Title: Evaluation of implementation of the primary, secondary and tertiary prevention measures of the Surveillance Program of Gestational and Congenital Toxoplasmosis in the city of Londrina-PR

Running Title: Program of Toxoplasmosis

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Abstract

Toxoplasmosis acquired during pregnancy is a serious disease. Thus, the objective was to evaluate the Toxoplasmosis Surveillance Program Acquired in Pregnant and Congenital 12 years after its implementation in the city of Londrina-PR. 424 pregnant women were interviewed regarding their knowledge of prevention measures in 2019. Secondary prevention measures were assessed on the results of anti-*Toxoplasma gondii* serological tests were collected from pregnant women, from 2015 to 2018. the tertiary prevention measures, we screened the babies of mothers who had recent suspected infections of *T. gondii* to verify the referrals to the reference service. As for the knowledge of pregnant women, 45.5% (192/424) reported that they received guidance from health professionals, however, only 35.4% (68/192) changed their risky habits. The variables education and age, prior guidance from health professionals and feline size were significant when associated with the notions of preventive measures. The retrospective analysis of assistance to pregnant women in the city, demonstrated the effectiveness of the program in terms of coverage and monitoring of the pregnant woman and her baby, since 90.2% (17,423 / 19,319) of pregnant women in the public health system underwent serology to detect the anti-T. gondii antibodies and 86.2% (225/261) performed the avidity test as prescribed in the protocol. On the other hand, there was an excess of requests for tests and medications for cases without clinical indication. women with suspected acute infection, it was observed that 40.6% (26/64) of the children were referred to the referral hospital. It is concluded that the execution of the Toxoplasmosis Program has positive results regarding the performance of serological screening in prenatal care, however it needs to improve the conduction and dissemination of knowledge regarding the diagnosis and prevention of toxoplasmosis and more active surveillance action to monitor women pregnant women with suspected or confirmed acute toxoplasmosis.

Keywords: *Toxoplasma gondii*, prenatal care, health education.

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Introduction

Toxoplasmosis, a cosmopolitan disease caused by the protozoan, *Toxoplasma gondii*, affects countless species of warm-blooded animals, including man (Dubey, 2010). While the majority of infected individuals (around 80-90%) are asymptomatic (L. de Moura et al., 2006), infection with the parasite has serious consequences in women who acquire the infection for the first time during pregnancy; depending on the gestational period of the infection and the mother's immune response; the fetus can be compromised with varying degrees of severity (Varella, 2007).

The main manifestations observed in the fetuses are chorioretinitis, hydrocephalus or microcephaly, brain calcifications, and neurological changes. Such signs were first described by Sabin em 1942 and are known as Sabin's tetrad. In addition, other aggravating factors such as abortion, neonatal death, premature birth, fetal growth restriction, hematological changes, and neurocognitive development deficits have also been observed (Montoya & Remington, 2008).

The health consequences for those infected with congenital toxoplasmosis, together with its socioeconomic impact on the population, created an impetus for the development of preventive disease prevention strategies, based on primary, secondary, and tertiary prevention measures. Primary prevention measures reduce the risk of exposure of pregnant women to the parasitic agent through the dissemination of information about the risk factors for the disease. Secondary prevention consists of the early identification of pregnant women in the acute phase of the infection and their subsequent treatment. Tertiary prevention consists of the diagnosis and treatment of children with congenital toxoplasmosis (Cook et al., 2000a; Foulon, Naessens, & Ho-Yen, 2000).

Such strategies are not uniform across countries or even within a country. France (Thulliez, 1992) adopts the maternal screening program, while Denmark and Poland employ strategies based on neonatal screening (Lebech et al., 1996; Paul, Petersen, Pawlowski, & Szczapa, 2000). The United Kingdom (Gilbert & Peckham, 2002) does not have a universal serological screening program. In the city of Londrina, Paraná, since 2006, the "Program for the Monitoring of Acquired and Congenital Toxoplasmosis" is in effect. It is based on serological screening during prenatal care, primary prevention measures, and the early diagnosis and treatment of both pregnant women and children (Mitsuka-Breganó et al., 2010).

This study aimed to evaluate the implementation of the primary, secondary, and tertiary measures of the Surveillance Program of Gestational and Congenital Toxoplasmosis.

Material and methods

This study was approved by the Human Research Ethics Committee of the State University of Londrina (approval number: 3215784) on March 22, 2019.

Assessment of primary prevention measures

A cross-sectional study was carried out using pregnant women who received prenatal care in the 42 Basic Health Units (BHU) in the urban region of Londrina, between April and November 2019. The municipality is located in the northern region of the state of Paraná (Figure 1). The sample size calculation was performed for an expected prevalence of 50%, error of 5%, and confidence level of 95%, giving a required sample size of at least 363 pregnant women (Raosoft®).

