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ORIGINAL ARTICLE

STUDY OF THE EPIDEMIOLOGICAL AND SEROLOGICAL ASPECTS OF TOXOPLASMOSIS IN PREGNANT WOMEN AND NEWBORNS TREATED IN THE CITY OF BLUMENAU, SANTA CATARINA, BRAZIL

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ABSTRACT

Toxoplasmosis is a disease caused by the protozoan parasite *Toxoplasma gondii*, which is of greater relevance in developing countries and can lead to severe complications in pregnant women and immunocompromised patients. Gestational toxoplasmosis, in most cases, occurs when a woman acquires a primary *T. gondii* infection during pregnancy, posing a risk of fetal sequelae or even fetal death, the disease being of compulsory notification in Brazil. The objectives of this study were to assess the epidemiological status of gestational and congenital toxoplasmosis in the city of Blumenau in the State of Santa Catarina and to increase knowledge about its prevalence in the region. To achieve this, an analysis of city data recorded in the Blumenau Epidemiological Surveillance Service database was conducted between the years 2019 and 2021. During the study period, the city of Blumenau presented a lower prevalence of gestational toxoplasmosis (2.93/ 1,000 pregnant women) and congenital toxoplasmosis (2.31/ 10,000 live births) than other regions of Brazil. Proper adherence to prenatal care and its screening protocols, as well as the early initiation of treatment, can result in a low incidence of congenital infections, even in tropical areas with high seroprevalence. Rapid and accessible immunoglobulin G avidity testing excludes suspected cases without requiring invasive tests or unnecessary treatments. On the other hand, education plays a crucial role in disease prevention and should focus on encouraging proper prenatal care and providing adequate information about the disease. Finally, treatment adherence should be monitored and promoted.

KEY WORDS: *Toxoplasma gondii*; pregnancy complications; parasitic infections; public health; toxoplasmosis, congenital; toxoplasmosis, epidemiology.

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INTRODUCTION

Toxoplasmosis is a disease caused by the protozoan *Toxoplasma gondii*, which is of greater relevance in developing countries and is capable of triggering severe complications in pregnant women and immunocompromised patients (Ferreira, 2021). In Brazil, it is estimated that anti-*T. gondii* Immunoglobulin G (IgG) is present in 53.8% of pregnant women, making Brazil the third country with the highest worldwide seroprevalence (Bigna et al., 2020). Vertical transmission (congenital) usually occurs when the primary infection occurs during pregnancy; nevertheless, there are recorded cases of vertical transmission being enabled by reinfections and exacerbation of chronic infections (Andrade et al., 2010).

Tachyzoites are capable of colonizing placental tissues and accessing the fetal compartment. Therefore, congenital toxoplasmosis is one of the most severe forms of protozoan infection, as it can lead to ocular, visceral, and intracranial lesions in the developing fetus. There is a risk of fetal death or severe sequelae (Ferreira, 2021), including seizures and visual and learning impairment (Robinson et al., 2021).

Because it is often an asymptomatic disease or presenting nonspecific symptoms (Bigna et al., 2020), the diagnosis is made using methods based on indirect quantification of IgG and IgM anti-*T. gondii* antibodies through serological tests (Marques et al., 2015). IgM serves as an indicator of acute toxoplasmosis infection; however, it is known that this immunoglobulin may remain in the blood for months or even years after the initial infection, making it challenging to differentiate between recent or past infection (Teimouri et al., 2020).

IgG avidity is an important complementary exam in the context of acute and chronic infection differentiation, and it measures the strength of binding between the toxoplasmosis antigen and the antibody, which increases over time after infection, classifying it as high, moderate, or low avidity. Before the 16th week of pregnancy and combined with the previously mentioned serological tests, IgG avidity can be used to accurately differentiate between acute infection in the ongoing pregnancy and infection before pregnancy (Brasil, 2022).

For pregnant women with positive IgM serology, suspected of having an acute gestational infection, notification of the infection to the Epidemiological Surveillance is mandatory and done by filling out the Notifiable Diseases Information System (SINAN) form; pregnant women with negative IgG and IgM should receive adequate guidance regarding the disease transmission and prevention, as they are susceptible to acute infection (Brasil, 2018).

