# Retrospective Evaluation of *Toxoplasma gondii* Test Results in Patients Applying to A Research Hospital

Bir Araştırma Hastanesine Başvuran Hastalarda Toxoplasma gondii Test Sonuçlarının Retrospektif Olarak Değerlendirilmesi

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## **ABSTRACT**

**Objective:** This study retrospectively evaluates our laboratory *Toxoplasma gondii* immunoglobulin (Ig)M, IgG antibody and avidity test results to determine the distribution of the pathogen according to sex, age, clinics and years.

**Methods:** The serum samples sent to Pamukkale University Healthcare Research and Practice Hospital's Medical Microbiology laboratory between January 2016 and December 2023 were evaluated for *T. gondii* seropositivity. *Toxoplasma* antibodies and avidity testing were studied using chemiluminescent microparticle immunological testing method (Abbott Architect i2000SR, Weisbaden, Germany).

**Results:** The study evaluated 42,817 tests ordered from patients with suspected toxoplasmosis. Five hundred-three (2.1%) were found to be anti-*T. gondii*-IgM positive and 4.406 (24.7%) to be anti-*T. gondii*-IgG positive. Anti-*T. gondii*-IgM and anti-*T. gondii*-IgM positivity were significantly higher in women than in men (p=0.0001; p=0.0001, respectively). Anti-*T. gondii*-IgM positivity was found to be high between the ages of 19 and 49 (p=0.0001). The anti-*T. gondii*-IgG positivity rate increased with age (p=0.0001). Anti-*T. gondii*-IgM and anti-*T. gondii*-IgG positivity rates have decreased significantly over the last four years (2020-2023) compared to the first four years (2016-2019) (p=0.0001; p=0.0001, respectively). When the results of 582 patients with *T. gondii*-avidity requests were evaluated, 38.1% of the anti-*T. gondii*-IgM positive patients and 7.8% of the anti-*T. gondii*-IgM negative patients had low avidity. **Conclusion:** *T. gondii* seroprevalence was found to be similar to the global average, with female sex, age and nationality among the risk factors. It has also been observed that seroprevalence has significantly decreased in recent years.

**Keywords:** *Toxoplasma gondii*, seroprevalence, age, change over years

# ÖZ

**Amaç:** Çalışmamızda laboratuvarımız *Toxoplasma gondii* immünoglobulin (Ig)M, IgG antikor ve IgG avidite test sonuçlarını retrospektif olarak değerlendirerek patojenin cinsiyete, yaşa, kliniklere ve yıllara göre dağılımının belirlenmesi amaçlanmıştır. **Yöntemler:** Pamukkale Üniversitesi Sağlık Araştırma Uygulama Hastanesi Tıbbi Mikrobiyoloji Laboratuvarı'na Ocak 2016-Aralık

**Yöntemler:** Pamukkale Universitesi Sağlık Araştırma Uygulama Hastanesi Tıbbi Mikrobiyoloji Laboratuvarı'na Ocak 2016-Aralık 2023 tarihleri arasında gönderilen serum örneklerinde *T. gondii* seropozitifliği değerlendirildi. Toxoplazma antikorları ve avidite testi kemolüminasan mikropartikül immünolojik test yöntemi (Abbott Architect i2000SR, Weisbaden, Germany) ile çalışıldı.

