FACTORS ASSOCIATED WITH TOXOPLASMOSIS IN PREGNANCY

FATORES ASSOCIADOS À TOXOPLASMOSE NA GESTAÇÃO

FACTORES ASOCIADOS A LA TOXOPLASMOSIS DURANTE LA GESTACIÓN

Marília Marcondes Campoamor[1]
Beatriz Conacci[2]
Maria José Bistafa[3]
Roberta Alvarenga[4]
Susana Muñoz[5]
Claudia Benedita dos Santos[6]

Objectives: to estimate seroprevalence and analyze factors associated with Toxoplasmosis in pregnancy. Method: epidemiological, analytical and cross-sectional investigation with women in prenatal care in Ribeirão Preto-SP. Data were obtained through serological tests and questionnaire. Logistic regression model was used, with the selection of independent variables performed using the Fisher's exact test, or Chi-square test, and Student's t, calculated crude and adjusted odds ratios, with significance level of 5%. Results: sample was composed of 165 women, with total seroprevalence, 34.5% [27.3; 41.8], reagents for IgG. The chance of being reactant to the IgG antitoxoplasm antibody is 1.09 times higher for each year of age; 19.48 for those with incomplete Elementary School I; 4.41 for direct contact with the earth. Conclusion: basic sanitation and the network of health services in the municipality studied favor the prevention of Toxoplasmosis in pregnancy.

Descriptors: Toxoplasmosis. Pregnancy. Risk factors. Prevalence. Susceptibility.

Objetivos: estimar a soroprevalência e analisar fatores associados a Toxoplasmose na gestação. Método: investigação epidemiológica, analítica e transversal com mulheres no pré-natal em Ribeirão Preto-SP. Os dados foram obtidos por meio de exames sorológicos e questionário. Modelo de regressão logística foi utilizado, com a seleção das variáveis independentes realizada por meio dos testes Exato de Fisher, ou Qui-quadrado, e t de Student, calculadas razões de chances brutas e ajustadas, com nível de significância de 5%. Resultados: amostra foi composta de 165 mulheres, com soroprevalência total, 34,5% [27,3; 41,8], reagentes para IgG. A chance de ser reagente ao anticorpo antitoxoplasma IgG é 1,09 vezes maior para cada ano a mais de idade; 19,48 para aquelas com Ensino Fundamental I incompleto; 4,41 para o contato direto com a terra. Conclusão: saneamento básico e a rede de serviços de saúde no município estudado favorecem a prevenção da Toxoplasmose na gestação.

Corresponding Author: Marília Marcondes Campoamor, mariliacampoamor52@gmail.com

[1] Universidade de Rio Verde. Rio Verde, GO, Brazil. https://orcid.org/0000-0001-8821-5919.
[2] Universidade de São Paulo. Ribeirão Preto, SP, Brazil. https://orcid.org/0000-0003-2034-230x.
[3] Universidade de São Paulo. Ribeirão Preto, SP, Brazil. https://orcid.org/0000-0002-6240-0048.
[4] Universidade Federal do Rio Grande do Sul. Porto Alegre, RS, Brazil. https://orcid.org/0000-0003-3286-6071.
[5] Universidade de São Paulo, Ribeirão Preto. SP, Brazil. https://orcid.org/0000-0002-6720-8231.
[6] Universidade de São Paulo, Ribeirão Preto. SP, Brazil. https://orcid.org/0000-0001-7241-7508.

Objetivos: estimar la seroprevalencia y analizar factores asociados a Toxoplasmosis en la gestación. Método: investigación epidemiológica, analítica y transversal con mujeres en el prenatal en Ribeirão Preto-SP. Los datos fueron obtenidos por medio de exámenes serológicos y cuestionario. Modelo de regresión logística fue utilizado, con la selección de las variables independientes realizada por medio de las pruebas Exacto de Fisher, o Chi-cuadrado, y t de Student, calculadas razones de probabilidades brutas y ajustadas, con nivel de significación del 5%. Resultados: muestra compuesta de 165 mujeres, con seroprevalencia total, 34,5% [27,3; 41,8], reactivos para IgG. La probabilidad de ser reactivo al anticuerpo antitoxoplasma IgG es 1,09 veces mayor para cada año a más de edad; 19,48 para aquellas con Enseñanza Fundamental I incompleto; 4,41 para el contacto directo con la tierra. Conclusión: saneamiento básico y la red de servicios de salud en el municipio estudiado favorecen la prevención de la Toxoplasmosis en la gestación.

