

RETROSPECTIVE SEROLOGICAL EVIDENCE OF HIGH EXPOSURE OF GLOBALLY RELEVANT ZONOTIC PARASITE *TOXOPLASMA GONDII* IN THE LATVIAN POPULATION

Gunita Dekšne^{1,2,#}, Vija Veisa³, Dace Rezeberga³, Ludmila Viksna⁴,
 and Angelika Krūmiņa^{1,4,5}

¹ Institute of Food Safety, Animal Health and Environment “BIOR”, 3 Leļupes Str., Rīga, LV-1076, LATVIA

² Faculty of Biology, University of Latvia, 1 Jelgavas Str., Rīga, LV-1004, LATVIA

³ Department of Obstetrics and Gynecology, Rīga Stradiņš University, 45 Miera Str., Rīga, LV-1001, LATVIA

⁴ Department of Infectology and Dermatology, Rīga Stradiņš University, 3 Linezera Str., Rīga, LV-1006, LATVIA

⁵ Rīga East Clinical University Hospital, Hipokrāta Str. 2, Rīga, LV-1079, LATVIA

Corresponding author, gunita.deksne@bior.lv

Contributed by Ludmila Viksna

Toxoplasmosis is an important infection caused by the single-celled parasite Toxoplasma gondii, which is a zoonotic parasite causing widespread human and animal diseases, mostly involving the central nervous system. Humans can acquire toxoplasmosis by ingestion of raw or undercooked meat containing T. gondii tissue cysts, ingestion of oocysts shed by infected felids via contaminated food or water, and by vertical transmission to the fetus through the placenta from the mother during pregnancy. The aim of the present study was to determine the seroprevalence of specific anti-T. gondii IgG and IgM antibodies using a large set of clinical diagnostic laboratory data obtained over a 14-year period. In total, 25 069 unique patients were included in the present study. The overall specific anti-T. gondii IgG prevalence were 36.3%, which was significantly ($p < 0.01$) higher than IgM prevalence (2.4%). Mean age for IgG antibody-positive patients was 33.7 ± 12.2 years. A significant positive correlation ($r = 0.99$; $p < 0.01$) was observed between age group and anti-T. gondii IgG antibody prevalence, which ranged from 4.2% to 66.7%. The most prevalent (69.9%; 95% CI 69.2–70.7) comorbidities of patients tested for presence of anti-T. gondii IgG and IgM antibodies were classified as factors affecting health status which includes also monitoring of normal pregnancy.

Key words: toxoplasmosis, large scale, seroprevalence, age, comorbidity, pregnancy.

INTRODUCTION

Infection by the Apicomplexan parasite, *Toxoplasma gondii* causes toxoplasmosis. The parasite infects almost all warm-blooded animals. Serological evidence indicates that it is one of the most common infection of humans throughout the world (Dubey, 2010). The disease is transmitted mainly by ingestion of an infective stage of the parasite, organ transplantation, blood transfusion, and transplacental transmission, which is very common (Tenter *et al.*, 2000). Most infected persons lack clinical manifestation of their infection and will never develop any symptoms. However,

disease severity is highly variable. Severe eye disease resulting in loss of sight is not uncommon, and immunocompromised patients may present with serious infections of the central nervous system. Toxoplasmosis in acquired immune deficiency syndrome (AIDS) patients and other immunocompromised patients may be life threatening. Disease in these individuals can be due to recently acquired infection or more commonly due to reactivation of a latent infection. Toxoplasmosis is one of the opportunistic infections that AIDS patients develop (Sonar and Brahmabhatt, 2010). Toxoplasmosis ranks high on the list of diseases that lead to death in patients with AIDS; approximately 10% of AIDS

