Risk Factors for *Toxoplasma gondii* Infection in Women of Childbearing Age

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Objectives: Determine the risk factors involved in toxoplasmosis transmission and determine whether pregnancy is a risk factor for toxoplasmosis infection. Study design: Cross-sectional study carried out on 2,242 women at childbearing age. An indirect immunofluorescence reaction was used to identify immunity to *Toxoplasma gondii*. Previous gestations were also analyzed as a possible risk factor. The results were analyzed by χ^2 and OR tests, and by variance analysis. The sample was statistically balanced according to social-economic risk factors. Results: Previously pregnant women were 1.74 times more frequently infected with toxoplasmosis, regardless of environmental conditions. Pregnant women living under unfavorable environmental conditions had an approximately two times increased risk of being infected for each risk factor (contact with host animals, presence of vehicles of oocyst transmission). Previous pregnancy was the risk factor that had the strongest influence on acquiring toxoplasmosis (variance analysis and statistical balancing). Discussion: The prevalence of this zoonosis is high in Goiânia-GO, Brazil (65.8%). Inadequate environmental sanitation was not significantly correlated with toxoplasmosis infection, except when associated with previous pregnancy, showing that the fundamental cause for infection is not environmental. Conclusion: The finding that pregnancy makes women more vulnerable to this protozoan, makes it important to implement prophylactic control of at-risk pregnant women.
Key Words: Toxoplasmosis in pregnancy; risk factor; host animals; means of transmission; contaminant elements.

Toxoplasmosis is important due to the possibility of transplacental transmission, harming the fetus. The rate of risk for pregnancy depends on infection prevalence among women at childbearing age, the types of meat that they eat, the degree of contact between vulnerable pregnant women and protozoan transmission sources, and the stage of gestation when transmission occurs; the earlier in gestation that transmission occurs, the greater the severity of sequelae in the fetus or newborn [1-9].

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Animals infected with *Toxoplasma gondii* transmit infective cysts to humans through ingestion of raw or undercooked meat. Oral transmission can also occur by ingestion of food or water contaminated with oocysts, which are eliminated in cat feces [10,11].

In different regions of the world, various types of factors have been implicated in toxoplasmosis transmission, however in the United States and France the ingestion of meat (beef, mutton or pork) containing parasite cysts is the most common source of human infection [2].

Oocysts can be carried or ingested by animals, such as invertebrate coprophages (flies and cockroaches), which can serve as a source of contamination of water and food [10], or of birds and rodents, which then infect cats [10,11]. *Toxoplasma gondii* has also been isolated from chicken eggs, but this is not considered an important route of oral transmission [2].

Unpasteurized goat's milk can also be a source of oral transmission of toxoplasmosis [2]. This epidemiological data is important in regions where this animal replaces cattle in the dairy industry (Northeast of Brazil) and for children who are allergic to cow's milk.

All the aforementioned sources of transmission are responsible for the worldwide distribution of this infection (40%-60%) and for the highly variable prevalence rates in several locations [12-20]. The incidence of infection depends on the immunological state of the population. It also depends on favorable environmental conditions, such as hot weather, for the survival of the oocysts, which are eliminated in cat feces [1,2,4,5].

The incidence of infection increases with age, and several studies have shown higher prevalence among women at childbearing age, although children are more exposed to the sources of transmission of this parasite [2]. This fact is intriguing and suggests that key factors that influence the vulnerability of the human organism to the parasite still need to be studied. Some studies have given conflicting results. A study in Australia could not relate exposure of women presenting seroconversion to any known risk factors [21]. In the Netherlands, there was an inexplicable rise of infection rate at the end of pregnancy, despite prophylactic measures [22]. Perhaps this was due to immunological alterations that occur in pregnant women, which can provoke immune suppression [23-27].

During pregnancy, especially at the end, there are alterations in the mother's T-lymphocyte sub-populations, with CD_4 lymphocytes decreasing and CD_8 lymphocytes increasing; the NK (natural killer) cells also have decreased immune functions of neutrophils, monocytes, and macrophages [24,25,27]. The phagocytic function and the chemotaxis of polymorphonuclear neutrophils are depleted in women between the 30th and 34th weeks of gestation, which would explain the higher vulnerability to the invasion of *T. gondii* during the third trimester of pregnancy [27]. Although there are many studies about risk factors to acquire toxoplasmosis and its effects on pregnancy, no studies on the risk of pregnancy itself to acquire this infection have been made.