Introduction

The health of women in the pregnancy-puerperal cycle is considered a priority in the guidelines of public policies directed to maternal and child health. Prenatal care comprises a series of clinical, psychosocial and educational actions that investigate the various ways of preventing health problems and diagnosing maternal and fetal complications, by monitoring gestational development and reducing maternal risk (1-2).

The National Policy for Women's Health Care, as well as the Program for Humanization in Prenatal and Birth, are results of the efforts of the Ministry of Health and the World Health Organization to improve the quality of women's and children's health care[3]. Quality prenatal care and access to health services can prevent maternal mortality in up to 92% of cases, and the principles and guidelines of the Unified Health System (UHS) favor such results[4].

At the national level, there is irregularity in the coverage of Primary Health Care (PHC), in access to services and in the effectiveness of prenatal, delivery and puerperium care due to inconsistencies in the results of the indicators of the pregnancy-puerperal cycle[5]. The municipalization of services through Basic Health Units (BHU) and Family Health Strategies (FHS) reorganized the entire Health Care Network (HCN) (5-6).

Toxoplasmosis is one of the main diseases that can affect women during pregnancy and that should be detected at the beginning of pregnancy, when performing prenatal screening[7].

Toxoplasma gondii is an obligate intracellular parasitic protozoan that causes toxoplasmosis, a zoonosis that infects homeothermal hosts, including humans. Felines are the definitive hosts, while other animals develop tissue cysts that ultimately compromise food security, regarding the consumption of raw or undercooked meat, making this important infection a public health issue, cause serious diseases in newborns[8].

Infection can occur by several ways, such as ingestion of contaminated water, raw or undercooked meat, unpasteurized milk and consumption of poorly washed vegetables and vegetables, contaminated with *T.gondii* that can be found in feces of cats and other felines (definitive or complete hosts), being able to stay in humans[9].

Infection with the protozoan *Toxoplasma gondii* during pregnancy requires greater attention from health authorities due to the risk of placental transmission. The result of congenital transmission is influenced by factors such as maternal immune status, maternal parasitemia and age, with the most severe infections in the first weeks of pregnancy due to ontogenetic development of the fetus[10].

Considered a pathology of sanitary importance and compulsory notification, according to the World Health Organization, Toxoplasmosis in pregnancy reflects sociodemographic vulnerability, characterized by the scarcity of basic sanitation, inaccessibility to pre-treatment,

Marília Marcondes Campoamor, Beatriz Conacci, Maria José Bistafa,
Roberta Alvarenga, Susana Muñoz, Claudia Benedita dos Santos

level of education, inappropriate conditions of hygiene for food consumption and cultural patterns. These conditions are still affected by climatic factors that intensify the dissemination of inoculum to the environment, favoring the contamination of individuals by the oocyst[11].

The conditions related to the appearance of clinical signs and late notifications are a consequence of the delay in laboratory confirmation, resulting in the difficulty of isolating the parasite in order to increase the knowledge about the contagion of the various infectious forms of the protozoa[12].

The prevalence of seropositive pregnant women for anti-Toxoplasma IgG, presents the following nationwide distribution: State of Mato Grosso do Sul, 50.2%; city of Porto Alegre-RS, 59.8%; Goiânia-GO, 67.7%; Porto Velho-RO, 73.4%; Vitória-ES, 73.5%; Caxias-MA, 77.9% demonstrating frequent exposure to protozoa in the country[13].

There are different techniques for the diagnosis of Toxoplasmosis, especially those that evaluate the serological profile of the disease. In pregnancy, this type of technique is extremely important for early diagnosis, through which it is possible to evaluate Immunoglobulin M (IgM) and Immunoglobulin G (IgG) antibodies[14].

The detection of IgG and absence of IgM indicates that the individual was previously exposed to *T.gondii*, while in the diagnosis of acute infection the positivity for IgM is observed. The use of other methods for differentiation of acute or chronic infection is verified through the IgG avidity test, in which the low avidity (< 30%) of IgG antibodies indicate the cases whose infection occurred in the last 12 weeks, while high avidity (> 60%) define cases more than 12 weeks ago, suggestive of a previous infection[15].

Due to the acquisition of primary infection during pregnancy and in the absence of treatment, the risk of intrauterine transmission is 25%, 54% and 65% in the first, second and third trimesters, respectively, causing serious changes that affect the fetus[16].

