

Toxoplasma gondii primary infection in pregnant women in Goiânia: a seroconversion study*

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Abstract

The purpose of this study was to test toxoplasmosis seroconversion in pregnant women in Goiânia-GO, a city in the Midwestern Region of Brazil. A prospective observational study of pregnant women vulnerable to *Toxoplasma gondii* was carried out using Indirect Immunofluorescent Antibody Test (IFAT) for serological screening and laboratorial surveillance of seroconversion. The immunoenzyme test (ELISA) was performed to confirm acute toxoplasmosis. The risk estimate used limits of reliability at 95% and the results were validated by χ^2 and RR tests. Acute infection found in the pregnant women participating in this study was 8.6% (45/522). Living in close contact with host animals and vehicles of oocyst transmission constituted statistical risks for pregnant women to acquire acute toxoplasmosis. This research revealed seroconversion (8.6%), pointing to the need of primary and secondary prevention for all pregnant women at risk (seronegative). This study originated a program for the protection of pregnant women in the state of Goiás.

Keywords: Seroconversion – Pregnant women – *Toxoplasma gondii*.

INTRODUCTION

Toxoplasmosis is a zoonosis widely spread in nature, caused by a coccidium protozoan, Toxoplasma gondii. Its importance lies in the possibility of transplacental transmission, which can cause abortion or congenital diseases, depending on the stage of organogenesis during which the mother acquires the infection. Damage to the fetus

during pregnancy depends on the infection prevalence among childbearing-aged women, on the animals these women eat, and on the level of contact between vulnerable pregnant women and the sources of protozoan transmission¹.

In most areas of the world, 40-60% of the population carries antibodies against this parasite, demonstrating previous exposure². The prevalence varies according to geographical region, weather

* Institutions associated with the research: Medical School of the Federal University of Goiás (FM-UFG), Institute of Tropical Pathology and Public Health of the Federal University of Goiás (IPTSP-UFG), National Foundation of Support to Research (FUNAPE), State Secretary of Health of Goiás, and Municipal Secretary of Health of Goiânia. Study accomplished between the years of 1997 to 1999, conducted in the Department of Pediatrics of the Health Science School, National University of Brasília-DF, Brazil, and concluded in Goiânia-GO, Brazil.

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characteristics, cultural factors, and food habits. Nevertheless, within the same region the infection can appear at different levels, as observed in São Paulo-SP³ and Goiânia-GO⁴. On the other hand, studies have shown: very low prevalence in Canada (0 to 20%), Thailand (3%), Japan (6%), and Australia (23%)⁵; low prevalence in Norway (10.9%)⁶, Stockholm (14%)⁷, London (18.8%)⁸, and São Paulo (32.4%)⁹; medium prevalence in Central Europe (40-60%)¹⁰, Salvador (42%)¹¹, and Porto Alegre (59.8%)¹²; high prevalence in Mato Grosso do Sul (92%)¹³, Rio de Janeiro (77.1%)¹⁴, El Salvador (77%)¹⁵, Paris (70%)¹⁶, and Goiânia (65.8%)⁴.

In different geographical regions, annual seroconversion rates range from 0.2 to 1.5%¹⁷⁻¹⁹. The incidence of infection increases with age and several studies have shown high prevalence among childbearing-aged women.

This study aimed at testing seroconversion in pregnant women in a city with high prevalence of toxoplasmosis among childbearing-aged women²⁰, even more important among pregnant women, who are more likely to develop this infection²¹.

MATERIAL AND METHODS

This longitudinal study, approved by the Ethics Committee of the Federal University of Goiás, was carried out between January 1997 and 1999, in Goiânia-GO, a city in the Midwestern Region of Brazil. The studied population was constituted by a group of 522 women identified via prenatal clinics and considered at risk of acquiring toxoplasmosis (seronegative). Women were considered pregnant when presenting delayed menstruation and confirmation by high blood level of β HCG.

Indirect Immunofluorescent Test (IFAT)²² was used for serological screening and laboratorial surveillance of seroconversion, and was prepared in the laboratory of the Department of Parasitology of the Institute of Tropical Pathology and Public Health of the Federal University of Goiás (IPTSP-UFG) using the conjugate of Biolab, the Fluoline G and M (France) and the strain RH of *T. gondii*. The presence of IgM specific for *T. gondii* was

confirmed by removing the rheumatoid factor from positive serum using reagents produced by Bio Merieux (France). Quality control was carried out by the laboratory of the Federal University of Goiás (IPTSP-UFG). Women seroconverting during the study period were submitted to a new serology using IFAT and the immunoenzyme test (ELISA) for confirmation²³. ELISA applied was produced by Clark (USA) for IgM and Salk (Brazil) for IgG, both prepared according to the manufacturers' instructions.