In the city of Blumenau in the State of Santa Catarina region, epidemiological studies on gestational and congenital toxoplasmosis are still scarce. This study will contribute to knowledge about its prevalence in the city and provide an understanding of the diagnosis and treatment carried out.

Furthermore, based on the obtained data, it will be possible to adopt more specific prevention and control measures.

MATERIAL AND METHODS

The present study is of retrospective cross-sectional and observational design with a quantitative approach to data analysis. To evaluate the occurrence of gestational and congenital toxoplasmosis, information from the city recorded in the Epidemiological Surveillance Service (EVS) database of Blumenau (SC) was analyzed between the years 2019 and 2021. The study population included all cases of suspected, probable, and confirmed gestational and congenital toxoplasmosis, as defined by the Gestational and Congenital Toxoplasmosis Notification and Investigation Protocol (Brasil, 2018), registered by the municipal EVS in the period in question.

The inclusion criteria considered notifications of congenital and gestational toxoplasmosis from residents of Blumenau/SC registered in the city EVS database between the years 2019 and 2021. Exclusions were made for notifications of gestational and congenital toxoplasmosis made before 2019, notifications in which the infection was classified as non-autochthonous to Blumenau, cases of duplicity, and notifications where the patient's city of residence was different.

In the present study, the gestational toxoplasmosis suspicion outcome was considered confirmed/ probable if one or more of the following criteria were met: i) anti-*T. gondii* IgM positive and IgG negative during pregnancy with evidence of IgG seroconversion found in repeated testing after 2-3 weeks; ii) IgM/IgG seroconversion occurring during pregnancy; iii) anti-*T. gondii* +IgG and + IgM if diagnosis cannot be discarded with IgG avidity testing; iv) confirmed/ probable congenital toxoplasmosis (Brasil, 2018).

The gestational toxoplasmosis suspicion outcome was considered discarded if one of the following criteria were found: i) IgM positive and IgG negative anti-*T. gondii* during pregnancy with no evidence of IgG seroconversion found in repeated testing after 2-3 weeks (false positive); ii) high IgG anti-*T. gondii* avidity (>60%) with ≤ 16 weeks of pregnancy; iii) IgG anti-*T. gondii* reagent more than three months before conception (Brasil, 2018).

The congenital toxoplasmosis suspicion outcome was considered confirmed/ probable if one or more of the following criteria were met: i) evidence of toxoplasmosis colonization in either amniotic liquid, fetal or placental tissue sample (PCR, direct search); ii) child with IgM and IgG anti-*T. gondii* reagent up to six months of age; iii) child with rising IgG anti-*T. gondii* serum levels in at least two serial samples with a minimum interval of three weeks during the first 12 months of life; iv) child with anti-*T. gondii* IgG persistently reactive after 12 months of age; v) child with clinical manifestations or imaging tests

compatible with congenital toxoplasmosis and IgG anti-*T. gondii* reagent (Brasil, 2018).

The congenital toxoplasmosis suspicion outcome was considered discarded if one or more of the following criteria were met: i) child with IgG anti-*T. gondii* negative titers before 12 months of age (at least two months after stopping antiparasitic drugs); ii) negative seroconversion of IgG anti-*T. gondii* after 12 months of age (Brasil, 2018).

The study used the Blumenau EVS database, identifying patients only by the initials of their name and date of birth. Data were organized into simple descriptive and associative tables containing absolute frequencies, relative frequencies (percentages), measures of central tendency (mean and median), measures of dispersion (standard deviation, quartile deviation), and estimates of mean and proportion with 95% confidence intervals.

For association and comparison, the following statistical tests were used: Fisher's Exact Test, Chi-square Test of Independence, Test of Two Independent Proportions, Student's *t*-test, and Analysis of Variance One-Way F-test. In all cases, statistical significance was considered when $p < 0.05$. Data analysis was performed using the Epi Info and Microsoft Excel 2016 software, with appropriate formulas for each situation.

The present study was approved by the Human Research Ethics Committee – CEPH, of the Universidade Regional de Blumenau (FURB) under protocol CAAE 65844522.2.0000.5370, with no damage to the integrity, individuality, and privacy of individuals involved in the research.

