

## Original Paper

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# Seroprevalence and risk factors of *Toxoplasma gondii* infection in oral cancer patients in China: a case-control prospective study

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**Abstract**

Over the recent years, potential associations between *Toxoplasma gondii* (*T. gondii*) infection and cancer risk have attracted a lot of attention. Nevertheless, the association between *T. gondii* infection and oral cancer remains relatively unexplored. We performed a case-control study of 861 oral cancer patients and 861 control subjects from eastern China with the aim to detect antibodies to *T. gondii* by enzyme-linked immunosorbent assay (ELISA) in these patients. The results showed that oral cancer patients (21.72%, 187/861) had a significantly higher seroprevalence than control subjects (8.25%, 71/861) ( $P < 0.001$ ). Among them, 144 (16.72%) oral cancer patients and 71 (8.25%) control subjects were positive for IgG antibodies to *T. gondii*, while 54 (6.27%) oral cancer patients and 9 (1.05%) controls were positive for IgM antibodies to *T. gondii*. In addition, multiple logistic analysis showed that *T. gondii* infection in oral cancer patients was associated with blood transfusion history, keeping cats at home, and oyster consumption. To our knowledge, this is the first study that provided a serological evidence of an association between *T. gondii* infection and oral cancer patients. However, further studies are necessary to elucidate the role of *T. gondii* in oral cancer patients.

**Introduction**

*Toxoplasma gondii* (*T. gondii*), an apicomplexan parasite with a global distribution, can infect all kinds of warm-blooded vertebrates, including humans [1]. Nearly one-third of the world's population is thought to be infected with this parasite [2]. The prevalence rate of *T. gondii* in China is over 7% and this rate is constantly rising due to the rapid growth of the number of cats (pet and stray cats) [3–5]. This parasite has three infective forms: the tachyzoite, the bradyzoite inside tissue cysts and the sporozoite in sporulated oocyst [6]. Humans are mainly infected through these ways: ingesting cysts from raw or uncooked meat, ingesting oocysts from food or water contaminated with cat faeces, and through vertical transmission from mother to foetus [2]. Latent toxoplasmosis is mainly associated with neurodegenerative disorders and autoimmune diseases [7, 8]. For individuals with the normal immune system, toxoplasmosis usually does not cause notably clinical symptoms and does not need to be treated. Benign and self-limited lymphadenopathy and lymphomonocytosis are found in nearly 20% of cases [9]. Nevertheless, for immunocompromised individuals, *T. gondii* might be fatal, especially for patients with neoplasia, transplant recipients and patients with AIDS [3, 6, 9]. Thus, more attention should be paid to these groups of individuals. Host inflammation responses may increase due to chronic infection with *T. gondii*, thus promoting mutations and enhancing cancer [10].

Cancer is the leading and the second leading cause of death in economically developed countries and developing countries, respectively [11]. It is estimated that deaths from cancer will continue to rise, and the number will reach 11 million by 2030 [12]. Currently, clinical toxoplasmosis in cancer patients has gradually turned into an important public health concern [13, 14]. Cancer patients who are seronegative for *T. gondii* infection could benefit from advice on precautionary measures, to avoid seroconversion that may induce active severe toxoplasmosis [15]. Therefore, potential associations between *T. gondii* infection and cancer have attracted a lot of attention [3, 4, 16–26]. Oral cancer is one of the most common malignant tumours. According to available statistical data, there were 300 000 new cases and 145 000 disease-related deaths in 2012 [27]. Still, the association between *T. gondii* infection and oral cancer remains unexplored. The following study examined the relationship between *T. gondii* infection and oral cancer in eastern China in order to identify associated potential risk factors and possible routes of infection for oral cancer patients.

## Materials and methods

### Study sites

The study was conducted at the Affiliated Hospital of Qingdao University, a large provincial comprehensive hospital located in Qingdao (35°35′–37°09′N, 119°30′–121°00′E), Shandong province, Eastern China.

