Seroepidemiology of *Toxoplasma gondii* infection in patients with liver disease in eastern China

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SUMMARY

The role of the protozoan parasite *Toxoplasma gondii* in the pathogenesis of liver disease has recently gained much interest. The aim of this study was to determine the prevalence and risk factors associated with *T. gondii* infection in patients with liver disease from three cities in Shandong and Henan provinces, China. A case-control study was conducted from December 2014 to November 2015 and included 1142 patients with liver disease and 1142 healthy controls. Serum samples were collected from all individuals and were examined with enzyme-linked immunosorbent assay for the presence of anti-*T. gondii* IgG and IgM antibodies. Information on the demographics, clinical and lifestyle characteristics of the participants was collected from the medical records and by the use of a questionnaire. The prevalence of anti-*T. gondii* IgG was 19.7% in patients with liver disease compared to 12.17% in the controls. Only 13 patients had anti-*T. gondii* IgM antibodies compared to 12 control individuals (1.14% *vs* 1.05%, respectively). The highest seroprevalence was detected in patients with liver cancer (22.13%), followed by hepatitis patients (20.86%), liver cirrhosis patients (20.42%), and steatosis patients (20%). Multivariate logistic regression analysis indicated that consumption of raw meat (odds ratio [OR] = 1.32; 95% CI, 1.01 to 1.71; $P = 0.03$) and source of drinking water from wells (odds ratio [OR] = 1.56; 95% CI, 1.08 to 2.27; $P = 0.01$) were independent risk factors for *T. gondii* infection in liver disease patients. These findings indicate that *T. gondii* infection is more likely to be present in patients with liver disease. Therefore, efforts should be directed toward health education of populations at high-risk of *T. gondii* infection and measures should be taken to protect vulnerable patients with liver disease.

Key words: *Toxoplasma gondii*, liver disease, case-control study, seroprevalence, risk factors.

INTRODUCTION

Toxoplasmosis is a parasitic disease caused by infection with the intracellular apicomplexan protozoan parasite *Toxoplasma gondii*. This parasite has a worldwide distribution and is able to infect any warm-blooded animal [1]. Infections caused by *T. gondii* continue to impose a significant public health threat because it can cause hydrocephaly, retinochoroiditis, mental retardation, and even death in developing fetus and life-threatening encephalitis in organ transplant recipients, individuals with AIDS, or receiving immunosuppressive therapy [2–4]. *T. gondii* is one of the most successful protozoan parasites owing to its ability to manipulate the immune system and because of the various means (e.g. waterborne, foodborne, transplacental, or organ transplant) by which the parasite can infect the host and establish a latent infection [5–9].

Although *T. gondii* possesses neurotropic and ocular affinities, this parasite can also infect other organs, such as liver, spleen, pancreas, heart, and lymph nodes [10, 11]. Several relatively small studies have detected association between *T. gondii* infection and various hepatic pathologies, such as hepatitis, granuloma, necrosis, hepatomegaly, jaundice, and cirrhosis [12–18]. *T. gondii* infection has also been linked to abnormal liver function tests [19, 20]. Liver has been shown to be the major site of tissue pathology during acute, lethal toxoplasmosis in mice [21]. An association between the amount of *T. gondii* antigens and increased number of stellate cells (HSCs) has been reported in the liver of acutely infected mice, suggesting a role for HSCs in the pathogenesis of *T. gondii*-induced hepatitis [22]. Also, *T. gondii* infection has been shown to induce several transcriptomic and proteomic changes in the liver of infected mice [23, 24].

In recent years, interest in assessing and understanding the relationship between *T. gondii* infection and liver disease in humans has surged [25, 26], probably due to the huge global burden and public health importance of both *T. gondii* infection [27] and liver diseases [28]. As considerable results exist linking *T. gondii* infection to hepatic damage, and there is no available data about the correlation between liver disease and *T. gondii* infection in China, it is important to identify any evidence linking *T. gondii* infection to liver disease in humans in China. Therefore, the present study was conducted to investigate the seroprevalence of and risk factors associated with *T. gondii* infection in patients with liver disease from three major cities in Shandong and Henan provinces in eastern China using ELISA. Our study advances the literature of the association between liver disease and *T. gondii* infection.