RESULTS

All 164 notifications during the period from 2019 to 2021 were analyzed. Among them, 18 cases (11%) were subsequently discarded because they were not autochthonous to the city of Blumenau, totaling 56 (38.4) notifications of congenital toxoplasmosis and 90 (61.6%) of gestational toxoplasmosis.

Using data from the last census conducted by the Brazilian Institute of Geography and Statistics (IBGE) in 2010, the confirmed gestational toxoplasmosis coefficient in the city from 2019 to 2021 was 1.22/ 10,000 inhabitants. For the prevalence calculation, both confirmed and probable cases were considered, following the criteria of the Protocol for Notification and Investigation of Gestational and Congenital Toxoplasmosis (Brasil, 2018). During the same period, the prevalence coefficient for confirmed congenital toxoplasmosis was 0.7716 cases/ 10,000 live births in the city of Blumenau.

Table 1 presents the general profile of sociodemographic and clinical characteristics of the patients in the gestational group. In this sample of 90 pregnant patients, with an average age of 26 years, the majority were of white race (85.6%).

Table 1. General profile of the sociodemographic and clinical characteristics of the patients in the gestational group.

Characteristics	*n* (%) (*n* = 90)	CI (95%)
Age (years)		
Mean (± SD)	26.3 (± 6.0)	(25.05 - 27.5)
Median (± QD)	26 (± 4.5)	
Year of notification		
2019	12 (13.3%)	(6.3 - 20.3)
2020	34 (37.8%)	(27.7 - 47.8)
2021	44 (48.9%)	(38.5 - 59.2)
Gestational trimester of acute toxoplasmosis diagnosis		
1st trimester	41 (45.6%)	(35.2 - 55.8)
2nd trimester	22 (24.4%)	(15.6 - 33.3)
3rd trimester	24 (26.7%)	(17.5 - 35.8)
Ignored	2 (2.2%)	(0 - 5.3)
Not Applicable	1 (1.1%)	(0 - 3.3)
Race		
White	77 (85.6%)	(78.3 - 92.8)
Black	0 (0%)	(0 - 0)
Asian	1 (1.1%)	(0 - 3.3)
Mixed-race	6 (6.7%)	(1.5 - 11.8)
Ignored	6 (6.7%)	(1.5 - 11.8)
Education		
Incomplete 1st to 4th grade	4 (4.4%)	(0.19 - 8.7)
Completed 1st to 4th grade	5 (5.6%)	(0.8 - 10.3)
Incomplete 5th to 8th grade	9 (10%)	(3.8 - 16.2)
Completed 5th to 8th grade	18 (20%)	(11.7 - 28.2)
Incomplete High School	4 (4.4%)	(0.2 - 8.7)
Completed High School	28 (31.1%)	(21.5 - 40.7)
Incomplete College	2 (2.2%)	(0 - 5.3)
Completed College	11 (12.2%)	(5.4 - 18.9)
Ignored	9 (10%)	(3.8 - 16.2)

Neighborhoods of Blumenau		
Itoupava Central	15 (16.7%)	(8.9 - 24.4)
Itoupavazinha	8 (8.9%)	(3.0 - 14.8)
Velha Central	7 (7.8%)	(2.2 - 13.3)
Velha	6 (6.7%)	(1.5 - 11.8)
Fortaleza	6 (6.7%)	(1.5 - 11.8)
Tribess	5 (5.6%)	(0.8 - 10.3)
Progresso	5 (5.6%)	(0.8 - 10.3)
Garcia	4 (4.4%)	(0.2 - 8.7)
Testo Salto	4 (4.4%)	(0.2 - 8.7)
Itoupava Norte	4 (4.4%)	(0.2 - 8.7)
Salto do Norte	4 (4.4%)	(0.2 - 8.7)
Ponta Aguda	3 (3.3%)	(0 - 7.0)
Passo Manso	3 (3.3%)	(0 - 7.0)
Escola Agrícola	3 (3.3%)	(0 - 7.0)
Salto	2 (2.2%)	(0 - 5.8)
Fortaleza Alta	2 (2.2%)	(0 - 5.8)
Glória	1 (1.1%)	(0 - 3.3)
Fidelis	1 (1.1%)	(0 - 3.3)
Boa Vista	1 (1.1%)	(0 - 3.3)
Vila Nova	1 (1.1%)	(0 - 3.3)
Vorstadt	1 (1.1%)	(0 - 3.3)
Badenfurt	1 (1.1%)	(0 - 3.3)
Água Verde	1 (1.1%)	(0 - 3.3)
Velha Grande	1 (1.1%)	(0 - 3.3)
Vila Itoupava	1 (1.1%)	(0 - 3.3)
Area		
Urban	89 (98.9%)	(96.7 - 101.0)
Rural	0 (0%)	(0 - 0)
Peri-urban	0 (0%)	(0 - 0)
Ignored	1 (1.1%)	(0 - 3.3)
Outcome of the notification		
Confirmed	38 (42.2%)	(32.0 - 52.4)
Discarded	49 (54.4%)	(44.1 - 64.7)
Missing follow-up	3 (3.3%)	(0 - 7.0)