patients in the USA and up to 30% in Europe are estimated to have died from toxoplasmosis (Luft and Remington, 1992). Congenital infection occurs only when a woman becomes infected during pregnancy. While the mother rarely has symptoms of infection, she does have a temporary parasitemia. Focal lesions develop in the placenta and the foetus may become infected. At first a generalised infection appears in the foetus. Later, the infection is cleared from the visceral tissues and may localise in the central nervous system. A wide spectrum of clinical diseases occurs in congenitally infected children. Mild disease may consist of slightly diminished vision, whereas severely diseased children may have the full spectrum of symptoms: retino-choroiditis, hydrocephalus, convulsions and intracerebral calcification. Of these, hydrocephalus is the least common, but most dramatic, lesion of toxoplasmosis. The most common sequel of congenital toxoplasmosis is ocular disease (Tenter *et al.*, 2000; Berger *et al.*, 2009; Dubey, 2010).

To our knowledge, there are no accurate, empirical data that have been used to estimate incidence of toxoplasmosis in Latvia. *Toxoplasma gondii* was recently ranked 2nd and 4th among zoonotic parasites evaluated for their relevance as foodborne pathogens in Europe and globally, respectively (Anonymous, 2014; Bouwknecht *et al.*, 2018). Estimates of infection prevalence are limited in scope. According to the Latvian Centre of Disease and Control, two to 16 acute toxoplasmosis cases per year were reported from 2002 to 2014. Starting from 2015 only congenital toxoplasmosis is reported and until August, 2018 zero cases were reported (Anonymous, 2018a). In 2014, 42 confirmed congenital toxoplasmosis cases were reported by 20 EU/EEA countries. The majority of cases in 2014 were reported from Poland (48%) and the United Kingdom (26%) (Anonymous, 2014).

In its acute infection form, toxoplasma spreads through all organs, and the parasite clears from the body in less than one week due to innate immunity and the rise of specific acquired immunity, including humoral immunity. Specific anti-*T. gondii* IgM antibodies are the first antibodies to appear, usually one week after the infection (Robert-Gangneux and Darde, 2012). Specific anti-*T. gondii* IgG antibodies appear after two weeks of infection and peaks at three months. The level then remains at a plateau for six months and after one year starts to slowly decrease to lower levels until the end of infected subject's life due to the persistence of latent cysts in immune privileged organs (Bessieres *et al.*, 1992). Because of this, for seroprevalence studies the prevalence of specific anti-*T. gondii* IgG antibodies is estimated. Seroprevalence estimates for human populations vary greatly among different countries, among different geographical areas within one country, and among different ethnic groups living in the same area. The global prevalence of toxoplasmosis is about 30% of the world population. However, the distribution of human exposure to the parasite varies from 0 to 100% (Tenter *et al.*, 2000; Dubey, 2010). Comparison of seroprevalence data of infections with *T. gondii* requires considering that the different

serological methods used to obtain these data are not standardized (Tenter *et al.*, 2000). For example, in Germany seroprevalence ranges from 20% (95% CI 17–23%) in the 18–29 age group to 77% (95% CI 73–81%) in the 70–79 age group (Wilking *et al.*, 2016). The prevalence of toxoplasma infection in pregnant women ranges from 10% in the United Kingdom and Norway to around 55% in France and Greece (Cook *et al.*, 2000). However, the weighted prevalence of toxoplasmosis in blood donors was determined to be 33% (95% CI, 28%–39%) (Foroutan-Rad *et al.*, 2016). There are previous fragmentary seroprevalence studies in Latvia, mainly in pregnant woman, which do not show toxoplasmosis seroprevalence in Latvian population.

Given the gaps in knowledge of *T. gondii* prevalence in the Latvia, uncertainties and comorbid conditions, the aim of the present study was to determine the seroprevalence of anti-*T. gondii* IgG and IgM antibodies using a large set of diagnostic laboratory data collected over a period of 14 years.