### Study design and sample collection

Here we investigated the *T. gondii* seroprevalence and identified potential risk factors of *T. gondii* infection as well as possible infection routes in oral cancer patients and control subjects in China from September 2013 to March 2017. Eight hundred and sixty-one oral cancer patients who presented to the Affiliated Hospital of Qingdao University were included in the study. In addition, 861 Control subjects were selected to match oral cancer patients by age, gender and residence.

Serum samples were randomly obtained from persons who participated in health screenings 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 incubated at room temperature overnight to allow 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 and then kept at –20 °C until further testing.

### Socio-demographic, clinical and behavioural data collection

Socio-demographic data including age, gender and area of residence were obtained from all participants. In addition, the following clinical data were collected from all patients: surgery history, blood transfusion history, chemotherapy history, differentiation degree and the TNM stage of cancer; and behavioural data including tobacco use, alcohol consumption, keeping cats at home, consumption of raw/undercooked meat, consumption of oyster, gardening or agricultural activities, exposure with soil apart from gardening or agricultural activities, source of drinking water and washing hands before meals. These variables were selected based on the previous literature. Data was obtained from the patients/guardians, medical examination records and informants. Patients were invited to provide veridical information and they were informed that data were used in a confidential manner.

### Serological assay

Sera were analysed for the presence of IgG and IgM antibodies to *T. gondii* using the commercially available enzyme-linked immunosorbent assay (ELISA) (Demeditec Diagnostics GmbH, Germany) according to the manufacturer's instructions. Positive and negative serum controls were included in every plate. To avoid biases, the serology test was done using double blinded approach. Samples from oral cancer patients and control subjects were randomly mixed, and the person performing the test did not know the source of samples in advance.

### Statistical analysis

The results were analysed with SPSS 18.0 software package. For the univariate analysis,  $\chi^2$  test or Fisher's exact test provided a comparison of the categorical variables. The Mantel–Haenszel

test was used to probe for any differences between patient and control groups. Multiple logistic regression models were used to adjust for potential confounders. Variables were included in the multiple logistic analysis if they had a *P*-value of equal or less than 0.25 in the univariate analysis [3]. Odds ratios (ORs) and the corresponding 95% confidence interval (CI) were calculated to identify independent risk factors for *T. gondii* infection. Results with a *P*-value of <0.05 were considered statistically significant.

### Ethics approval and consent to participate

The study protocol was reviewed and approved by the Ethics Committee of the Affiliated Hospital of Qingdao University. Patients were made aware of the aim of the study. Each individual provided written consent for their participation in the study. Control sera were collected from volunteers.

## Results

The overall seroprevalence of *T. gondii* infection in oral cancer patients and control subjects were 21.72% (187/861) and 8.25% (71/861), respectively. Of these, IgG antibodies to *T. gondii* were found in 144 (16.72%) out of 861 oral cancer patients and in 71 (8.25%) out of 861 control subjects (*P* < 0.001). Fifty-four (6.27%) oral cancer patients and 9 (1.05%) controls were positive for IgM antibodies to *T. gondii* (*P* < 0.001). Among oral cancer patients, 133 (15.45%) were positive for IgG antibodies compared to 62 (7.20%) controls. 43 (4.99%) oral cancer patients and 0 (0%) controls were positive for IgM antibodies only, while 1.28% oral cancer patients and 1.05% controls were positive for both IgG and IgM antibodies. Detailed information is summarised in Table 1. Univariate analysis of socio-demographic and risk factors for oral cancer patients and controls identified some factors with a *P*-value of <0.25 that may be related to infection (Table 1). Three of these, i.e. blood transfusion history, keep cats at home, and consumption of oysters were found to be significantly associated with *T. gondii* infection in the multivariable logistic analysis (Table 2).