The pregnant women were selected according to the order of their arrival at each BHU for a prenatal consultation. The number of women selected was proportional to the demand for care at each BHU. Data was obtained from individual interviews, in which a semi-structured epidemiological questionnaire was used, with questions about family income, basic sanitation habits, education levels, and eating and hygiene habits. In addition, their knowledge of information on the prevention of toxoplasmosis was assessed; reporting knowledge of one or more correct preventive measure for the prevention of toxoplasmosis in women was considered as knowledge. To assess the effectiveness of the guidelines on changing risky behaviors, only those who had demonstrated risky behavior and who had already undergone the first prenatal consultation were considered.

Assessment of secondary prevention measures

The identification of pregnant women in the acute phase of the infection was carried out for all pregnant women who underwent prenatal care at the BHU, from January 2015 to December 2018 using the municipality's WebSaúde platform; this platform collected all data regarding the date of birth, address, serological tests for toxoplasmosis, and requests for the drugs spiramycin, pyrimethamine, and sulfadiazine.

The pregnant women were classified into four groups according to the results obtained from the serological tests for toxoplasmosis and analyzed according to the protocol specified in the Manual of Gestational and Congenital Toxoplasmosis (Mitsuka-Breganó et al., 2010).

In addition, to verify the existence of a distribution pattern, an analysis of the spatial distribution of the places of residence of pregnant women of different immunological status

was carried out, categorizing these women into three groups (1) those suspected of acute infection - reagents IgG and IgM antibodies, (2) those who were chronically infected - reactive IgG and non-reactive IgM, and (3) – those who were susceptible to infection - non-reactive IgG and IgM.

Assessment of tertiary prevention measures

In this phase, the referral of children born to mothers with suspected or confirmed toxoplasmosis (Group 1) was evaluated by analyzing the medical records obtained at the reference service (Hospital Universitário de Londrina) from January 2015 to December 2018.

Statistical analysis

The data obtained from the interviews and the database of the WebSaúde platform were analyzed using the Epi Info7 software (CDC-Atlanta). Chi-square or Fisher's exact tests and multiple logistic regressions with a significance level of 5% were employed. The odds ratio with a 95% confidence interval was used as a measure of association.

The areas of residence of the pregnant women were mapped using the MMQGIS plugin of the Qgis 3.1 software, with which a kernel density analysis was also carried out.

Results

Primary prevention measures

A total of 424 pregnant women, with a median age of 27-years (ranging from 15 to 44-years), were interviewed. Of these, 45.3% (192/424) reported having received **1.** guidance on measures to prevent toxoplasmosis from health professionals with the remaining receiving advice after the interview. The most cited preventive measures were adequate washing of fruits and vegetables, 48.0% (92/192); no ingestion of undercooked or raw meats, 39.1% (75/192); and avoiding contact with cat waste, 36.0% (69/192). However, of this group of respondents, 19.3% (37/192) said that avoiding direct contact with cats was also mentioned and 24.5% (47/192) did not remember any guidance. These results are shown in Table 1.

All the pregnant women interviewed reported having one or more risky habits such as handling sand or earth, 9.4% (40/424); eating raw or undercooked meat, 44.6% (189/424); poorly cleaned fruits and vegetables, 98.6% (418/424); and raw milk, cheese, and fresh sausage, 72.1% (306/424).

Of the pregnant women who received guidance from the health team, only 35.4% (68/192) indicated that they had changed their habits, with 61.8% (42/68) having stopped eating undercooked meat or raw, 20.6% (14/68) having improved the hygiene of fruits and

vegetables, 4.4% (3/68) avoiding being in gardens, and 13.2% (9/68) having changed two or more risky behaviors.

For the performance of anti-*T. gondii* serological examination, 74.5% (316/424) reported having taken the test, 17.6% (74/424) did not remember, and 8.0% (34/424) reported not having taken the exam, while 85.4% (362/424) indicated that they had not had toxoplasmosis.

Secondary prevention measures

A total of 19,319 pregnant women who underwent prenatal care in the public health service from January 2015 to December 2018 in the city of Londrina were identified. Of these, only 1,896 (9.8%) did not undergo serological tests for toxoplasmosis. The analysis of the need for these tests is described in Figure 2.

An IgG antibody seropositivity of 42.6% (7,415 / 17,423) was observed, which was associated with age over 19 years and place of residence in the rural region (Table 2).

Requests for medication for the treatment of T. gondii infections were made by 102 pregnant women, of whom 4.0% (4/102) did not have any anti-*T. gondii* serological results registered in the health database, 18.6% (19/102) had non-reactive IgG and IgM, 19.6% (20/102) had reactive IgG and non-reactive IgM, 57.8% (59/102) had IgM and IgG reagents, and 22.0% (13/59) had IgG with high avidity, 18.6% (11/59) had IgG with intermediate avidity, and 59.3% (35/59) had IgG with low avidity. Of the 64 pregnant women with low or intermediate avidity, 18 (28.1%) had no record of medication requests.

Figure 3 shows the analysis of the Kernel density of the spatial distribution of the areas of residence of the pregnant women according to their serological status (IgG and IgM). Of the 19,319 pregnant women, only 11,200 pregnant women in the urban area were geocoded due to the lack of availability of their complete addresses.