MATERIALS AND METHODS

Ethics statement

The protocol of the study was reviewed and approved by the Ethics Committee of the Medical College of Qingdao University, Wenhaiwei People's Hospital and Henan Provincial People's Hospital. Patients were informed about the aim of the study, and they all provided written consent for participation in the study. Also, informed consent was provided by parents or guardians on behalf of all child participants. The control sera were collected with agreement from volunteers.

Study sites

The study was conducted in two provinces (Shandong and Henan) located in eastern China (Figure 1). Two cities were selected in Shandong province, namely Qingdao and Weihai. Qingdao is located at the south-eastern tip of Shandong province, eastern China (35°35′–37°09′N, 119°30′–121°00′E), and is a key economic center, famous for its cultural heritage and attractive scenery. Weihai is located at the eastern tip of Shandong province (36°41′–37°35′N, 121°11′–122°42′E). The annual average temperature is 12°C and the average precipitation is over 800mm. Zhengzhou (31°23′–36°22′N, 110°21′–116°39′E) is the capital of Henan province, which has a subtropical semi-humid monsoon climate with a hot, humid and rainy summer, long and cold winter, and an annual average temperature of 14°C.

Study design and data collection

A case-control study was designed to identify prevalence and risk factors associated with *T. gondii* infection. The study was conducted from December 2014 to November 2015 and included 1142 inpatients hospitalized for the diagnosis or treatment of liver disease. The clinical cases included patients with various forms of hepatic disease, including liver cirrhosis (n = 333), hepatitis (n = 326), liver cancer (n = 253), steatosis (n = 205), and other hepatic disease (n = 25). An equivalent number of seronegative control subjects (n = 1142), of similar age and gender from each of the three geographic regions were included in the study. Demographics, such as age, gender and area of residence were obtained from the computerized inpatient registry of cases or by asking the control participants. A questionnaire was distributed to all participants and to the parents of children or to adult individuals in order to obtain information about their lifestyle and feeding habits, including the history of close contact with cats or dogs at home (Yes/No), consumption of raw vegetables (Yes/No), exposure to soil (Yes/No), consumption of raw/undercooked meat (Yes/No), and the source of drinking water (None, running water, wells). All personal information was anonymized and treated as strictly confidential.

Serum collection and serological testing

Around 5-mL of venous blood were drawn from each participant. Blood samples were kept overnight at ambient temperature in order to allow clot formation and were centrifuged at 1000g for 10min. The sera were collected in 2-mL Eppendorf tubes and were stored at 4°C for 24–72h until transportation in an icebox to State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Gansu

Province, where samples were kept at -20°C until analysis. Serum samples were analyzed for the presence of anti-*T. gondii* IgG and IgM antibodies (using commercially available ELISA kits (Demeditec Diagnostics GmbH, Germany) according to the manufacturer's instructions. Positive and negative serum controls were included in every plate.

Statistical analysis

Comparison between cases and controls for continuous variables such as age used one-way ANOVA and are reported as mean ±S.D. For categorical variables, the Chi-square test or the Fisher's exact test was used and results are expressed either as percentage of total (number of cases or control) or median (IQR), as appropriate. To identify factors associated with *T. gondii* IgG seropositivity a univariate approach was first used with a cut-off of ≤ 0.10 to identify contributing factors to include in a multivariate analysis. Logistic regression was then used to identify significantly contributing factors with all variables included from univariate analyses. Only variables significant at $p < 0.05$ were retained in multivariate analysis. The statistical model included age and gender (fitted first, to account for minor variation in the datasets; adjustment used the Mantel-Haenszel test). All statistical analysis was performed using Genstat v18 (VSNi, Rothampsted, UK). Odds ratios (ORs) with 95% Confidence Intervals (CI) were calculated to indicate effect size of predictive variables. A *P*-value < 0.05 was considered to be statistically significant.

RESULTS

Toxoplasma gondii IgG antibodies were detected in 225 (19.7%) of patients with liver disease, which was significantly higher ($P < 0.001$) than the level detected in the control individuals (139; 12.17%). In contrast, *T. gondii* IgM antibodies were only detected in 13 patients and 12 controls (1.14% *vs* 1.05%, respectively, $P = 0.84$). Table 1 describes the seroprevalance according to patients' age, gender, geographic region and residence area. The highest seroprevalence of *T. gondii* infection was found in patients with liver disease whose ages ranged from 41–50 (Table 1). The seroprevalence of *T. gondii* infection among patients with liver disease from rural areas (20.49%) versus urban areas (20.30%) was not significantly different ($P = 0.71$).