We examined the risk factors associated with infection by *T. gondii* and whether pregnancy can be considered a risk factor for acquiring this protozoan infection.

Material and Methods

This study was carried out from 1997 to 1999, in Goiânia-GO, Brazil, a state capital in the Midwestern region of Brazil. A cross-sectional community-based or nested study (in a longitudinal study) was made of acute toxoplasmosis in women at childbearing age (12 to 49 years old).

Women at childbearing age were identified via parental clinics, birth control groups, communitarian work groups, religious congregations, or in public elementary and high schools, and they were invited to meetings. After explaining the program, informed consent was obtained from volunteers and blood samples were collected. Serological screening showed that 65.8% of the women were seropositive.Trained interviewers interviewed 2,563 of these women.

Women negative for toxoplasma-specific IgG, or with levels lower than 1/20, and negative for toxoplasma -pecific IgM, or with levels lower than 1/5, based on an indirect immunofluorescent antibody test (IFAT), were considered at risk. The indirect immunofluorescent antibody test was processed in the Department of Parasitology of the Institute of Tropical Pathology and Public Health of the Federal University of Goiás (IPTESP-UFG). The immune reaction detected IgG and IgM antibodies specific for *T. gondii*, labeled with fluorescein, using the Biolab (France) conjugate, the G and M Fluoline, and the RH strain of *T. gondii*. The finding of IgM specific for toxoplasma was confirmed by removing the rheumatoid factor from positive serum, using reagents manufactured by Bio Merrier (France). Quality control was carried out by the Immunology Departments of IPTESP and the Hospital das Clínicas (Federal University of Goiás), using the immunoenzyme test (ELISA), produced by Clark (USA) for IgM and Salk (Brazil) for IgG, both prepared according to the manufacturers' instructions.

The results of the laboratory exams indicated seronegative and seropositive women depending on whether IgG levels were higher than 1/20, since this was the initial level. On the other hand, being pregnant or not at the moment of the exam did not influence the presence or absence of immunity at the time of diagnostic screening, and the women were then subdivided into two more groups: those who had not been previously pregnant (nulliparous or primiparous) and those with previous pregnancies.

Essential criteria for inclusion in the study were: a) interview to collect information about risk indicators of toxoplasmosis infection; b) absence of IgM antibodies specific for *T. gondii;* and c) medical records with sufficient and clear information about age and previous pregnancies.

Criteria for exclusion were the following: a) presence of IgG antibodies at levels ≥1/4,096, with or without IgM; b) IgG level ≤ 1/40 but with presence of IgM; c) seronegativity detected by IFAT but not confirmed by ELISA; and d) questionnaires that did not contain essential information to the analysis (prior gestations and age).

Among the initial group of women, who were submitted to the above-mentioned criteria, 55 were suspected to have acute toxoplasmosis, 105 presented false-negative reactions and for 161 there was a lack of basic information in the medical records, and therefore they were excluded.

The following were analyzed as possible risk factors for toxoplasmosis: a) deficiencies in environmental sanitation (water and sewage treatment, garbage collection); b) presence of host animals in the house (cats and dogs); c) contact with vehicles of oocyst transmission (flies, cockroaches and rats); d) consumption of potentially contaminated food (raw or undercooked meat, raw or undercooked eggs, raw or undercooked, inappropriately washed vegetables, and unpasteurized goat's milk); e) pica, geophagia, or inadequate soil handling; g) low level of formal education (≤ four years); h) low family income (≤ two minimum wages); i) previous pregnancy.

Different known risk factors to acquire toxoplasmosis were compared in two groups: women who had already had toxoplasmosis (infected) and those who had never had the infection (non-infected). In both groups, it was investigated whether a previous pregnancy resulted in higher risk for acquiring the disease.

The women were divided into five year age groups, except for the extremes: adolescents (< 20 years old) and older women (35 years or more). We analyzed the different risk factors involved in toxoplasmosis infection and the possible association between these factors with previous pregnancy.

The behavior of these variables was analyzed with the c^2 test, using the presence of infection as the dependent variable. Data were analyzed using Excel version 97 (Microsoft, Brasil), EPI-INFO (version 6.0, CDC, USA) and SPSS (version 93, USA). The significance level at 5% and the limits of reliability at 95% were used. The Odds Ratio (OR) for the risk factors to which the women were exposed was calculated for each risk factor and for age group.