MATERIALS AND METHODS

For the present study data from the largest clinical laboratory in Latvia were used. All unique patients, who were tested for presence of specific anti-*T. gondii* IgG and/or IgM antibodies with appropriate serological screening methods like enzyme-linked immunosorbent assay in the period from 1 January 2004 to 31 December 2017, were included in the study. Use of this data to characterise epidemiology of infectious disease presents a unique opportunity to assess prevalence of infection with minimal cost, while allowing for characterisation of occurrence across time. Additionally, this approach facilitates identification of patterns of infection with respect to patient age, gender, and common comorbidities. The study was approved by the Institute of Food Safety, Animal Health and Environment “BIOR” Ethical committee.

All unique patients were evaluated for the presence of specific anti-*T. gondii* IgG/IgM antibodies at first diagnosis, age, gender and comorbidity were recorded. The patient age was divided into six groups as suggested by the European Centre for Disease Prevention and Control: 0 – younger than one year; 1 – 1–6 years, 2 – 7–14 years, 3 – 15–24 years, 4 – 25–44 years, 5 – 45–64 years, 6 – older than 65 years. Comorbidity diagnoses were divided by disease code into 23 groups (Anonymous, 2018b). The confidence intervals (CI) of the prevalence estimates and the two-tailed *p* values of the two-by-two table comparisons were calculated using the Mid-P Exact in open source software OpenEpi v.2.3.1 (Dean *et al.*, 2015). Spearman's rank-order correlation calculations for *T. gondii* seroprevalence and patients age group were performed with SPSS Statistics Version 21 (IBM Corporation, Chicago, Illinois). *p* < 0.05 was considered statistically significant.

Table 1

PREVALENCE OF ANTI-TOXOPLASMA GONDII IGG AND IGM ANTIBODIES, MIN-MAX AND MEAN AGE OF PATIENTS

	No. of tested	No. of positive	Prevalence, %	95% Confidence Interval	Mean age of patient \pm SD	Min-Max age of patient
Anti- <i>T. gondii</i> IgG antibodies	20 428	7 411	36.3	35.6–37.0	33.7 \pm 12.2	0–86
Anti- <i>T. gondii</i> IgM antibodies	19 349	470	2.4	2.2–2.7	30.5 \pm 11.1	1–72

RESULTS

In total, 25 069 unique patients were included in the present study. From those, 18.5% (n = 4641) were tested for presence of anti-*T. gondii* IgM antibodies, 22.8% (n = 5720) were tested for presence of anti-*T. gondii* IgG antibodies and 58.7% (n = 14 708) were tested for presence of anti-*T. gondii* IgG and IgM antibodies. Patients ranged in age from 0 to 86 years (mean = 30.3, median = 30.0), and 82.1% (n = 20 586) were females and 17.9% (n = 4483) were males. The overall anti-*T. gondii* IgG prevalence (36.3%) and was significantly ($p < 0.01$) higher than IgM prevalence (2.4%) (Table 1).

During the studied time period, a slight decrease of anti-*T. gondii* IgG prevalence was observed, but it remained stable (Table 2). However, there was a significant decrease ($p < 0.01$) of specific anti-*T. gondii* IgM prevalence during the study period (Table 2). A significant difference were observed in seroprevalence by sex; females had 1.149 (95% CI 1.061–1.244) times higher risk of being specific anti-*T. gondii* IgG positive than males, while there was no significant difference of specific anti-*T. gondii* IgM prevalence by sex (Table 3).

In the analysis of seroprevalence by age groups, the age group under one year was excluded from further analyses as positive IgG antibody findings (23.3%; 95% CI 19.6–27.4) in this group were assumed to be maternal antibodies. None of the samples were positive for IgM antibodies in the age group under age of one year. A significant positive correlation ($r = 0.99$; $p < 0.01$) was observed between age group and anti-*T. gondii* IgG antibody prevalence, which ranged from 4.2% (95% CI 2.9–6.0) in patients from one to six years to 66.7% (95% CI 61.4–71.6) in patients older than 65 years (Fig. 1A). There were no significant difference of anti-*T. gondii* IgM antibody prevalence by age group, however results shows that higher risk for first toxoplasmosis infection is until age of 24 years (Fig. 1B).

