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Risk and other factors associated with toxoplasmosis and toxocariasis in pregnant women from southern Brazil

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Abstract

Toxoplasmosis causes complications during pregnancy that have serious effects on fetal development. Thus far, toxocariasis has been reported to spread only via vertical transmission. Nonetheless, the population of pregnant women is also exposed to this infection. Co-infection with both *Toxoplasma gondii* and *Toxocara* spp. has been reported in children, but there are no reports of co-infection in the population of pregnant women. The aim of this study was to determine the prevalence of co-infection with *T. gondii* and *Toxocara* spp. in pregnant women at a university hospital in southern Brazil, and to identify the risk factors associated with infection by both parasites. Two hundred pregnant women were tested for the presence of anti-*T. gondii* and anti-*Toxocara* spp. antibodies and were asked to complete an epidemiological questionnaire. In this study, the co-infection rate observed in the total population of pregnant women was 8%. In addition, women with a positive result for a serology test for *Toxocara* spp. were at increased risk of infection by *T. gondii* ($P = 0.019$). Co-infection with both parasites in pregnant women was associated with low birth weights in neonates. The similar modes of transmission of both parasites could explain the co-infection. Only a few previous studies have investigated this phenomenon. The findings of the present study emphasize the importance of serological diagnosis during prenatal care and further research in this area to identify risk factors associated with this co-infection, and the possible implications of this co-infection during pregnancy and on the health of newborns.

Introduction

Toxoplasmosis, which stands out as a major public health problem and a serious disorder during pregnancy, can cause abortion, fetal death, impaired fetal growth and congenital infection, with serious consequences to the neonate (Tenter *et al.*, 2000; Pappas *et al.*, 2009). Although vertical transmission of toxocariasis has been recorded only once (Maffrand *et al.*, 2006), its importance in the transmission of *Toxocara canis* has been widely proven, as has the potential risk of complications it poses

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during pregnancy (abortion and impaired fetal growth), according to studies involving experimental models (Schoenardie *et al.*, 2013a; Aguiar *et al.*, 2015; Telmo *et al.*, 2015). Moreover, the seroprevalence of *Toxocara* spp. in pregnant women was registered as 6.4% in Brazil, which indicates that this population is also exposed to the infection (Santos *et al.*, 2015).

The aetiological agents of toxoplasmosis and human toxocariasis are different; however, the risk factors for infection, related to social, economic, cultural and dietary habits, are the same for both of these diseases (Campos *et al.*, 2003; Tenter, 2009).

In toxoplasmosis, it has been proven that the main mode of transmission is through the consumption of sausages or raw or undercooked meat that contains intermediate hosts of the parasite *Toxoplasma gondii* and tissue cysts (Sukthana, 2006). However, in toxocariasis this type of transmission is still neglected, even though there have been reports of toxocariasis associated with the consumption of raw or undercooked meat or viscera of paratenic hosts of the helminth *Toxocara* spp., which contains infective encapsulated larvae (Hoffmeister *et al.*, 2007; Choi *et al.*, 2012). Another common mode of infection by these parasites is through the accidental ingestion of contaminated soil. Sporulated oocysts of *T. gondii* (Tenter *et al.*, 2000) are transmitted through soil contaminated with cat faeces, while embryonated eggs of *Toxocara* spp. (Despommier, 2003) are transmitted through soil contaminated with dog faeces. Studies show that the prevalence of co-infection with *T. gondii* and *Toxocara* spp. in children ranges from 3% (Marchioro *et al.*, 2015) to 12% (Franscisco *et al.*, 2006). In addition, experimental and human studies regarding the co-infection with both parasites have been conducted to investigate behavioural changes in individuals (Corrêa *et al.*, 2014; Khademvatan *et al.*, 2014). However, research on the implications of co-infection during pregnancy is rare. The objective of this study was to determine the co-infection with *T. gondii* and *Toxocara* spp. in pregnant women at a university hospital in southern Brazil and to identify the risk factors associated with infection by both parasites.