Tertiary prevention measures

Of the 64 pregnant women with a suspected or confirmed diagnosis of acute infection by *T. gondii* (IgG and IgM reagents with low or intermediate avidity), only 40.6% (26/64) of the children born to these mothers, registered at the reference service (University Hospital of Londrina). As for the other children, it was not possible to identify the situation because it is not known whether these mothers delivered the child in the municipality or not.

Discussion

Since there is no vaccine and treatment is not completely effective, health professionals play a fundamental role in the primary prevention of toxoplasmosis, as they are responsible for

providing health education to pregnant women, providing guidance on preventive measures against infection during prenatal care as a strategy to reduce the risk of exposure to the agent (Conyn-Van Spaendonck & van Knapen, 1992; Martins, Dantes, Almeida, & Santos, 2012). In this study, this role was highlighted, since the guidelines provided by the health team were a significant factor in increasing the knowledge about preventive measures against toxoplasmosis among pregnant women.

However, less than half (45.5%) of the respondents reported having received guidance from doctors and/or nurses, a result similar to the study carried out in the municipality of Cascavel, state of Paraná (PR), where 46.5% respondents reported having received any guidance (n = 330) (Contiero-Toninato et al., 2014). In Niterói, the state of Rio de Janeiro (RJ), the percentage of pregnant women who received orientation was even lower, with only 27.8% reporting having received guidance (n = 289) (Millar et al., 2014). This difference may be associated with the implementation of a Gestational and Congenital Toxoplasmosis Surveillance Program based on the protocol of the municipality of Londrina, in the municipality of Cascavel, in 2007. In addition, the high turnover of public health professionals and the lack of interest or time on their part toward providing more detailed care can negatively influence the orientation of these patients (Pawlowski et al., 2001).

With regard to changing risky habits, only 35.4% of women reported a change in their habits after receiving the guidelines, demonstrating that knowledge alone does not translate into hygienic sanitary and food habits. A study carried out in Belgium, for 12 consecutive years, observed a 63.0% reduction in seroconversion of acute infections after the establishment of a health education program on toxoplasmosis (Foulon, Naessens, & Derde, 1994). In this instance, it was assumed that the effectiveness of the primary prevention was linked to the continued awareness of the population, impacting both current behavioral changes and future generations (Bobić, Nikolić, Klun, & Djurković–Djaković, 2011). Thus, changes are needed in the way the health team promotes the guidelines emphasizing the consequences of congenital toxoplasmosis in the child's life

Of the participants who were instructed, the majority mentioned correct preventive measures in relation to toxoplasmosis and only a small portion (19.4%) mentioned avoiding contact with cats. However, Cook (Cook et al., 2000b) and Lopis-Mori (Lopes-Mori et al., 2013) highlighted that contact with places contaminated with cat feces, rather than contact with cats themselves, is a risk factor for infection, since the self-cleaning habit of this species

eliminates hair oocysts before their sporulation (Dubey, 1995), Thus, there is a need for the reinforcement of periodic training of health professionals so that correct information is provided to pregnant women.

The socio-demographic condition of a population is strongly linked to toxoplasmosis, since low income and education have been identified as risk factors for infection by several authors (Andiappan et al., 2014; Costa et al., 2012; da Silva, Vinaud, & de Castro, 2015). In the present study, it was observed that such variables influenced the lack of knowledge about the preventive measures of infection. In this context, investments in education can contribute to the development of health; therefore, the rise in the educational level becomes a protective factor not only against toxoplasmosis, but also against other diseases (J. B. Avelar et al., 2018; M. v Avelar et al., 2017; Passos et al., 2018).

Another association observed was with respect to age, with those over 19 years of age having greater knowledge compared to those under 19 years of age. Corroborating research was carried out by Moura (F. L. de Moura et al., 2016) in the city of Niterói, RJ. With regard to the evaluation of the knowledge of Polish pregnant women, young women (under 19 years old) were the ones with the most information (Smereka et al., 2018). The discrepancy between these results may be linked to the profiles of the socially-vulnerable young population in the present study, who experience socioeconomic, cultural, and political challenges (Ayres, Júnior, Calazans, & Filho, 2012). Thus, the study reinforces the need for doctors and nurses to intensify instructions on preventive measures in women under 19-years old.

With respect to secondary prevention measures, it was observed that almost all pregnant women (90.2%) had undergone serological tests for toxoplasmosis. Of those classified as susceptible (non-reactive IgG and IgM), 50% of the women repeated the serological test and 80.2% with suspected acute infection (IgG and IgM reagents) performed the avidity test according to the protocol, revealing that the Gestational and Congenital Toxoplasmosis Surveillance Program in the city of Londrina-PR has excellent coverage and good monitoring of its patients. In contrast, a 44.4% increase in the repetition of serological tests was detected in the categories of women with chronic infection (IgG reagent and IgM non-reagent) and suspected acute infection (IgG and IgM reagent), resulting in an unnecessary expenditure of public money. Thus, reviews of the program's implementation should be made by health professionals to minimize these errors.