Pregnant women that did not present specific anti-*T. gondii* antibodies (IgG and/or IgM), identified by IFAT, were considered seronegative or at risk of acquiring acute toxoplasmosis during pregnancy. The following antibody titres were considered identifiers of previous exposure to *T. gondii*: IFAT IgG \geq 1/20 and IgM \geq 1/5. Furthermore, using ELISA, IgM was considered negative when the sample presented IRS \leq 0.90, which means IgM class anti-*T. gondii* antibodies were not detected. Values between 0.91 and 1.09 represented doubtful results and these samples were tested again using the same technique. When the doubtful results remained so, they were compared to the ones using IFAT in order to interpret them. For values \geq 1:10, the results were considered positive, corresponding to a significant level of detectable anti-*T. gondii* IgM.

The sensibility to identify IgG anti-*T. gondii* by IFAT was just 56.9%; specificity 98.85%; positive predictive value 71.42% and negative predictive value 97.85% in the study.

The sensibility to identify IgM anti-*T. gondii* by IFAT was just 59.6%; specificity 91.7%; positive predictive value 93.3% and negative predictive value 53.7% (laboratory of the Department of Parasitology of the Institute of Tropical Pathology and Public Health of the Federal University of Goiás - IPTSP-UFG)²⁴.

For women presenting specific anti-*T. gondii* antibodies during the surveillance period, new blood samples were collected within a period ranging from one to three months to confirm seroconversion using IFAT and ELISA. Also, the serology of these women's previous samples (seronegative and suspicious of seroconversion) were tested again using ELISA. Pregnant women were considered positive for acute toxoplasmosis when their initial serology was

confirmed as seronegative (IFAT and ELISA) and presented IgG class specific anti-*T. gondii* antibodies in increasing titres compared to the previous sample and/or IgM. Previously non-reactive women who acquired IgG antibodies (IFAT, ELISA) in increasing titres and/or the presence of IgM confirmed by ELISA (in all samples) were considered infected.

Essential criteria of inclusion in the study were pregnancy, vulnerability to the infection (seronegativity), and permission to collect subsequent blood samples to allow evaluation of seroconversion. Presence of immunity against the protozoan, lack of serological surveillance, and loss of their babies before the end of the proposed time of surveillance during this study were the criteria of exclusion from this research. During the study, 21 women (4%) were excluded: 6 due to abortion and 15 because their surveillance was not concluded.

Serology tests were performed within surveillance period monthly, bimonthly or trimonthly, depending on the gestation period of the woman when she started participating in this research. Thus, 11.1% of the infected pregnant women (5/45) were submitted to serological control five times during gestation; 40% (18/45) of them had four samples collected; 44.4% (20/45), three samples; and 4.4% (2/45) of them, just two samples. On the other hand, 22.2% (10/45) of the participants underwent the last serological control during the puerperal period so as to confirm a previously detected seroconversion not confirmed due to failure of the participant to return and repeat the test of suspicious seroconversion. Most part of the serological surveillance was possible through home visits that were necessary due to lack of awareness regarding the need to closely watch toxoplasmosis seroconversion among pregnant women at risk.

The following were considered potential risk factors for acute toxoplasmosis: environmental sanitation (water and sewage treatment); presence of host animals in the house (cats and dogs); contact with vehicles of oocyst transmission (flies, cockroaches, and rats); consumption of potentially contaminated food products (raw meat, raw eggs, inappropriately washed vegetables, and unpasteurized goat's milk); pica, geophagia, or inadequate soil handling; low level of formal

education (\leq four years); low family income (\leq two minimum salaries).

The behavior of the variables was validated by the χ^2 test using the presence of infection as a dependent variable (effect), a significance level at 5% and limits of reliability at 95%. The relative risk (RR) of the probable risk factors the women were exposed to during the study period was calculated for each analyzed risk factor, and for the statistics computation the age was considered in risk bands: adolescents; between 20 and 29 years; between 30 and 34 years; and over 35 years.

RESULTS

Table 1 shows the age distribution of the women studied and the percentage of seroconversion in each age group.

It can be observed in Table 2 that adolescents and women below 30 years living in contact with cats showed statistically significant risk to acquire the infection, the same happening to women between 20 and 30 years when in contact with rats.

