



Toxoplasmosis in the Autonomous Province of Vojvodina, Serbia: a 29-year surveillance data analysis (1997–2025)

Toksoplazmoza u Autonomnoj pokrajini Vojvodini, Srbija: analiza podataka nadzora tokom 29 godina (1997–2025)

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Abstract

Background/Aim. Toxoplasmosis is a widespread zoonotic infection with significant public health implications, particularly for pregnant women and immunocompromised individuals. The aim of the study was to analyze the epidemiological characteristics and temporal trends of toxoplasmosis in the Autonomous Province (AP) of Vojvodina, Serbia, over 29 years (1997–2025). **Methods.** A descriptive, population-based study was conducted using routine surveillance data on reported toxoplasmosis cases for the 1997–2025 period. Incidence rates were calculated *per* 100,000 inhabitants. Demographic, topographic, and seasonal differences among patients were analyzed. The association between the reported incidence of toxoplasmosis and climatic variables was evaluated using Spearman's correlation coefficient. **Results.** A total of 389 cases of toxoplasmosis were reported during the study period, with no fatal outcomes registered. The annual reported incidence ranged from 0.06 to 1.49 *per* 100,000 inhabitants. Segmented log-linear regression analysis identified two statistically significant trend changes in the reported toxoplasmosis incidence: a significant increase during the 2001–2007 period [annual percent change (APC) = 32.1%, 95% confidence interval (CI): 14.0–53.0; $p = 0.005$] and a decrease during the 2015–2019 period (APC = -35.1%,

95% CI: -49.8–-16.2; $p = 0.013$). The highest proportion of cases was observed in the 15–29 years (34.19%) age group, followed by 0–14 years (23.14%) and 30–39 years (21.59%). Females accounted for 60.15% of cases and were significantly older than males (30.56 ± 14.74 vs. 22.88 ± 15.02 years; $p < 0.001$). The majority of cases (66.07%) were recorded in urban areas. No statistically significant difference was found in seasonal distribution, although the highest proportion of cases (38.30%) occurred in the period January–March. A statistically significant negative correlation was found between reported incidence of toxoplasmosis and average temperature ($\rho = -0.5111$, $p = 0.0046$), whereas correlations with precipitation, humidity, and the number of rainy days were not statistically significant ($p > 0.05$). **Conclusion.** The reported incidence of toxoplasmosis in the AP of Vojvodina showed a declining trend over the observed period. Despite the decrease in reported incidence, enhancing surveillance, improving the recognition of congenital toxoplasmosis, and considering standardized prenatal screening could contribute to more effective prevention and early detection of congenital infection.

Keywords: epidemiology; incidence; serbia; toxoplasmosis; zoonoses.

Apstrakt

Uvod/Cilj. Toksoplazmoza je široko rasprostranjena zoonoza od značaja za javno zdravlje, naročito kod trudnica i imunokompromitovanih osoba. Cilj rada bio je da se analiziraju epidemiološke karakteristike i vremenski trendovi toksoplazmoze u Autonomnoj Pokrajini (AP) Vojvodini, Srbija, u periodu od 29 godina (1997–2025). **Metode.** Sprovedena je deskriptivna populaciona studija korišćenjem podataka o prijavljenim slučajevima toksoplazmoze iz rutinskog sistema nadzora za period 1997–2025. Stope incidencije izračunate su na 100 000

stanovnika. Analizirane su demografske, topografske i sezonske razlike među obolelima. Povezanost između utvrđene učestalosti toksoplazmoze i klimatskih varijabli procenjena je primenom Spearman-ovog koeficijenta korelacije. **Rezultati.** Registrovano je ukupno 389 slučajeva toksoplazmoze tokom studije, bez registrovanih smrtnih ishoda. Godišnja utvrđena incidencija kretala se od 0,06 do 1,49 na 100 000 stanovnika. Segmentiranjem log-linearnom regresionom analizom utvrđene su dve statistički značajne promene trenda registrovane incidencije toksoplazmoze: značajan porast u periodu 2001–2007. godine [godišnja procentualna promena (*annual percent*

change – APC) = 32,1%; 95% interval poverenja (confidence interval – CI): 14,0–53,0; $p = 0,005$] i pad u periodu 2015–2019. godine (APC = -35,1%; 95% CI: -49,8–-16,2; $p = 0,013$). Najveći udeo registrovanih slučajeva zabeležen je u grupi životnog doba 15–29 godina (34,19%), zatim 0–14 godina (23,14%) i 30–39 godina (21,59%). Žene su činile 60,15% svih slučajeva i bile su statistički značajno starije od muškaraca ($30,56 \pm 14,74$ naspram $22,88 \pm 15,02$ godina; $p < 0,001$). Većina slučajeva (66,07%) registrovana je u urbanim sredinama. Nije nađena statistički značajna razlika u sezonskoj distribuciji, mada je najveći udeo (38,30%) slučajeva zabeležen u periodu januar–mart. Nađena je statistički značajna negativna povezanost između prijavljene incidencije toksoplazmoze i prosečne temperature ($\rho = -0,5111$; $p = 0,0046$),

dok povezanost sa padavinama, vlažnošću i brojem kišnih dana nije bila statistički značajna ($p > 0,05$). **Zaključak.** Prijavljena incidencija toksoplazmoze u AP Vojvodini pokazala je opadajući trend tokom posmatranog perioda. Uprkos sniženju prijavljene učestalosti, unapređenje nadzora, poboljšanje prepoznavanja kongenitalne toksoplazmoze i razmatranje standardizovanog prenatalnog skrininga mogu doprineti efikasnijoj prevenciji i ranom otkrivanju kongenitalne infekcije.

Cljučne reči:
epidemiologija; incidencija; srbija; toksoplazmoza; zoonoze.

Introduction

Toxoplasma (T.) gondii is a ubiquitous intracellular protozoan parasite with a global distribution, capable of infecting virtually all warm-blooded animals, including humans^{1, 2}. Humans typically acquire infection by ingesting tissue cysts in undercooked meat, consuming food or water contaminated with oocysts shed by felids, or through congenital transmission from mother to fetus^{2–4}. Although infection is often asymptomatic in immunocompetent individuals, toxoplasmosis represents a significant public health concern due to its potential to cause severe disease in immunocompromised persons and adverse pregnancy outcomes, including miscarriage, stillbirth, and congenital anomalies^{1, 5}.

Globally, it is estimated that approximately one-third of the human population has been exposed to *T. gondii*, although seroprevalence varies widely depending on geographic, climatic, socioeconomic, and cultural factors^{3, 6, 7}. Higher prevalence rates have been reported in regions with warm and humid climates, as these conditions favor the survival of oocysts in the environment^{8, 9}. In contrast, lower prevalence is typically observed in high-income countries with improved hygiene standards and food safety practices¹⁰. In recent decades, several studies have suggested a declining trend in seroprevalence in many developed countries, likely reflecting changes in dietary habits, meat production, and public health measures^{7, 11}.