Confirmation/discard criteria		
Laboratory	89 (98.9%)	(96.7 - 101.0)
Clinical/epidemiological	1 (1.1%)	(0 - 3.3)
Autochthonous		
Yes	88 (97.8%)	(94.7 - 100.8)
No	0 (0%)	(0 - 0)
Undetermined	2 (2.2%)	(0 - 5.3)
Case evolution		
Recovery/improvement	82 (91,1%)	(85.2 - 96.9)
Death from the disease	0 (0%)	(0 - 0)
Death from another cause	0 (0%)	(0 - 0)
No information	8 (8.9%)	(3.0 - 14.8)
Immunoglobulin G (IgG)		
Positive	82 (91.1%)	(85.2 - 96.9)
Negative	2 (2.2%)	(0 - 5.3)
Undetermined	2 (2.2%)	(0 - 5.3)
Not done	4 (4.4%)	(0.2 - 8.7)
Immunoglobulin M (IgM)		
Positive	76 (84.4%)	(76.9 - 91.9)
Negative	4 (4.4%)	(0.2 - 8.7)
Undetermined	8 (8.9%)	(3.0 - 14.8)
Not done	2 (2.2%)	(0 - 5.3)
IgG Avidity		
Low	10 (11.1%)	(4,62 - 17.6)
Moderate	3 (3.3%)	(0 - 7.0)
High	46 (51.1%)	(40.8 - 61.4)
Not Done	31 (34.4%)	(24.6 - 44.3)

SD: Standard deviation; QD: quartile deviation; CI: Confidence intervals for the mean and proportion (prevalence) with 95% confidence.

From a total of 56 newborns with suspected congenital toxoplasmosis, only one case (1.8%) had a positive diagnosis confirmed by serology. Table 2 shows the overall profile of sociodemographic and clinical characteristics of newborns reported for congenital toxoplasmosis. The sample consisted of 56 children, 23 (41.1%) female and 33 (58.9%) male. Regarding race, 98.2% were white, with only one (1.8%) described as mixed racial origins.

Table 2. General Profile of Sociodemographic and Clinical Characteristics of Patients in the Congenital Group.

Characteristics	*n* (%) (*n* = 56)	CI (95%)
Sex		
Female	23 (41.1%)	(28.2 - 53.9)
Male	33 (58.9%)	(46.0 - 71.8)
Year of notification		
2019	17 (30.4%)	(18.3 - 42.4)
2020	22 (39.3%)	(26.5 - 52.1)
2021	17 (30.4%)	(18.3 - 42.4)
Race		
White	55 (98.2%)	(94.7 - 101.7)
Mixed-race	1 (1.8%)	(0 - 5.2)
Neighborhoods of Blumenau		
Itoupava Central	11 (19.6%)	(9.2 - 30.0)
Velha	8 (14.3%)	(5.1 - 23.4)
Progresso	5 (8.9%)	(1.4 - 16.4)
Garci a	4 (7.1%)	(0.4 - 13.9)
Fortaleza	4 (7.1%)	(0.4 - 13.9)
Badenfurt	3 (5.3%)	(0 - 11.2)
Passo Manso	3 (5.3%)	(0 - 11.2)
Testo Salto	3 (5.3%)	(0 - 11.2)
Itoupavazinha	3 (5.3%)	(0 - 11.2)
Velha Central	2 (3.6%)	(0 - 8.4)
Escola Agrícola	2 (3.6%)	(0 - 8.4)
Salto	1 (1.8%)	(0 - 5.2)
Glória	1 (1.8%)	(0 - 5.2)
Boa Vista	1 (1.8%)	(0 - 5.2)
Água Verde	1 (1.8%)	(0 - 5.2)
Ponta Aguda	1 (1.8%)	(0 - 5.2)
Vila Itoupava	1 (1.8%)	(0 - 5.2)
Itoupava Norte	1 (1.8%)	(0 - 5.2)
Salto do Norte	1 (1.8%)	(0 - 5.2)
Area		
Urban	56 (100%)	(100 - 100)