No information was obtained regarding the ordering of medication for women with suspected acute infections; however, requests for medication for cases without clinical indications were

observed. It is known that both situations can be harmful to the pregnant woman and the fetus, as the absence of adequate provision of therapy during pregnancy increases the risk of sequelae in the fetus, causing neurological changes such as diffuse brain calcifications, chorioretinitis, seizures, hydrocephalus, or microcephaly (Wilson, Nizet, Maldonado, Remington, & Klein, 2015) However, despite the benefits that treatment offers, the drugs have a potential for toxicity. Pyrimethamine causes the gradual depression of the bone marrow hematopoietic system and is also teratogenic in the first trimester (Remington, Klein, Wilson, & Baker, 2007), which makes treatment in patients without clinical indications extremely risky for the health of both the pregnant woman and her baby. From this perspective, a more effective surveillance system can reduce these errors by following up on all suspected or confirmed cases.

Tertiary prevention measures ensure that all children born to mothers with suspected or confirmed acute toxoplasmosis are referred to the referral service. In this study, the low numbers indicate that either children were referred to another service besides the University Hospital, or there was a failure in their targeting processes. In the latter case, the situation can be serious, as studies show that treatment during the first year of life is essential to reduce the sequelae caused by the infection, as observed in the study by Phan (Phan et al., 2008), in which more than 70% of the children developed new eye injuries due to lack of treatment. Thus, the treatment and monitoring of children are essential to reduce the consequences of toxoplasmosis, contributing to an improvement in their quality of life.

In the retrospective analysis of the pregnant women seen at the public health service in the city, age was a significant factor for seropositivity to anti-*T. gondii* IgG, and adult pregnant women (> 19 years old) were 1.48 times more likely to be seropositive than young pregnant women (≤ 19 years old). Similar results were found in Ivaiporã, PR, and Porto Alegre, state of Rio Grande do Sul. Such an association may be related to the greater exposure to the parasitic agent with age, since the chance of being infected over the years increases (Engroff et al., 2014; Mareze et al., 2019). It was also observed that pregnant women who live in rural areas were more likely to become infected when compared to pregnant women living in urban areas. The habits present in the rural area can favor contact with the agent. Research carried out in the states of Rio de Janeiro, Minas Gerais, and Paraná show soil manipulation and the consumption of unfiltered water, recurrent habits in rural life, as important risk factors associated with toxoplasmosis (Bahia-Oliveira et al., 2003; Carellos et al., 2014; Lopes-Mori

et al., 2013). Thus, prevention measures adapted to the reality of the rural population need to be considered when planning strategies for the control of congenital toxoplasmosis.

In the analysis of kernel density, the distribution of pregnant women with acute (reactive IgG and IgM), chronic (IgG reactive and IgM non-reactive), and susceptible (non-reactive IgG and IgM) infection in the urban area was homogeneous, and it was not possible to obtain evidence for any region in the municipality with significantly higher distribution in the groups studied. This was similar to a study by Benitez (Benitez et al., 2017) regarding the seroprevalence of anti-*T. gondii* antibodies in the population of dog owners in Londrina, which showed no difference between regions. It should be noted that the same analysis was not possible for the rural area due to insufficient data.

In addition, there is evidence that the prevalence of toxoplasmosis in Londrina has been decreasing in recent years. When comparing its prevalence in the present study (42.6%) with serological surveys carried out in previous years involving the urban and rural regions, we observed a 56.6% prevalence in 2003 and a 49.2% prevalence in 2006 (Lopes et al., 2009; Mandai, Lopes, & Mitsuka-Breganó, 2007). This decrease may be associated, among other factors, with the increase in the consumption of frozen meat by the population. When subjected to temperatures of -10 °C for 3 days or at -20 °C for 2 days existing cysts can be deactivated (Dubey & Jones, 2008; El-Nawawi, Tawfik, & Shaapan, 2008; Kijlstra & Jongert, 2008), In addition to the improvement of livestock systems, reducing tissue cysts in meat through good management practices and, consequently, the prevalence of toxoplasmosis, has been observed in the prevalence studies carried out on pigs on farms in Londrina (Tsutsui et al., 2003; Vidotto, Navarro, Mitsuka, & Freire, 1990).

The constant evaluation of the program to identify and correct weaknesses is extremely important, so that it remains effective and efficient for each reality. It is noteworthy that the implementation of the program promotes the standardization of conduct, saving of resources, and the improvement of the health of the population, especially with respect to toxoplasmosis, has been reported (Mitsuka-Breganó et al., 2010).

Conclusion

It can be concluded that the implementation of the Program for the Monitoring of Acquired and Congenital Toxoplasmosis has yielded positive results regarding the delivery and dissemination of knowledge. However, it also presented some flaws with respect to the primary preventive measures, regarding the awareness of pregnant women to changes in risky

habits; to the secondary prevention measures regarding the process of requesting serological tests; and to the tertiary prevention measures regarding the problems in referring patients at risk to the reference service. Thus, to minimize the identified challenges and errors, regular training workshops for health professionals and the resumption of surveillance actions may be necessary.