In Europe, toxoplasmosis remains an important zoonotic infection, with considerable heterogeneity in both seroprevalence and incidence across countries^{8, 12}. Cases of congenital toxoplasmosis (CT) across 30 European Union and European Economic Area countries increased slightly in 2021 after the decrease from 2017 to 2020. This was mainly due to the increased number of cases reported by France, which accounted for the majority (78%) of all the reported cases during that period. Surveillance systems across Europe are not uniform, and toxoplasmosis is not mandatorily reported in all countries, which complicates direct comparisons¹². Nevertheless, CT continues to be a major concern, and some countries have implemented systematic prenatal screening programs to reduce disease burden^{5, 13}.

Environmental and climatic factors, including temperature and humidity, as well as food consumption patterns (e.g., raw or undercooked meat), are recognized as key determinants of infection risk in European populations^{8, 9}.

In Serbia, data on toxoplasmosis are relatively limited and primarily derived from seroepidemiological studies^{14–16}. However, comprehensive long-term analyses of incidence trends, age-specific patterns, and environmental determinants of toxoplasmosis in Serbia remain scarce¹⁷.

Similar to other European countries, changes in lifestyle, dietary habits, and improved hygienic conditions have likely contributed to a gradual decline in infection rates over time. However, this trend may increase the proportion of susceptible women of reproductive age, thereby raising the risk of primary infection during pregnancy and, consequently, CT¹⁶. Surveillance of toxoplasmosis has been conducted since 1991. Notably, no cases of CT were officially reported in the Autonomous Province (AP) of Vojvodina, Serbia, during the period 1991–2024¹⁸.

Understanding temporal trends and epidemiological characteristics of toxoplasmosis at the regional level is essential for guiding public health interventions, particularly in settings where systematic surveillance data are available over extended periods.

By providing a comprehensive assessment of toxoplasmosis epidemiology in this region, the study aims to enhance understanding of disease dynamics in Southeast Europe and inform future prevention and control strategies.

Therefore, the aim of this study was to analyze the epidemiological characteristics of toxoplasmosis in the AP of Vojvodina, Serbia, over a 29-year period, including temporal trends, age- and gender-specific distribution, seasonal patterns, topographic variation, gender differences, and associations with selected meteorological variables.

Methods

This descriptive study was conducted in the AP of Vojvodina, Serbia, a northern region of Serbia with approximately 1.7 million inhabitants¹⁹.

Data were retrieved from the regional communicable disease surveillance system, coordinated by the Institute of Public Health of Vojvodina (IPHV), Novi Sad, Serbia.

The study was based on anonymized surveillance data routinely collected for public health purposes. Therefore, approval from an Ethics Committee was not required under Serbian regulations.

Reporting of toxoplasmosis is mandatory in Serbia and is based on standardized notification forms completed by healthcare providers. All toxoplasmosis cases reported between 1 January 1997 and 31 December 2025 were included in the comprehensive analysis. Given that the majority of toxoplasmosis infections are asymptomatic^{1–7}, it is likely that most reported cases were detected through routine screening programs, testing requested by primary care physicians or gynecologists before or during pregnancy, or patient-initiated testing.

Laboratory testing was performed following referral by a primary care physician, gynecologist, or infectious disease specialist, as well as upon the patient's own request. Confirmation was based on serological evidence of acute or recent *T. gondii* infection, characterized by the presence of toxoplasma-specific immunoglobulin (Ig) M antibodies, low-avidity IgG antibodies, or both. All serological testing was performed at the Center for Virology of the IPHV. During the study period, different serological methods were used for the laboratory diagnosis of toxoplasmosis. From 1997 to 2002, diagnosis was based on the complement fixation test (CFT). Between 2002 and 2007, serological testing was performed using the SERION enzyme-linked immunosorbent assays (ELISA) classic *T. gondii* IgG and IgM assays (SERION Diagnostics GmbH, Würzburg, Germany). From 2007 to 2025, serum samples were analyzed for *T. gondii*-specific IgM and IgG antibodies using ELISA with EUROIMMUN reagents (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany). Assessment of IgG antibody avidity was not routinely available throughout the study period. IgG avidity testing was introduced in 2016 and was subsequently performed only in selected pregnant women upon referral by their primary care physician or gynecologist^{18, 20}.

Demographic data (age, gender, place of residence), topographic information (district of residence), and temporal data (year and month of diagnosis) were extracted for all reported cases.

Annual population data for the AP of Vojvodina were obtained from official census data and inter-census estimates provided by the Statistical Office of the Republic of Serbia¹⁹. Crude incidence rates (IRs) were calculated as the number of reported cases *per* 100,000 inhabitants *per* year. Age-specific IRs were calculated for the following groups: 0–14, 15–29, 30–39, 40–49, 50–59, and ≥ 60 years.

Data on meteorological variables, including average annual temperature (°C), total annual precipitation (mm), average relative humidity (%), and number of rainy days (≥ 0.1 mm precipitation) for the period 1997–2025, were obtained from the Republic Hydrometeorological Service of Serbia²¹. These variables were analyzed to assess their potential association with reported annual toxoplasmosis incidence.

Statistical analysis

Descriptive statistics were used to summarize the demographic and epidemiological characteristics of toxoplasmosis cases. Continuous variables were expressed as means $\pm$ standard deviations, while categorical variables were presented as frequencies and percentages. Differences between groups were assessed using the chi-square (χ^2) test for categorical variables and the independent samples *t*-test for continuous variables.

Correlation between reported annual IRs and climatic variables was assessed using Spearman's rank correlation coefficient (ρ), given the non-normal distribution of the data.

All statistical analyses were conducted using SPSS software, version 21 (IBM Corp., Armonk, NY, USA).

Temporal trends in toxoplasmosis IRs were assessed using segmented log-linear regression analysis²². Annual IRs *per* 100,000 inhabitants were logarithmically transformed and modeled separately for predefined time intervals corresponding to visually identified changes in the direction of the trend. For each segment, the annual percent change (APC) was estimated from the slope of the regression line according to the formula: $APC = [\exp(\beta) - 1] \times 100$, where β represents the regression coefficient for calendar year. Statistical significance of temporal trends was evaluated using *t*-tests of the regression coefficients. Corresponding 95% confidence interval (CI) and *p*-values were calculated for each APC estimate.

A two-sided value of $p < 0.05$ was considered statistically significant.

Results

Between 1997 and 2025, a total of 389 toxoplasmosis cases were reported in the AP of Vojvodina, Serbia, with no fatal outcomes registered. In addition, no cases of CT were reported through the surveillance system during the study period. The overall reported annual IR fluctuated between 0.06 and 1.49 *per* 100,000 inhabitants, without a consistent long-term increasing trend.

