Research Article

Toxoplasma gondii Infection in Diabetes Mellitus Patients in China: Seroprevalence, Risk Factors, and Case-Control Studies

Yong-Xin Li,1 Hai Xin,1 Xiang-Yan Zhang,2 Cui-Ying Wei,3 Yu-He Duan,4 Hao-Fu Wang1 and Hai-Tao Niu5

1Department of Vascular Surgery, The Affiliated Hospital of Qingdao University, Qingdao, China
2Department of Pathology, The Affiliated Hospital of Qingdao University, Qingdao, China
3Department of Pneumology, The Affiliated Hospital of Qingdao University, Qingdao, China
4Department of Pediatric Surgery, The Affiliated Hospital of Qingdao University, Qingdao, China
5Department of Urinary Surgery, The Affiliated Hospital of Qingdao University, Qingdao, China

Correspondence should be addressed to Hao-Fu Wang; wanghaof2018@163.com and Hai-Tao Niu; niuhaitao@qduhospital.cn

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The association between Toxoplasma gondii (T. gondii) infection and diabetes mellitus remains controversial. With the improvement of living standards, the prevalence rate of diabetes is steadily increasing in China. Thus, it is necessary to explore the possible association between toxoplasmosis and diabetes mellitus in China. Hence, case-control studies were conducted to explore the T. gondii seroprevalence and identify the risk factors and possible transmission routes of T. gondii infection in different types of diabetes, including type 1 diabetes (T1DM), type 2 diabetes (T2DM), and gestational diabetes (GDM) patients in China. Four hundred serum samples for each type of diabetes mellitus, matched with 400 control subjects for each group, were collected and examined for anti-T. gondii IgG and IgM antibodies using commercially available enzyme immunoassay kits. The total T. gondii seroprevalence in T1DM, T2DM, and GDM patients was 16.50%, 23.50%, and 21.25%, respectively. Each type of diabetes mellitus patients had a significantly higher T. gondii seroprevalence than the control subjects. Multivariate regression identified three variables as risk factors for T. gondii infection in diabetes patients, including keeping cats at home and consumption of raw oysters for T1DM patients and consumption of raw/undercooked meat and raw oysters for T2DM patients, which may help to guide future research and control policies in diabetes mellitus patients.

1. Introduction

Toxoplasma gondii, an obligate intracellular opportunistic parasite, can infect nearly all types of warm-blood animals, including humans [1]. Notably, nearly one-third of the human population worldwide have been estimated to be infected with this parasite, and over 7% of Chinese have chronic T. gondii infection [1–3]. In general, most T. gondii infections do not cause significant clinical symptoms [4]. But in some cases, infected persons may present clinical symptoms of toxoplasmosis such as lymphangitis, cerebral, and eye diseases [4–6]. In some extreme cases, T. gondii infection can be reactivated and lead to a life-threatening disease with involvement of the central nervous system in immunocompromised patients [4, 5]. T. gondii can reach many organs of the host after infection [7], including the pancreas [8].

Diabetes mellitus is a common chronic metabolic disease and more than 300 million persons worldwide are projected to be affected by this disease in 2030 [9]. With the improvement of living standards, the prevalence rate of diabetes has steadily increased in China. The sensitivity and susceptibility to various infections can be higher in diabetes mellitus patients [10]. In some cases, the Apicomplexan parasite, T. gondii, has been proposed as a possible cause of diabetes, and current information is nearly predicated on this issue [11–13]. Meanwhile, chronic toxoplasmosis has been considered as a potential risk factor for type 2 diabetes (T2DM) identified by a meta-analysis of studies on the association between chronic toxoplasmosis and diabetes mellitus [13]. However,
type 1 diabetes (T1DM) patients in Colombia were found to have significantly lower *T. gondii* seroprevalence [14]. In USA, no association was found between *T. gondii* infection and diabetes mellitus in a prospective cohort of elderly Latinos [15]. Since data of previous studies on the association between *T. gondii* infection and diabetes mellitus remain controversial, we conducted matched case-control studies to determine whether *T. gondii* seropositivity is associated with different clinical types of diabetes mellitus, including T1DM, T2DM, and gestational diabetes (GDM), and explore the risk factors for *T. gondii* infection in diabetes patients for the first time in China.

2. Materials and Methods

2.1. Study Sites. The study was conducted in The Affiliated Hospital of Qingdao University, Qingdao (35°35’–37°09’N, 119°30’–121°00’E), Shandong province, eastern China. The Affiliated Hospital of Qingdao University is a large comprehensive hospital in Shandong province that occupies an important position in the national medical system. The patients at this hospital mainly come from five provinces (Shandong, Jiangsu, Liaoning, Jilin, and Heilongjiang).