**Bulgular:** Çalışmada, toksoplazmozis şüphesi olan hastalardan istenen 42.817 test sonucu değerlendirilmiştir. Beş yüz üçünde (%2,1) anti-*T. gondii*-IgM ve 4406'sında (%24,7) anti-*T. gondii*-IgG pozitif olarak saptanmıştır. Anti-*T. gondii*-IgM ve anti-*T. gondii*-IgM pozitifliği kadınlarda erkeklere göre anlamlı şekilde yüksek bulunmuştur (sırasıyla p=0,0001; p=0,0001). Anti-*T. gondii*-IgM pozitifliğinin 19-49 yaş aralığında yüksek olduğu görülmüştür (p=0,0001). Anti-*T. gondii*-IgG pozitiflik oranı ise yaş ilerledikçe artmıştır (p=0,0001). Anti-*T. gondii*-IgM ve anti-*T. gondii*-IgG pozitiflik oranı son dört yılda (2020-2023) ilk dört yıla (2016-2019) göre anlamlı şekilde azalmıştır (sırasıyla p=0,0001; p=0,0001). IgG avidite istemi olan 582 hastanın sonuçları değerlendirildiğinde; anti-Toxo IgM pozitif hastaların %38,1'inin ve anti-Toxo IgM negatif hastaların %7,8'inin düşük aviditeye sahip olduğu görüldü. **Sonuc:** *T. gondii* seroprevalansının küresel ortalama ile benzerlik gösterdiği, kadın cinsiyet, yasın ve uyruğun risk faktörleri

**Sonuç:** *T. gondii* seroprevalansının küresel ortalama ile benzerlik gösterdiği, kadın cinsiyet, yaşın ve uyruğun risk faktörleri arasında yer aldığı bulunmuştur. Aynı zamanda son yıllarda seroprevalansın anlamlı şekilde azaldığı görülmüştür.

Anahtar Kelimeler: Toxoplasma gondii, seroprevalans, yaş, yıllar arası değişim



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## INTRODUCTION

Toxoplasmosis caused by *Toxoplasma gondii* (*T. gondii*) is a common infection worldwide (1). *T. gondii* is an obligate intracellular protozoan parasite in the Apicomplexa family. Cats are the definitive hosts and they can spread a large number of oocysts containing two sporocysts with four sporozoites each. Alongside humans, intermediate hosts consist of many other warm-blooded animals, including birds, rodents, pigs, chickens, goats, sheep (2). Transmission to intermediate hosts can occur by consuming fruit, vegetables and water contaminated with sporulated oocysts, eating raw or uncooked meats containing bradyzoite forms of the parasite, congenitally from mother to baby via the placenta, blood transfusion and transplantation of organs containing cysts or tachyzoites (3).

Most cases of toxoplasmosis are asymptomatic or have mild symptoms. Infection can result in congenital toxoplasmosis and sometimes death of the fetus. The most common clinical manifestations in congenital toxoplasmosis are hydrocephalus, cerebral calcification and chorioretinitis. In some cases, it can be a cause of chorioretinitis in patients with sufficient immunity. In patients with suppressed immune systems, encephalitis may occur because of activation of latent infection (3-5).

There are differences in *T. gondii* seroprevalence rates between countries. This difference is caused by the complex interaction of human and environmental factors (6). This study retrospectively evaluates our laboratory *T. gondii* immunoglobulin (Ig)M, IgG antibody and avidity test results to determine the distribution of the pathogen according to sex, age, nationality, clinics and years.

# **METHODS**

# **Ethically Approval**

This study was approved by the Ethics Board for Non-Invasive Clinical Research at Pamukkale University (date: 06/03/2024, number: E.501178).

# **Study Design**

The results of the samples delivered to Pamukkale University Healthcare Research and Practice Hospital's Medical Microbiology Laboratory between January 01, 2016 and December 31, 2023 with *T. gondii* IgM, IgG antibody and avidity requests were evaluated retrospectively. In case patients had recurrent results, only the first results were included in the study. Toxoplasma antibodies and avidity testing were studied with chemiluminescent microparticle immunological testing method (Abbott Architect i2000SR, Weisbaden, Germany). Index values of toxoplasmosis

IgM antibodies were reported to be <0.50 non-reactive,  $\leq$ 0.50-<0.60 gray zone,  $\geq$ 0.60 reactive; and Toxo IgG antibodies were reported to be <1.6 IU/mL non-reactive, 1.6-<3 IU/mL gray zone, and  $\geq$ 3 IU/mL reactive. The studied avidity results were evaluated according to clinical request. Those with an avidity index value of 50% were considered low avidity (infection within the last four months) 50% to <60% gray zone, and  $\geq$ 60% were considered high avidity (infection acquired at least four months ago). The results of the patients were grouped according to the age groups 0-18, 19-49, >49 years, the clinics where they were referred, and years. In addition, the first four-year and last four-year periods were compared.