## Discussion

Oral cancer is considered to be the sixth most common malignancy worldwide [28]. Nearly 145 000 patients die from oral cancer each year [27]. It is generally recognised that the development of oral cancer is mainly caused by tobacco consumption including smokeless tobacco and heavy alcohol consumption [29]. Apart from these risk factors, human papilloma virus has been found to be associated with oral cancer [30, 31]. Yet, the connection between oral cancer and parasitic infection remains unexplored. In this study, we first explored the relationship between oral cancer and *T. gondii* infection. Briefly, we found that oral cancer patients (21.72%, 187/861) have a significantly higher *T. gondii* seroprevalence compared to control subjects (8.25%, 71/861) (*P* < 0.001). Thus, our findings based on serological methods support a potential association between oral cancer and *T. gondii* infection.

After infection, *T. gondii* can disseminate into each organ of the infected host through blood circulation [32]. Previous studies have shown that intracellular tachyzoites can disseminate into the whole body through the blood circulation, while extracellular tachyzoites do not have this ability [33, 34]. In this study, oral

**Table 1.** Seroprevalence of *T. gondii* infection in oral cancer patients and control subjects in China

Characteristic	Oral cancer (N = 861)				Controls (N = 861)				Oral cancer vs. controls P-value
	No. tested	No. positive	%	P-value	No. tested	No. positive	%	P-value	
<b>Age</b>									
≤40	100	26	26.00	0.711	65	5	7.69	0.208	0.003
41–50	119	23	19.33		114	6	5.26		0.001
51–60	218	45	20.64		244	16	6.56		<0.001
61–70	255	53	20.78		331	30	9.06		<0.001
>70	169	40	23.67		107	14	13.08		0.031
<b>Gender</b>									
Male	538	118	21.93	0.844	475	43	9.05	0.340	<0.001
Female	323	69	21.36		386	28	7.25		<0.001
<b>Area of residence</b>									
Rural	522	90	17.24	<0.001	300	39	13.00	<0.001	0.108
Urban	339	97	28.61		561	32	5.70		<0.001
<b>Surgery history</b>									
Yes	521	111	21.31	0.716	103	3	2.91	0.036	<0.001
No	340	76	22.35		758	68	8.97		<0.001
<b>Blood transfusion history</b>									
Yes	315	86	27.30	0.003	28	4	14.29	0.238	0.134
No	546	101	18.50		833	67	8.04		<0.001
<b>Chemotherapy history</b>									
Yes	261	51	19.54	0.307	–	–	–	–	–
No	600	136	22.67		–	–	–	–	–
<b>Differentiation degree</b>									
Low	95	19	20.00	<0.001	–	–	–	–	–
Middle	221	69	31.22		–	–	–	–	–
High	545	99	18.17		–	–	–	–	–
<b>The TNM stage of cancer</b>									
I	292	61	20.89	0.846	–	–	–	–	–
II	324	68	20.99		–	–	–	–	–
III	159	37	23.27		–	–	–	–	–
IV	86	21	24.42		–	–	–	–	–
<b>Tobacco use</b>									
Yes	288	63	21.88	0.937	284	15	5.28	0.027	<0.001
No	573	124	21.64		577	56	9.71		<0.001
<b>Alcohol consumption</b>									
Yes	249	48	19.28	0.268	242	17	7.02	0.415	<0.001
No	612	139	22.71		619	54	8.72		<0.001
<b>Keeping cats at home</b>									
Yes	137	42	30.66	0.006	122	22	18.03	<0.001	0.019
No	724	145	20.03		739	49	6.63		<0.001

(Continued)