Association of *T. gondii* infection with clinical liver disease is shown in Table 2. Patients with liver disease had a higher frequency of *T. gondii* infection *per se*, with those patients with a diagnosis of liver cancer > cirrhosis > hepatitis having greatest seropositivity. Multivariate analysis revealed that consumption of raw meat (odds ratio [OR] = 1.32; 95% CI, 1.01 to 1.71; $P = 0.03$) and source of drinking water from wells (odds ratio [OR] = 1.56; 95% CI, 1.08 to 2.27; $P = 0.01$) were the only independent risk factors for *T. gondii* infection in liver disease patients (Table 3). Other variables did not show any significant association with *T. gondii* infection.

DISCUSSION

The present work revealed a higher prevalence of anti-*T. gondii* IgG antibodies in patients with liver disease compared with the control subjects (12.17%). It is possible that the immune-compromised status associated with liver disease might make the patients become more vulnerable to infection with *T. gondii* compared to healthy individuals [29, 30]. One previous study did not detect an association between *T. gondii* infection and liver disease in patients from Mexico [26]. However, the prevalence rate obtained in our study was lower than that reported in Egypt in patients with hepatic disease 65.5% *vs* 27% in the controls [31], in late-stage cirrhotic patients 92.6% *vs* 15% controls, and chronic HCV non-cirrhotic patients 76.9% *vs* 40% controls [32]. Another study in patients with chronic liver disease form Egypt reported 30% *vs* 6% in controls [25]. Differences in prevalence rates among these studies could be attributed to geographic variability, heterogeneity of the studied populations or in the diagnostic approaches employed. In our study, the result of *T. gondii* IgM antibodies (1.14% *vs* 1.05%, respectively, $P = 0.84$) was not significantly different between cases and control. This finding is consistent with the results reported in patients with liver disease in Egypt where no significant difference *(*$P = 0.610$) in the level of anti-*T. gondii* IgM antibodies was detected in late stage (13.6%) and early stage (12.8%) patients compared to 7.5% controls [32].

Recent transcriptomic [23] and proteomic [24] findings showed that *T. gondii* infection can dysregulate several signaling pathways especially immune response pathway in the liver of infected mice. Also, acute *T. gondii* can reduce the activity of hepatic enzyme butyrylcholinesterase, which plays a role in immune response and lipid synthesis [33]. Additionally, infection with *T. gondii* RH strain can elicit strong inflammatory response and

high levels of T helper cell type 1 (Th1) cytokines, which damage the liver [21]. Therefore, it is sensible to assume that the pathogenesis of liver damage during *T. gondii* infection can be induced by parasite-derived factors that disrupt host signaling pathways and/or host-mediated factors (and other as yet unknown mechanisms).

Further, we found that patients with liver cancer had the highest *T. gondii* seroprevalence compared to other forms of liver diseases. This is expected because patients treated with antineoplastic agents are more likely to be infected with *T. gondii* [34, 35]. The immune-suppression associated with chemotherapeutic treatment of patients with malignancies can result in the reactivation of latent *T. gondii* infection [36]. An association between *T. gondii* seropositivity and consumption of sheep meat in patients with live disease has been reported in Mexico (OR = 8.69; 95% CI: 1.02-73.71; $P = 0.04$) [26]. However, in our study none of the lifestyle variables, including contact with cats or dogs at home, consumption of raw vegetables, consumption of raw/undercooked meat, or exposure to soil, was found to have a significant association with *T. gondii* seropositivity. Interestingly, multivariate analysis revealed only two independent risk factors for *T. gondii* infection, which were the consumption of raw meat (odds ratio [OR] = 1.32; 95% CI, 1.01 to 1.71; $P = 0.03$) and source of drinking water from wells (odds ratio [OR] = 1.56; 95% CI, 1.08 to 2.27; $P = 0.01$).