Among newborns infected with Congenital Toxoplasmosis, about 85% of cases do not present evident clinical signs at birth. However, some discrete clinical manifestations may be associated, such as intrauterine growth restriction, prematurity, lighter visual and neurological abnormalities[17].

Among the newborns with clinical manifestations (10-15%), the most frequent are: visual impairment in varying degrees, mental retardation, motor abnormalities and deafness, and in more severe cases may present hepatomegaly, splenomegaly, severe changes in the nervous system and (dilation of cerebral ventricles and cerebral calcification) and more extensive damage to vision[18].

In the gestational period, proper monitoring increases the possibilities of ensuring the healthy development of the fetus. In this sense, prenatal serological screening for Toxoplasmosis helps the adoption of preventive and therapeutic procedures in order to minimize the possibilities of vertical transmission and harm to the child[19].

In this context, the present study aimed to estimate the seroprevalence of Toxoplasmosis in pregnant women in the city of Ribeirão Preto, in the state of São Paulo, and to analyze the socio-environmental factors associated with confirmed cases of Toxoplasmosis in pregnancy.

Method

An analytical, observational, cross-sectional epidemiological study developed in Ribeirão Preto, São Paulo, Brazil, extracted from the doctoral thesis "Prevalence and factors associated with Toxoplasmosis in pregnant women in a municipality in the interior of the state of São Paulo"[20].

The estimated population of the municipality is 703,293 inhabitants (IBGE, 2019) and has a primary health care network (PHC) with 25 Basic Health Units (BHU), of which 21 are units of the Family Health Strategy (FHS).

These are distributed in five sanitary districts, and in three of these there is a 24-hour Emergency Unit (UPA - *Unidade de Pronto Atendimento*), with 85% of the population registered with e-SUS. This set of units has 25% coverage of the FHS and a prenatal coverage of 95%, with the prospect of expansion of 40% for the 2022-2025

quadrennium[21]. Among the various health care programs and projects offered to the population, the coordinatory of comprehensive assistance to women's health, responsible for structuring the care to this population group, stands out. The screening protocol for Toxoplasmosis in pregnancy occurs as recommended by the Ministry of Health, Brazil. For positive cases are indicated treatment with spiramycin and referral of pregnant women to perform high-risk prenatal care at the *Hospital das Clínicas* of the *Faculdade de Medicina de Ribeirão Preto* of the *Universidade de São Paulo* (HCFMRP – USP).

The study population consists of pregnant women who performed prenatal consultation in the PHC network of the city of Ribeirão Preto-SP in 2019. We included pregnant women who underwent prenatal consultation in Basic Health Units (UBS) in the western, eastern, eastern, and of the city of Ribeirão Preto in the course of 2019 and excluded who did not accept to participate in the research and with inconclusive serological tests.

The sample number was obtained from the formula:

$n = N. p. q(Z\alpha/2)^2 / [p.q(Z\alpha/2)^2 + (N - 1). d^2]$, where

p: seroprevalence of Toxoplasmosis in Ribeirão Preto of 46% (p = 0.46; q = 0.54)[21];

d: maximum estimation error of 7% (d = 0.07);

α: 5% significance level (α = 0.05);

Z: standardized Normal variable (Zα/2 = 1.96)

N: finite population of 800 pregnant women who attended prenatal consultations in the PHC network in the city of Ribeirão Preto-SP in 2018.

Considering a sample loss equal to 5%, the sample number was estimated in at least 157 pregnant women.

The information was collected in the BHU chosen randomly considering the 15-day interval between one unit and another, selected as follows: CSE of the Ipiranga neighborhood; BHU of Vila Recreio; BHU Parque Ribeirão; BHU Vila Abranches; UBS São José; CSE JD Aeroporto. The data collection questionnaire that included

sociodemographic and socio-environmental variables was elaborated based on the scientific literature and validated in content by three expert judges on the subject addressed. The pregnant women answered the questionnaire at the time they waited for the consultation during prenatal care in PHC. The electronic tool Research Electronic Data Capture (Redcap) was used to store the information.

The variable dependent on the serology result was dichotomized between Group 1: Seroimmune pregnant women, positive IgG and negative IgM and Pregnant women with acute infection, positive IgG and IgM; Group 2: Susceptible pregnant women with negative IgG and IgM or pregnant women with inconclusive IgG negative and IgM positive.