The outcome of the notification		
Confirmed	1 (1.8%)	(0 - 5.2)
Discarded	55 (98.2%)	(94.7 - 101.7)
Confirmation/discard criteria		
Laboratory	56 (100%)	(100 - 100)
Autochthonous		
Yes	56 (100%)	(100 - 100)
No	0 (0%)	(0 - 0)
Case evolution		
Recovery/improvement	55 (98.2%)	(94.7 - 101.7)
No information	1 (1.8%)	(0 - 5.2)
Immunoglobulin G (IgG)		
Positive	47 (83.9%)	(74.3 - 93.5)
Negative	6 (10.7%)	(2.6 - 18.8)
Not done	3 (5.4%)	(0 - 11.2)
Immunoglobulin M (IgM)		
Positive	1 (1.8%)	(0 - 5.2)
Negative	53 (94.6%)	(88.7 - 100.5)
Not done	2 (3.6%)	(0 - 8.4)

I - CI: Confidence intervals for the mean and proportion (prevalence) with 95% confidence.

From notifications of gestational toxoplasmosis (n= 90), four (4.4%) resulted in subsequent miscarriage. Among these four pregnancies, only one of them did not have the diagnosis of gestational toxoplasmosis discarded. However, it was not possible to confirm whether the protozoan infection was a triggering factor for this miscarriage.

Table 3 presents the comparison between the gestational trimesters of acute toxoplasmosis diagnosis in relation to the age of the pregnant women. According to statistical tests, the age of the pregnant women diagnosed within the 1st trimester of gestation was significantly higher than in the other two trimesters, with $p< 0.05$, indicating that the older the mother, the earlier the diagnosis. It is important to note that the forms of three patients did not contain information regarding the gestational trimester; therefore, these patients were not included in this analysis.

Table 3. Comparison between gestational trimesters of diagnosis of suspected gestational toxoplasmosis in relation to the age of the pregnant women.

Age (years)	Gestational Trimester of Diagnosis of Suspected Gestational Toxoplasmosis			*p*
	1st trimester (*n* = 41)	2nd trimester (*n* = 22)	3rd trimester (*n* = 24)	
Mean (± SD)	28.05 (± 5.9)[a]	24.55 (± 5.9)[b]	25.08(+-5.8)[b]	0.0428
Median (± QD)	28 (± 4)	24 (± 4.4)	24 (± 3.7)	

I - SD: Standard deviation; QD: Interquartile range. II - P: P-value of the One-Way Analysis of Variance (ANOVA - parametric) F-test. Lowercase letters represent significant differences between groups (Tukey's test - Multiple comparison test). If $p < 0.05$, then there are significant differences between groups.

Using population data provided by the Management and Decision Support Information System (SIGAD) of Blumenau, a city map indicating the prevalence of toxoplasmosis notifications by individual neighborhoods was created and is shown in Figure 1.