# **Statistical Analysis**

All statistical analyses were performed using SPSS 25.0 (IBM SPSS Statistics 25 software (Armonk, NY: IBM Corp.). Categorical variables were defined by number and percent. Chi-square test was used for categorical variables comparisons. Bonferroni adjustment method was used for the examinations of multiple comparisons (clinics). Statistical significance was determined as p < 0.05.

#### **RESULTS**

In the study, there were 42,817 test requests from patients with suspected toxoplasmosis. 24,395 were evaluated for anti-T. gondii-IgM, 17,840 for anti-T. gondii-IgG and 582 for T. gondii-avidity. The number of samples evaluated was 18,656 (43.6%) between 2016 and 2019 and 24,161 (56.4%) between 2020 and 2023. Of the samples, 26,717 (62.4%) belonged to female patients, 16,100 (37.6%) to male patients and 42,270 (98.7%) to Turkish nationals and 547 (1.3%) to foreign patients. There were 15,748 (36.8%) samples aged 0-18, 18,518 (43.2%) samples aged 19-49 and 8,551 (20.0%) samples aged 50 and older. Anti-T. gondii-IgM and anti-T. gondii-IgG were detected positive in 503 (2.1%) and 4406 (24.7%) samples, respectively.

Anti-*T. gondii*-IgM and anti-*T. gondii*-IgG positivity were higher in women (2.6%; 26.3%, respectively) than in men (1.1%; 22.4%, respectively) (p=0.0001; p=0.0001, respectively). The anti-*T. gondii*-IgM positivity rate was higher in the 19-49 age (3.3%) range than in other age groups (1.1%, 1.1%) (p=0.0001). The anti-*T. gondii*-IgG positivity rate increased with age (p=0.0001). Anti-*T. gondii*-IgM and anti-*T. gondii*-IgG positivity rates were found to have decreased significantly over the last four years (2020-2023) (1.8%; 23.1%, respectively) compared to the first four years (2016-2019) (2.4%; 26.9%, respectively) (p=0.0001; p=0.0001, respectively) (Table 1).

		IgM			IgG			
		Positive	Negative	p	Positive	Negative	p	
Sex	Female	403 (2.6%)	15,218 (97.4%)	0.0001*	2,797 (26.3%)	7,858 (73.7%)	0.0001* (cs=34.321)	
	Male	100 (1.1%)	8,674 (98.9%)	(cs=57.702)	1,609 (22.4%)	5,576 (77.6%)		
Age	0-18	85 (1%)	8,562 (99%)		538 (7.6%)	6,524 (92.4%)	0.0001* (cs=2513.024)	
	19-49	364 (3.3%)	10,609 (96.7%)	0.0001* (cs=155.969)	2,018 (28.2%)	5,141 (71.8%)		
	50 and above	54 (1.1%)	4721 (98.9%)	(03=133.303)	1,850 (51.1%)	1,769 (48.9%)		
Years	2016-2019	266 (2.4%)	10,592 (97.6%)	0.0001*	2,026 (26.9%)	5,493 (73.1%)	0.0001*	
	2020-2023	237 (1.8%)	13,300 (98.2%)	(cs=14.58)	2,380 (23.1%)	7,941 (76.9%)	(cs=35.308)	

Anti-T. gondii-IgM and anti-T. gondii-IgG positivity did not differ by sex in patients aged 0-18 (female 1.1%; 8.1%, male 0.9; 7.2; respectively) (p=0.464; p=0.142, respectively). Although statistically significant differences were not observed, women's positivity (3.5%) was higher than men's in clinical terms. Anti-T. gondii-IgM positivity was found to be higher in women (3.5%) than in men (2.2%) in the 19-49 age group, whereas anti-T. gondii-IgG positivity did not differ by gender (female and male; 28.2%) (p=0.003; p=0.992, respectively). In patients aged 50 years and older, anti-T. gondii-IgM and anti-T. gondii-IgG positivity rates were higher in women (1.5%; 53.9, respectively) (male; 0.8%, 48.3% respectively) (p=0.689; p=0.067, respectively) (Table 2).