The independent variables considered were: age; educational level (illiterate or Incomplete Elementary I, Complete Elementary I, Incomplete or Complete Elementary II, Technician or Graduated); presence of animals at home (yes, no); water treatment at home (tap, filtration); consumption of raw or undercooked meat (yes, no); direct contact with the soil (yes, no); consumption of fresh cheese (yes, no) and consumption of artisanal sausage (yes, no).

Regarding total seroprevalence, 57 (34.5%), CI [95%]: [27.3; 41.8], were reagents for IgG, being seroprevalent 54 (32.7%) and 3 (1.8%) seroreactive. Among pregnant women, 108 (65.5%) presented susceptibility to the absence of anti-oxoplasm IgG and IgM antibodies. None of the tests were inconclusive, i.e., positive for IgM exclusively.

The results of Logistic Regression, with the insertion of the variables: age; schooling (illiterate or Incomplete Elementary I, Elementary I or Incomplete Elementary II, Complete Elementary II, Technician or Graduated); presence of animals at home (yes, no); direct contact with the soil (yes, no) and consumption of artisanal sausage (yes, no).

The bivariate analysis, selection of the independent variables for the composition of

Marília Marcondes Campoamor, Beatriz Conacci, Maria José Bistafa,
Roberta Alvarenga, Susana Muñoz, Claudia Benedita dos Santos

the logistic model, was performed using the Fisher's exact or Chi-square tests, with Yates correction, in the case of categorical variables and Student's t test, for the numerical variables. Those who presented values below 0.25 for the Wald statistic (p<0.25) were considered in the final regression model. The respective crude and adjusted odds ratios (OR) were calculated, with 95% Confidence Intervals. In all analyses the significance level adopted was 5% (α = 0.05). The program used for statistical analysis was R Core Team, 2018, version 3.6.1.

The project was approved by a Research Ethics Committee under CAAE 9909418100005393. All participants over 18 years of age signed the Informed Consent Form (ICF) and participants, underage, were included by signing the ICF for the legal guardian, along with the Informed Assent Form (IAF) signed by the component itself under 18 years.

Results

Serology results were analyzed for the antitoxoplasm antibodies of the IgG and IgM class of 165 pregnant women who started prenatal care in the basic health units selected for the study. The mean age for the group of pregnant women with serology results reagent for the antibody antitoxoplasm IgG was 29.4 years with standard deviation of 6.2 years CI[95%]: [27.9; 30.9] and, for the group not reagents for the antibody antitoxoplasm IgG, the mean age was 26 years with standard deviation equal to 6 years CI[95%]: [25.0; 27.0]. Student's t-test showed a statistically significant difference between mean ages (p = 0.001).

Table 1 shows the estimated values for seroprevalence, seroreactivity and susceptibility and the respective confidence intervals.

Table 1: Estimated values for total seroprevalence, seroprevalence, seroreactivity and susceptibility to Toxoplasmosis in pregnant women, as well as respective 95% confidence intervals. Ribeirão Preto, SP, Brazil 2019 (N=165)

	n	%	CI[95%]		n	%	CI[95%]
Seroprevalence Total (IgG+)	57	34.5	[27.3;41.8]	Seroprevalent (IgM-)	54	32.7	[25.6;39.9]
				Seroreactive (IgM+)	3	1.8	[0; 3.9]
[a]IgG-				Susceptibility (IgM-)	108	65.5	[58.2; 72.7]
Total					165	100	---

Source: created by the authors

There was no case of indeterminate diagnosis, that is, IgG- and IgM+

Of the total, 57 (34.5%), CI[95%]:[27.3; 41.8] were reagents for IgG, being seroprevalent 54 (32.7%), CI[95%]:[25.6; 39.9] and 3 (1.8%), CI[95%]:[0; 3.9] reactive. Among pregnant women, 108 (65.5%), CI[95%]:[58.2; 72.7] were susceptible to the absence of IgG and IgM antitoxoplasm antibodies. None of the tests were inconclusive, i.e., positive for IgM exclusively. Table 2 describes the distributions of pregnant women according to the results of serology for Toxoplasmosis antibody antitoxoplasm IgG and sociodemographic variable educational level. The results indicate that the majority (84.2%) of the participants had educational level between Complete Elementary I and Complete II (49.7%). The socio-environmental variables with presence of animals in the household (86.1%) without direct contact with the land (73.3%) consume drinking water from the public network, highlighting that 99% of households

in Ribeirão Preto are supplied with water that meets all the criteria of potability, 33% consume rare or raw meat, 64.2% fresh cheese and 58.2% artisanal sausage.