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Conflict of interest statement

Veterinarian, specialist in parasitic diseases and a master in animal science from London State University, Paraná, Brazil.

References

Andiappan, H., Nissapatorn, V., Sawangjaroen, N., Khaing, S.-L., Salibay, C. C., Cheung, M. M. M., … Mat Adenan, N. A.
(2014). Knowledge and practice on Toxoplasma infection in pregnant women from Mala ysia, Philippines, and Thailand. *Frontiers in Microbiology*, *5*(JUN), 291. https://doi.org/10.3389/fmicb.2014.00291

Avelar, J. B., da Silva, M. G., Rezende, H. H. A., Storchilo, H. R., do Amaral, W. N., Xavier, I. R., … de Castro, A. M.
(2018). Epidemiological factors associated with toxoplasma gondii infection in postpartu m women treated in the public healthcare system of goiânia, state of Goiás, Brazil. *Revista Da Sociedade Brasileira de Medicina Tropical*, *51*(1), 57–62. https://doi.org/10.1590/0037-8682-0112-2017

Avelar, M. v, Martinez, V. O., de Moura, D. L., Barros, I. A., Primo, A. A. da S., Duarte, A. O., … Lima, F. W. de M. (2017). Association between seroprevalence of IgG anti-Toxoplasma gondii and risk factors for infection among pregnant women in climério de

oliveira maternity, Salvador, Bahia, Brazil. *Revista Do Instituto de Medicina Tropical de Sao Paulo*, *59*. https://doi.org/10.1590/S1678-9946201759090

Ayres, J. R. de C. M., Júnior, I. F., Calazans, G. J., & Filho, H. C. S. (2012). O conceito de Vulnerabilidade e as Práticas de Saúde. In *Promoção da saúde: conceitos, reflexões, tendência* (2nd ed., pp. 1–176). Rio de Janeiro: FIOCRUZ.

Bahia-Oliveira, L. M. G., Jones, J. L., Azevedo-Silva, J., Alves, C. C. F., Oréfice, F., & Addiss, D. G. (2003). Highly endemic, waterborne toxoplasmosis in North Rio de Janeiro State, Brazil. *Emerging Infectious Diseases*, *9*(1), 55–62. https://doi.org/10.3201/eid0901.020160

Benitez, A. do N., Martins, F. D. C., Mareze, M., Santos, N. J. R., Ferreira, F. P., Martins, C. M., … Navarro, I. T.
(2017). Spatial and simultaneous representative seroprevalence of anti-Toxoplasma gond ii antibodies in owners and their domiciled dogs in a major city of southern Brazil. *PLOS ONE*, *12*(7), e0180906. https://doi.org/10.1371/journal.pone.0180906

Bobić, B., Nikolić, A., Klun, I., & Djurković–Djaković, O.
(2011, September 22). Kinetics of Toxoplasma infection in the Balkans. *Wiener Klinisch e Wochenschrift*, *123*(SUPPL. 1), 2–6. https://doi.org/10.1007/s00508-011-0052-6

Carellos, E. V. M., de Andrade, G. M. Q., Vasconcelos-Santos, D. v, Januário, J. N., Romanelli, R. M. C., Abreu, M. S., … Caiaffa, W. T. (2014).
Adverse socioeconomic conditions and oocyst-related factors are associated with congen ital toxoplasmosis in a population-based study in Minas
Gerais, Brazil. *PLoS ONE*, *9*(2). https://doi.org/10.1371/journal.pone.0088588

Contiero-Toninato, A. P., Cavalli, H. O., Marchioro, A. A., Ferreira, É. C., Caniatti, M. C. da C. L., Breganó, R. M., … Falavigna-Guilherme, A. L.
(2014). Toxoplasmosis: An examination of knowledge among health professionals and p regnant women in a municipality of the State of Paraná. *Revista Da Sociedade Brasileira de Medicina Tropical*, *47*(2), 198–203. https://doi.org/10.1590/0037-8682-0016-2014

Conyn-Van Spaendonck, M. A., & van Knapen, F.
(1992, January 1). Choices in preventive strategies:
Experience with the prevention of congenital toxoplasmosis in the Netherlands. *Scandin avian Journal of Infectious Diseases*, *23*(84), 51–58.

Cook, A. J. C., Gilbert, R. E., Buffolano, W., Zufferey, J., Petersen, E., Jenum, P. A., … Dunn, D. T.

(2000a). Sources of toxoplasma infection in pregnant women: European multicentre case -control study. *British Medical Journal*, *321*(7254), 142–147. https://doi.org/10.1136/bmj.321.7254.142

Cook, A. J. C., Gilbert, R. E., Buffolano, W., Zufferey, J., Petersen, E., Jenum, P. A., … Dunn, D. T.