In China, cats are popular companion animals, but the public are not well-informed of the risk of environmental contamination with *T. gondii* oocysts [37]. Humans can be infected with *T. gondii* oocysts through drinking water contaminated with cat faeces [38], which has been identified as a public health threat [39]. Felids are the only definitive hosts for *T. gondii*, and primary infection results in shedding of millions of environmentally resistant non-sporulated

oocysts within approximately 2 weeks. Oocysts become infectious within 1–5 days, depending on the climatic conditions [40]. The identification of the source of drinking water as one of the two independent risk factors for *T. gondii* infection in our study necessitates that measures should be taken to protect the public in general and immunocompromised individuals in particular from the risk of water-borne infection with *T. gondii* oocysts. Likewise, the identification of consumption of raw meat as a significant risk factor for *T. gondii* infection confirms the overwhelming evidence regarding the epidemiological relevance of meat-borne infection with *T. gondii* [2, 41–43]. There is a clear need for increased education of health professionals and the public about the potential transmission of *T. gondii* via drinking water from wells or consumption of raw meat in order to reduce the risk and burden of toxoplasmosis in China.

CONCLUSION

To our knowledge, this is the largest study that specifically investigates the epidemiology of *T. gondii* infection in association with liver disease. Additionally, our study examined patients with various clinical forms of liver disease and utilized multivariate analysis to determine which factors are independently associated with risk of *T. gondii* infection in patients with liver disease. Our results underscore the need for monitoring the presence of *T. gondii* infection in patients with liver disease especially those with immunocompromised status. Also, efficient lifestyle and environmental intervention strategies are needed in order to reduce the risk of water-borne and meat-borne transmission of *T. gondii* oocysts. Additional studies are needed to define which *T. gondii* genotype is most likely to be associated with hepathopathy in China.

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DECLARATION OF INTEREST

None.

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Figure legend:

Figure 1. Location maps of the study sites in eastern China showing the location of Qingdao, Weihai and Zhengzhou cities targeted in the present prevalence study.