Table 2: Distribution of pregnant women according to serology results for Toxoplasmosis anti-Toxoplasma antibody to IgG and socio-environmental variables with respective p-values associated with bivariate analyses. Ribeirão Preto, 2019 (N=165)

	IgG +	IgG -	Total
Illiterate/Incomplete Elementary I	6	8	14
Complete Elementary I	27	47	74
Elementary II	23	42	65
Technical level and/or Higher education	1	11	12
p = 0.2220; Fisher's Exact Test			
Presence of animals in the home- peridomicile			
No	22	59	81
Yes	34	48	82
p = 0.0788; Chi-square test			
Direct contact with the soil			
No	44	98	142
Yes	12	6	18
p = 0.0064; Chi-square test			
Water treatment at home			
Potable water from the public network	45	76	121
Potable water from the public network + Filtration	12	32	44
p = 0.3175; Chi-square test			
Consumption of undercooked or raw meat			
No	71	39	110
Yes	37	18	55
p = 0.8621; Chi-square test			
Consumption of fresh cheese			
No	35	21	56
Yes	72	34	106
p = 0.6038; Chi-square test			
Consumption of artisanal sausage			
No	50	18	68
Yes	57	39	96
p = 0.0875; Chi-square test			

Source: created by the authors

By analyzing the p values were inserted into the final logistic regression model the variables schooling; presence of animals in the household; direct contact with the land and consumption of artisanal sausage (p <0.25).

The results of the logistic regression model are presented in Table 3.

Marília Marcondes Campoamor, Beatriz Conacci, Maria José Bistafa,
Roberta Alvarenga, Susana Muñoz, Claudia Benedita dos Santos

Table 3: Results of the Logistic Regression model. Ribeirão Preto, 2019 (N=165)

Variables	Brute ODDS	CI [95%]	p		CI [95%]	p
Age	1.09	[1.03; 1.15]	0.002	1.09	[1.03; 1.16]	0.004
Illiterate/Incomplete Elementary I	6.75	[0.66; 68.77]	0.107	19.68	[1.47;262.82]	0.024
Complete Elementary I	5.00	[0.60; 41.78]	0.137	12.34	[1.19;128.19]	0.035
Elementary II	5.17	[0.62; 43.48]	0.130	12.13	[1.17;125.8]	0.036
Direct contact with the soil	4.00	[1.39; 11.51]	0.010	4.41	[1.24;15.73]	0.022
Presence of animals in the home	2.13	[1.09; 4.17]	0.027	2.03	[0.98; 4.20]	0.056
Consumption of artisinal sausage	1.91	[0.96; 3.82]	0.067	1.47	[0.69; 3.16]	0.320

Source: created by the authors.

The results show that Illiterate/Incomplete Elementary I increases the chance in 19.68 times of the pregnant woman to be IgG reagent in relation to those with Technical level and/or Higher Education, being 12.34 times higher for those with Complete Elementary I and 12.13 times higher for those with elementary II. Direct contact with the soil was 4.41 times more likely to present reagent IgG. The increase in age represents the chance of being reagent to the antibody antitoxoplasm IgG 1.09 times higher.

Discussion

The total seroprevalence for Toxoplasmosis in pregnant women identified in this study is 34.5%, with CI[95%]: [27.3; 41.8] lower than the other Brazilian states that register high prevalence, such as Amazonas (73.5%), Sergipe (69.3%) and Alagoas (43.9%)climatic variations and social imbalance that facilitate the increase of protozoan contamination[22]. This study identified a risk for the seroprevalence of antibodies antitoxoplasm of the IgG class with increased age of pregnant women participating in this study, and the average age for those with IgG-reactive results was 34 years CI[95%]: [33; 35.2]. This result was verified in other studies at the national level, relating the age of pregnant women older than 30 years, with the longest time of exposure and susceptibility to protozoa[23].

When it comes to schooling, the association between the lowest degree and the absence of habits that prevent infection in pregnancy is frequent. This study corroborated this result demonstrating that pregnant women with incomplete elementary school have a risk of 31.2 times higher seroprevalence compared to those with technical or higher education level. Pregnant women who have nine years of schooling or more are more protected from positivity of Toxoplasmosis serology, as a result of a greater understanding of the information[24].