In the late 1990s and early 2000s, reported IRs were relatively variable, with an initial peak observed in 1998 (1.49 *per* 100,000), followed by a decline in the period 1999–2002 (range: 0.25–0.45 *per* 100,000). A second period of elevated reported IR was recorded between 2004 and 2008, with values exceeding 1.0 *per* 100,000 in most years and reaching 1.38 *per* 100,000 in 2007.

From 2009 onwards, a gradual decline in reported incidence was observed, with rates generally remaining below 1.0 *per* 100,000. This downward trend became more pronounced after 2016, with consistently low reported incidence levels recorded between 2017 and 2025 (range: 0.06–0.57 *per* 100,000), including the lowest value in 2025 (0.06 *per* 100,000) (Figure 1).

The overall long-term trend for the entire study period (1997–2025) indicated a decreasing pattern (average APC = -7.9% *per* year).

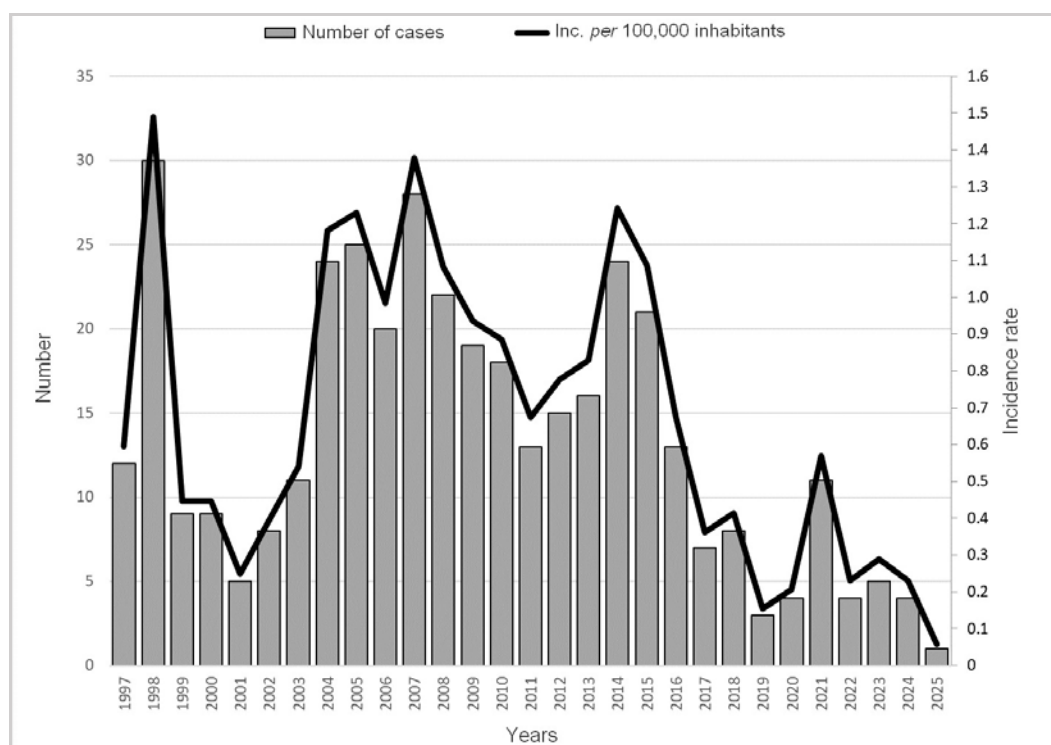


**Fig. 1 – Toxoplasmosis in the Autonomous Province of Vojvodina, Serbia (1997–2025).
Inc. – incidence.**

Table 1

**Segment-specific annual percent changes in toxoplasmosis incidence rates
in the Autonomous Province of Vojvodina, Serbia (1997–2025)**

Period	Trend phase	APC	95% CI	<i>p</i> -value	Interpretation
1997–2001	Early decline	-25.5	-56.2–26.5	0.175	Rapid initial decrease in incidence
2001–2007	Epidemic increase	32.1	14.0–53.0	0.005	Marked increase with epidemic-like fluctuations
2007–2015	Plateau/mild decline	-1.5	-8.5–6.1	0.643	Relative stabilization with slight downward trend
2015–2019	Sharp decline	-35.1	-49.8–-16.2	0.013	Pronounced and sustained decrease
2019–2021	Rebound increase	88.7	-86.9–2,612.2	0.203	Short-term resurgence, possibly outbreak-related
2021–2025	Final decline	-36.3	-60.1–-1.7	0.055	Strong decline to lowest observed incidence

APC – annual percent change; CI – confidence interval.

Values that differ significantly ($p < 0.05$) are marked in bold.

Segmented log-linear regression analysis revealed a significant increase in toxoplasmosis incidence during the 2001–2007 period (APC = 32.1%; 95% CI: 14.0–53.0; $p = 0.005$), followed by a period of relative stability during the 2007–2015 period (APC = -1.5%; 95% CI: -8.5–6.1; $p = 0.643$). A significant decline was observed during the 2015–2019 period (APC = -35.1%; 95% CI: -49.8–-16.2; $p = 0.013$). No statistically significant changes were identified during the remaining periods (Table 1).

Among children aged 0–14 years, reported IRs fluctuated markedly, with several pronounced peaks, including the

highest value observed in 2013 (2.88 *per* 100,000), followed by 2010 (2.48 *per* 100,000), and 2005 and 2008 (both 2.17 *per* 100,000). After 2016, reported incidence in this age group declined substantially, reaching zero in 2022 and 2023, and remaining low thereafter (Figure 2A).

In the 15–29 age group, reported IRs were generally more stable but showed intermittent increases, with notable peaks in 2011 (2.20 *per* 100,000), 2014 (2.48 *per* 100,000), and a maximum in 2015 (3.30 *per* 100,000). Following this peak, a declining trend was observed, with low reported incidence values recorded in the most recent years (Figure 2A).