There was no significant difference of seroprevalence of anti-*T. gondii* IgG antibodies by sex in age groups, except in the fourth age group where females were observed to have significantly higher seroprevalence and 1.4 (95% CI 1.2–1.5) times higher risk of testing specific anti-*T. gondii* IgG positive than males (Table 4).

Comorbidities to patients tested for presence of anti-*T. gondii* IgG and/or IgM antibodies in 69.9% (95% CI 69.2–70.7) of cases were classified as factors affecting health status or suggesting past contact with health services, which includes also monitoring of normal pregnancy. Another 4.6 (95% CI

Table 2

PREVALENCE OF SPECIFIC ANTI-TOXOPLASMA GONDII ANTIBODIES IN THE STUDY PERIOD FROM 2004 TO 2017 IN THE LATVIAN POPULATION

Year	Anti- <i>T. gondii</i> IgG antibodies			Anti- <i>T. gondii</i> IgM antibodies		
	No. of tested	Prevalence	95% CI	No. of tested	Prevalence	95% CI
2004	729	38.6	35.1–42.1	613	8.8	6.8–11.3
2005	814	40.9	37.6–44.3	719	6.4	4.8–8.4
2006	1191	40.2	37.5–43.0	1040	3.1	2.2–4.3
2007	1347	39.0	36.4–41.6	1297	4.5	3.5–5.7
2008	1667	39.7	37.3–42.0	1698	3.3	2.5–4.3
2009	1574	26.8	24.9–28.7	1744	2.2	1.6–3.1
2010	1249	35.7	33.1–38.4	1404	1.8	1.2–2.6
2011	1441	35.4	33.0–37.9	1353	1.5	0.9–2.3
2012	1680	36.5	34.2–38.8	1483	1.9	1.3–2.7
2013	1565	34.9	32.6–37.3	1452	1.6	1.0–2.4
2014	1599	34.5	32.2–36.9	1546	1.5	1.0–2.2
2015	1727	34.0	31.8–36.3	1675	1.2	0.8–1.8
2016	1729	32.4	30.2–34.6	1512	1.7	1.1–2.4
2017	2116	35.1	33.1–37.2	1813	1.2	0.7–1.8
Total	20 428	36.3	35.6–37.0	19 349	2.4	2.2–2.7

Table 3

PREVALENCE OF ANTI-TOXOPLASMA GONDII ANTIBODIES BY GENDER IN LATVIA FROM 2004 TO 2017

Gender	Anti- <i>T. gondii</i> IgG antibodies			Anti- <i>T. gondii</i> IgM antibodies		
	No. of tested	Prevalence	95% CI	No. of tested	Prevalence	95% CI
Female	17 235	36.8	36.1–37.5	15 838	2.5	2.2–2.7
Male	3 193	33.6	32.0–35.3	3 511	2.3	1.9–2.9

4.3–5.0) cases were classified as pregnancy, childbirth or the puerperium and 3.4% (95% CI 3.1–3.6) cases were classified as diseases of the genitourinary system. However, cases from other classification ranged from 0.0% to 2.8%.

DISCUSSION

Use of the large database presented an opportunity to assess prevalence of medical diagnosis of toxoplasmosis in the Latvian population, confirmed some trends highlighted in earlier literature, and yielded some unexpected results. Within the present study we found evidence of high exposure (36.3%) to *T. gondii*, which is a zoonotic parasite presenting a threat to human health and life quality, food