2.2. Study Design and Sample Collection. Through case-control studies, we studied *T. gondii* seroprevalence and identified the risk factors and possible transmission routes of *T. gondii* infection in diabetes mellitus patients and control subjects in China between September 2014 and January 2017. A total of 1200 diabetes mellitus patients who visited the Affiliated Hospital of Qingdao University were included in the study. Three types of diabetes mellitus patients (T1DM, T2DM, and GDM) were invited to participate in this study. The number of patients of each type was 400 (Tables 1–3).

A total of 1200 control subjects, matched with diabetes mellitus patients by age, gender, and residence, were included in the study. Serum samples were obtained from persons who participated in health screening at the Affiliated Hospital of Qingdao University.

Approximately 5 mL of venous blood samples was drawn from participants who gave their consent to participate in this study. Blood samples were left overnight at room temperature to allow clotting and centrifuged at 3000 rpm for 10 minutes. The sera were collected in Eppendorf tubes and stored at 4°C for 24-72 hours and then kept at -20°C until testing.

2.3. Sociodemographic, Clinical, and Behavioral Data Collection. Sociodemographic data including age, gender, area of residence, and employment were obtained from all participants. Clinical data including the type of diabetes and behavioral data including obesity and overweight, keeping cats at home, consumption of raw/undercooked meat, fish, oysters, raw vegetables and fruits, gardening or agricultural activities, exposure to soil, source of drinking water, and washing hands before meals were collected from the participants. These variables were selected based on published literature [15, 16]. Data was obtained from the patients/guardians, medical examination records, and informants. Patients were invited to provide veridical information and were informed that their data were used in a confidential manner.

2.4. Serological Assay. Sera were analyzed 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. Clinical specificity and sensitivity of IgG kit were 99% and 98%, respectively. Clinical specificity and sensitivity of IgM kit were 99% and 100%, respectively [16, 17]. Positive and negative serum controls were included in every plate. To avoid bias of results, the serology test was performed double-blinded. Samples from diabetes mellitus patients and control group were randomly mixed, and the person performing the test did not know the source of samples in advance [16, 17].

2.5. Statistical Analysis. Results were analyzed with SPSS 18.0 software package. For the univariate analysis, Chi-square test was used to compare the categorical variables. The Mantel-Haenszel test was used to probe any differences between the patient and control groups. Multivariate regression models were used to adjust for potential confounders. Variables were included in the multivariate analysis if they had a *p* value ≤0.25 in the univariate analysis [17, 18]. Odds ratios (ORs) and the corresponding 95% confidence interval (CI) were calculated, in order to identify the independent risk factors for *T. gondii* infection. Results with a *p* value <0.05 were considered as statistically significant.

2.6. Ethics Approval and Consent to Participate. The study protocol was reviewed and approved by the Ethics Committee of the Affiliated Hospital of Qingdao University. The aim of the study was explained to the patients and they provided written consent for their participation in the study. Control sera were collected from volunteers.

3. Results

3.1. Epidemiology of T1DM Patients with *T. gondii* Infection. T1DM patients (16.50%) had a significantly higher *T. gondii* seroprevalence than the control subjects (11.50%) (*p*=0.042). Of these, 53 T1DM patients (13.25%) were found to be positive for *T. gondii* IgG antibodies, as compared to 40 controls (10.00%), and the difference was not statistically significant (*p*=0.152). *T. gondii* IgM antibodies were detected in 15 of the 400 T1DM patients and in seven of the 400 controls (3.75% versus 1.75%, respectively, *p*=0.084). The details of T1DM patients and control subjects, including age distribution, gender, employment, and area of residence, are shown in Table 1. The highest seroprevalence of *T. gondii* infection was detected in T1DM patients in the age range of 30 years (24.19%). T1DM patients living in Shandong province (20.00%) had a higher *T. gondii* seroprevalence than those living in Jilin (14.89%) and Heilongjiang (12.36%) provinces, but the difference was not significant (*p*=0.237). There were no significant differences between female (18.13%) and male (14.98%) T1DM patients (*p*=0.395). The seroprevalence of *T. gondii* infection among the T1DM patients who lived in rural areas (18.18%) was slightly higher than those who lived...
Table 1: Seroprevalence of *T. gondii* infection by sociodemographic factors in type 1 diabetes (T1DM) patients and controls in eastern China.