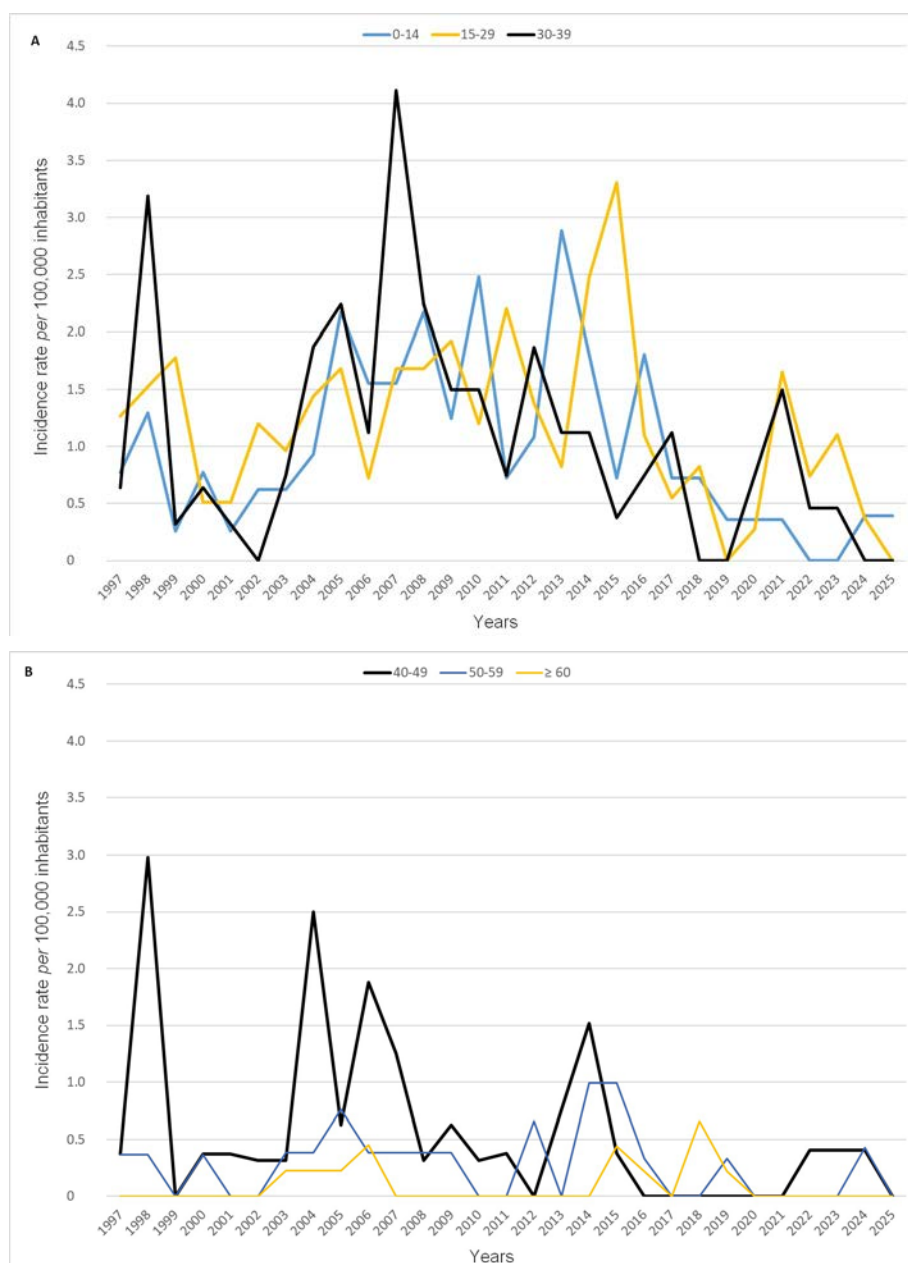


Fig. 2 – Age-specific incidence rates of toxoplasmosis among individuals aged: A) 0–14, 15–29, and 30–39 years and B) 40–49, 50–59, and ≥ 60 years, in the Autonomous Province of Vojvodina, Serbia (1997–2025).

The 30–39 age group exhibited the greatest variability, including the highest single-year reported incidence overall in 2007 (4.11 *per* 100,000). Additional elevated values were recorded in 1998 (3.19 *per* 100,000) and 2005 and 2008 (both 2.24 *per* 100,000). However, reported incidence declined markedly after 2012, with several years of zero reported incidence (2018, 2019, 2024, and 2025) (Figure 2A).

In the 40–49 age group, reported IRs showed intermittent peaks, most notably in 1998 (2.98 *per* 100,000) and 2004 (2.50 *per* 100,000), followed by moderately elevated values in 2006 (1.88 *per* 100,000) and 2014 (1.52 *per* 100,000). After 2016, reported incidence declined markedly, with predominantly zero values and only occasional low rates recorded between 2022 and 2024 (0.40 *per* 100,000) (Figure 2B).

In the 50–59 age group, reported incidence remained consistently low. The highest values were observed in 2014 and 2015 (0.99 *per* 100,000), while most other years were characterized by either very low or zero reported incidence (Figure 2B).

Among individuals aged ≥ 60 years, reported incidence was minimal throughout the entire period, with only sporadic cases resulting in low reported incidence values. The highest recorded rate was 0.66 *per* 100,000 in 2018, while most years showed zero reported incidence, particularly after 2020 (Figure 2B).

The highest proportion of toxoplasmosis cases was recorded in January (19%), followed by February (12%) and November (9%). In contrast, the lowest proportion was observed in December (5%). During the spring and summer

months, the distribution was relatively stable, with monthly proportions ranging from 6% to 7% between March and October, suggesting a more even occurrence of cases during this period (Figure 3).

Age-specific distribution of toxoplasmosis cases showed marked monthly variation (Figure 4), with the 15–29 age group predominating in most months. This group accounted for the highest proportions in February (21/46; 45.7%), May (9/29; 31.0%), July (9/25; 36.0%), August

(10/25; 40.0%), September (11/28; 39.3%), November (14/35; 40.0%), and December (9/20; 45.0%), indicating consistent dominance throughout the year, particularly in late summer and winter.

The 0–14 age group predominated only in October (11/28; 39.3%), while the 30–39 years was leading in June (9/23; 39.1%). In January, the 15–29 and 30–39 years shared the highest proportions (21/74; 28.4% each), with a substantial contribution from children (19/74; 25.7%) (Figure 4).

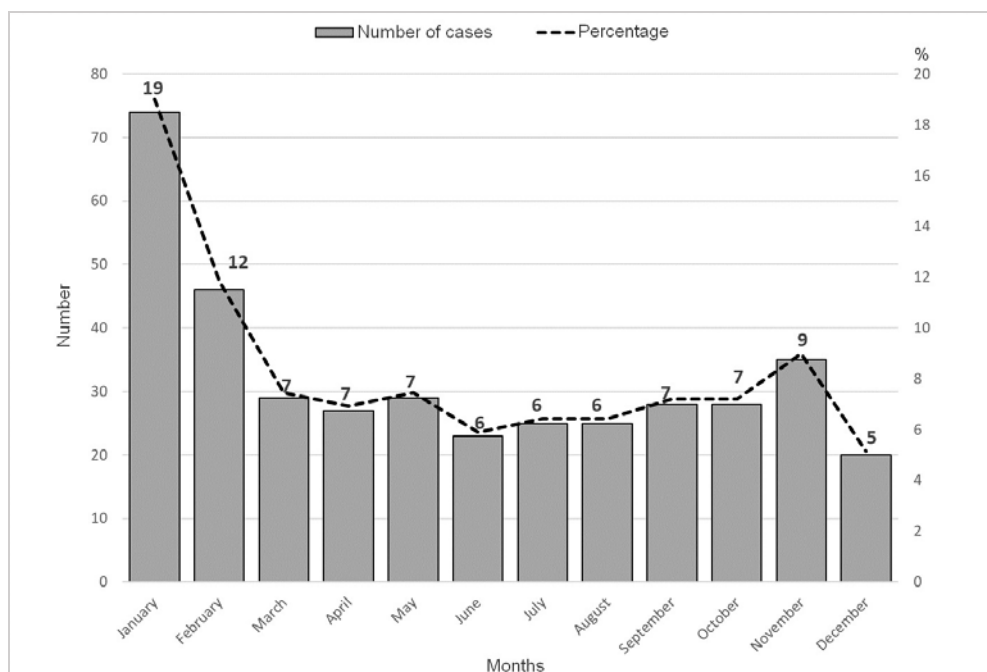


Fig. 3 – Seasonal distribution of toxoplasmosis in the Autonomous Province of Vojvodina, Serbia (1997–2025).