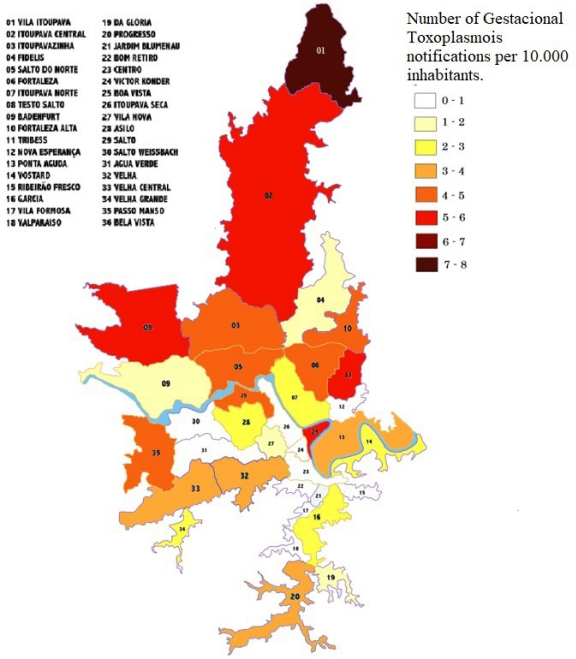

Figure 1. The city map indicates the prevalence of toxoplasmosis notifications by individual neighborhoods in the city of Blumenau in the State of Santa Catarina.

No significant statistical associations were found in the analysis of gestational trimesters of notification with education level, as well as the results of the IgG avidity test and the age of the pregnant women.

According to the Chi-square test conducted, it can be stated that there is a significant relationship between late diagnosis and higher rates of positivity for gestational toxoplasmosis. The diagnostic confirmation of 22.2% in the 1st trimester increased to 50% in the 3rd trimester (Table 4).

Table 4. Association between gestational trimester and notification outcome of the evaluated patients.

Gestational Trimester	Notification Outcome		*p*
	Confirmed	Discarded	
1st	8 (22.2%)	32 (66.7%)	0.00001
2nd	10 (27.8%)	12 (25%)	
3rd	18 (50%)	4 (8.3%)	
Total	36 (100%)	48 (100%)	

I - P: *p*-value of the Chi-square Test of Independence. If $p < 0.05$, then a significant association.

There was no significant association between the notification outcome and the age of the pregnant women. According to the statistical test applied, age did not significantly influence the notification outcome result, meaning that younger pregnant women did not have a higher likelihood of a confirmed diagnosis ($p = 0.1179$).

DISCUSSION

Observing the profile of the analyzed patients, the age of IgM-positive pregnant women in this study ranged from 14 to 40 years (mean 26.5 ± 4.9 years), and the prevalence of positive IgM antibodies was 5.85/ 1,000 pregnant women. In a study in the State of Mato Grosso do Sul in Brazil, the age of patients ranged from 14 to 39 years with an average of 23 (±5.9) years, and the IgM prevalence was 4.2/1,000 pregnant women (Figueiró-Filho et al., 2005). On the other hand, in the city of Porto Alegre in the State of Rio Grande do Sul in Brazil, the observed average age was 26.3 years, with seropositivity varying among pregnant women aged 13 to 45 years (Varella et al., 2009). Meanwhile, in the city of Chapecó (SC), the prevalence of positive IgM in pregnant women was 0.94/ 1,000 (Sandrin et al., 2012).

Studies show higher rates of IgG seropositivity as age increases due to longer exposure time to *T. gondii*, suggesting that acute infections should be more common within younger patient groups. However, there was no significant correlation between acute infection and the age group of the pregnant women (p= 0.11). However, a significant relationship was observed between maternal age and early notification (p< 0.05), indicating that older pregnant women had higher rates of notification during the first trimester; similar findings occurred in the survey conducted in Chapecó (Sandrin et al., 2012).

The prevalence of autochthonous gestational toxoplasmosis in Blumenau between 2019 and 2021 was 2.93/ 1,000 pregnant women. This prevalence was lower than surveys conducted in other parts of the country, such as Mato Grosso do Sul 4.2/ 1,000 (Figueiró-Filho et al., 2005), Sergipe 4.6/ 1,000 (Alves et al., 2009), Porto Alegre/RS 4.8/ 1,000 (Varella et al., 2009), and Brasília 5.7/ 1,000 (Nóbrega & Karnikowski, 2005). It is important to note that underreporting is a relevant factor in determining disease prevalence, as Sandrin et al. (2012) suggested in their study in Chapecó between 2005 and 2009, where the prevalence found was 0.57 per 1,000 pregnant women in the city.