The data were also submitted to variance analysis and the sample was statistically balanced according to social-economic risk factors, which were not homogeneous. Inadequate environmental sanitation and low levels of education, which were prevalent, but not homogeneous, were considered social risks in our study. In order to balance the sample and make it homogeneous, 123 women were excluded: three were illiterate and lived in houses without environmental sanitation; 15 were in houses without environmental sanitation, but with up to four years education; 22 lived in houses without environmental sanitation, but with for more than four years education; nine were in houses with water and sewage treatment but without garbage collection and with for more than four years education; and 74 were in houses with environmental sanitation but they a low level of education. The cuts were made randomly with a raffle.

Results

The sample of 2,242 women at childbearing age lived in the surroundings of Goiânia, GO. The social

organization investigation showed that 61.8% of the women were included in a stable family context (44.4% married and 17.4% living in concubinage).

According to the social-economic investigation, 41.4% (909/2,195) of the women earned less than two minimum salaries (one minimum salary was about US$90 at the time of the study) and lived with financial difficulty. Previous residence in a rural area was found to be a risk factor for acquiring toxoplasmosis (χ^2 = 16.7 and p=0.000004). Living in houses with just a few rooms and in crowded conditions was not a risk factor for *T. gondii* infection.

The exposure of this population to vehicles of oocyst transmission was high: 77.4% of the women were in contact with flies, 79.0% with cockroaches, and 40.7% with rats.

The ingestion of raw or undercooked meat was a frequent habit in theis population, practiced by 42.1% of the women.

Table 1 shows the distribution of the women studied, divided according to previous occurrence of pregnancy, among infected (1,148) and non-infected (1,094) individuals. A significant association was found in this case, with a 1.74 times higher risk of acquiring toxoplasmosis in previously pregnant women.

The relationship between low income and presence of toxoplasmosis in women at childbearing age for different age groups was examined (Table 2). A significant association was found in this case; adolescents with low income exhibited a greater risk of acquiring toxoplasmosis.

The relationship between level of formal education and occurrence of toxoplasmosis in women at childbearing age for different age groups was also examined (Table 3). significant association was demonstrated for women in general, and moreover for women between 20 and 25 years old, who showed risk 3.24 times higher to acquire toxoplasmosis.

We investigated the relationship between environmental risk factors and toxoplasmosis (Table 4). A significant statistical association was with toxoplasmosis only found when women indicated a previous pregnancy, with an approximately two times higher risk for previously pregnant, compared to nulliparous women, all of whom had contact with cats, dogs, flies, cockroaches, rats, and/or inadequate soil handling.

A significant association between toxoplasmosis infection and lack of water and sewage treatment was only found for adolescents. The presence of vehicles of oocyst transmission was a significant contributing factor for acquiring this infection only in those adolescents who reported a previous pregnancy (Table 5).

The risk of infection was very high only in previously pregnant women (Table 6), who were almost twice as likely to be infected among those who consumed raw or undercooked meat or eggs, or raw or undercooked, inappropriately washed, vegetables. There was no significant association of alimentary risk factors with toxoplasmosis in adolescents (Table 7).

Table 8 shows the relationship between several risk factors and toxoplasmosis after balancing the sample, which did not modify the conclusion that pregnancy is a risk factor for infection.

After randomized cut-off to balance the sample as to the risk factors that showed to be different in both groups of women (infected and non-infected), which could make the results interpretation difficult for developed countries, the statistical analysis continued showing that pregnancy was the greatest risk factor, followed by low level of education, low income, living previously in rural areas, lack of environmental sanitation, ingestion of raw or underdone meat, ingestion of raw or underdone eggs, unpasteurized goat's milk, and geophagia (Table 8).

Discussion

The prevalence of this infection varies in different regions of the world and even of the same city, which could be observed in Goiânia during the period of this study [28]. The northwestern region of the city, where the population has lower family income, had already shown a statistical risk to acquire toxoplasmosis before the prevention program started. This city is located in the Midwestern Region of Brazil and presents tropical

Table 1. Distribution of the study population according to previous occurrence of pregnancy in Goiânia-GO, Brazil