Materials and methods

Population study

This was a cross-sectional study of 280 pregnant women treated at the University Hospital of the Federal University of Rio Grande (FURG), Rio Grande do Sul, Brazil. The sample size was calculated using the Epi Info 3.5.2 software (CDC, Atlanta, Georgia, USA) and an expected co-infection rate of 3.2% (Marchioro *et al.*, 2015) with a confidence interval of 95% and 10% losses. This study was performed from May 2013 to April 2014. The prevalence of IgG antibodies to *Toxocara* spp. in this population was 6.4% (Santos *et al.*, 2015).

Pregnant women were invited to participate in the study and those who agreed signed an informed consent form. Participants were required to allow researchers to analyse blood samples collected during routine prenatal care and access medical records, and to answer an epidemiological questionnaire. For pregnant women under the age of 18 years, a legal guardian was required to sign the informed consent form.

Serology

Serology tests for the detection of anti-*T. gondii* antibodies were performed during prenatal care using indirect immunofluorescence assays in the clinical analysis laboratory at the University Hospital, Rio Grande, Rio Grande do Sul. To conduct research on the IgG anti-*Toxocara* spp. antibodies, indirect enzyme-linked immunosorbent assays (ELISAs) were performed using the antigen excretion and secretion of *T. canis*. Serum samples were pre-adsorbed with the *Ascaris suum* antigen (Santos *et al.*, 2015).

Data analysis

Two trained researchers used a structured questionnaire to survey participants after parturition in the maternity ward of University Hospital. This questionnaire covered the obstetric history (a history of abortions, premature births, number of pregnancies and number of births), general aspects (contact with pets, type of food), and social and economic characteristics (social, economic and level of education) of the participants. The questionnaires were double-entered using the Epidata 3.1 program (http://www.epidata.dk).

To verify the association of infection with *T. gondii* and with *Toxocara* spp. and the association of co-infection with socio-demographic characteristics, obstetric history, eating habits and contact with animals, we performed an analysis using the Chi-square test with a categorical comparison between variables. The prevalence ratio (RP) was calculated for each variable with a significant difference of $P < 0.05$ and confidence interval (CI) of 95%. Multivariate analysis was performed via logistic regression, followed by the construction of a hierarchical linear model, which incorporated variables with $P \leq 0.20$ in the crude analysis. The first level was composed of demographic and socio-economic variables (family income and place of residence), while the second level was composed of the risk factors for infection with *Toxocara* spp. (contact with a domesticated dog, contact with a domesticated cat and vegetable consumption). All analyses were performed using SPSS (SPSS Inc., Chicago, Illinois, USA) and Epi-info 3.5.2 programs.

Results

The prevalence of IgG antibodies to *Toxocara* spp. was 6.4% in the population under study (Santos *et al.*, 2015). Serology tests for toxoplasmosis were performed prenatally in 71.4% of women, and 28.6% were not tested for toxoplasmosis. From the group of pregnant women who were tested for toxoplasmosis, 62.5% (125) were positive for IgG antibodies to *T. gondii*. According to these results, of all the women who participated in our study, 200 were tested for both toxoplasmosis and human toxocariasis.

In this study, a co-infection rate of 8% was observed in the total population of pregnant women and, among women who were seropositive for *T. gondii*, the co-infection rate was found to be 12.8%. In addition, seropositivity for *Toxocara* spp. increased the risk of infection

Table 1. The proportion (%) of risk factors associated with co-infection with *Toxocara* spp. and *T. gondii* in pregnant women treated in a hospital in Rio Grande – Rio Grande do Sul. *N*, Number of samples examined; CI, 95% confidence intervals; the prevalence ratio denotes comparisons between variables using the Chi-square test.