Regarding direct contact with the soil, this study showed 5.82 times more chance of pregnant women with IgG reagent, compared to those who did not have direct contact. The ingestion of the oocyst form of the protozoan *T.gondii* can occur during the handling of the earth without the use of gloves, shovels and also by inadequate hand hygiene[25].

A relevant situation, pointed out in the present study, was that 65.5% of the pregnant women presented serological results not reacting to the antibodies indicating that most of them were not infected by the protozoan *T.gondii* until the time of sample collection. This situation refers to a sociodemographic

and health context that combines conditions of protection against infection[24].

Access to quality water is a protective factor for contamination of the protozoan *T.Gondii,* considering that outbreaks of the disease caused by water transmission affect a greater number of individuals when compared to those of food origin, due to its permanent transmission, until the infective form of the oocyst protozoan has been eliminated from the source[25].

The city of Ribeirão Preto, scenario of this study, has a favorable sanitary structure that justifies the high prevalence of susceptible pregnant women identified. The water that supplies the city comes from an immense groundwater reservoir called *Guarani Aquifer*, requiring only the addition of chlorine and fluoride, constantly undergoing bacteriological and physical analysis laboratories of the Water and Sewage Department of Ribeirão Preto (SAERP) in order to maintain the quality control of potability[21]. In addition to issues related to water quality, the municipality is privileged, since 99.19% of the population is served with basic sanitation[21].

Municipalities such as Riberão Preto, with wide coverage of basic sanitation and capillarity of the health services network, advance to meet the United Nations Sustainable Development Goals, specifically what estimates the reduction of perinatal and neonatal mortality up to 12 cases per 1000 live births, referring to preventable and preventable diseases, such as Gestational Toxoplasmosis, mediated by equitable access to health services and sanitation infrastructure[22].

The results of this study reinforce the need to implement educational health programs aimed at preventing Toxoplasmosis in pregnancy. As well as robust strategies at the national and international level, which include the training of health teams to perform and interpret diagnostic tests, treatment and clinical and serological monitoring of pregnant women during pre-natal care.

In addition, it is emphasized the need for coordination of managers at the Municipal, State and Federal level, for the reduction of risks, given the example of the municipality of Ribeirão Preto, which presented low prevalence and high susceptibility to Toxoplasmosis as a result of the coverage of public drinking water supply and the coverage of the prenatal program implemented in the health services of the municipality.

The cross-sectional design used in this study is a limitation of it, since it does not allow to confirm causality or estimate risk factors for toxoplasmosis in pregnancy. Despite the regional character of this research, it is considered that the results provide knowledge of social and scientific relevance, and may support the implementation of actions to prevent Toxoplasmosis.

Conclusion

The results indicate a total seroprevalence for Toxoplasmosis in pregnant women, estimated at 34.5%, CI[95%]: [27.3; 41.8]. Age, education and contact with land were associated factors. On the other hand, the favorable scenario of the city of Ribeirão Preto, in relation to access to drinking water, collection and treatment of sewage, as well as the network of health services, favors the prevention of Toxoplasmosis in pregnancy.

Thus, like the municipality in question, the implementation and implementation of surveillance and screening actions in the gestational period should be considered as strategies to reduce Toxoplasmosis in pregnancy, condition that compromises the growth and development of the newborn, with irreversible sequelae, such as microftalmia, strabismus, destruction of the retina, leading to blindness and neuromotor retardation caused by hydrocephalus.

Collaborations:

1 – conception and planning of the project: Marilia Marcondes Campoamor and Claudia Benedita dos Santos;

2 – analysis and interpretation of data: Claudia Benedita dos Santos and Marilia Marcondes;

3 – writing and/or critical review: Beatriz Conacci; Maria José Bistafa, Roberta Alvarenga, Susana Muñoz and Claudia Benedita dos Santos;

9

Marília Marcondes Campoamor, Beatriz Conacci, Maria José Bistafa,
Roberta Alvarenga, Susana Muñoz, Claudia Benedita dos Santos

4 – approval of the final version: Marilia Marcondes Campoamor, Beatriz Conacci, Maria José Bistafa, Roberta Alvarenga, Susana Muñoz, Roberta Alvarenga and Claudia Benedita dos Santos.

Conflicts of interests

There are no conflicts of interests.

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