Age (years)	Infected			Non-infected			Total
	Previously pregnant	Never pregnant	Subtotal	Previously pregnant	Never pregnant	Subtotal	
<20	82 (27.2)	219 (72.8)	301 (43.5)	71 (18.2)	319 (81.8)	390 (56.5)	691 (30.8)
≥20 and <25	185 (60.6)	120 (39.4)	305 (50.3)	161 (53.5)	140 (46.5)	301 (49.7)	606 (27.1)
≥25 and <30	155 (78.7)	42 (21.3)	197 (52.8)	129 (73.3)	47 (26.7)	176 (47.2)	373 (16.5)
≥30 and <35	114 (85.1)	20 (14.9)	134 (51.9)	89 (71.8)	35 (28.2)	124 (48.1)	258 (11.5%)
≥35	186 (88.2)	25 (11.8)	211 (67.2)	90 (87.4)	13 (12.6)	103 (32.8)	314 (14.0)
Total	722 (62.9)	426 (37.1)	1148 (51.2)	540 (49.4)	554 (50.6)	1094 (48.8)	2242 (100.00)

* OR = 1.74 (1.46 < OR < 2.07).
P=0.00000001*

Table 2. Low income as a risk factor to acquire toxoplasmosis in Goiânia-GO, Brazil

Age (years) Toxoplasmosis	Income		
	≤2minimum wages*		>2minimum wages
	Previously pregnant	Never pregnant	
<20			
Infected	54/163 (33.1%)	109/163 (66.9%)	130/293 (44.4%)
Non-infected	34/155 (21.9%)	121/155 (65.7%)	228/383 (59.6%)
≥20 and <25			
Infected	87/143 (60.8%)	56/143 (39.2%)	151/294 (51.4%)
Non-infected	81/141 (54.5%)	60/141 (42.5%)	157/298 (52.7%)
≥ 25 and <30			
Infected	74/84 (88.1%)	10/84 (11.9%)	106/190 (55.8%)
Non-infected	55/72 (76.4%)	17/72 (23.6%)	100/172 (58.1%)
≥ 30 and <35			
Infected	33/39 (84.6%)	6/39 (15.4%)	91/130 (70.0%)
Non-infected	20/31 (64.5%)	11/31 (35.5%)	92/123 (74.8%)
≥35			
Infected	45/53(84.9%)	8/53 (15.1%)	156/209 (74.6%)
Non-infected	22/28 (78.6%)	6/28 (21.4%)	75/103 (72.8%)
Total			
Infected	**293/482 (60.8%)**	**189/482 (39.2%)**	**634/1116 (56.8%)**
Non-infected	**212/427 (49.6%)**	**215/427 (50.4%)**	**652/1079(60.4%)**

* At the time of this study, the minimum wage was equivalent to about US$90.

Table 3. Low level of education as a risk factor to acquire toxoplasmosis in Goiânia-GO, Brazil

Age (years) / **Toxoplasmosis**	**Education**		
	≤ 4 years		**> 4 years**
	Previously pregnant	**Never pregnant**	
<20			
Infected	39/125 (31.2%) (P=0.0000000[1])	86/125 (68.2%)	176/301 (58.5%)
Non-infected	24/70 (34.3%)	46/70 (65.7%)	319/389 (82.0%)
≥ 20 and <25			
Infected	75/105 (71.4%) (p=0.003[2])	30/105 (28.6%)	200/305 (65.6%)
Non-infected	48/78 (61.5%)	30/78 (38.5%)	219/297 (73.7%)
≥ 25 and <30			
Infected	52/62 (83.9%)	10/62 (16.1%)	135/197 (68.5%)
Non-infected	43/56 (76.8%)	13/56 (23.2%)	118/174 (67.8%)
≥ 30 and <35			
Infected	43/50 (86.0%)	7/50 (14%)	84/134 (62.7%)
Non-infected	30/36 (83.4%)	6/36 (16.6%)	87/123 (70.7%)
≥35			
Infected	71/80 (88.8%)	9/80 (11.2%)	131/211 (62.1%)
Non-infected	29/32 (90.6%)	3/32 (9.4%)	70/102 (68.6%)
Total			
Infected	**280/422 (66.4%)**	**142/422 (33.6%)**	**726/1148 (63.2%)**
Non-infected	**174/272 (63.9%)**	**98/272 (36.1%)**	**813/1085 (74.9%)**

[1]OR (odds ratio) = 1.47 (1.02<OR<2.12).
[2]OR = 3.24 (2.26<OR<2.09).

weather, which favors the survival of *T. gondii* oocysts, a fact that increases the chances of acquiring this infection when the women live in contaminated environments. Furthermore, the high level of migratory flux favors the contamination of women originally from other places presenting lower prevalence of the disease [1,2]. The high percentage of women at childbearing age who are vulnerable to this parasite (34.2%) favors primo-infection during pregnancy.