(2000b). Sources of toxoplasma infection in pregnant women: European multicentre case -control study. *British Medical Journal*, *321*(7254), 142–147. https://doi.org/10.1136/bmj.321.7254.142

Costa, F., Soares G, A., de Lima, M., Braga, J., Vieira, L. J. E. S., & Araújo, M. L.

(2012). Preventive behavior for toxoplasmosis in pregnant adolescents in the state of Cea ra, Brazil. *BMC Public Health*, *12*(1), 73. https://doi.org/10.1186/1471-2458-12-73

da Silva, M. G., Vinaud, M. C., & de Castro, A. M.

(2015). Prevalence of toxoplasmosis in pregnant women and vertical transmission of Tox oplasma gondii in patients from basic units of health from Gurupi, Tocantins, Brazil, from 2012 to 2014. *PLoS ONE*, *10*(11). https://doi.org/10.1371/journal.pone.0141700

de Moura, L., Bahia-Oliveira, L. M. G., Wada, M. Y., Jones, J. L., Tuboi, S. H., Carmo, E. H., … Garrett, D. O.

(2006). Waterborne toxoplasmosis, Brazil, from field to gene. *Emerging Infectious Disea ses*, *12*(2), 326–329. https://doi.org/10.3201/eid1202.041115

Dubey, J. P.

(1995). Duration of immunity to shedding of Toxoplasma gondii oocysts by cats. *Journa l of Parasitology*, *81*(3), 410–415. https://doi.org/10.2307/3283823

Dubey, J. P. (2010). *Toxoplasmosis of Animals and Humans* (2nd ed.). United States: CRC Press.

Dubey, J. P., & Jones, J. L. (2008, September).

Toxoplasma gondii infection in humans and animals in the United States. *International Journal for Parasitology*, Vol. 38, pp. 1257–1278. Int J Parasitol. https://doi.org/10.1016/j.ijpara.2008.03.007

El-Nawawi, F. A., Tawfik, M. A., & Shaapan, R. M.

(2008). Methods for inactivation of Toxoplasma gondii cysts in meat and tissues of exper imentally infected sheep. *Foodborne Pathogens and Disease*, *5*(5), 687–690. https://doi.org/10.1089/fpd.2007.0060

Engroff, P., Ely, L. S., Guiselli, S. R., Goularte, F. H., Gomes, I., Viegas, K., & de Carli, G. A. (2014). Soroepidemiologia de toxoplasma gondii em idosos atendidos pela estratégia saúde da família, Porto alegre, Rio Grande do sul, Brazil. *Ciencia e Saude Coletiva*, *19*(8), 3385–3394. https://doi.org/10.1590/1413-81232014198.12402013

Foulon, W., Naessens, A., & Derde, M. P. (1994). Evaluation of the Possibilities for Preventing Congenital Toxoplasmosis. *Americ an Journal of Perinatology*, *11*(01), 57–62. https://doi.org/10.1055/s-2007-994537

Foulon, W., Naessens, A., & Ho-Yen, D. (2000). Prevention of congenital toxoplasmosis. *Journal of Perinatal Medicine*, *28*(5), 337–345. https://doi.org/10.1515/JPM.2000.043

Gilbert, R. E., & Peckham, C. S. (2002). Congenital toxoplasmosis in the United Kingdom: To screen or not to screen? *Journal of Medical Screening*, *9*(3), 135–141. https://doi.org/10.1136/jms.9.3.135

Kijlstra, A., & Jongert, E. (2008, October). Control of the risk of human toxoplasmosis transmitted by meat. *Intern ational Journal for Parasitology*, Vol. 38, pp. 1359–1370. Int J Parasitol. https://doi.org/10.1016/j.ijpara.2008.06.002

Lebech, M., Joynson, D. H. M., Seitz, H. M., Thulliez, P., Gilbert, R. E., Dutton, G. N., … Petersen, E. (1996). Classification system and case definitions of Toxoplasma gondii infection in im munocompetent pregnant women and their congenitally infected offspring. *European Jo urnal of Clinical Microbiology and Infectious Diseases*, *15*(10), 799–805. https://doi.org/10.1007/BF01701522

Lopes, F. M. R., Mitsuka-Breganó, R., Gonçalves, D. D., Freire, R. L., Karigyo, C. J. T., Wedy, G. F., … Navarro, I. T. (2009). Factors associated with seropositivity for anti-*Toxoplasma gondii* antibodies in pregnant women of Londrina, Paraná, Brazil. *Memorias Do Instituto Oswaldo Cruz*, *104*(2), 378–382. https://doi.org/10.1590/S0074-02762009000200036

Lopes-Mori, F. M. R., Mitsuka-Breganó, R., Bittencourt, L. H. F. de B., Dias, R. C. F., Gonçalves, D. D., Capobiango, J. D., … Navarro, I. T. (2013). Gestational toxoplasmosis in Paraná State, Brazil: Prevalence of IgG antibodies and associated risk factors. *Brazilian J*

ournal of Infectious Diseases, *17*(4), 405–409. https://doi.org/10.1016/j.bjid.2012.12.003

Mandai, O. N., Lopes, F. M. R., & Mitsuka-Breganó, R. (2007). Prevalência de anticorpos igG e igM anti-Toxoplasma gondii em gestantes atendidas nas unidades básicas de saúde do município de Londrina - Paraná, no período de 2003 e 2004. *Rev. Bras. Anal. Clin*, *39*(4), 247–249.