Another relevant finding in this study was the prevalence of congenital toxoplasmosis of 0.77/ 10,000 live births, a figure lower than the national literature. In maternity wards of three major hospitals in Porto Alegre, congenital toxoplasmosis prevalences of 9/ 10,000 (Varella et al., 2009), 6/ 10,000 (Lago et al., 2007), and 4.5/ 10,000 live births (Andrade et al., 2018) were found. Similar prevalence discrepancy was found in studies conducted in Sergipe (4/ 10,000), Belém (10/10,000), Minas Gerais (12.99/ 10,000), and Goiânia (60/ 10,000) (Vasconcelos-Santos et al., 2009; Bichara et al., 2012; Melo Inagaki et al., 2012; Rodrigues et al., 2014).

Looking at global congenital toxoplasmosis prevalence rates, such as in Mexico City, with 20/10,000 live births (Vela-Amieva et al., 2005), Australia, with 2.3/10,000 live births (Walpole et al., 1991), Colombia, with 25-38/10,000 live births (Gómez-Marin et al., 2011), and Morocco, with 3.9/10,000 live births (El Mansouri et al., 2007), the same trend is observed. Based on the relationship established between toxoplasmosis seroprevalence and environmental variables, such as humidity and heat in different regions of the world (Belfort & Souza, 2014), other factors, such as the quality of basic sanitation and cultural dietary habits like consumption of undercooked meats or organic vegetables and other sociodemographic conditions may play a more significant role on toxoplasmosis prevalence (Rostami et al., 2019).

By using neonatal screening samples from private laboratories between 1995 and 2009, Eurico Neto et al. (2010) estimated the prevalence of congenital toxoplasmosis in all Brazilian States. For the States of Paraná and Rio Grande do Sul, the prevalence was 4/ 10,000 and 7/ 10,000, respectively, while the estimated prevalence of congenital toxoplasmosis in Santa Catarina was 10/ 10,000. It should be noted that the population of neonates tested in this

study came from private laboratories and may not be fully representative of the general population of newborns.

The low prevalence of congenital toxoplasmosis found in this study may have been influenced by early diagnosis and treatment of infected pregnant women or by the period of analysis. In the sample evaluated here, the annual prevalence of confirmed congenital toxoplasmosis varied between 0 and 2.31/ 10,000 live births. From 2000 to 2015, in Porto Alegre, Andrade et al. (2018) conducted a retrospective cross-sectional study and found variations between zero and five cases per year of confirmed congenital toxoplasmosis. Consequently, the annual prevalence ranged from 0 to 18.3/ 10,000 live births during the period under review.

Regarding laboratory confirmation, the IgG avidity test identifies the affinity between IgG anti-*T. gondii* antibodies and their target antigens, which progressively increase over months after activation of immunity against the disease (Brasil, 2018). It measures the binding strength between the antigen and antibody and classifies it as high, moderate, or low avidity (Emelia et al., 2014). This analysis constitutes a critical complementary exam for evaluating pregnant women suspected of having an acute infection, being part of the disease investigation and notification protocol (Brasil, 2018).

In pregnant women with IgM and IgG anti-*T. gondii* reagent before the 16th week of pregnancy, IgG avidity can be used to safely differentiate between acute infection in the ongoing pregnancy and infection before pregnancy, which poses a reduced risk to the fetus (Brasil, 2022). According to the Gestational and Congenital Toxoplasmosis Notification and Investigation Protocol (Brasil, 2018), the presence of a high IgG anti-*T. gondii* avidity result prior to 16 weeks of gestation discards the suspicion of gestational toxoplasmosis.

The 16-week limit for the avidity test is often unmet in the diagnostic routine due to unawareness of pregnancy, difficulties in scheduling prenatal care, or delays in the flow of tests performed in the public health system. In personal communication with EVS professionals, it was inferred that such logistics require effective coordination between the Brazilian Single Health System (SHS) and the laboratories involved.

In this study, there was a relevant and progressive increase in discarded notifications during the study period. In 2019, none of the 11 cases of gestational toxoplasmosis were discarded; in 2020, 16 (47%) of the 34 cases were discarded, while in 2021, 33 (75%) out of 44 suspected cases were discarded.