| Characteristic                          | TIDM (N=400) | Controls (N=400) | TIDM vs. Controls |
|----------------------------------------|--------------|------------------|------------------|
|                                        | Prevalence of *T. gondii* infection | Prevalence of *T. gondii* infection | Prevalence of *T. gondii* infection |
|                                        | No. tested  | No. positive  | %     | P-value | No. tested  | No. positive  | %     | P-value | No. tested  | No. positive  | %     | P-value |
| Age                                    |             |                 |       |         |             |                 |       |         |             |                 |       |         |
| ≤ 30                                   | 62          | 15              | 24.19 | 0.187   | 62          | 10              | 16.12 | 0.591   | 0.263       |
| 31-40                                   | 70          | 12              | 17.14 |          | 85          | 12              | 14.12 |          | 0.604       |
| 41-50                                   | 100         | 13              | 13.00 |          | 90          | 8               | 8.89  |          | 0.367       |
| 51-60                                   | 92          | 18              | 19.57 |          | 86          | 8               | 9.30  |          | 0.053       |
| >60                                     | 76          | 8               | 10.53 |          | 67          | 8               | 11.94 |          | 0.789       |
| Region                                  |             |                 |       |         |             |                 |       |         |             |                 |       |         |
| Shandong                                | 170         | 34              | 20.00 | 0.237   | 170         | 23              | 13.53 | 0.493   | 0.110       |
| Jilin                                   | 141         | 21              | 14.89 |          | 141         | 13              | 9.22  |          | 0.144       |
| Heilongjiang                            | 89          | 11              | 12.36 |          | 89          | 10              | 11.24 |          | 0.816       |
| Gender                                  |             |                 |       |         |             |                 |       |         |             |                 |       |         |
| Male                                    | 207         | 31              | 14.98 | 0.395   | 221         | 23              | 10.41 | 0.447   | 0.155       |
| Female                                  | 193         | 35              | 18.13 |          | 179         | 23              | 12.85 |          | 0.160       |
| Area of residence                       |             |                 |       |         |             |                 |       |         |             |                 |       |         |
| Urban                                   | 202         | 30              | 14.85 | 0.370   | 211         | 16              | 7.85  | 0.031   | 0.019       |
| Rural                                   | 198         | 36              | 18.18 |          | 189         | 27              | 14.29 |          | 0.299       |
| Employment                              |             |                 |       |         |             |                 |       |         |             |                 |       |         |
| Unemployed                              | 156         | 28              | 17.95 | 0.261   | 137         | 21              | 15.33 | 0.147   | 0.549       |
| Employed part-time                      | 139         | 26              | 18.71 |          | 161         | 13              | 8.07  |          | 0.006       |
| Employed full-time                      | 105         | 12              | 11.43 |          | 102         | 12              | 11.76 |          | 0.940       |
| Obesity and overweight                  |             |                 |       |         |             |                 |       |         |             |                 |       |         |
| Yes                                     | 156         | 21              | 13.46 | 0.191   | 194         | 24              | 12.37 | 0.596   | 0.762       |
| No                                      | 244         | 45              | 18.44 |          | 206         | 22              | 10.68 |          | 0.021       |
| Keeping cats at home                    |             |                 |       |         |             |                 |       |         |             |                 |       |         |
| Yes                                     | 50          | 16              | 32.00 | 0.002   | 58          | 10              | 17.24 | 0.138   | 0.074       |
| No                                      | 350         | 50              | 14.29 |          | 342         | 36              | 10.53 |          | 0.134       |
| Consumption of raw/undercooked meat     |             |                 |       |         |             |                 |       |         |             |                 |       |         |
| Yes                                     | 26          | 8               | 30.77 | 0.043   | 60          | 10              | 16.67 | 0.174   | 0.140       |
| No                                      | 374         | 58              | 15.51 |          | 340         | 36              | 10.59 |          | 0.052       |
| Consumption of oyster                   |             |                 |       |         |             |                 |       |         |             |                 |       |         |
| Raw                                     | 94          | 32              | 34.04 | <0.001  | 134         | 16              | 11.94 | 0.845   | <0.001     |
| Boiled                                  | 306         | 34              | 11.11 |          | 266         | 30              | 11.28 |          | 0.950       |
| Consumption of fish                     |             |                 |       |         |             |                 |       |         |             |                 |       |         |
| Raw                                     | 147         | 34              | 23.13 | 0.007   | 167         | 19              | 11.38 | 0.948   | 0.006       |
| Boiled                                  | 253         | 32              | 12.65 |          | 233         | 27              | 11.59 |          | 0.721       |
| Consumption of raw vegetables and fruits|             |                 |       |         |             |                 |       |         |             |                 |       |         |
| Yes                                     | 204         | 37              | 18.14 | 0.368   | 228         | 27              | 11.84 | 0.805   | 0.066       |
| No                                      | 196         | 29              | 14.80 |          | 172         | 19              | 11.05 |          | 0.287       |
| Exposure to soil                        |             |                 |       |         |             |                 |       |         |             |                 |       |         |
| Yes                                     | 232         | 40              | 17.24 | 0.639   | 247         | 32              | 12.96 | 0.246   | 0.190       |
| No                                      | 168         | 26              | 15.48 |          | 153         | 14              | 9.15  |          | 0.182       |
| Gardening or agricultural activities    |             |                 |       |         |             |                 |       |         |             |                 |       |         |
| Yes                                     | 257         | 42              | 16.34 | 0.909   | 257         | 33              | 12.84 | 0.260   | 0.261       |
| No                                      | 143         | 24              | 16.78 |          | 143         | 13              | 9.09  |          | 0.053       |
| Washing hands before meals              |             |                 |       |         |             |                 |       |         |             |                 |       |         |
| Yes                                     | 262         | 44              | 16.79 | 0.827   | 266         | 28              | 10.53 | 0.390   | 0.036       |
| No                                      | 138         | 22              | 15.94 |          | 134         | 18              | 13.43 |          | 0.559       |
| Source of drinking water                |             |                 |       |         |             |                 |       |         |             |                 |       |         |
| Spring/well                             | 85          | 19              | 22.35 | 0.101   | 68          | 13              | 19.12 | 0.031   | 0.625       |
| Tap                                     | 315         | 47              | 14.92 |          | 332         | 33              | 9.94  |          | 0.054       |
| Total                                   | 400         | 66              | 16.50 |          | 400         | 46              | 11.50 |          | 0.042       |
in urban areas (14.85%), but the difference was not statistically significant \((p=0.370)\). Moreover, *T. gondii* infection seroprevalence was not significantly different among T1DM patients with different types of employment \((p=0.261)\).