Improper environmental sanitation, consumption of potentially contaminated food products, pica or geophagia, inadequate soil handling, low level of formal education, and low family income showed no statistically significant risk to acquire acute toxoplasmosis, except for women older than 30 with low family income ($p = 0.01$) (Table 3) or that consume unpasteurized goat's milk ($p = 0.005$) (Table 4).

Seroconversion rate was: 11.1% (5/45) in the first trimester of gestation, 57.8% (26/45) in the second, and 31.3% (14/45) in the third. Among the participants, 95.5% (43/45) presented increasing IgG titres and 55.5% (25/45), positive IgM. Permanence of IgM in the blood stream was transitory for most pregnant women analyzed: in 44% (11/25) of them it remained for less than 30 days; in 24% (6/25), it persisted for 2 months; in 20% (5/25) of them, for 3 months; in 4% (1/25), for 4 months; and in 4% (1/25), for 5 months; for one of the patients it was not possible to determine the time of permanence of IgM.

The rate of congenital transmission was calculated as 34.5/1,000, considering serological conversion for 45 pregnant women out of 522,

Table 1. Age distribution of the groups of pregnant women under study and its relation with acute toxoplasmosis in Goiânia-GO, Brazil.

Age (years)	Infected		Non-infected		Total	
	N	%	N	%	N	%
<20	21	9.37	203	90.63	224	42.91
≥20<30	19	7.63	230	92.37	249	47.70
≥30	5	10.20	44	89.80	49	9.39
Total	45	8.62	477	91.38	522	100.00

Table 2. Environmental risk factors for pregnant women to acquire acute toxoplasmosis in Goiânia-GO, Brazil.

Age (years)	Environmental risk factor	Living with risk factor		Not living with risk factor	
		Seroconverted %	Seronegative %	Seroconverted %	Seronegative %
<20	Cats	20.45 (9/44) P=0.009 ¹	70.55 (35/44)	6.66 (12/180)	93.34(168/180)
	Dogs	10.77 (14/130)	89.23(116/130)	5.31 (5/94)	94.69 (89/94)
	Flies	9.55 (17/178)	90.45(161/178)	4.34 (2/46)	95.66 (44/46)
	Cockroaches	10.33 (19/184)	89.67(165/184)	45.00 (18/40)	55.00 (22/40)
	Rats	10.59 (9/85)	89.41 (76/85)	8.63 (12/139)	91.37(127/139)
	Cats	20.45 (9/44) P=0.001 ²	79.55 (35/44)	4.87 (10/205)	95.13(195/205)
	Dogs	8.73 (11/126)	91.27(115/126)	6.50 (8/123)	93.50(115/123)
≥20<30	Flies	9.55 (17/178)	90.45(161/178)	2.81 (2/71)	97.19 (69/71)
	Cockroaches	6.56 (13/198)	93.44(185/198)	11.76 (6/51)	88.24 (45/51)
	Rats	12.37 (12/97) P=0.04 ³	87.63 (85/97)	4.60 (7/152)	95.40(145/152)
	Cats	25.00 (2/8)	75.00 (6/8)	7.31 (3/41)	92.69 (38/41)
≥30	Dogs	3.79 (4/29)	86.21 (25/29)	5.00 (1/20)	95.00 (19/20)
	Flies	11.90 (5/42)	88.10 (37/42)	0 (0/7)	100.00 (7/7)
	Cockroaches	9.09 (4/44)	90.91 (40/44)	20.00 (1/5)	80.00 (4/5)
	Rats	10.71 (3/28)	89.29 (25/28)	9.52 (2/21)	90.48 (19/21)

Note: ¹RR = 3.07 (1.38<RR<6.82); ²RR = 4.19 (1.81<RR<9.71); ³RR = 2.69 (1,10<RR<6.59)

Table 3. Low income and low level of education as risk factors for pregnant women to acquire acute toxoplasmosis in Goiânia-GO, Brazil.

Age (years)	Demographics	Living with risk factor		Not living with risk factor	
		Seroconverted %	Seronegative %	Seroconverted %	Seronegative %
<20	Low income	10.34 (12/116)	89.66(104/116)	8.33 (9/108)	92.67(99/108)
	Low level of education	3.03 (2/66)	96.97 (64/66)	12.02 (19/158)	87.98(139/158)
≥20<30	Low income	7.56 (9/119)	92.44(110/119)	7.69 (10/130)	92.31(120/130)
	Low level of education	10.59 (9/85)	89.41 (76/85)	6.10 (10/164)	93.90(154/164)
≥30	Low income	23.81 (5/21) p ¹ =0.01	76.19 (16/21)	0 (0/28)	100.00 (28/28)
	Low level of education	21.05 (4/19)	78.95 (15/19)	3.33 (1/30)	96.67 (29/30)
Total	Low income	10.15 (26/256)	89.85(230/256)	7.14 (19/266)	92.86(247/266)
	Low level of education	8.82 (15/170)	91.18(155/170)	8.52 (30/352)	91.48(322/352)

Note: RR¹= undefined.

and the risk of congenital transmission during pregnancy was 40%.