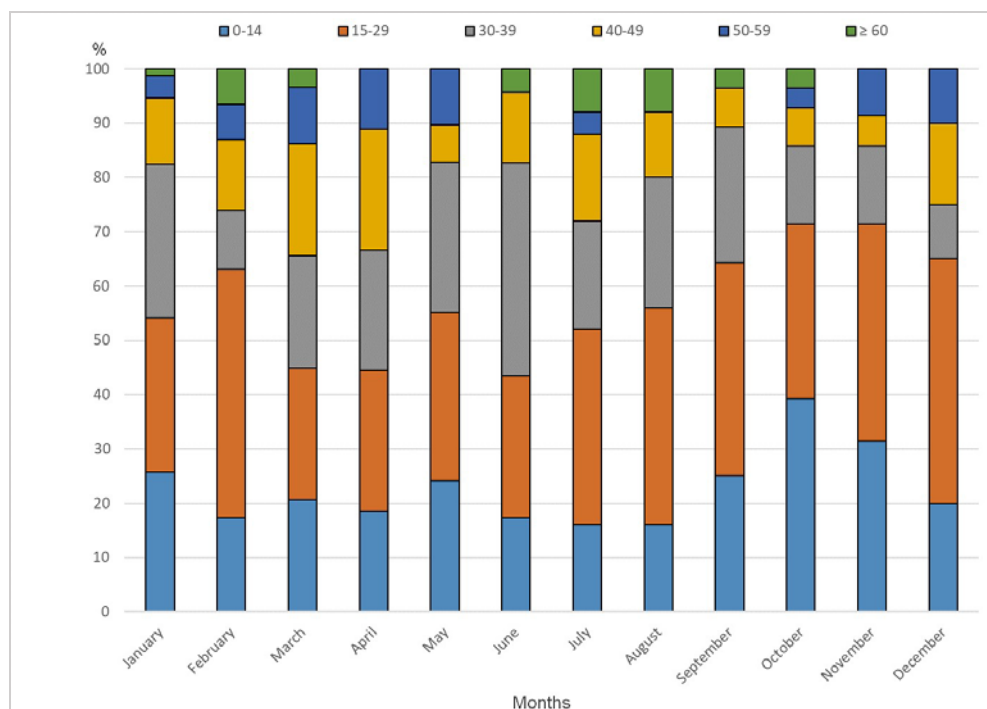


Fig. 4 – Monthly predominance of age groups in toxoplasmosis cases in the Autonomous Province of Vojvodina, Serbia (1997–2025).

Older age groups (40–49, 50–59, and ≥ 60 years) did not predominate in any month and consistently accounted for lower proportions of cases (Figure 4).

Gender-specific characteristics of toxoplasmosis cases during the period of 1997–2025 are shown in Table 2.

A total of 389 cases of toxoplasmosis were analyzed, including 155 (39.85%) males and 234 (60.15%) females. The mean age of all cases was 27.50 ± 15.32 years, with males being significantly younger than females (22.88 ± 15.02 vs. 30.56 ± 14.74 years, respectively; $p < 0.001$).

Regarding age distribution, the highest proportion of cases was observed in the 15–29 age group (34.19%), followed by the 0–14 (23.14%) and 30–39 (21.59%) age groups. Significant differences in age distribution between males and females were observed ($p = 0.0001$), with males more frequently represented in younger age groups.

A statistically significant difference in place of residence was observed between sexes ($p = 0.0048$), with a higher proportion of urban residence among females (71.79%) compared to males (57.42%).

Regarding topographic distribution within the AP of Vojvodina, the highest proportion of cases was recorded in South Bačka District (36.76%), followed by West Bačka (25.19%) and North Banat (11.31%). Distribution across districts differed significantly between males and females ($p = 0.0027$).

Seasonal distribution showed no statistically significant variation ($p = 0.7817$), with the highest proportion of cases observed in the January–March period (38.30%), followed by relatively similar proportions across the remaining quarters of the year.

A statistically significant moderate inverse correlation was observed between annual toxoplasmosis incidence and average temperature ($\rho = -0.5111$, $p = 0.004608$), indicating that higher temperatures were associated with lower reported IRs over the study period (Figure 5A).

No statistically significant association was observed between reported annual toxoplasmosis incidence and average precipitation ($\rho = 0.2216$, $p = 0.247987$), indicating a weak and non-significant positive correlation over the study period (Figure 5B).

Although this association did not reach statistical significance ($p = 0.051243$), a moderate positive correlation was observed between reported annual toxoplasmosis incidence and average relative humidity ($\rho = 0.3654$), suggesting a borderline relationship between humidity levels and reported incidence over the study period (Figure 5C).

A weak positive correlation was found between reported annual toxoplasmosis incidence and the number of rainy days ($\rho = 0.2985$), which did not reach statistical significance ($p = 0.115758$), indicating no clear association between precipitation frequency and disease-reported incidence over the study period (Figure 5D).

Table 2

**Gender-specific characteristics of toxoplasmosis cases
in the Autonomous Province of Vojvodina, Serbia (1997–2025)**

Variable	Total (n = 389)	Male (n = 155)	Female (n = 234)	p-value
Age, years	27.50 ± 15.32	22.88 ± 15.02	30.56 ± 14.74	< 0.001
Age, range				
0–14	90 (23.14)	51 (32.90)	39 (16.67)	0.0001
15–29	133 (34.19)	60 (38.71)	73 (31.20)	
30–39	84 (21.59)	20 (12.90)	64 (27.35)	
40–49	48 (12.34)	15 (9.68)	33 (14.10)	
50–59	22 (5.66)	6 (3.87)	16 (6.84)	
≥ 60	12 (3.08)	3 (1.94)	9 (3.85)	
Place of residence				
urban	257 (66.07)	89 (57.42)	168 (71.79)	0.0048
rural	132 (33.93)	66 (42.58)	66 (28.21)	
District of AP of Vojvodina				
North Bačka	33 (8.48)	18 (11.61)	15 (6.41)	0.0027
West Bačka	98 (25.19)	42 (27.10)	56 (23.93)	
South Bačka	143 (36.76)	42 (27.10)	101 (43.16)	
North Banat	44 (11.31)	23 (14.84)	21 (8.97)	
Central Banat	16 (4.11)	3 (1.94)	13 (5.56)	
South Banat	36 (9.25)	15 (9.68)	21 (8.97)	
Srem	19 (4.88)	12 (7.74)	7 (2.99)	
Quarter of the year				
January–March	149 (38.30)	60 (38.71)	89 (38.03)	0.7817
April–June	79 (20.31)	28 (18.06)	51 (21.79)	
July–September	78 (20.05)	31 (20.00)	47 (20.09)	
October–December	83 (21.34)	36 (23.23)	47 (20.09)	

n – number; AP – Autonomous Province.