3.2. Epidemiology of T2DM Patients with *T. gondii* Infection. T2DM patients (25.50%) had a significantly higher *T. gondii* seroprevalence than the control subjects (11.75%) \((p<0.001)\). A total of 77 T2DM patients (19.25%) were found to be positive for *T. gondii* IgG antibodies, as compared to 37 controls (9.25%), and the difference was statistically significant \((p<0.001)\). *T. gondii* IgM antibodies were detected in 19 of the 400 T2DM patients and in 11 of the 400 controls (4.75% versus 2.75%, respectively, \(p=0.137\)). The details of T2DM patients and control subjects, including age distribution, gender, employment, and area of residence, are shown in Table 2. The highest seroprevalence of *T. gondii* infection was detected in T1DM patients in the age range of 51-60 years (25.81%). T2DM patients living in Shandong province (28.85%) had a higher *T. gondii* seroprevalence than those living in Jilin (22.54%) and Heilongjiang (16.67%) provinces, but the difference was not significant \((p=0.074)\). There were no significant differences between male (24.75%) and female (22.22%) T2DM patients \((p = 0.551)\). The seroprevalence of *T. gondii* infection among the T2DM patients who lived in urban areas (25.00%) was slightly higher than those who lived in rural areas (22.00%), but the difference was not statistically significant \((p = 0.479)\). Moreover, *T. gondii* infection seroprevalence was not significantly different among T2DM patients with different types of employment \((p=0.118)\).

3.3. Epidemiology of GDM Patients with *T. gondii* Infection. GDM patients (21.25%) had a significantly higher *T. gondii* seroprevalence than the control subjects (12.00%) \((p=0.042)\). A total of 70 GDM patients (17.50%) were found to be positive for *T. gondii* IgG antibodies, as compared to 37 controls (9.25%), and the difference was statistically significant \((p<0.001)\). *T. gondii* IgM antibodies were detected in 18 of the 400 GDM patients and in 11 of the 400 controls (4.50% versus 2.75%, respectively, \(p=0.186\)). The details of GDM patients and control subjects, including age distribution, gender, employment, and area of residence, are shown in Table 3. The highest seroprevalence of *T. gondii* infection was detected in GDM patients in the age range of >40 years (38.71%), and significant difference was found among different age groups \((p=0.043)\). GDM patients living in Shandong province (24.69%) had a higher *T. gondii* seroprevalence than those living in Jilin (12.31%) and Heilongjiang (18.95%) provinces, but the difference was not significant \((p=0.079)\). The seroprevalence of *T. gondii* infection among the GDM patients who lived in rural areas (20.20%) was slightly lower than those who lived in urban areas (22.28%), but the difference was not statistically significant \((p=0.612)\). Moreover, *T. gondii* seroprevalence was not significantly different among GDM patients with different types of employment \((p=0.081)\).

3.4. Risk Factors Associated with *T. gondii* Infection. Univariate analysis showed that some lifestyle variables of T1DM patients had a \(p\) value \(\leq 0.25\), including obesity and overweight, keeping cats at home, consumption of raw/undercooked meat, oysters, and fish, and source of drinking water. Six lifestyle variables of T2DM patients had a \(p\) value \(\leq 0.25\) through univariate analysis. They are obesity and overweight, keeping cats at home, consumption of raw/undercooked meat, fish, oysters, and raw vegetables and fruits. In GDM patients, keeping cats at home and consumption of raw/undercooked meat, fish, and oysters had a \(p\) value \(\leq 0.25\) by univariate analysis. In the multivariate analysis, keeping cats at home \((\text{OR}=2.885; 95\% \text{CI}: 1.37-6.07; p = 0.005)\) and consumption of fish \((\text{OR}=13.19; 95\% \text{CI}: 2.91-59.82; p = 0.001)\) were associated with significantly increased odds of *T. gondii* infection in T1DM patients (Table 4).