No significant differences were found in anti-T. gondii-IgM and anti-T. gondii-IgG positivity rates for age groups between the first four years (2016-2019) and the last four years (2020-2023) (p>0.05). However, despite the absence of significant difference, the anti-T. gondii-IgM (1.1%; 0.9%, respectively) and anti-T. gondii-IgG (8.4%; 7.2%, respectively) positivity rates in the first four years between the ages of 0-18 were higher than the positivity rate in the last four years. Similarly, despite the absence of significant difference, the anti-T. gondii-IgM (3.6%; 3%, respectively) and anti-T. gondii-IgG (29.2%; 27.2%, respectively) positivity rates in the first four years between the ages of 19-49 were higher than the positivity rate in the last four years. The anti-T. gondii-IgM positivity rate (1.2%) in the first four years of the age group 50 and older was found to be high relative to the positivity rate (1.1%) in the last four years, and the anti-T. gondii-IgG positivity rate was low (50.7%, 51.1%, respectively) (Table 2).

When the anti-T. gondii-IgM and anti-T. gondii-IgG requests of clinics were evaluated, the highest number of requests according to the frequency were found in pediatric health and diseases with 32.9% (13,885/42,235), followed by internal diseases with 28.4% (11,993/42,235), obstetrics and gynecology with 19.5% (8,241/42,235), infectious diseases with 5.4% (2,266/42,235), organ transplant clinic with 3.9% (1,638/42,235) and eye diseases with 2.5% (1,052/42,235), respectively. An evaluation of the anti-T. gondii-IgM results showed that the clinics with the highest rate of positivity were infectious diseases (18.6%) and psychiatry (3.6%) clinics, respectively. Anti-T. gondii-IgG positivity was highest in cardiology (54.1%) chest disease (52.6%) and adult intensive care (45.4%) samples, respectively (Figure 1). In samples sent from internal medicine clinic and adult intensive care, no statistically significant difference was found between anti-T. gondii-IgM and anti-T. gondii-IgG positivity rates (p=0.028; p=0.352, respectively). When compared anti-T. gondii-IgM positivity rates assessed in the pediatric health and diseases clinic and in the child intensive care departments, there was no statistically significant difference (p>0.05). Anti-T. gondii-IgG positivity rates were higher in samples sent from the pediatric health and diseases clinic than in samples sent from the child intensive care department (p=0.082; p=0.0001, respectively).

Anti-T. gondii-IgM positivity rates were evaluated according to years, a statistically significant difference was found. There is a significant difference between the years; 2016-2017 (p<0.0001) 2016-2018 (p<0.0001), 2016-2019 (p<0.0001), 2016-2020 (p=0.0005), 2016-2021 (p<0.0001), 2016-2022 (p<0.0001), 2016-2023 (p<0.0001), 2017-2022 (p<0.0001), 2018-2020 (p<0.0001), 2019-2020 (p<0.0001), 2020-2022 (p<0.0001), 2020-2023 (p<0.0001). Anti-T. gondii-IgG positivity rates were evaluated according to years, a statistically significant difference was found. There is a significant difference between the years; 2016-2017 (p<0.0001), 2016-2018 (p<0.0001), 2016-2020 (p=0.0001), 2017-2021 (p<0.0001), 2017-2022 (p<0.0001), 2017-2023 (p<0.0001), 2018-2021 (p<0.0001), 2018-2022