Variable	Sample		Co-infection		Prevalence ratio	CI	*P* value
	N	%	*N*	%			
Dog ownership							
No	83	41.5	6	7.2	1		
Yes	117	58.5	10	8.5	1.41	0.50–3.90	0.692
Cat ownership							
No	156	78	13	8.3	1		
Yes	44	22	3	6.8	0.54	0.12–2.32	0.604
Onychophagia							
No	139	69.5	12	8.6	1		
Yes	61	30.5	4	6.5	0.56	0.16–1.94	0.530
Consumption of raw and/or undercooked meat							
No	130	65	8	6.2	1		
Yes	70	35	8	11.4	1.48	0.87–2.52	0.189
Consumption of processed food (sausage)							
No	17	8.5	1	5.9	1		
Yes	183	91.5	15	8.19	1.30	0.18–9.29	0.791
Consumption of raw vegetables							
No	50	25	6	12	1		
Yes	150	75	10	6.6	0.50	0.18–1.33	0.277
Contact with sand							
No	139	69.5	11	7.91	1		
Yes	61	30.5	5	8.2	1.13	0.40–3.19	0.804

by *T. gondii* ($P = 0.019$) and seropositivity for *T. gondii* increased the risk of infection by *Toxocara* spp. (RP: 5.35; 95% confidence interval (CI): 1.19–24.0; $P = 0.015$).

There was no association between the socio-demographic characteristics of the study population and co-infection with *T. gondii* and *Toxocara* spp., nor between risk factors and co-infection (table 1).

In the multivariate analysis of the obstetric history of pregnant women who tested positive for IgG antibodies to *T. gondii* and *Toxocara* spp., there was no significant relationship between abortions and premature birth and co-infection; however, we observed that co-infection increases the chances of an infant being born with a low birth weight ($P = 0.011$) (table 2).

Discussion

Helminth infections during pregnancy have been associated with low birth weights in newborns, neonatal mortality (Friedman *et al.*, 2007; Imhoff-Kunsch & Briggs, 2012) and metabolic disorders in pregnancy (Tweyongyere *et al.*, 2011). Among helminth infections, toxocariasis is a parasitic zoonosis with worldwide distribution (Smith *et al.*, 2009) and, according to other studies, its impact on children's health has been observed, with a registered prevalence rate of 50% (Schoenardie *et al.*, 2013b). However, only a few studies have been conducted on pregnant women, even though the registered seroprevalence of *T. canis* in Brazil is 6.4%, which shows that this population is also exposed to infection (Santos *et al.*, 2015). Furthermore, the intracellular parasite *T. gondii* is responsible for disorders during pregnancy and complications during fetal development (Hill *et al.*, 2005). In a serological study of pregnant women in Paraná, the prevalence of IgG antibodies to *T. gondii* was found to be 59% (Ferezin *et al.*, 2013). Few studies have evaluated co-infection with *T. gondii* and *Toxocara* spp. and its potential for harm during pregnancy and fetal development. However, the present study showed that 88.9% of the pregnant women who were seropositive for *T. canis* were also seropositive for *T. gondii*.

During helminthiasis an increased T-helper 2 (Th2) immune response occurs (Hernández *et al.*, 2010), and the same occurs during pregnancy, which negatively affects the immune response to intracellular pathogens, as these pathogens promote a Th1 immune response (Spellberg & Edwards, 2001).

In this study, the total population presented a co-infection rate of 8%, which is lower than the 12.4% rate found in a study of 339 children from São Paulo (Franscisco *et al.*, 2006) and higher than the 3.2% rate found in a study on children from Paraná, Brazil. However, both of the studies on children showed that positivity for *Toxocara* spp. increases the risk of infection by *T. gondii* in children (Franscisco *et al.*, 2006; Marchioro *et al.*, 2015). This association was also observed in the population of pregnant women in this study. However, we also observed that seropositivity for *T. gondii* increases the risk of infection by *Toxocara* spp., which was not observed by Marchioro *et al.* (2015).