Consumption of raw/undercooked meat \((\text{OR}=2.663; 95\% \text{CI}: 1.08-6.56; p = 0.033)\) and oysters \((\text{OR}=4.785; 95\% \text{CI}: 1.98-11.45; p<0.001)\) was associated with significantly increased odds of *T. gondii* infection in T2DM patients (Table 4). There was no evidence of a significant association between *T. gondii* status and the selected variables in GDM patients (Table 4).

4. Discussion

The association between *T. gondii* infection and diabetes mellitus remains controversial, with few studies reporting conflicting results [19, 20]. Thus, the present study was conducted to determine whether *T. gondii* infection is associated with different types of diabetes mellitus in eastern China. The results showed that diabetes mellitus patients had higher frequencies of antibodies against *T. gondii* as compared to control subjects. Thus, our findings based on serological methods supported an association between diabetes mellitus and *T. gondii* infection.

Type 1 diabetes mellitus (T1DM) is an autoimmune disease with complex interactions between genetic and environmental factors [14]. The enteroviruses and other infectious agents were found to be associated with T1DM [21]. In the present study, T1DM patients had a significantly higher *T. gondii* seroprevalence than the controls \((p=0.042)\), suggesting that T1DM patients are more likely to be infected with *T. gondii*. However, further targeted studies should be conducted to explore and confirm the association between T1DM and *T. gondii* infection.

Type 2 diabetes mellitus (T2DM), a major global health problem, is a complex metabolic disease [11]. The incidence of T2DM has notably increased in recent years, in both developed and developing countries [22]. Various infections, including *T. gondii*, may easily appear in T2DM patients because they are immunocompromised [23]. In the present study, T2DM patients had a significantly higher *T. gondii* seroprevalence than the control subjects \((p<0.001)\). These evidences indicated a potential association between *T. gondii* infection and T2DM implying that *T. gondii* infection may increase susceptibility to T2DM, while T2DM patients are more vulnerable to opportunistic infections such as *T. gondii*.

Importantly, the clinician should pay more attention to *T. gondii* infection when they diagnose and treat T2DM patients given the high prevalence of *T. gondii* infection in T2DM patients, and *T. gondii* infection may aggravate the T2DM status.
Table 2: Seroprevalence of *T. gondii* infection by sociodemographic factors in type 2 diabetes (T2DM) patients and controls in eastern China.