Values are given as mean $\pm$ standard deviation and numbers (percentages).

Values that differ significantly ($p < 0.05$) are marked in bold.

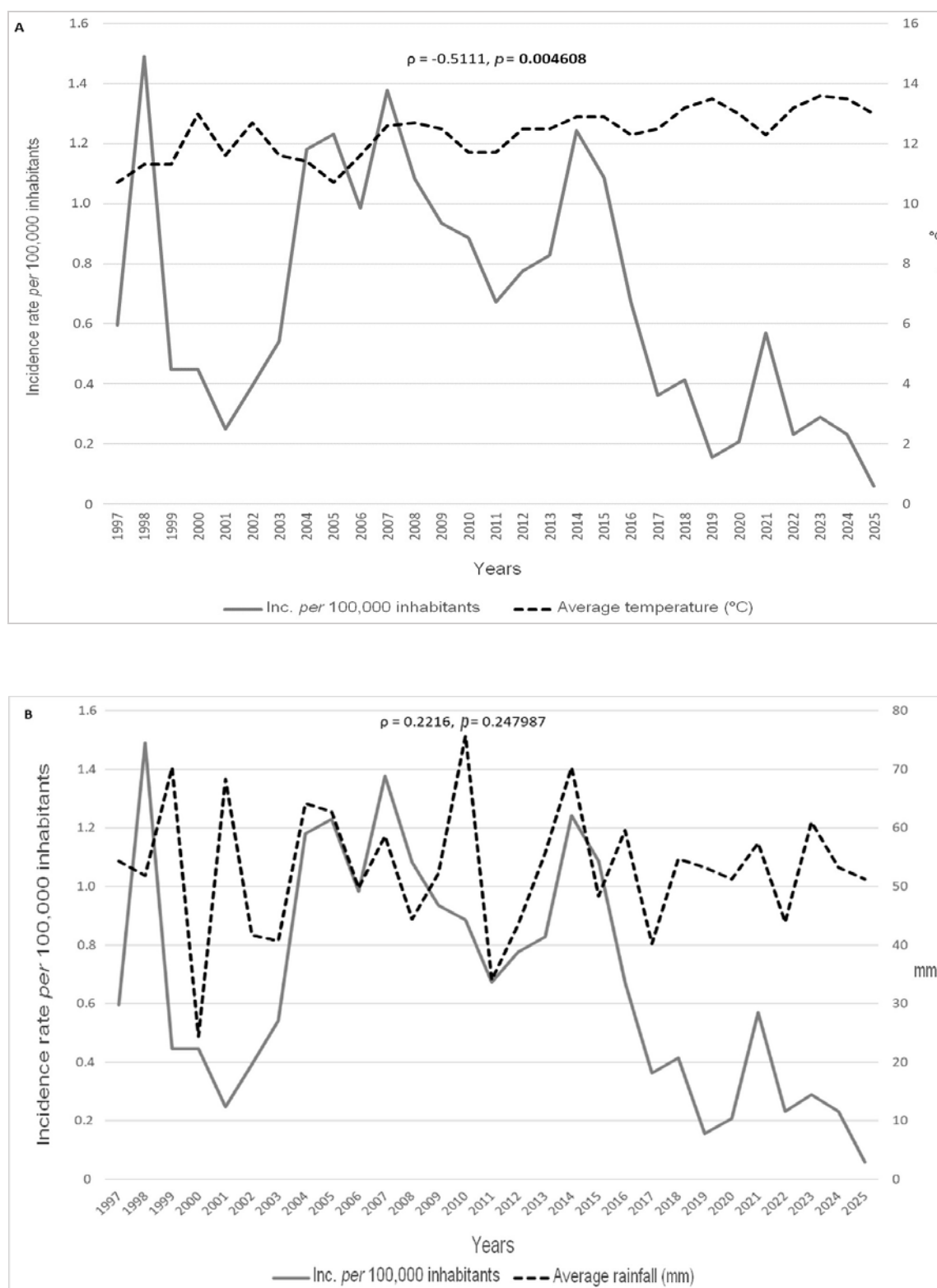


Fig. 5 – Temporal trends in toxoplasmosis incidence and association with: A) average annual temperature; B) average annual precipitation; C) average annual relative humidity; and D) average annual rainy days, in the Autonomous Province of Vojvodina, Serbia (1997–2025). Inc. – incidence.

Note: Fig. 5 continued on next page.