			IgM			IgG		
			Negative	Positive	p	Negative	Positive	p
0-18	Sex	Female	4091 (98.9%)	44 (1.1%)	0,464 (cs=0.535)	3,084 (91.9%)	272 (8.1%)	0,142 (cs=2.152)
		Male	4,471 (99.1%)	41 (0.9%)		3,440 (92.8%)	266 (7.2%)	
	Total		8,562 (99%)	85 (1%)		6,524 (92.4%)	538 (7.6%)	
19-49	Sex	Female	8,807 (96.5%)	324 (3.5%)	0,003* (cs=9.059)	3938 (71.8%)	1546 (28.2%)	0,992 (cs=0)
		Male	1,802 (97.8%)	40 (2.2%)		1203 (71.8%)	472 (28.2%)	
	Total		10,609 (96.7%)	364 (3.3%)		5,141 (71.8%)	2,018 (28.2%)	
	Sex	Female	2,320 (98.5%)	35 (1.5%)	0,022*	836 (46.1%)	979 (53.9%)	0,001* (cs=11.59)
50 and above		Male	2,401 (99.2%)	19 (0.8%)	(cs=5.247)	933 (51.7%)	871 (48.3%)	
	Total		4721 (98.9%)	54 (1.1%)		1,769 (48.9%)	1,850 (51.1%)	
	Years	2016-2019	3048 (98.9%)	33 (1.1%)	0,537 (cs=0.382)	2183 (91.6%)	200 (8.4%)	0.08 (cs=3.066)
0-18		2020-2023	5514 (99.1%)	52 (0.9%)		4341 (92.8%)	338 (7.2%)	
	Total		8,562 (99%)	85 (1%)		6,524 (92.4%)	538 (7.6%)	
	**	2016-2019	5,626 (96.4%)	210 (3.6%)	0.08 (cs=3.072)	2,558 (70.8%)	1,053 (29.2%)	0,065 (cs=3.405)
19-49	Years	2020-2023	4,983 (97%)	154 (3%)		2,583 (72.8%)	965 (27.2%)	
	Total		10,609 (96.7%)	364 (3.3%)		5,141 (71.8%)	2,018 (28.2%)	
50 and above	77	2016-2019	1,918 (98.8%)	23 (1.2%)	0.77	752 (49.3%)	773 (50.7%)	0,658 (cs=0.196)
	Years	2020-2023	2803 (98.9%)	31 (1.1%)	(cs=0.086)	1,017 (48.6%)	1,077 (51.4%)	
	Total		4721 (98.9%)	54 (1.1%)		1,769 (48.9%)	1,850 (51.1%)	

When the results of 582 patients with *T. gondii*-avidity requests were evaluated, 38.1% of the anti-*T. gondii*-IgM positive patients and 7.8% of the anti-*T. gondii*-IgM negative patients had low avidity. Of the 124 patients with low avidity, 105 (84.7%) were female patients, of whom 90 (85.7%) were in the fertility age group. It was found that 44.4% of anti-*T. gondii*-IgM positive patients and 87.3% of anti-Toxo IgM-negative patients had high avidity (Table 3).

## **DISCUSSION**

*T. gondii* causes toxoplasmosis and is found all over the world. It can remain in the body for life in humans and animals. Most people with a healthy immune system have no symptoms. Toxoplasmosis is usually diagnosed by the detection of Toxoplasma-specific IgG, IgM, IgA or IgE antibodies. These antibodies are detected by dye test, Indirect fluorescent antibody test, enzyme immunoassays (immunoblots), Avidity test and Agglutination test (1).

This study examined *T. gondi* prevalence and the changes in prevalence by sex, age, clinics and over years. Anti-*T. gondii*-IgG and anti-*T. gondii*-IgM positivity rates were 24.7% and 2.1%, respectively. The global *T. gondii* seroprevalence ranges widely

from 0.5% to 87.7%, and the average seroprevalence is 25.7% (6). Seroprevalence is influenced by geographical location and there are differences in the prevalence rates between continents. Africa (61.4%) and Oceania (38.5%) have high prevalence, while Asia (16.4%) presents low prevalence (6). The overall seroprevalence of the European continent is estimated at 32.1% (7). The seroprevalence of *T. gondii* in neighboring countries of Türkiye with similar geography has been reported as 27% in Bulgaria, 2.3% for anti-*T. gondii*-IgM and 31.1% for anti-*T. gondii*-IgG in Iran, and 37.6% in the city of Basra in Iraq (8-10). The prevalence in Türkiye ranges from 17.5% to 69.5% (4). Türkiye is located in Europe and Asia, and our study was conducted in a province of the Aegean region in the western part of Türkiye. The prevalence of *T. gondii* in our study is similar to the global average.