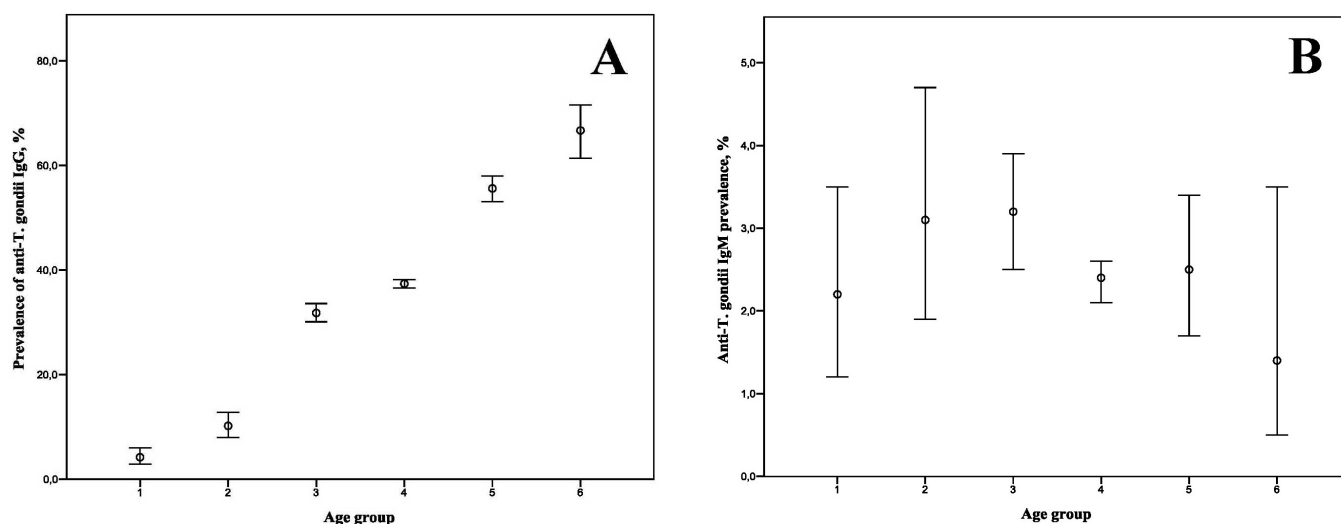


Fig. 1. Prevalence of specific anti-*Toxoplasma gondii* seroprevalence by age groups in Latvia from 2004 to 2017. A, IgG antibodies; B, IgM antibodies. Age groups: 1, 1–6 years, 2, 7–14 years, 3, 15–24 years, 4, 25–44 years, 5, 45–64 years, 6, older than 65 years.

Table 4

PREVALENCE OF FIRST DIAGNOSIS OF ANTI-*TOXOPLASMA GONDII* IGG ANTIBODIES BY AGE AND GENDER AND ODDS RATIO ESTIMATES IN LATVIA FROM 2004 TO 2017

Age group	Anti- <i>T. gondii</i> IgG prevalence in females				Anti- <i>T. gondii</i> IgG prevalence in males				Odds ratio estimates	
	No of tested	No of positive	%	95% CI	No of tested	No of positive	%	95% CI	<i>p</i>	OR (95% CI)
1	353	13	3.7	2.1–6.3	461	16	3.5	2.1–5.6	0.02*	1.1 (0.5–2.2)
2	358	35	9.8	7.1–13.3	368	28	7.6	5.3–10.8	0.15	1.3 (0.8–2.2)
3	2 827	756	26.7	25.1–28.4	509	126	24.8	21.2–28.7	0.17	1.1 (0.1–1.4)
4	15 072	4690	31.1	30.4–31.7	2124	531	25.0	23.2–26.9	.01*	1.4 (1.2–1.5)
5	1 411	642	45.5	43.0–48.1	573	238	41.5	37.6–45.6	0.05	1.2 (1.0–1.4)
6	266	147	55.3	49.3–61.1	128	73	57.0	48.4–65.3	0.3	0.9 (0.6–1.4)

Age groups: 1, 1–6 years, 2, 7–14 years, 3, 15–24 years, 4, 25–44 years, 5, 45–64 years, 6, older than 65 years.

safety, the economy and the environment (Bouwknegt *et al.*, 2018; Anonymous, 2014; Torgerson, 2013).