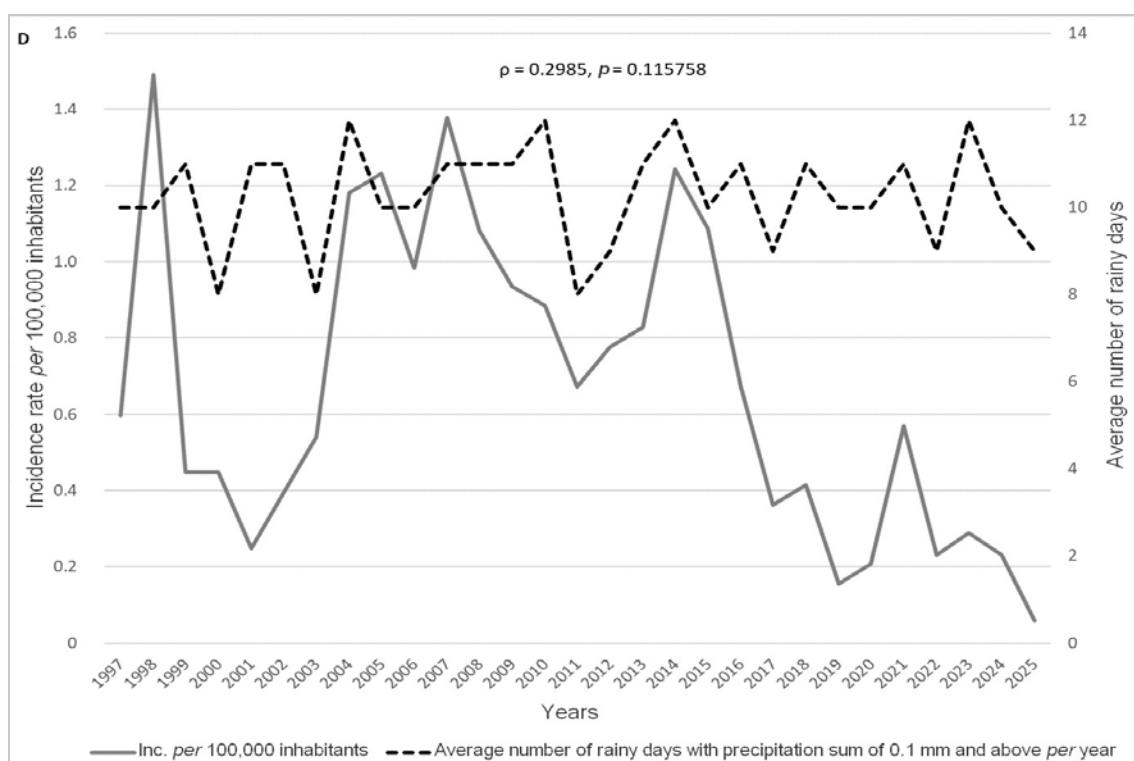
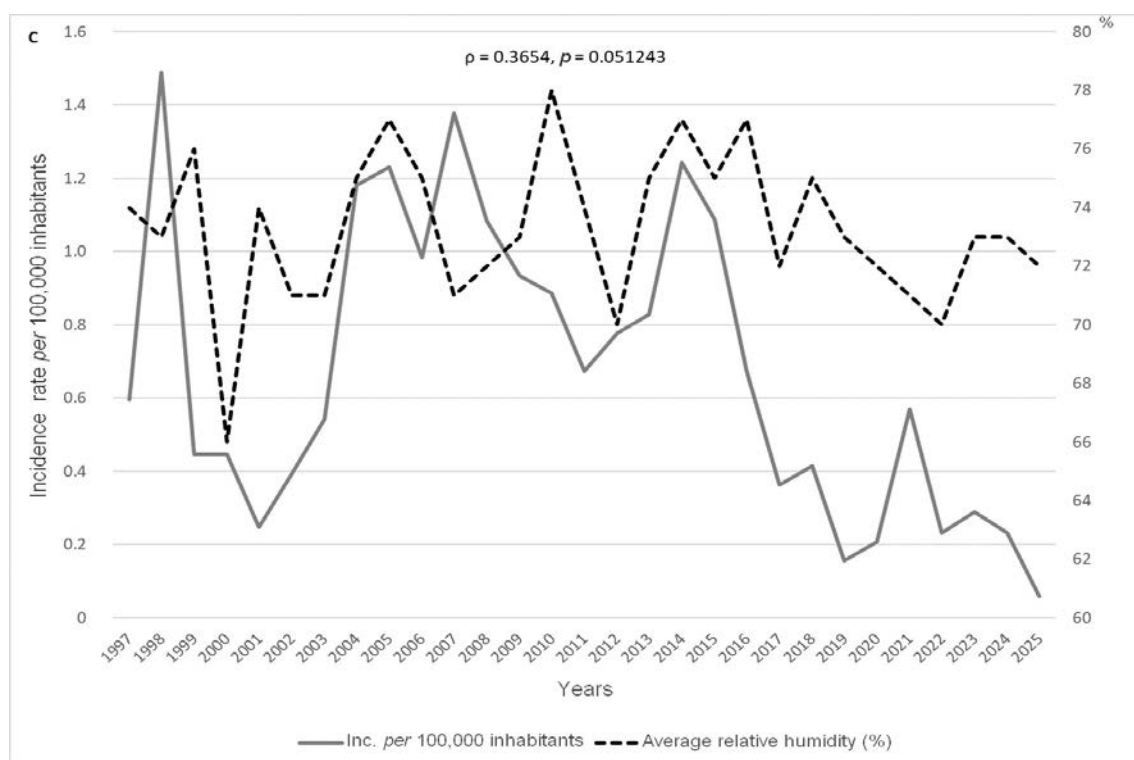


Fig. 5 (Continued) – Temporal trends in toxoplasmosis incidence and association with: A) average annual temperature; B) average annual precipitation; C) average annual relative humidity; and D) average annual rainy days, in the Autonomous Province of Vojvodina, Serbia (1997–2025). Inc. – incidence.

Discussion

Our findings indicate that, despite a sustained downward trend, toxoplasmosis epidemiology remains dynamic and influenced by changes in exposure pathways, surveillance practices, and environmental conditions, consistent with a previous study¹⁰.

Segmented log-linear regression analysis revealed substantial fluctuations in toxoplasmosis incidence over the study period. Incidence increased significantly during the 2001–2007 period, remained relatively stable between 2007 and 2015, and declined markedly during the 2015–2019 period. Although a temporary increase was observed between 2019 and 2021, this trend was not statistically significant and should be interpreted cautiously because of the small number of reported cases. A further decline after 2021 resulted in the lowest IRs recorded during the study period. The observed temporal variations likely reflect a combination of factors, including changes in diagnostic practices, testing intensity, healthcare-seeking behavior, surveillance sensitivity, and reporting completeness, rather than true changes in transmission alone. Given that toxoplasmosis is predominantly asymptomatic, reported incidence is strongly influenced by screening activities, particularly among women before and during pregnancy. Therefore, fluctuations in reported incidence may partly represent changes in case detection rather than actual changes in disease occurrence.

Similar to our findings, the observed decline in incidence is consistent with broader European trends and is likely attributable to improvements in food safety, meat processing, and hygiene standards^{2, 3, 12}. However, reported incidence represents only a fraction of total infections, as most cases remain asymptomatic and are not captured through routine surveillance systems. Consequently, reductions in notified cases do not necessarily reflect proportional declines in population-level exposure, particularly given the lifelong persistence of latent infection. This discrepancy between reported incidence and true prevalence has been well documented across European settings^{2, 5, 12, 23, 24}.

The findings of this study should also be considered in the context of the broader epidemiological role of latent toxoplasmosis, as highlighted by Flegr et al.²⁵, who demonstrated substantial global variation in seroprevalence across 88 countries and its potential association with population-level disease burden. The marked decline in reported incidence observed after 2015, together with consistently low rates in recent years, may reflect improvements in hygiene, food safety, and public awareness—the key determinants of transmission also emphasized in global analyses. However, as latent infection persists lifelong, reductions in reported incidence do not necessarily correspond to immediate declines in population-level prevalence^{1, 3–5}.

Recent European evidence supports the declining trend observed in our study. A systematic review and meta-analysis covering the period 2000–2020 reported an overall

seroprevalence of 32.1% in Europe, with marked heterogeneity between countries and a general decrease over time²⁶. More recent modeling data (2000–2021) confirm substantial regional variation with higher seroprevalence in Western (48%) and Eastern (50%) Europe and considerably lower levels in Northern Europe (18%), reflecting differences in environmental conditions, dietary practices, and exposure risk²⁷.