Consequently, in the evaluated sample, the avidity test discarded the suspicion of toxoplasmosis in 77.97% of pregnant women, reaching a discard rate of 82.1% for those who underwent the test in the first trimester of pregnancy. This increase in the number of discarded cases may result from a recent change in the routine of laboratory tests for toxoplasmosis in Blumenau. Before 2020, the test was performed after payment approval by a

single laboratory located in a distant neighborhood from the central region of the city, which likely reduced adherence to sample collection due to distance and travel time, which were often unfeasible for pregnant women residing in other neighborhoods. Furthermore, the patient had to wait for SHS approval for the test to be performed, which usually did not leave enough time for testing, especially in cases of pregnancies close to 16 weeks. In these cases, the delay in conducting the test would make it challenging to utilize the avidity test as a discarding tool for suspected cases (Brasil, 2022).

Then, in 2020, the avidity test started receiving prior approval to be performed; whenever a positive IgM sample from a pregnancy with less than 16 weeks was detected, the laboratory could use the same sample collected for IgM and IgG measurement to perform avidity testing. This was made possible by the municipal hiring a laboratory through a bidding process with multiple collection points, streamlining the prior approval for the tests to be conducted. Thus, suspected pregnant women began to undergo the avidity test in a timely manner, allowing the municipal surveillance service to optimize toxoplasmosis diagnosis.

With this information, it was possible to understand the sudden increase in notifications and discards from 2020 onwards. The exclusion of suspected cases allows the suspension of treatment medication, preventing mothers and newborns from being exposed for extended periods of time to possible side effects of the drugs. Economically, despite increased costs for laboratory tests, implementing the avidity test in the protocol has previously been shown to reduce overall expenses, especially in medication prescriptions (Margonato et al., 2007).

Regarding the trimester of investigation and diagnosis, it was observed that 45.6% of pregnant women were assessed in the first trimester and 24.4% in the second trimester. This shows good adherence to prenatal care compared to other Brazilian regions, such as Mato Grosso do Sul, where 45.8% of pregnant women were diagnosed in the second trimester (Figueiró-Filho et al., 2005). It is important and desirable for this rate to be even higher, allowing for better control and earlier identification of discards, preventing unnecessary medication use.

The absence of a statistical relationship between the patients' education and early diagnosis does not corroborate with the literature. Another study conducted in Blumenau showed that 69.2% of pregnant women treated by SHS in the city had no basic knowledge about the disease and its transmission, and 26.9% of them did not have toxoplasmosis serology results recorded in their prenatal care booklet (Kohler et al., 2022). Similarly, a study conducted in the city of Niterói in the State of Rio de Janeiro showed that 57.3% of pregnant women did not know about the protozoan infection (Moura et al., 2016). The importance of well-executed prenatal care is evident, encouraging

correct examination, attendance at appointments, and proper education about the disease and its prevention in susceptible pregnant women.

Therefore, despite the low prevalence of gestational and congenital toxoplasmosis in the city compared to other regions of Brazil, it is clear how knowledge about the disease and its correct guidance and assessment during prenatal care are still lacking. Given the data collected during this study period, the importance of maintaining measures that facilitate the carrying out of screening and confirmation exams is highlighted, ensuring that these pregnant women have their acute infection suspicion either promptly discarded or that their treatment is instituted early to prevent vertical transmission.

As a recent advancement in congenital toxoplasmosis diagnosis, serological tests for congenital toxoplasmosis are now included within the recent expansion of the heel prick test and provided as a screening test for all newborns (Brasil, 2021), and yet the interpretation of these tests continues to be challenging. Neonatal IgM/IgA testing lacks sensitivity, being non-reagent in 25% of infected newborns aged two to five days (Brasil, 2014); as for IgG, in the majority of cases, confirmation through this method relies on periodic monitoring of IgG titers through the first year of life (Brasil, 2018), despite these difficulties, the implementation of congenital toxoplasmosis screening seems to be a positive advancement, especially for newborns born to mothers who did not undergo prenatal screening.

The current treatment of gestational and congenital toxoplasmosis involves the combined use of medications for an extended period. Thus, while research has not yet been carried out to assess the degree of adherence of pregnant women and mothers to the treatment of gestational and congenital toxoplasmosis in Brazil, it is understood that shared monitoring between the high-risk prenatal care and the basic health unit is essential to encourage adherence and control the adverse effects of treatment.

Finally, it is crucial to reinforce the need for continuous monitoring of prevalence rates in the city through new studies and frequent reassessment so that new measures can be taken to reduce infection rates by this zoonotic protozoan.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest to disclose.

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