| Characteristic                        | T2DM (N=400) | Controls (N=400) | T2DM vs. Controls |
|---------------------------------------|--------------|------------------|-------------------|
|                                       | Prevalence of *T. gondii* infection | Prevalence of *T. gondii* infection | P-value |
|                                       | No. tested  | No. positive | %  | P-value | No. tested  | No. positive | %  | P-value | P-value |
| **Age**                               |             |             |    |         |             |             |    |         |         |
| ≤ 30                                  | 37          | 9           | 24.32 | 0.956 | 37          | 4           | 10.81 | 0.793 | 0.127   |
| 31-40                                 | 83          | 19          | 22.89 | 0.500 | 87          | 11          | 12.64 | 0.080 |         |
| 41-50                                 | 96          | 23          | 23.96 | 0.000 | 99          | 13          | 13.13 | 0.052 |         |
| 51-60                                 | 93          | 24          | 25.81 | 0.000 | 97          | 12          | 12.37 | 0.018 |         |
| >60                                   | 91          | 19          | 20.88 | 0.000 | 90          | 7           | 7.78  | 0.012 |         |
| **Region**                            |             |             |    |         |             |             |    |         |         |
| Shandong                              | 156         | 45          | 28.85 | 0.074 | 156         | 25          | 16.03 | 0.025 | 0.007   |
| Jilin                                 | 142         | 32          | 22.54 | 0.000 | 142         | 17          | 11.97 | 0.019 |         |
| Heilongjiang                          | 102         | 17          | 16.67 | 0.000 | 102         | 5           | 4.90  | 0.000 |         |
| **Gender**                            |             |             |    |         |             |             |    |         |         |
| Male                                  | 202         | 50          | 24.75 | 0.551 | 211         | 27          | 12.80 | 0.492 | 0.002   |
| Female                                | 198         | 44          | 22.22 | 0.000 | 189         | 20          | 10.58 | 0.002 |         |
| **Area of residence**                 |             |             |    |         |             |             |    |         |         |
| Urban                                 | 200         | 50          | 25.00 | 0.479 | 229         | 18          | 7.86  | 0.005 | <0.001  |
| Rural                                 | 200         | 44          | 22.00 | 0.000 | 171         | 27          | 12.86 | 0.223 |         |
| **Employment**                        |             |             |    |         |             |             |    |         |         |
| Unemployed                            | 163         | 43          | 26.38 | 0.118 | 169         | 24          | 14.20 | 0.326 | 0.006   |
| Employed part-time                    | 133         | 23          | 17.29 | 0.000 | 148         | 13          | 8.78  | 0.033 |         |
| Employed full-time                    | 104         | 28          | 26.92 | 0.000 | 83          | 10          | 12.05 | 0.012 |         |
| **Obesity and overweight**            |             |             |    |         |             |             |    |         |         |
| Yes                                   | 152         | 30          | 19.74 | 0.152 | 190         | 20          | 10.53 | 0.470 | 0.017   |
| No                                    | 248         | 64          | 25.81 | 0.000 | 210         | 27          | 12.86 | 0.001 |         |
| **Keeping cats at home**              |             |             |    |         |             |             |    |         |         |
| Yes                                   | 52          | 19          | 36.54 | 0.017 | 56          | 9           | 16.07 | 0.279 | 0.015   |
| No                                    | 348         | 75          | 21.55 | 0.000 | 344         | 38          | 11.05 | 0.001 |         |
| **Consumption of raw/undercooked meat**|             |             |    |         |             |             |    |         |         |
| Yes                                   | 28          | 14          | 50.00 | <0.001 | 161         | 5           | 3.11  | <0.001 | <0.001  |
| No                                    | 372         | 80          | 21.51 | 0.000 | 339         | 42          | 12.39 | 0.001 |         |
| **Consumption of oyster**             |             |             |    |         |             |             |    |         |         |
| Raw                                   | 91          | 41          | 45.05 | <0.001 | 143         | 15          | 10.49 | 0.559 | <0.001  |
| Boiled                               | 309         | 53          | 17.15 | 0.000 | 257         | 32          | 12.45 | 0.119 |         |
| **Consumption of fish**               |             |             |    |         |             |             |    |         |         |
| Raw                                   | 150         | 50          | 33.33 | <0.001 | 167         | 22          | 13.17 | 0.454 | <0.001  |
| Boiled                               | 250         | 44          | 17.60 | 0.000 | 233         | 25          | 10.73 | 0.031 |         |
| **Consumption of raw vegetables and fruits** |     |             |    |         |             |             |    |         |         |
| Yes                                   | 253         | 66          | 26.09 | 0.109 | 286         | 32          | 11.19 | 0.581 | <0.001  |
| No                                    | 147         | 28          | 19.05 | 0.000 | 114         | 15          | 13.16 | 0.203 |         |
| **Exposure to soil**                  |             |             |    |         |             |             |    |         |         |
| Yes                                   | 228         | 54          | 23.68 | 0.920 | 248         | 34          | 13.71 | 0.120 | 0.005   |
| No                                    | 172         | 40          | 23.26 | 0.000 | 152         | 13          | 8.55  | 0.001 |         |
| **Gardening or agricultural activities** |             |             |    |         |             |             |    |         |         |
| Yes                                   | 248         | 56          | 22.58 | 0.580 | 254         | 35          | 13.78 | 0.096 | 0.011   |
| No                                    | 152         | 38          | 25.00 | 0.000 | 146         | 12          | 8.22  | 0.001 |         |
| **Washing hands before meals**        |             |             |    |         |             |             |    |         |         |
| Yes                                   | 270         | 63          | 23.33 | 0.910 | 285         | 30          | 10.53 | 0.232 | <0.001  |
| No                                    | 130         | 31          | 23.85 | 0.000 | 115         | 17          | 14.78 | 0.075 |         |
| **Source of drinking water**          |             |             |    |         |             |             |    |         |         |
| Spring/well                           | 90          | 20          | 22.22 | 0.745 | 64          | 13          | 20.31 | 0.020 | 0.776   |
| Tap                                   | 310         | 74          | 23.87 | 0.000 | 336         | 34          | 10.12 | <0.001 |         |
| **Total**                             | 400         | 94          | 23.50 | 0.000 | 400         | 47          | 11.75 | <0.001 |         |
| Characteristic                  | GDM (N=400) Prevalence of *T. gondii* infection | Controls (N=400) Prevalence of *T. gondii* infection | GDM vs. Controls |
|--------------------------------|-----------------------------------------------|-----------------------------------------------|------------------|
|                                | No. tested | No. positive | % | P-value | No. tested | No. positive | % | P-value | P-value |
| Age                           |            |              |   |         |            |              |   |         |         |
| ≤ 30                          | 185        | 35           | 18.92 | 0.043   | 201        | 26           | 12.94 | 0.017   | 0.107   |
| 31-40                         | 184        | 38           | 20.65 | 0.294   | 71         | 19           | 26.76 | <0.001  |         |
| >40                           | 31         | 12           | 38.71 |          | 28         | 3            | 10.71 |          | 0.014   |
| Region                        |            |              |   |         |            |              |   |         |         |
| Shandong                      | 239        | 59           | 24.69 | 0.079   | 239        | 32           | 13.39 | 0.451   | 0.002   |
| Jilin                         | 65         | 8            | 12.31 |          | 65         | 8            | 12.31 |          | 1.00    |
| Heilongjiang                  | 95         | 18           | 18.95 |          | 95         | 8            | 8.42  |          | 0.035   |
| Area of residence             |            |              |   |         |            |              |   |         |         |
| Urban                         | 202        | 45           | 22.28 | 0.612   | 245        | 25           | 10.20 | 0.165   | 0.001   |
| Rural                         | 198        | 40           | 20.20 |          | 155        | 23           | 14.84 |          | 0.192   |
| Employment                    |            |              |   |         |            |              |   |         |         |
| Unemployed                    | 126        | 24           | 19.05 | 0.081   | 98         | 15           | 15.31 | 0.470   | 0.464   |
| Employed part-time            | 143        | 39           | 27.27 |          | 197        | 20           | 10.15 | <0.001  |         |
| Employed full-time            | 131        | 22           | 16.79 |          | 105        | 13           | 12.38 |          | 0.343   |
| Obesity and overweight        |            |              |   |         |            |              |   |         |         |
| Yes                           | 79         | 15           | 18.99 | 0.582   | 117        | 9            | 7.69  | 0.086   | 0.018   |
| No                            | 321        | 70           | 21.81 |          | 283        | 39           | 13.78 |          | 0.011   |
| Keeping cats at home          |            |              |   |         |            |              |   |         |         |
| Yes                           | 40         | 12           | 30.00 | 0.135   | 47         | 4            | 8.51  | 0.433   | 0.010   |
| No                            | 360        | 73           | 20.28 |          | 353        | 44           | 12.46 |          | 0.005   |
| Consumption of raw/undercooked meat |        |              |   |         |            |              |   |         |         |
| Yes                           | 33         | 12           | 36.36 | 0.027   | 77         | 5            | 6.49  | 0.098   | <0.001  |
| No                            | 367        | 73           | 19.89 |          | 323        | 43           | 13.31 |          | 0.021   |
| Consumption of oyster         |            |              |   |         |            |              |   |         |         |
| Raw                           | 84         | 30           | 35.71 | <0.001  | 153        | 18           | 11.76 | 0.909   | <0.001  |
| Boiled                        | 316        | 55           | 17.41 |          | 247        | 30           | 12.15 |          | 0.084   |
| Consumption of fish           |            |              |   |         |            |              |   |         |         |
| Raw                           | 137        | 42           | 30.66 | <0.001  | 158        | 17           | 10.76 | 0.086   | <0.001  |
| Boiled                        | 263        | 43           | 16.35 |          | 242        | 41           | 16.94 |          | 0.858   |
| Consumption of raw vegetables and fruits |  |            |   |         |            |              |   |         |         |
| Yes                           | 282        | 64           | 22.70 | 0.275   | 299        | 37           | 12.37 | 0.692   | 0.001   |
| No                            | 118        | 21           | 17.80 |          | 101        | 11           | 10.89 |          | 0.149   |
| Exposure to soil              |            |              |   |         |            |              |   |         |         |
| Yes                           | 233        | 47           | 20.17 | 0.534   | 254        | 34           | 13.39 | 0.261   | 0.045   |
| No                            | 167        | 38           | 22.75 |          | 146        | 14           | 9.59  |          | 0.002   |
| Gardening or agricultural activities |        |            |   |         |            |              |   |         |         |
| Yes                           | 257        | 52           | 20.23 | 0.505   | 263        | 35           | 13.31 | 0.265   | 0.034   |
| No                            | 143        | 33           | 23.08 |          | 137        | 13           | 9.49  |          | 0.002   |
| Washing hands before meals    |            |              |   |         |            |              |   |         |         |
| Yes                           | 266        | 54           | 20.30 | 0.513   | 302        | 38           | 12.58 | 0.529   | 0.013   |
| No                            | 134        | 31           | 23.13 |          | 98         | 10           | 10.20 |          | 0.011   |
| Source of drinking water      |            |              |   |         |            |              |   |         |         |
| Spring/well                   | 78         | 14           | 17.95 | 0.840   | 66         | 15           | 22.73 | 0.003   | 0.476   |
| Tap                           | 322        | 61           | 18.94 |          | 334        | 33           | 9.88  |          | <0.001  |
| Total                         | 400        | 85           | 21.25 |          | 400        | 48           | 12.00 |          | <0.001  |
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Table 4: Multivariate analysis of selected characteristics of diabetic patients and their association with *Toxoplasma gondii* infection.