In addition, the decreasing reported incidence observed in this study is consistent with previously reported declines in seroprevalence in our country. Earlier data from the AP of Vojvodina (2006–2008), including the study by Brkić et al.²⁸, indicated substantially higher seroprevalence levels (38.1%) in the general population. In contrast, more recent studies conducted between 2017 and 2018¹⁴ and between 2008 and 2021²⁰ have reported lower levels (20.5–23.5%), supporting a long-term reduction in transmission. This concordance between decreasing seroprevalence and declining reported incidence suggests a sustained epidemiological shift towards lower exposure to toxoplasmosis in the population.

At the same time, the predominance of cases among younger age groups in our study (34.19% in the 15–29 age group and 21.59% in the 30–39 age group) is consistent with evidence of lower seroprevalence in younger cohorts and increasing seropositivity with age, indicating delayed primary infection. From a public health perspective, this shift may result in a growing proportion of susceptible individuals, particularly among women of reproductive age, potentially increasing the risk of primary infection during pregnancy and consequent congenital infection, as highlighted in global burden estimates^{5–7}. No cases of CT were reported through the surveillance system during the study period; however, this finding may reflect underdiagnosis or insufficient systematic screening rather than the true absence of congenital infection.

The predominance of females (60.15%) among reported cases is likely influenced by differences in healthcare-seeking behavior and targeted prenatal screening, as women of reproductive age are routinely tested due to the risk of CT in Europe^{29–31}. In our study, females were also significantly older than males, which likely reflects detection bias rather than true gender-specific differences in infection risk. Women are more frequently diagnosed through screening in adulthood, particularly during pregnancy, whereas men are more likely to be tested only when symptomatic, contributing to the lower average age observed among males. This pattern is consistent with findings from other European settings^{32, 33}. In countries with systematic screening programs, such as Austria and France, women account for the majority of diagnosed cases, reflecting the impact of antenatal testing on case detection^{31, 34}.

An interesting finding of this study was the significant difference in the distribution of cases by gender and place of residence. Women living in urban areas accounted for nearly three-quarters of all female cases, whereas the distribution among men was more balanced between urban and rural settings. This pattern is unlikely to reflect a true difference in

susceptibility to *T. gondii* infection. Rather, it may be explained by differences in healthcare utilization and diagnostic practices, as women of reproductive age in urban areas are more likely to undergo routine serological testing during prenatal care or infertility evaluations and generally have better access to specialized healthcare services. Nevertheless, differences in exposure to environmental or dietary risk factors between urban and rural populations cannot be excluded and warrant further investigation.

Seasonal variation in reported toxoplasmosis incidence is not consistently demonstrated across European studies and may differ depending on transmission route, surveillance sensitivity, and population behavior. Although environmental survival of *T. gondii* oocysts is generally favored by moderate temperatures and higher humidity, human infection is predominantly driven by foodborne exposure, particularly consumption of contaminated meat, which may not follow strict seasonal patterns^{2, 8, 12}. Although the observed difference was not statistically significant, the higher proportion of cases during winter months may reflect increased consumption of undercooked meat during holiday periods, as well as potential clustering of healthcare utilization and diagnostic testing following symptom onset in colder months. Similar weak or inconsistent seasonality has been reported in European surveillance systems, suggesting that climatic effects are limited in regions with stable food supply chains and widespread refrigeration practices¹². Consistent with this observation, we found an inverse correlation between reported incidence and temperature, indicating higher incidence during colder periods. Although the possibility of an “ecological fallacy” cannot be excluded, this finding may suggest that low winter temperatures in the AP of Vojvodina are associated with certain cultural practices, such as traditional pig slaughtering and the consumption of insufficiently cooked meat products during this period. While environmental survival of *T. gondii* oocysts is generally favored by moderate temperatures and humidity, the relationship between climate and human infection remains complex and context-dependent. This likely reflects the predominance of foodborne transmission pathways over direct environmental exposure in contemporary European settings^{20, 25–27}.

We observed topographical differences across the AP of Vojvodina, with the highest proportion (36.76%) of cases reported in the South Bačka District. This finding is likely influenced, at least in part, by referral patterns, as the South Bačka District is home to the University Clinical Center of Vojvodina, the only tertiary-level healthcare facility in the province, which serves as a regional referral center for more severe or complex cases³⁵. In addition, South Bačka is the most populous district in the AP of Vojvodina, accounting for 607,454 inhabitants in 2022 (18.7% of the provincial population)¹⁹, which may further contribute to the higher number of reported cases.

The main strength of this study lies in its long observation period (29 years) and the use of population-based surveillance data, enabling robust analysis of temporal trends and epidemiological patterns. The inclusion of age-

specific, seasonal, topographic, and climatic analyses provides a comprehensive overview of toxoplasmosis epidemiology in the region.

Limitations of the study

Several limitations should be acknowledged. First, the study relies on passive surveillance data, which may be subject to underreporting, particularly regarding asymptomatic or mild cases that seroepidemiological research can better detect. Second, changes in diagnostic methods over the study period, including the transition from the CFT to ELISA-based assays and the later introduction of IgG avidity testing, may have influenced case detection. In addition, because information on testing indications was not available in the surveillance database, the potential impact of changes in testing practices over time on the observed temporal trends cannot be excluded. Third, the lack of individual-level data on risk factors (e.g., dietary habits, contact with animals, socioeconomic status) limits the ability to identify specific determinants of infection. Fourth, the official infectious disease surveillance records lacked data on comorbidities (e.g., immunodeficiency) and pregnancy status, precluding the evaluation of these variables in the analysis. Fifth, our findings suggest the presence of potential detection bias and a demographic artifact. Specifically, women accounted for the majority of reported cases (60.15%), were significantly older than men, and most infections occurred among individuals aged 15–39 years. This distribution is unlikely to reflect a true biological susceptibility or greater exposure to infection among women; rather, it is probably a consequence of differences in testing practices. Women of reproductive age are routinely screened during prenatal care or infertility evaluations, whereas men are generally tested only when clinical symptoms, such as lymphadenopathy, develop. Therefore, the observed demographic patterns should be interpreted in light of these differences in diagnostic ascertainment. Finally, ecological analysis of climatic variables does not allow for causal inference and may be influenced by unmeasured confounders.

Conclusion

The epidemiology of toxoplasmosis in the Autonomous Province of Vojvodina reflects a broader European transition characterized by declining reported incidence alongside persistent underlying transmission and population susceptibility. The absence of reported congenital toxoplasmosis cases during the study period may reflect underrecognition rather than the true absence of disease. Strengthening surveillance, improving the detection and reporting of congenital toxoplasmosis, and considering the implementation of standardized prenatal screening may contribute to earlier diagnosis and more effective prevention of congenital infection. Further research integrating epidemiological, environmental, and behavioral data is needed to better understand disease transmission and inform evidence-based public health strategies.

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