In our study, *T. gondii* IgM and IgG positivity was higher in female sex (female, 2.6%; 26.3%; male, 1.1%; 22.4%, respectively). Some studies considered male sex to be an independent risk factor (10,11). The high prevalence of antibody positivity in the female sex in our study may have been mostly due to women preparing meals and more encounters with raw meat and raw vegetables (12).

It has been suggested that 1.1% of adults and 1.3% of women between the ages of 18 and 49 experience seroconversion each year (11). A recent study in Iraq reported that the IgM positivity was higher between the ages of 15 and 44 than other ages (10). In our study, the IgM positivity was higher in the 19-49 age interval than in other age groups. Especially in female patients,

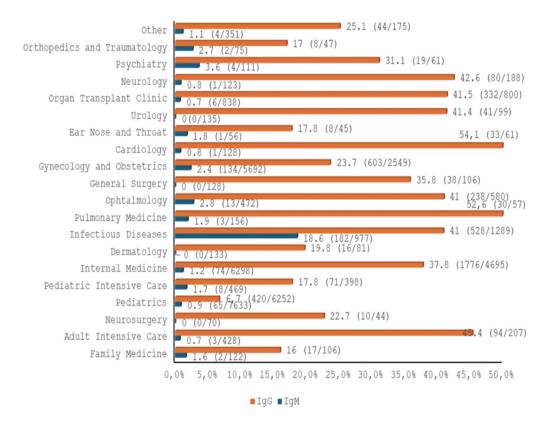
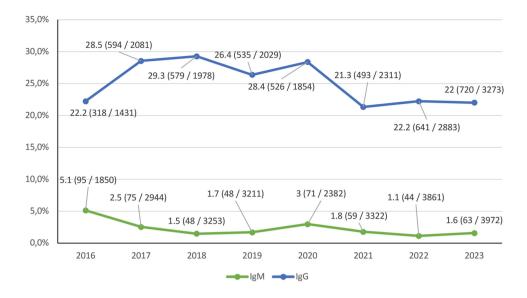


Figure 1. Anti-T. gondii-IgM and anti-T. gondii-IgG positivity rates in patient samples sent from different clinics % (positive/total)

Ig: Immunoglobulin, Other (Emergency Service, Anesthesia, Physical Therapy and Rehabilitation, Cardiovascular Surgery, Chest Surgery, Plastic Surgery, Pediatric Surgery, Nuclear Medicine, Genetics, Pharmacology, Pathology outpatient clinics), Adult Intensive Care (Anesthesia, Brain Surgery, Internal Diseases, Cardiovascular Surgery, Chest Diseases, Cardiology, Neurology), Pediatric Intensive Care (Newborn, Pediatric Surgery, Child)



**Figure 2.** Anti-*T. gondii*-IgM and anti-*T. gondii*-IgG positivity rates per year % (positive/total) *Ig: Immunoglobulin* 

Table 3. T. gondii-avidity results n (%)							
T gondii eviditu	IgM	IgM					
T.gondii-avidity	Positive	Interpolation	Negative				
Low	96 (38.1)	4 (19.1)	24 (7.8)				
Gray zone	44 (17.5)	2 (9.5)	15 (4.9)				
High	112 (44.4)	15 (71.4)	270 (87.3)				
Total	252 (100)	21 (100)	309 (100)				
Ig: Immunoglobulin							

IgM positivity at this age interval (fertility age) is very important as it can cause congenital toxoplasmosis in the case of pregnancy. Seroconversion monitoring and avidity testing are recommended in the case of pregnancy (13).