The similar mode of transmission of both parasites, which includes the consumption of raw or undercooked meat and the ingestion of embryonated eggs of *Toxocara* spp. (Smith *et al.*, 2009) and oocysts of *T. gondii* (Montoya & Remington, 2008), may explain the co-infection rate observed in our study. Thus, it is important to emphasize that co-infection with these parasites may occur because the intermediate hosts of *T. gondii* and the paratenic hosts of *Toxocara* spp. include the same species of animals, such

Table 2. The proportion (%) of obstetric disorders associated with co-infection by *Toxocara* spp. and *T. gondii* in pregnant women treated at a hospital in Rio Grande – Rio Grande do Sul. *N*, Number of samples examined; CI, 95% confidence intervals; the prevalence ratio denotes comparisons between variables using the Chi-square test.

	Samples		Co-infection				
Variable	N	(%)	N	(%)	Prevalence ratio	CI	*P* value
Abortion							
No	154	77	12	7.8	1		
Yes	46	23	4	8.7	1.21	0.40–3.64	0.725
History of premature parturition							
No	168	84	11	6.5	1		
Yes	32	16	5	15.6	2.62	0.96–7.16	0.069
History of low birth weight							
No	169	84.5	10	5.9	1		
Yes	31	15.5	6	19.3	3.48	1.27–11.42	0.011*

*Level of significance with $P < 0.05$.

as cattle, sheep and pigs (Sukthana, 2006; Hoffmeister *et al.*, 2007; Choi *et al.*, 2012). Therefore, these animals may be considered sources of infection as they may carry both tissue cysts containing bradyzoites of *T. gondii* (Sukthana, 2006) and infective larvae of *Toxocara* spp., which are found in the animal's muscle tissue or viscera (Choi *et al.*, 2012).

Another common mode of infection of these parasites is the accidental ingestion of sporulated oocysts of *T. gondii* (Montoya & Remington, 2008) and embryonated eggs of *Toxocara* spp. (Smith *et al.*, 2009), both of which are developmental forms of the parasites that may be found in soil, on fomites and on poorly cleaned vegetables. In toxoplasmosis, even though the ingestion of oocysts is a well-known mode of infection, it is not the most significant mode in the epidemiology of the disease (Dubey, 2004), while in human toxocariasis, ingestion of embryonated eggs is considered to be the main mode of infection of *Toxocara* spp. (Smith *et al.*, 2009).

In addition to factors related to the similar modes of transmission of these parasites that may facilitate co-infection, helminthiasis can increase the severity and incidence of infections by intracellular parasites as it causes an increased Th2 response and decreased Th1 response. Such immunomodulation is also observed in pregnancy, and Hernández *et al.* (2010) discuss the possibility of a synergistic immune response to helminthiasis during pregnancy. A previous study showed that patients with a co-infection with helminths are at increased risk for malaria, which may increase the risk of complications during pregnancy and harm to the fetus (Gallagher *et al.*, 2005).

Epidemiological studies on helminth infections often relate infection to low birth weights in newborns (Imhoff-Kunsch & Briggs, 2012), which was confirmed by experimental studies on these infections (Anderson, 1996). The only study that considered a relationship between seropositivity for *Toxocara* spp. and low birth weights of newborns found no association between these variables (Santos *et al.*, 2015). However, in the population under study, we found a significant association between co-infection with *T. gondii* and *Toxocara* spp. and low birth weights in newborns.

The association between co-infection with *T. gondii* and *Toxocara* spp. in pregnant women and low birth weights in neonates demonstrates the importance of serological diagnosis during prenatal care. It also demonstrates the importance of further research in this area to identify risk factors associated with co-infection, and the possible implications of co-infection during pregnancy and on the health of the newborn.

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Conflict of interest

None.

Ethical standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation. This study was approved by the Ethics Committee on Research in Health, FURG (CEPAS no. 33/2011).

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