DISCUSSION

The prevalence of this infection varies in different regions of the world and even within the same city, which could be observed in Goiânia during the period of this study⁴. The city presents tropical weather, which favors the survival of *T. gondii* oocysts, a fact that increases the chances of acquiring acute infection when women live in contaminated environments. Besides, the high level of human migratory flux favors the contamination of women originally from other places with lower prevalence of the disease^{1,17}. The high percentage of women (34.2%) that reach childbearing age presenting vulnerability to this parasite²⁰ favors primo-infection during pregnancy.

In different geographical regions, annual seroconversion rates range from 0.6 to 1.5%²⁵⁻²⁹. This research revealed high rate of seroconversion (8.6%), even higher than the ones found in Niger (5.4%) by Olusi, Gross and Ajayi³⁰, and in Belgium between 1966 and 1975, before they introduced the prevention program³¹. In that last

country, after implementing such a program, it was observed a decrease in these rates, from 1.43% (1979-1982) to 0.53% (1983-1990), and finally to 0.09% (1991-2001)³², proving the importance of controlling this infection in pregnant women.

In Brazil, most studies involving *T. gondii* seroconversion estimated rates find results that differ among the analyzed regions: 0.3% in the state of São Paulo³; 1.7% in the state of Santa Catarina³³; 1.8% in the state of Paraná³⁴, and 0.6% in Brasília-DF³⁵.

In this study, improper environmental sanitation, pica or geophagia, and soil handling were not considered as risk factors to acquire toxoplasmosis, which is not in compliance with the results found in the cross-sectional study carried out before the introduction of primary measures of prevention²⁰. The presence of cats in the house was confirmed to be a risk factor to acquire acute toxoplasmosis, which is consistent with scientific knowledge already published¹. Wallace³⁶ also found higher prevalence of toxoplasmosis when there was contact with dogs, cats, coprophagous invertebrates, and rats, the last ones also observed in the present research. Furthermore, in this study, the consumption of unpasteurized goat's milk was

Table 4. Dietary risk factors for pregnant women to acquire acute toxoplasmosis in Goiânia-GO, Brazil.

Age (years)	Dietary risk factor	Living with risk factor		Not living with risk factor	
		Seroconverted %	Seronegative %	Seroconverted %	Seronegative %
<20	Raw meat	8.45 (6/71)	91.55 (65/71)	9.80 (15/153)	90.20(138/153)
	Raw egg	14.70 (5/34)	85.30 (29/34)	8.42 (16/190)	91.58(174/190)
	Inappropriately washed vegetable	11.70 (11/94)	89.30(10/130)	7.69 (10/130)	92.3 (120/130)
	Non-pasteurized goat's milk	25.00 (1/4)	75.00 (3/4)	9.10 (20/220)	90.90(200/220)
	Raw meat	3.12 (3/96)	93.88 (93/96)	10.46 (16/153)	89.54 (137/153)
	Raw egg	5.80 (4/69)	94.20 (65/69)	8.33 (15/180)	91.67(165/180)
?20< 30	Inappropriately washed vegetable	5.04 (6/119)	94.96(113/119)	10.00 (13/130)	90.00(117/130)
	Non-pasteurized goat's milk	0 (0/10)	100.00 (10/10)	8.26 (19/230)	91.73(211/230)
	Raw meat	13.04 (3/23)	86.96 (20/23)	7.69 (2/26)	92.31 (24/26)
	Raw egg	10.00 (3/30)	90.00 (27/30)	10.52 (2/19)	89.48 (17/19)
?30	Inappropriately washed vegetable	7.69 (1/13)	92.31 (12/13)	11.11 (4/36)	88.89 (32/36)
	Non-pasteurized goat's milk	60.00 (3/5) p=0.005	40.00 (2/5)	4.54 (2/44)	95.46 (42/44)

Note: $^1RR = 13.20$ ($2.85 < RR < 61.06$)

considered a risk factor to acquire this infection for women older than 30 years.