The *T. gondii*-avidity test can predict when the infection has been acquired. A low avidity index indicates a recent infection, while a high avidity index excludes a recent infection (before four months) (14). In our study, avidity test results were analyzed in all IgM-positive or negative patients with an avidity test request. Similarly, the Toxoplasmosis Reference Centre of France, one of the few countries that has long had a mandatory screening program for pregnant women, recommends that all patients with anti-*T. gondii*-IgG positive be screened for avidity (13). In our study, it was determined that 24 (7.8%) of the patients who were negative for anti-*T. gondii*-IgM had low avidity, and thus the primary infections in these patients were not missed. In addition, it was thought that the reason for the high avidity observed in anti-*T. gondii*-IgM positive patients in our study could be the IgM positivity that can persist for months in some patients (14).

In the present study, it was understood that IgG positivity increases with age as reported in some studies (11,15,16). Hypothetically, it may be suggested that increased age and prolonged exposure to infection and risk factors in individuals lead to a higher prevalence (15,16).

It is seen that all clinics of the hospital are requested for diagnosis. In addition, it has been determined that there were more requests from children's health and diseases (32.9%), internal diseases (28.4%), obstetrics and gynecology (19.5%), infectious diseases (5.4%), organ transplant clinic (3.9%) and eye diseases clinic (2.5%) compared to other departments. More requests from these departments by physicians may indicate that the organ involvement of the risk groups for T. gondii infection (congenital toxoplasmosis, pregnancy, immunosuppressive patient groups) and the clinical picture are fully understood. Some studies suggest that routine monitoring of risk groups for this parasite is also valuable in terms of reactivation/reinfection (17).

As a result of the public health practices of countries, changes have occurred in the prevalence of toxoplasmosis over the years. A study in the Netherlands showed no decrease in prevalence in the last 10 years (18). However, a study in Portugal found that the prevalence decreased over the years (16). In our study, the *T. gondii* IgG and IgM test positivity rate was significantly lower in the last four years (2020-2023) than in the first four years (2016-2019) (p=0.0001; p=0.0001, respectively). The main reasons for this decrease in the positivity rate include that, as in the whole world, more importance has been given to the hygiene rules in our country with Coronavirus disease-2019, after which hygiene habits has become permanent.

For an effective fight against toxoplasmosis, it is crucial to evaluate environmental, animal and human health from a holistic perspective (19). Recommended methods for the control of toxoplasmosis include training on the subject, prevention of

contact with contagious materials, food and water sanitation, vaccinations to prevent the spread of oocysts to cats, and the use of immunotherapy and chemotherapy for the treatment of diseases (5).

## **Study Limitations**

The limitations of this study include the fact that the data reflects a single center and risk factors could not be evaluated due to the retrospective nature of the study. But that it covers a long period of eight years and has a high number of samples represent its strengths.

# **CONCLUSION**

In conclusion, *T. gondii* seroprevalence was found to be similar to the global average, with female sex and age among the risk factors. It has also been observed that seroprevalence has significantly decreased in recent years.

#### \*Ethics

**Ethics Committee Approval:** This study was approved by the Ethics Board for Non-Invasive Clinical Research at Pamukkale University (date: 06/03/2024, number: E.501178).

Informed Consent: Retrospective study.

# \*Footnotes

## **Authorship Contributions**

Concept: S.Z.Ö., A.Ç., M.D., Ç.E., İ.K., Design: Ü.Ç., M.G.Ö., Data Collection or Processing: S.Z.Ö., Ç.E., M.D., E.M., Analysis or Interpretation: S.Z.Ö., M.D., A.Ç., H.Ş., İ.K., Literature Search: S.Z.Ö., A.Ç., M.D., Ç.E., Writing: S.Z.Ö.

**Conflict of Interest**: No conflict of interest was declared by the authors.

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