| Type of diabetic patients | Characteristica | Adjusted Odds ratio b | 95% Confidence interval | P value |
|--------------------------|----------------|------------------------|-------------------------|---------|
| T1DM                     | Obesity and overweight | 0.584 | 0.32-1.08 | 0.086 |
| T1DM                     | Keeping cats at home | 2.885 | 1.37-6.07 | 0.005 |
| T1DM                     | Consumption of raw/undercooked meat | 2.177 | 0.81-5.88 | 0.125 |
| T1DM                     | Consumption of oyster | 13.39 | 2.91-59.82 | 0.001 |
| T1DM                     | Consumption of fish | 0.295 | 0.06-1.36 | 0.117 |
| T1DM                     | Source of drinking water | 1.713 | 0.78-3.77 | 0.181 |
| T1DM                     | Obesity and overweight | 0.673 | 0.39-1.15 | 0.147 |
| T2DM                     | Consumption of raw/undercooked meat | 2.663 | 1.08-6.56 | 0.033 |
| T2DM                     | Consumption of oyster | 4.758 | 1.98-11.45 | <0.001 |
| T2DM                     | Consumption of fish | 0.747 | 0.32-1.72 | 0.493 |
| GDM                      | Consumption of raw vegetables and fruits | 1.296 | 0.72-2.33 | 0.385 |
| GDM                      | Consumption of fish | 1.376 | 0.63-2.99 | 0.420 |
| GDM                      | Consumption of raw/undercooked meat | 1.706 | 0.78-3.99 | 0.173 |
| GDM                      | Consumption of oyster | 1.516 | 0.72-3.18 | 0.271 |