Cook et al.³⁷ could not demonstrate that living in contact with cats (host animals) meant a risk to acquire toxoplasmosis, showing that the risk factors change according to the food habits of the population, the geographic region considered, and the immunological condition of the host. The facts that the pregnant woman's organism is at risk of infection by *T. gondii*, as already demonstrated by Avelino et al.²¹, and that the fetus is immunodeficient justify the importance of controlling this infection in pregnant women, regardless of the regions of higher

prevalence, because it is vital to invest in quality of life.

Primary prophylaxis reduced the incidence of toxoplasmosis in pregnant women, which is in accordance with other researches, such as the ones conducted by Aspöck and Pollack³⁸, Henri, Jacques and Rene³¹, Thulliez²⁷, and Foulon et al.³⁹, who reported decreases of as much as 63% in the incidence of this infection when preventive measures were adopted. Some communities, differently from the one under study, have a high number of vulnerable inhabitants and very low prevalence of this infection. In Finland, Koskiniemi et al.²⁹

showed that, despite the observation of a low prevalence of the disease (20.3%), there is a great number of women at risk that can get pregnant, increasing the possibility of primo-infection, which has justified the continuity of the prevention program held in that country.

In this study, 44% (11/25) of the pregnant women acutely infected by *T. gondii* presented a transitory permanence of IgM in their blood stream (less than 30 days), which shows how difficult it can be to diagnose previous exposure to this parasite when just one serological exam is performed. This also highlights the need to repeat serology after two or three weeks for pregnant women presenting very low or very high IgG titres regardless of the presence of IgM. Furthermore, this evidence confirms the importance of IgM as a marker for acute toxoplasmosis, since it was possible to detect its presence for up to 4 months in the blood of 96% of the women participating in this study, as already pointed out in the literature. This finding is of great importance for during pregnancy the IgM marker will hardly be observed out of the acute phase of toxoplasmosis, a fact that has not been observed using more sensitive techniques to detect IgM. Also, the identification of IgM can make the prescription of antitoxoplasmic medication safer⁴.

The high risk of pregnant women to acquire acute toxoplasmosis found in the present research justified the introduction of a governmental program for pre-natal assistance, which was started in

September 2002. The preventive measures adopted will soon be evaluated as to their impact on decreasing the incidence of toxoplasmosis during pregnancy in the state of Goiás. The program is also intended to decrease the high vertical transmission rate, currently estimated in 35/1,000, higher than the one observed in Guatemala (18/1,000) by Sinibaldi and Ramirez⁴⁰.

ACKNOWLEDGEMENTS

The authors would like to thank all the institutions and the people that have helped in the course of this research, providing financial support and assisting with data collection and sample processing.

Financial support: National Foundation for Research Support, State Health Secretary of Goiás, Municipal Health Secretary of Goiânia.

Technical laboratory support: Department of Parasitology, Institute of Tropical Pathology and Public Health, Federal University of Goiás, Laboratory of Immunology, Institute of Tropical Pathology and Public Health, Federal University of Goiás, Laboratory of the Clinical Hospital, Medical School, Federal University of Goiás

Our special gratitude goes to the community leaders, who were vitally important in helping to assemble the women's group that was researched here.

Infecção primária de *Toxoplasma gondii* em mulheres grávidas em Goiânia: um estudo de soroconversão

O objetivo deste estudo foi testar a soroconversão para toxoplasmose em gestantes em Goiânia-GO, cidade na Região Centro-Oeste do Brasil. Um estudo observacional prospectivo de gestantes mais vulneráveis para *Toxoplasma gondii* foi realizado utilizando Reação de Imunofluorescência Indireta (IFI) para a triagem sorológica e vigilância laboratorial de soroconversão. O teste imunoenzimático (ELISA) foi realizado para confirmar a toxoplasmose aguda. O risco estimado usou os limites de confiança de 95% e os resultados foram validados por testes χ^2 e RR. A infecção aguda encontrada nas mulheres grávidas que participaram deste estudo foi de 8,6% (45/522). A vida em estreito contato com animais hospedeiros e veículos de transmissão de oocistos constituíram os riscos estatísticos para as mulheres grávidas em adquirir toxoplasmose aguda. Esta pesquisa revelou soroconversão (8,6%), apontando para a necessidade de prevenção primária e secundária para todas as gestantes de risco (soronegativas). Este estudo se originou de um programa para a proteção das mulheres grávidas no estado de Goiás.

Palavras-chave: Soroconversão – Gestantes – *Toxoplasma gondii*.

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Recebido em / Received: 22/07/2009
 Aceito em / Accepted: 26/11/2009