor of *T. gondii* seropositivity in the Mongol group (aOR 2·490, 95% CI 1·275–4·861) (Table 3).

DISCUSSION

Our cross-sectional study estimated the seroprevalence of *T. gondii* infection in the Korean, Manchu, Mongol and Han ethnic groups in eastern and northeastern China. To our knowledge, this is the first report of *T. gondii* infection in the Korean and Manchu ethnic groups in eastern and northeastern China. *T. gondii* seroprevalence in the Mongol, Korean, and Manchu groups was significantly higher than that in the Han group. Apart from the impact of the limited number of samples in this study, lifestyle, eating habits and living environment may have contributed to high

Table 1. *IgG and IgM anti-*Toxoplasma gondii *antibodies in different ethnic groups in eastern and northeastern China*

	Han ethnicity ($N = 802$)		Korean ethnicity ($N = 520$)		Manchu ethnicity ($N = 303$)		Mongol ethnicity ($N = 217$)		
Seroreaction	Pos.	%	Pos.	%	Pos.	%	Pos.	%	*P* value
IgG only	89	11·10	80	15·38	38	12·54	41	18·89	<0·001
IgM only	7	0·87	4	0·77	3	0·99	3	1·38	0·880
IgG and IgM	2	0·25	2	0·38	1	0·33	1	0·46	0·956
Total	98	12·22	86	16·54	42	13·86	45	20·74	0·008

Pos., Positive.

T. gondii seroprevalence in the Mongol, Korean and Manchu groups. However, the *T. gondii* seroprevalence in the present study was much higher (7·9%) than the national average estimated for the general population [19], but lower than that in the Bai ethnic group, which presents a 32·3% seroprevalence [18]. These differences may have been caused by several factors, including geographical conditions, the type and size of the population evaluated, lifestyle, the number of cats, and the specificity and sensitivity of the detection methods used.

T. gondii seropositivity has been reported to be related to age [18, 20, 21]. It is well known that *T. gondii* is an opportunistic pathogen; thus, the acquisition of *T. gondii* infection is a result of immunosuppression due to a higher probability of contact with the infective forms of the parasite throughout the years. However, for subjects in several age groups, the present data were inconclusive because there was a limited number of samples in this study. Thus, further studies with more samples under controlled conditions are necessary to further define the potential morbidity associated with *T. gondii* infection.

Cats, definitive hosts for *T. gondii*, play a crucial role in transmitting *T. gondii* because they have the ability to discharge oocysts in their faeces [22, 23]. In China, cats' excretion of *T. gondii* oocysts into the environment has been considered to increase the possibility of human infection [23]. Casual contact with cats may not necessarily be a risk factor, but continuous exposure to feline faeces or neglect of preventive measures (i.e. not washing hands or wearing gloves) may increase the risk of infection to an appreciable level. In China, with the continuous development of society and the improvement of human wellbeing, more and more people are starting to keep pets, including cats and dogs. This, together with inadequate inspection and quarantine measures, could enhance the potential risk to pet owners of zoonotic hazards, such as *Toxoplasma* [24].

Several previous studies have demonstrated that humans can acquire *T. gondii* infection via ingesting tissue cysts in undercooked or raw meat from an infected intermediate host, or by ingesting oocytes via unwashed vegetables and fruit or drinking water polluted by the excrement of infected cats [25–28]. Similarly, our study found that the consumption of raw/undercooked meat was highly associated with *T. gondii* seropositivity in the Han and Mongol groups. Meanwhile, the consumption of unwashed vegetables and fruits and the source of drinking water were also highly associated with *T. gondii* seropositivity in the Han group. Therefore, it is very important to publicize the knowledge of disease prevention to the public, with particular emphasis on the important role cats play in the transmission of *T. gondii* and the association between *T. gondii* infection and behavioural characteristics.

In contrast to other regions of the world, where the prevalence of *T. gondii* infection has been declining over the past several decades, in eastern and northeastern China the prevalence seems to be increasing [8, 12–18]. The increasing number of pets in recent decades may have contributed to an increased *T. gondii* prevalence. Thus, further studies should be conducted to investigate the possible sources of infection and the health burden that toxoplasmosis imposes on the population of China.

CONCLUSION

Using a cross-sectional design, the present study revealed that infection with *T. gondii* in Korean, Manchu, Mongol and Han ethnic groups is common in eastern and northeastern China. *T. gondii* seroprevalence in the Mongol, Korean, Manchu groups

Table 2. *Socio-demographic factors associated with* Toxoplasma gondii *seropositivity in different ethnic groups by univariate analysis*