Mareze, M., Benitez, A. do N., Brandão, A. P. D., Pinto-Ferreira, F., Miura, A. C., Martins, F. D. C., … Navarro, I. T. (2019). Socioeconomic vulnerability associated to Toxoplasma gondii exposure in south ern Brazil. *PLOS ONE*, *14*(2), e0212375. https://doi.org/10.1371/journal.pone.0212375

Martins, J. S. A., Dantes, F. de A., Almeida, T. F., & Santos, M. B. R. (2012). A assistência de enfermagem no pré-natal: enfoque na estratégia da saúde da família | Martins | Revista Uniabeu.

Millar, P. R., de Moura, F. L., Bastos, O. M. P., de Mattos, D. P. B. G., Fonseca, A. B. M., Sudré, A. P., … Amendoeira, M. R. R. (2014). Conhecimento sobre toxoplasmose entre gestantes e puérperas atendidas na rede pública de saúde do município de Niterói, Rio de Janeiro, Brasil. *Revista Do Instituto de Medicina Tropical de Sao Paulo*, *56*(5), 433–438. https://doi.org/10.1590/S0036-46652014000500011

Mitsuka-Breganó, R., Lopes-Mori, F. M. R., Navarro, I. T., Casella, A. M. B., Reiche, E. M. v, Lago, E. G., … Narcis, S. G. (2010). *Toxoplasmose Adquirida na Gestação e Cogênita*. Londrina: Eduel . Retrieved from www.uel.br/editora

Montoya, J. G., & Remington, J. S. (2008). Management of Toxoplasma gondii Infection during Pregnancy. *Clinical Infectious Dise ases*, 554–566. https://doi.org/10.1086/590149

Moura, F. L. de, Goulart, P. R. M., Moura, A. P. P. de, Souza, T. S. de, Fonseca, A. B. M., & Amendoeira, M. R. R. (2016). Fatores associados ao conhecimento sobre a toxoplasmose entre gestantes atendidas na rede pública de saúde do município de Niterói, Rio de Janeiro, 2013-2015. *Epidemiologia e Servicos de Saude : Revista Do Sistema Unico de Saude Do Brasil*, *25*(3), 655–661. https://doi.org/10.5123/S1679-49742016000300022

Passos, A. D. C., Bollela, V. R., Furtado, J. M. F., de Lucena, M. M., Bellissimo-Rodrigues, F., Paula, J. S., … Rodrigues, M. de L. v. (2018). Prevalence and risk factors of toxoplasmosis among adults in

a small Brazilian city. *Revista Da Sociedade Brasileira de Medicina Tropical*, *51*(6), 781–787. https://doi.org/10.1590/0037-8682-0214-2017

Paul, M., Petersen, E., Pawlowski, Z. S., & Szczapa, J. (2000). Neonatal screening for congenital toxoplasmosis in the Poznan region of Poland by analysis of Toxoplasma gon dii-specific IgM antibodies eluted from filter paper blood spots. *Pediatric Infectious Dise ase Journal*, *19*(1), 30–36. https://doi.org/10.1097/00006454-200001000-00007

Pawlowski, Z. S., Gromadecka-Sutkiewicz, M., Skommer, J., Paul, M., Rokossowski, H., Suchocka, E., & Schantz, P. M.
(2001). Impact of health education on knowledge and prevention behavior for congenital toxoplasmosis:
The experience in Poznań, Poland. *Health Education Research*, *16*(4), 493–502. https://doi.org/10.1093/her/16.4.493

Phan, L., Kasza, K., Jalbrzikowski, J., Noble, A. G., Latkany, P., Kuo, A., … Mcleod, R. (2008). Longitudinal Study of New
Eye Lesions in Children with Toxoplasmosis Who Were Not Treated During the First Ye ar of Life. *American Journal of Ophthalmology*, *146*(3), 375. https://doi.org/10.1016/j.ajo.2008.04.033

Remington, J. S., Klein, J. O., Wilson, C. B., & Baker, C. J.
(2007). Infectious diseases of the fetus and newborn infant, 6th edn.
In *Archives of Disease in Childhood - Fetal and Neonatal Edition* (Vol. 92). BMJ. https://doi.org/10.1136/adc.2006.102566

Smereka, J., Szarpak, L., Ruetzler, K., Schacham, Y., Smereka, A., Dabrowski, M., … Adam, I. (2018).
A multicenter survey on toxoplasmosis knowledge among pregnant women in Poland (th e TOWER study). *BMC Pregnancy and Childbirth*, *18*(1), 389. https://doi.org/10.1186/s12884-018-2031-7