The observed seroprevalence in the present study was comparable to that observed in the Netherlands (40.5%), Italy (42%), Germany (49.1%), Estonia (55.8%), and Belgium (up to 67%) (Kortbeek *et al.*, 2004; Pappas *et al.*, 2009; Lassen *et al.* 2016; Wilking *et al.* 2016). During the last decades, *T. gondii* seroprevalence in general has decreased (Kortbeek *et al.*, 2004; Nowakowska *et al.*, 2006; Dubey 2010). This tendency was observed in a study from the Netherlands where overall seroprevalence decreased from 40.5% in 1995/1996 to 26.0% in 2006/2007 and France, where seroprevalence decreased from 64.5% in 1997 to 54.7% in 2013 (Hofhuis *et al.*, 2011; Guigue *et al.*, 2018). However, there was no temporal trend of anti-*T. gondii* IgG prevalence in the present study and it remained stable ranging from 32.4% to 40.9%, while there was a significant decrease of anti-*T. gondii* IgM prevalence from 8.8% to 1.1% during the study period from 2004 to 2017. The decreasing trend might be related to significant improvement in social economic condition, which includes consuming filtered water, good quality meat and improvement in hygienic condition in general (Tenter *et al.*, 2000).

Toxoplasma gondii seroprevalence typically increases with age, indicating acquired infection. In the present study the observed specific anti-*T. gondii* IgG seroprevalence increased from 4.2% in children from one to six years and reached 66.7% in patients under 65 years. Some studies suggest that the main risk factors for acquired toxoplasmosis among humans in different age groups and regions are eating undercooked lamb, beef or game, consumption of unwashed vegetables, contact with soil and travel outside Europe (Cook *et al.*, 2000; Ertug *et al.*, 2005; Robert-Gangneux and Dardé, 2012; Wilking *et al.*, 2016). There are controversial results on close contact with cats as a risk factor, as some show that close contact with cats has no risk while others shows that it is positively associated with seropositivity (Cook *et al.*, 2000; Wilking *et al.*, 2016). The high *T. gondii* seroprevalence in children (37.5%, age 14–18 years) suggests that the infection pressure is substantial (Lassen *et al.* 2016). In Russia, the *T. gondii* seroprevalence observed in children from six to 12 years of age was 13.4% which is close to that observed in present study (10.2%) (Janse *et al.*, 2014).

A significant interaction was noted between age and gender ($p = 0.023$) in a representative, cross-sectional, serological

study in Germany, since higher seroprevalence was observed among younger males and older females. Although not associated in univariable analysis, multivariate analysis showed that men have a 1.76-times higher chance of being seropositive (Wilking *et al.*, 2016). In a similar study in United States, more females than males were identified with toxoplasmosis, respectively, 59% vs 41% of the total number of cases. The number of male to female cases was most similar at early age, became increasingly different with age, toward increased incidence in females (Lykins *et al.*, 2016). These results correspond to those observed in the present study where no difference in seroprevalence by age and gender was observed, except at age from 25 to 44 years when females had significantly higher seroprevalence than in males. This might be explained by screening of women of reproductive age, or could potentially indicate a difference in risk of exposure to the pathogen (Lykins *et al.*, 2016). Indeed, the present study identified almost 70% of patients as cases classified as factors influencing health status or suggesting past contact with health services, which includes also monitoring of normal pregnancy. The mean age of *T. gondii*-infected patients was 33.7 years. The mean age identifies the age when humans become infected for the first time. The high seroprevalence observed in males can be explained by use of twice as much meat and meat products in the diet than for females (Bremer *et al.*, 2005). The seroprevalence observed in females can be explained by reproduction and by socio-economic status, and cat holding (Lykins *et al.*, 2016, Wilking *et al.*, 2016). High *T. gondii* seroprevalence in domestic cats in Latvia indicates that the environment has been contaminated with oocysts, which is supported by results on *T. gondii* prevalence in other wild and domestic animals in Latvia (Deksne and Kirjušina, 2013; Deksne *et al.*, 2013; Deksne *et al.*, 2017).