**Table 1.** (Continued.)

Characteristic	Oral cancer (N = 861)				Controls (N = 861)				Oral cancer vs. controls P-value
	Prevalence of <i>T. gondii</i> infection				Prevalence of <i>T. gondii</i> infection				
	No. tested	No. positive	%	P-value	No. tested	No. positive	%	P-value	
Consumption of raw/undercooked meat									
Yes	185	45	24.32	0.332	90	14	15.56	0.008	0.097
No	676	142	21.01		771	57	7.39		<0.001
Consumption of oyster									
Raw	138	55	39.86	<0.001	159	12	7.55	0.723	<0.001
Boiled	723	132	18.26		702	59	8.40		<0.001
Gardening or agricultural activities									
Yes	200	44	22.00	0.912	210	13	6.19	0.213	<0.001
No	661	143	21.63		651	58	8.91		<0.001
Exposure with soil apart from gardening or agricultural activities									
Yes	272	59	21.69	0.989	194	23	11.86	0.038	0.006
No	589	128	21.73		667	48	7.20		<0.001
Washing hands before meals									
Yes	448	99	22.10	0.779	517	48	9.28	0.175	<0.001
No	413	88	21.31		344	23	6.69		<0.001
Total	861	187	21.72		861	71	8.25		<0.001

**Table 2.** Multiple logistic analysis of selected characteristics of oral cancer patients and their association with *T. gondii* infection

Characteristic <sup>a</sup>	Adjusted OR <sup>b</sup>	95% CI	P-value
Area of residence	1.22	0.84–1.71	0.240
Blood transfusion history	1.66	1.18–2.32	0.003
Differentiation degree (middle vs. high)	0.97	0.60–1.55	0.882
Differentiation degree (low vs. high)	1.03	0.71–1.50	0.872
Keep cats at home	1.70	1.12–2.59	0.013
Consumption of oysters	2.94	1.98–4.37	<0.001

<sup>a</sup>The variables included were those with a  $P < 0.25$  obtained in the univariate analysis.

<sup>b</sup>Adjusted by age.

cancer patients with blood transfusion history had a significantly higher seroprevalence than control subjects (adjusted OR = 1.66, 95% CI: 1.18–2.32,  $P = 0.003$ ). Although the current epidemiological knowledge suggests that *T. gondii* can be transmitted by blood transfusion in oral cancer patients, further studies should be conducted to explore the magnitude of the association between *T. gondii* infection and blood transfusion in oral cancer patients.

With respect to behavioural characteristics, the multivariable logistic analysis showed that keeping cats at home and fresh oyster consumption were associated with *T. gondii* seropositivity. As the definitive hosts for *T. gondii*, cats have a vital role in maintaining *T. gondii* in nature [35]. In China, cats are prevalently kept as pets

because they are very easy to take care of and they provide company thus enriching people lives. Still, little attention has been given to the fact that potentially pollute the environment with *T. gondii* [36]. In this study, we found that keeping cats at home was highly associated with *T. gondii* seropositivity (adjusted OR = 1.70; 95% CI: 1.12–2.59;  $P = 0.013$ ). Moreover, we found that fresh oyster consumption (adjusted OR = 2.94; 95% CI: 1.98–4.37;  $P < 0.001$ ) was another potential risk factor for *T. gondii* infection in oral cancer patients, which was similar to the study from the United States [37]. Previous studies have shown that *T. gondii* oocysts can be washed into the sea through rain-wash and runoff [38, 39], and oocysts may be stored in shellfish such as oysters, clams and mussels [38–43]. Similarly, *T. gondii* oocysts have been found in oysters in China [44], and fresh oyster consumption is becoming ever more popular over recent years. This phenomenon may contribute to the higher *T. gondii* seroprevalence in the oral cancer patients with the habit of fresh oyster consumption. Therefore, knowledge of these risk factors can be of great help in prevention efforts. So, having in mind the public health, it is very important to publicise the knowledge of disease prevention.

## Conclusion

In this study, we reported a serological evidence of an association between *T. gondii* infection and oral cancer patients. Moreover, blood transfusion history, keeping cats at home and consumption of raw oysters were identified as risk factors for *T. gondii* positivity. This information may be used to guide future research and control policies. Beyond that, further studies are necessary to elucidate the role of *T. gondii* in oral cancer patients.

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**Declaration of interest.** The authors declare that they have no competing interests.

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