a The variables with a *p* <0.25 in the univariate analysis were included.

b Adjusted by age and the other characteristics included in this table.

*T. gondii* infection during pregnancy may cause serious consequences such as miscarriage, microcephaly, hydrocephalus, and severe neurological disorders in the fetus [5]. In addition, in immunodeficient individuals, released bradyzoites from tissue cysts switching back into rapidly multiplying tachyzoites could cause reactivation of latent infection and dissemination throughout the body [24]. The immune system in diabetes mellitus patients is affected, and GDM patients are more susceptible to *T. gondii* infection. In the present study, GDM patients had a significantly higher *T. gondii* seroprevalence than the control subjects (*p*<0.001). Thus, serological screening of GDM patients is needed, followed by proper treatment of the *T. gondii* infection [25]. Moreover, information about toxoplasmosis and its transmission routes should be given to GDM patients as part of prenatal care.

The first epidemiological investigation on *T. gondii* infection in humans in China was conducted in Guangxi Zhuang Autonomous Region in 1978 [26]. Now, toxoplasmosis has become a notifiable disease in China. However, there are no national guidelines for the prevention of toxoplasmosis in China. Humans acquire the infection through three major routes: consumption of undercooked meat containing *T. gondii* tissue cysts, ingesting oocysts-contaminated water, soil, vegetables, and fruits, and transmission from mother to fetus during pregnancy [1, 27]. As expected, we found that keeping cats at home and consumption of raw/undercooked meat were associated with significantly increased odds of *T. gondii* infection in diabetic patients. These two risk factors have been identified in many studies in China [16, 28–30]. Interestingly, fresh oyster consumption was also a potential risk factor for *T. gondii* infection in T1DM and T2DM patients, which was similar to a study reported from the United States [31]. *T. gondii* oocysts can be washed into the sea via rainwash and runoff [32, 33], and shellfish including oysters, clams, and mussels can ingest the oocysts directly from seawater [1, 32–37]. In China, *T. gondii* oocysts have been detected in oysters [38] and consumption of fresh oysters is common in recent years, which may explain the higher *T. gondii* seroprevalence in the diabetes mellitus patients who consume raw oysters than those who do not consume raw oysters. Thus, knowledge of these risk factors will help in prevention efforts. Some limitations of the present study should be kept in mind. First, our study participants might not represent the general clinically healthy individuals, pregnant women, and diabetes mellitus population due to the potential limitation of enrollment methods. Therefore, potential selection bias should be considered when interpreting our results. Second, serology could not clearly indicate the infection status as current infection or past infection; potential bias caused by such misclassification could not be eliminated. Moreover, molecular identification, taxonomy, genetic variation, and diagnosis of *T. gondii* should be considered in further studies. Third, more effective statistical analysis methods should be used to confirm the association between diabetes mellitus and *T. gondii* infection. Therefore, our results need to be proved in further studies.

5. Conclusion

This study provided serological evidence of an association between *T. gondii* infection and three types of diabetes mellitus (T1DM, T2DM, and GDM). Moreover, keeping cats at home and consumption of raw/undercooked meat and raw oysters were risk factors for *T. gondii* positivity using
multivariate regression, which may help to guide future research and control policies. Further studies should be conducted to elucidate the role of T. gondii in diabetes mellitus.

Data Availability
The clinical and behavioral data used to support the findings of this study are included within the article.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

Authors’ Contributions
Yong-Xin Li and Hai Xin are equal contributors.

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