This study demonstrated that women presenting previous pregnancy have a 1.74 times higher risk of toxoplasmosis than non-previously pregnant women (Table 1). This greater vulnerability of pregnant women to the parasite is probably due to alterations in the immune mechanisms inherent to gestation, resulting from supression of immune response because of the necessity of tolerance to the graft (fetus) and/or as a consequence of hormone imbalances characteristic of the gestational condition [29].

This risky situation (pregnancy) adds to the unfavorable conditions of life of the population that lives in underdeveloped countries, increasing the chance of contamination of women, mainly during pregnancy, when they become more vulnerable to the parasitic infection.

Water contamination as a result of low level of education increases the possibility of ingestion of contaminated food. In fact, untreated water was

Table 4. Environmental risk factors and their relationship with toxoplasmosis in Goiânia-GO, Brazil

Risk factor	Without previous pregnancy		With previous pregnancy	
	Infected[a] (%)	Non-infected[b] (%)	Infected[c] (%)	Non-infected[d] (%)
Cats	24.2 (265/1094)	21.7 (236/1090)	63.8 (169/265) p = 0.00007[1]	46.2 (109/236)
Dogs	68.0 (708/1041)	66.6 (693/1041)	60.3 (427/708) p = 0.000003[2]	47.9 (332/693)
Flies	77.0 (876/1137)	78.6 (860/1094)	64.4 (564/876) p = 0.000001[3]	51.4 (442/860)
Cockroaches	79.4 (903/1137)	82.6 (896/1084)	64.2 (580/903) p = 0.0000001[4]	51.1 (458/896)
Rats	42.8 (482/1126)	39.5 (432/1093)	65.6 (316/482) p = 0.00007[5]	50.9 (220/432)
Lack of water treatment	24.2 (265/1094)	21.7 (236/1090)	63.8 (169/265)	46.2 (109/236)
Lack of sewage treatment	43.4 (495/1140)	36.9 (403/1093)	65,6 (325/495)	60.3 (243/403)
Geophagy	19.3 (210/1086)	23.4 (254/1085)	38.5 (430/1116)	37.4 (407/1087)
Soil handling	38.5 (430/1116)	37.4 (407/1087)	69.1 (297/430) p=0.00001[6]	54.5 (222/407)

[1]OR = 2.05 (1.41 < OR < 2.98); [4]OR = 1.72 (1.42 < OR < 2.08);
[2]OR = 1.65 (1.33 < OR < 2.05); [5]OR = 1.83 (1.39 < OR < 2.42);
[3]OR = 1.71 (1.40 < OR < 2.08); [6]OR = 1.86 (1.39 < OR < 2.49).

a. Numerator: number of infected women living in contact with the risk factor; Denominator: total number of infected adolescents;
b. Numerator: number of infected women living in contact with the risk factor; Denominator: total number of infected adolescents;
c. Numerator: number of infected women living in contact with the risk factor, depending on previous pregnancy; Denominator: total number of infected women living in contact with the risk factor;
d. Numerator: number of infected women living in contact with the risk factor, depending on previous pregnancy; Denominator: total number of infected women living in contact with the risk factor.
OR = odds ratio.

consumed by 22.9% of the women; 40.2% of them lived in houses lacking sewage system, and 7% lacking garbage collection. However, comparing the group exposed to inadequate sanitary conditions with the presence or absence of immunity to the protozoan, there were noticeable differences only in women younger than 20 years in relation to untreated water and lack of sewage treatment (Table 5). The lack of garbage collection represented a higher risk, increasing in women at young age. This is in agreement with several studies found in the literature, which indicate higher chances of contamination when living in contact with such risk factors [1,2,5].

The presence of host animals (cats) in the house was confirmed to be a risk for toxoplasmosis only when women reported previous pregnancy, a fact not mentioned in the literature. This finding shows that the simple fact of having animals at home is not enough to acquire the protozoan infection, and that it is imperative to have contact with other infected sources to be contaminated and present higher vulnerability to the invasor organism. A multicenter case-control study carried out in Europe [30] did not identify cats as a risk factor for seroconversion during pregnancy.

The exposure to vehicles of oocyst transmission was high in the studied population in general (Table 4).