The present study showed that 2.4% of all tested patients had acute acquired infection of *T. gondii*. However, the present study does not provide information on whether these patients were symptomatic or asymptomatic. In previous studies, a part of the patient population (9–27%) has been found to exhibit a persistent IgM antibody response that remains for two years or more (Gras *et al.*, 2004).

Prevalence of comorbidities associated with toxoplasmosis was also assessed; offering insight into how commonly individuals with this infection suffer from other conditions. The present study shows that factors influencing health status or past contact with health services which includes also monitoring of normal pregnancy, pregnancy, childbirth or the puerperium and diseases of the genitourinary system, were the most prevalent disease classifications. However, the present study do not provide data to understand these relationships directionality and causality with toxoplasmosis. Previous studies showed that, compared to controls matched for age, geography, and health, patients with classifications for toxoplasmosis had greater odds of suffering from conditions including HIV, benign and malignant brain neoplasm, epilepsy, autoimmune diseases including lupus and multiple sclerosis, and psychiatric conditions including substance

abuse, anxiety, bipolar disorder, and schizophrenia (Lykins *et al.*, 2016).

These results provide baseline data showing high and stable *T. gondii* seroprevalence in the Latvian population. This calls for greater awareness of zoonotic parasite infection in Latvia. Antibodies against *T. gondii* appear to be formed already in childhood, indicating considerable infection pressure. In further studies to evaluate toxoplasmosis risk factors and their influence on prevalence in different age groups, gender and other significant factors, a questionnaire together with blood sample testing should be incorporated to obtain more precise information about patient lifestyles and comorbid diseases.

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RETROSPEKTĪVI SEROLOĢISKIE PIERĀDĪJUMI PAR PASAULĒ NOZĪMĪGA ZOOTISKĀ PARAZĪTA *TOXOPLASMA GONDII* BIEŽO SASTOPAMĪBU LATVIJAS IEDZĪVOTĀJU POPULĀCIJĀ

Toksoplazmoze ir nozīmīga infekcija, ko ierosina vienšūnas parazīts *Toxoplasma gondii*, zoonotisks parazīts, kas ierosina plaša spektra cilvēku un dzīvnieku saslimšanas, galvenokārt saistītas ar centrālo nervu sistēmu. Cilvēks var iegūt toksoplazmozi, uzņemot neapstrādātu vai nepietiekami apstrādātu gaļu, kas satur *T. gondii* audu cistas, uzņemot oocistus ar kontaminētu pārtiku vai ūdeni, ko izplata invadētie kaķi, un vertikāli pārnēsot auglim caur placentu no mātes grūtniecības laikā. Šī pētījuma mērķis bija novērot specifisko pret *T. gondii* IgG un IgM antivielu seroprevalenci, izmantojot plašu klīniskās diagnostikas laboratorijas datu kopu 14 gadu periodā. Kopumā šajā pētījumā tika iekļauti 25 069 pacienti. Kopējā specifisko pret-*T. gondii* IgG antivielu prevalence tika novērota 36,3% un bija būtiski ($p < 0,01$) augstāka nekā IgM antivielu prevalence, kas bija 2,4%. IgG antivielu pozitīvo pacientu vidējais vecums bija $33,7 \pm 12,2$ gadi. Būtiska pozitīva korelācija ($r = 0,99$; $p < 0,01$) tika novērota starp pacientu vecuma grupām un pret-*T. gondii* IgG antivielu prevalenci, un tā svārstījās attiecīgi no 4,2% līdz 66,7%. Visbiežākās (69,9%; 95% CI 69,2–70,7) blakus diagnozes pacientiem, kam tika veikti izmeklējumi, lai noteiktu pret-*T. gondii* IgG un IgM antivielu klātbūtni, tika klasificētas kā faktori, kas ietekmē veselības stāvokli, tostarp arī normālas grūtniecības uzraudzību.