Thulliez, P. (1992). Screening programme for congenital toxoplasmosis in France. *Scandinavian Journal of Infectious Diseases*, *82*, 5–43. Retrieved from https://pubmed.ncbi.nlm.nih.gov/1290073/

Tsutsui, V. S., Navarro, I. T., Freire, R. L., Freitas, J. C., Prudencio, L. B., Delbem, A. C. B., & Marana, E. R. M. (2003). Soroepidemiologia e fatores associados à transmissão do Toxoplasma gondii em suínos do norte do
Paraná. *Archives of Veterinary Science*, *8*(2). https://doi.org/10.5380/avs.v8i2.4030

Varella, I. R. dos S. (2007). *Prevalência de toxoplasmose aguda em gestantes, incidência de toxoplasmose congênita e desempenho de testes diagnósticos em toxoplasmose congênita*. https://doi.org/http://hdl.handle.net/10183/11351

Vidotto, O., Navarro, I. T., Mitsuka, R., & Freire, R. L. (1990). Estudos epidemiológicos da toxoplasmose em suínos da região de Londrina – PR. *Semina: Ciências Agrárias*, *11*(1), 53. https://doi.org/10.5433/1679-0359.1990v11n1p53

Wilson, C., Nizet, V., Maldonado, Y., Remington, J., & Klein, J. (2015). *Remington and Klein's Infectious Diseases of the Fetus and Newborn Infant* (8th ed.). Retrieved from https://www.elsevier.com/books/remington-and-kleins-infectious-diseases-of-the-fetus-and-newborn-infant/wilson/978-0-323-24147-2

Table 1: Factors associated with knowledge about toxoplasmosis prevention measures reported by pregnant women (n = 424) attended by the public health service in the city of Londrina-PR, 2019.

Variables	Total n (%)	Has knowledge of preventive measures n (%)	Bivariate		Multiple logistic regression	
			p	OR (IC95%)	p	OR adjusted (IC95%)
Education level						
Up to 8 years	105 (24,8)	16 (15,2)	< 0,001		< 0,001	
Above 8	319 (75,2)	126 (39,5)		3,64 (2,04-6,49)		3,40 (1,84-6,30)
Income *						
Above 481,0	22 (5,3)	12 (54,5)	< 0,001		> 0,05	
Up to 481,0	126 (30,3)	55 (43,6)		0,65 (0,26-1,60)		
Up to 240,5	268 (64,4)	72 (26,9)		0,31 (0,13-0,74)		
Age						
Young (≤ 19 years)	47 (11,1)	7 (14,9)	< 0,001		> 0,05	
Adult (> 19 years)	376 (88,9)	134 (35,6)		3,18 (1,39-7,30)		
N° gestetion						
Primiparous	151 (35,7)	58 (38,4)	0,060		> 0,05	
Multiparous	272 (64,3)	84 (30,9)				
Pregnancy trimester						
First	39 (9,2)	16 (41)	0,096		> 0,05	
Second	157 (37)	60 (38,2)				
Third	228 (53,8)	66 (28,9)				
Has cats						
No	362 (85,4)	111 (30,7)	< 0,001		< 0,001	
Yes	62 (14,6)	31 (50)		2,28 (1,32-3,94)		2,98 (1,58-5,65)
Received guidance						
No	231 (54,5)	37 (16)	< 0,001		< 0,001	
Yes	193 (45,5)	105 (54,4)		5,92 (3,76-9,32)		6,11 (3,80-9,84)
Housing region						
Center	43 (10,1)	22 (51,2)	0,076		> 0,05	
North	131 (30,9)	45 (34,4)				
South	95 (22,4)	27 (28,4)				
East	79 (18,6)	24 (30,4)				
West	76 (17,9)	24 (31,6)				

* Table footnotes: coverage, 1 dollar for 4.32 reais. (income: value per capita, in class)

Table 2: Factors associated with the detection of anti-*T. gondii* IgG antibodies in pregnant who attended UBS between January 2015 and December 2018 in Londrina-PR.

Variables	Total n (%)	Reagents	No reagents	p	OR (IC 95%)
Age					
Young (≤ 19 years)	1.083 (7,8)	484 (44,7)	599 (55,3)		
Adult (> 19 years)	12.809 (92,2)	6976 (54,5)	5833 (45,5)	< 0,001	1,48 (1,31-1,68)
Housing region					
Urban	13.191 (95)	6035 (45,8)	7156 (54,2)		
Rural	701 (5)	397 (56,6)	304 (43,4)	< 0,001	1,55 (1,33-1,81)

Figure 1. Location of the city of Londrina, Paraná showing its respective districts and regions.

Figure 2. Algorithm for analyzing serological results for anti-*T. gondii* IgG and IgM in pregnant women who attended UBS between January 2015 and December 2018 in Londrina-PR.

Figure 3. Kernel density analysis of the spatial distribution of the areas of residence of the 11,200 pregnant women in the urban area who attended UBS between January 2015 and December 2018 in Londrina-PR, according to the group that they belonged to (suspected of acute infection, chronically infected and susceptible) based on their serological status (IgG and IgM).