Table 5. Environmental risk factors for adolescents and their relationship with toxoplasmosis in Goiânia-GO, Brazil

Risk factor	Without previous pregnancy		With previous pregnancy	
	[a]Infected (%)	[b]Non-infected (%)	[c]Infected (%)	[d]Non-infected (%)
Cats	21.5 (62/289)	24.1 (93/386)	32.3 (20/62)	20.4 (19/93)
Dogs	67.4 (186/276)	71.5 (264/369)	25.3 (47/186)	17.8 (47/264)
Flies	79.9 (239/299)	79.7 (311/390)	27.6 (66/239) p = 0.03[1]	19.9 (62/311)
Cockroaches	77.5 (231/298)	82.6 (322/390)	27.3 (63/231) p = 0.01[2]	18.6 (60/322)
Rats	40.1 (118/294)	39.3 (153/389)	27.1 (32/118) p = 0.04[3]	16.3 (25/153)
Lack of water treatment	27.3 (82/300) p = 0.004[4]	18.2 (71/389)	40.2 (33/82) p = 0.03[5]	22.5 (16/71)
Lack of sewage treatment	49.3 (148/300) p = 0.00001[6]	31.0 (121/390)	33.8 (50/148)	26.4 (32/121)
Geophagy	20.5 (59/287)	25.7 (99/385)	34.1 (98/287)	30.9 (119/385)
Soil handling	33.4 (98/293)	30.8 (119/386)	33.7 (33/98)	15.1 (18/119)

[1]OR = 2.05 (1.41 < OR < 2.98); [4]OR = 1.72 (1.42 < OR < 2.08);
[2]OR = 1.65 (1.33 < OR < 2.05); [5]OR = 1.83 (1.39 < OR < 2.42);
[3]OR = 1.71 (1.40 < OR < 2.08); [6]OR = 1.86 (1.39 < OR < 2.49).
a. Numerator: number of infected women living in contact with the risk factor; Denominator: total number of infected adolescents;
b. Numerator: number of infected women living in contact with the risk factor; Denominator: total number of infected adolescents;
c. Numerator: number of infected women living in contact with the risk factor, depending on previous pregnancy; Denominator: total number of infected women living in contact with the risk factor;
d. Numerator: number of infected women living in contact with the risk factor, depending on previous pregnancy; Denominator: total number of infected women living in contact with the risk factor.
OR = odds ratio.

Table 6. Contact with alimentary risk factors and their relationship with toxoplasmosis infection in Goiânia-GO, Brazil

Risk factor	Without previous pregnancy		With previous pregnancy	
	Infected (%)	Non-infected (%)	Infected (%)	Non-infected (%)
Raw or underdone meat	42.2 (472/1118)	41.9 (456/1088)	62.7 (296/472) p = 0.00003[1]	49.1 (224/456)
Inappropriately washed vegetables	38.3 (429/1120)	34.4 (375/1089)	61.1 (262/429) p = 0.0001[2]	47.5 (178/375)
Raw or underdone eggs	22.6 (252/1115)	27.5 (298/1084)	63.1 (159/252) p = 0.002[3]	50.3 (150/298)
Unpasteurized goat's milk	14.0 (156/1113)	11.6 (126/1088)	66.7 (104/156)	56.3 (71/126)

[1]OR = 1.74 (1.33 < OR < 2.28); [2]OR = 1.62 (1.23 < OR < 2.14); [3]OR = 1.69 (1.18 < OR > 2.41).

Table 7. Contact of adolescents with alimentary risk factors and their relationship with toxoplasmosis infection in Goiânia-GO, Brazil

Risk factor	Without previous pregnancy		With previous pregnancy	
	Infected (%)	Non-infected (%)	Infected (%)	Non-infected (%)
Raw or underdone meat	44.1 (127/288)	43.1 (166/385)	18.9 (24/127)	14.4 (24/166)
Inappropriately washed vegetables	37.3 (108/288)	39.9 (154/386)	25.0 (27/108)	14.9 (23/154)
Raw or underdone eggs	22.2 (64/288)	25.5 (98/384)	21.9 (14/64)	14.3 (14/98)
Unpasteurized goat's milk	14.0 (156/1113)	11.6 (126/1088)	66.7 (104/156)	56.3 (71/126)

Table 8. Results of the statistical analysis before and after balancing the sample for the non-homogeneous social risk factors