	Han ethnicity				Korean ethnicity				Manchu ethnicity				Mongol ethnicity			
Variable	No. tested	No. pos.	%	*P* value	No. tested	No. pos.	%	*P* value	No. tested	No. pos.	%	*P* value	No. tested	No. pos.	%	*P* value
Age group, years																
⩽19	90	8	8·89	0·434	58	6	10·34	0·566	53	5	9·43	0·741	27	3	11·11	0·529
20–39	173	17	9·83		118	16	13·56		64	10	15·63		57	11	19·30	
40–59	356	48	13·48		324	43	13·27		144	20	13·89		95	22	23·16	
⩾60	183	25	13·66		120	21	17·50		42	7	16·67		37	9	24·32	
Gender																
Male	370	48	12·97	0·547	244	42	17·21	0·697	137	20	14·60	0·736	95	25	28·42	0·074
Female	432	50	11·57		276	44	15·94		166	22	13·25		122	20	14·75	
Location																
Changchun	131	17	12·98	0·904	113	19	16·81	0·896	148	20	13·51	0·734	116	23	19·83	0·877
Qingdao	210	24	11·43		190	33	17·37		89	11	12·36		29	7	24·14	
Weihai	461	57	12·36		217	34	15·67		66	11	16·67		72	15	20·83	
Area of residence																
Urban	487	53	10·88	0·151	300	41	13·67	0·040	170	22	12·94	0·151	123	22	17·89	0·236
Rural	315	45	14·29		220	45	20·45		103	20	19·42		94	23	24·47	
Cat at home																
Yes	174	19	10·92	0·554	122	40	32·79	<0·001	59	15	25·42	0·004	47	18	38·30	<0·001
No	628	79	12·58		398	46	11·56		244	27	11·06		170	27	15·88	
Dog at home																
Yes	164	19	11·59	0·781	99	12	12·12	0·583	69	7	10·14	0·309	33	7	21·21	0·578
No	638	79	12·38		421	74	17·58		234	35	14·96		184	38	20·65	
Consumption of unwashed vegetables and fruits																
Yes	322	53	16·46	0·003	211	39	18·48	0·324	122	22	18·03	0·085	88	23	26·14	0·105
No	480	45	9·38		309	47	15·21		181	20	11·05		129	22	17·05	
Consumption of raw/undercooked meat																
Yes	330	55	16·67	0·001	242	37	15·29	0·474	136	18	13·24	0·776	87	25	21·84	0·017
No	472	43	9·11		278	49	17·63		167	24	14·37		130	20	20·00	
Exposure to soil																
Yes	401	58	14·46	0·052	273	51	18·68	0·167	161	26	16·15	0·220	115	24	20·87	0·873
No	401	40	9·98		247	35	14·17		142	16	11·27		102	21	20·59	
Source of drinking water																
Tap	536	54	10·07	0·009	352	54	15·34	0·287	187	23	12·30	0·318	156	30	19·23	0·381
Well + river	266	44	16·54		168	32	19·05		116	19	16·38		61	15	24·59	

Pos., Positive.

Table 3. *Multiple analysis of selected characteristics of the participants and their association with* Toxoplasma gondii *infection*

Ethnicity	Variable[a]	Category	aOR[b]	95% CI	*P* value
Han	Area of residence	Urban	Ref.		
		Rural	0·805	0·526–1·234	0·320
	Consumption of raw vegetables and fruits	No	Ref.		
		Yes	1·654	1·083–2·528	0·019
	Consumption of raw/undercooked meat	No	Ref.		
		Yes	2·092	1·364–3·210	<0·001
	Exposure to soil	No	Ref.		
		Yes	0·792	0·518–1·211	0·281
	Source of drinking water	Tap	Ref.		
		Well + river	1·607	1·045–2·472	0·030
Korean	Area of residence	Urban	Ref.		
		Rural	0·770	0·484–1·226	0·270
	Cat at home	No	Ref.		
		Yes	2·913	1·786–4·752	<0·001
	Exposure to soil	No	Ref.		
		Yes	0·805	0·505–1·284	0·363
Manchu	Area of residence	Urban	Ref.		
		Rural	0·776	0·398–1·513	0·456
	Cat at home	No	Ref.		
		Yes	2·400	1·171–4·918	0·015
	Consumption of raw vegetables and fruits	No	Ref.		
		Yes	0·631	0·328–1·214	0·166
	Exposure to soil	No	Ref.		
		Yes	0·829	0·429–1·599	0·575
Mongol	Gender	Male	Ref.		
		Female	1·452	0·752–2·805	0·265
	Area of residence	Urban	Ref.		
		Rural	0·753	0·390–1·454	0·397
	Cat at home	No	Ref.		
		Yes	2·188	1·055–4·535	0·033
	Consumption of raw vegetables and fruits	No	Ref.		
		Yes	0·767	0·398–1·479	0·428
	Consumption of raw/undercooked meat	No	Ref.		
		Yes	2·490	1·275–4·861	0·007

aOR, Adjusted odds ratio; CI, confidence interval; Ref., reference.
[a] The included variables were those with a $P \leqslant 0{\cdot}25$ obtained in the bivariate analysis.
[b] Adjusted by region and the rest of the characteristics included in this table.

was higher than that in the Han group. Several behavioural characteristics were important risk factors for the acquisition of *T. gondii* infection, including having cats at home, the consumption of raw vegetables and fruits, the consumption of raw/undercooked meat and the source of drinking water. Considering the increasing number of pets over the past several decades, it is essential to implement integrated strategies with efficient management measures to prevent and control *T. gondii* infection in this region and elsewhere in China.

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DECLARATION OF INTEREST

None.

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