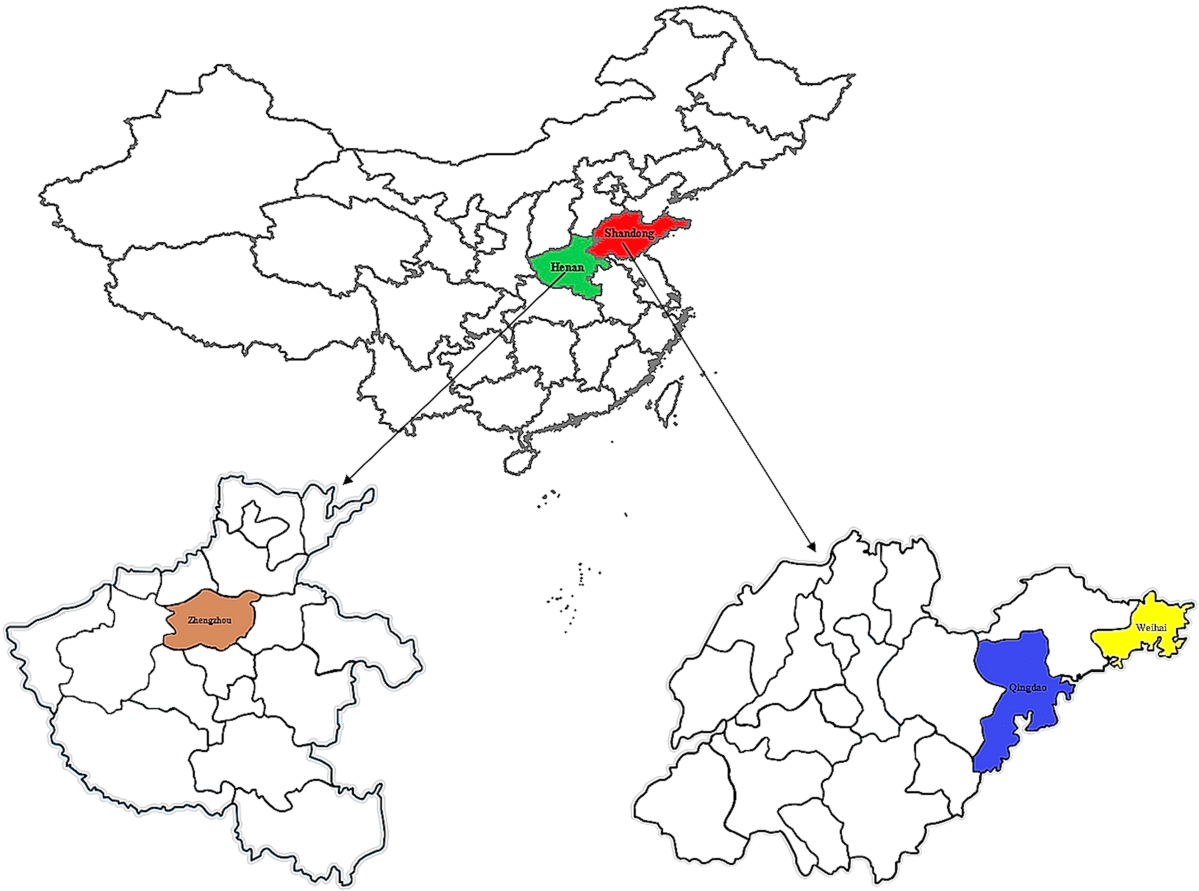

Table 1. *Demographics of the study population and seroprevalence of Toxoplasma gondii infection*

Variable	Cases with liver diseases (n = 1142)			Control group (n = 1142)			*P*-value
	No. tested	No. positive	Prevalence %	No. tested	No. positive	Prevalence %	
Age group (years)							0.10
30 or less	103	23	22.33	254	40	15.75	
31–40	153	29	18.95	169	16	9.47	
41–50	309	74	23.95	297	38	12.79	
51–60	243	38	15.64	150	13	8.67	
61–70	190	36	18.95	132	17	12.88	
＞70	144	33	22.92	140	26	18.57	
Gender							0.008
Male	595	114	19.16	532	67	12.59	
Female	547	119	21.76	610	83	13.61	
Geographic region							< .001
Qingdao	67395	87	22.03	382	44	11.52	
Weihai	3439	95	27.22	494	67	13.56	
Zhengzhou	398	51	12.81	266	39	14.66	
Residence area							0.71
Urban	532	108	20.30	649	66	10.17	
Rural	610	125	20.49	493	84	17.04	

Table 2. *The seroprevalence of Toxoplasma gondii in patients with various hepatic disorders in China*

Clinical disease	Patients with anti-*T. gondii* IgG antibodies			
	No. tested	No. positive (%)	OR (95% CI)	*p*-value
None	1003	139 (13.8)	reference	-
Liver cirrhosis	267	66 (24.7)	2.05 (1.46–2.86)	< 0.001
Hepatitis	259	67 (25.8)	1.85 (1.34–2.56)	< 0.001
Liver cancer	199	54 (27.1)	2.48 (1.69–3.65)	< 0.001
Steatosis	167	38 (22.7)	1.59 (1.06–3.39)	0.02
Other	25	0 (0)	-	-
Total	1920	364 (18.9)	1.91 (1.51–2.42)	< 0.001

* Include amoebic liver abscess, hepatic hemangioma, hepatic cyst, focal liver lesions and hepatocellular liver disease.

Table 3. *Multivariate analysis of risk factors for Toxoplasma gondii infection in patients with liver disease*

Variable	Number tested	Number positive	Prevalence %	Univariate analysis[1] OR (95% CI) *	p-value	Multivariate analysis[2] OR (95% CI) *	p-value
Cats at home	744	35	4.7	1.08 (0.77–1.51)	0.61	-	-
Dogs at home	744	29	3.9	1.13 (0.78–1.63)	0.49	-	-
Consumption of raw vegetables	744	73	9.81	0.90 (0.69–1.18)	0.48	-	-
Exposure to soil	744	97	13.04	1.13 (0.87–1.46)	0.34	-	-
Consumption of raw/undercooked meat	744	81	10.89	1.30 (1.00-1.69)	0.04	1.32 (1.01–1.71)	0.03
Source of drinking water: None	398	48	12.06	Reference	-	-	-
Running water	587	135	23.0	1.27 (0.95–1.68)	0.09	1.09 (0.79–1.50)	0.56
Wells	157	42	26.75	1.79 (1.26–2.54)	< .001	1.56 (1.08–2.27)	0.01

* OR, odds ratio; CI, 95% Confidence interval.

[1], Statistical model tested individual effect, after correction for Age, Gender and Geographic region.

[2], Statistical model excluded factors not-significant on univariate analysis, but included consumption of raw/undercooked meat and source of drinking water from wells, after correction for Age, Gender and Geographic region.