Risk factor	Before balancing sample		After balancing sample	
	χ^2	P (CI = 95%)	χ^2	P (CI = 95%)
Without previous pregnancy				
Living in rural area	16.66	< 0.0001	13.29	< 0.0001
Pregnancy	41.69	< 0.0001	50.92	< 0.0001
Low level of education	46.35	< 0.0001	32.85	< 0.0001
Low income	15.32	< 0.001	14.45	< 0.0001
Lack of sewage system	9.96	0.001	9.53	0.002
Geophagy	5.35	0.02	3.95	0.04
With previous pregnancy				
Soil handling	18.72	< 0.0001	0.27	0.59
Raw or underdone meat	17.38	< 0.0001	8.08	0.004
Raw or underdone eggs	7.00	0.008	6.02	0.01
Inappropriately washed vegetables	9.03	0.002	0.02	0.95
Unpasteurized goat's milk	3.15	0.07	5.99	0.01

However, this fact was statistically significant only when women reported previous pregnancy (Tables 4 and 5). It was also found in another study higher prevalence of seroconversion when there was contact with rats, cats and coprophagous invertebrates [10].

In the present case, the findings are corroborated by studies found in the literature [1,2,4,5] only for women that mentioned previous pregnancy, probably due to physiological immunosuppression.

The ingestion of raw or underdone meat was a frequent habit in the analyzed population, as commented before (Table 6). However, there was no difference in exposure to this factor in both groups (infected and non-infected), not showing to be a risk factor for the studied population, except when the woman mentioned previous pregnancy. This happening can be explained by the lower virulence of the *T. gondii* strain that infects animals and also by the presence of some environmental factors that increase the resistance of women to the parasite.

The ingestion of raw or underdone inappropriately washed vegetables was also a frequent habit among the studied population, corresponding to 36.4% of the

women. The exposure was similar for infected and non-infected women, though, differing only among the women that mentioned prior pregnancy.

The ingestion of unpasteurized goat's milk was not usual, being observed in 12.8% of the population and the exposure to this factor did not show statistical differences between the two groups of women.

Geophagia during childhood was not frequent among the studied population, being referred by 21.3% of the women; it was not found statistical difference as to this exposure for both groups of women. Geophagia during pregnancy was reported by 38% of the studied women, but the two groups showed no differences in exposure to this factor (Table 6), a fact that was modified when the sample was balanced (Table 8). The present results, after balancing the sample, do not differ from those found in the literature [1,2,4], which mention geophagia as a risk factor.

It was not observed statistical significance between soil handling in gardens and risk to acquire toxoplasmosis when the women did not mention previous pregnancy. This can be explained by the adequate defense of the woman's body, by the low significance of the inoculum amount or by the lower aggressiveness of the existing *T. gondii* strain in the soil, which would require a weak organism in order to cause infection. In case of previous pregnancy, the contamination risk was higher in women younger than 20 years (Tables 5 and 6).

Susceptible individuals submitted to contaminated environments can be easily infected, as shown in this study. Pregnant women present with higher probability of being contaminated, regardless their life conditions. Finding this situation, the protozoan sources of infection present greater importance, because the pregnant woman is more vulnerable and a lower inoculum of the parasite or a less aggressive strain can infect her.

There are controversial studies in the literature, which evaluated the validity of the preventive program mainly in countries where this infection is not highly frequent. The researches tend to analyze the operational costs of the governmental programs and show divergent opinions concerning the secondary prevention. The lower cost of primary prevention is not questioned, because the investments are related to education and this is an obligation of the State. However, when secondary prevention is mentioned, with identification and follow-up of the women at risk, the cost-benefit analysis is imposed.

The present study demonstrates a high vulnerability of the pregnant women to the protozoan and suggests that the public health policy should include primary and secondary prevention for all pregnant women at high risk. The benefits resulting from lower incidence of congenital toxoplasmosis with the adoption of these measures justify the costs, because it guarantees good quality life to infected fetuses. The early identification of the fetus infection and the introduction of immediate appropriate therapy open an opportunity for the individuals to have better life expectation and a worthy future.

In conclusion, previous pregnancy showed to be the greatest risk factor to toxoplasmosis infection in the studied population. This has already been confirmed in another study carried out for the seronegative women found among the same population analyzed in the present study [31]. In that research, the presence of gestation increased the risk to acquire toxoplasmosis, being 2.2 times higher for pregnant women in general and 7.7 times higher for pregnant adolescents.

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