Rev Soc Bras Med Trop 49(5):641-643, September-October, 2016
doi:10.1590/0037-8682-0106-2016

Short Communication

Toxocara spp. seroprevalence in pregnant women in Brasília, Brazil

***Lívia Custódio Pereira*[1], *Guita Rubinsky Elefant*[2], *Yanna Medeiros Nóbrega*[3], *Tamires Vital*[3], *Nadjar Nitz*[3], *Lenora Gandolfi*[3], *Riccardo Pratesi*[3] *and Mariana Hecht*[3]**

[1]. Hospital Universitário de Brasília, Universidade de Brasília, Brasília, Distrito Federal, Brasil.
[2]. Instituto de Medicina Tropical de São Paulo, Universidade de São Paulo, São Paulo, Brasil.
[3]. Laboratório Interdisciplinar de Biociências, Faculdade de Medicina, Universidade de Brasília, Brasília, Distrito Federal, Brasil.

Abstract

Introduction: The impact of gestational toxocariasis is an understudied topic on female reproductive health. We estimated anti-*Toxocara* IgG prevalence among pregnant women in Brasília, Brazil, and investigated the association of the infection with history of abortion and contact with pets. **Methods:** Infection was diagnosed using ELISA with excretory/secretory antigens. Participant information was obtained via questionnaires. **Results:** Of 311 pregnant women, 23 were anti-*Toxocara* IgG positive. Twenty-two percent of anti-*Toxocara* IgG-positive participants and 26% had previously miscarried. Previous contact with pets was associated with higher toxocariasis prevalence. **Conclusions:** A direct relationship between toxocariasis and contact with pets was observed, but there was no relationship with the miscarriage prevalence.

Keywords: *Toxocara* spp. Pregnancy. Animal contact.

Toxocariasis is a disease caused by accidental infection of man by *Toxocara canis* or *Toxocara cati*, roundworms found in dogs and cats, respectively. This zoonosis is widespread throughout the globe and is transmitted to humans through food or water contaminated with feces containing parasite eggs. Larvae released in the small intestine may result in damage to several tissues and systemic inflammatory responses, showing a wide range of symptoms[1]. The diagnosis can be made by using histological or serological methods. However, because of the difficulty of demonstrating the presence of larva in tissue obtained by biopsy, immunodiagnosis is the first-choice test. Enzyme-linked immunosorbent assay (ELISA) using secretory-excretory antigens (TES) of *Toxocara* spp. larvae is the most recommended method, due its low cost and high sensitivity[2].

Gestational toxocariasis is an understudied topic, and few articles have been published on this subject. It has been estimated that the infection rate in pregnant women can reach 39%, depending on the analyzed area. Only one study has investigated the prevalence of toxocariasis among pregnant women in Brazil, reporting an infection rate of 6.4%[3]. Data on the impact of the disease on women's reproductive health are also scarce, but there is evidence of increased infertility with tubal blockage and abortions[4]. Furthermore, congenital transmission of this parasite has been reported and has been linked to newborn ocular injury[5].

Thus, toxocariasis is a major public health problem that has not received the attention needed to improve identification, treatment, and control. The current study aimed to determine the prevalence of *Toxocara* spp. in a low-income population of pregnant women in Brasília (Federal District), Brazil. In addition, this study investigated the association of the infection with abortion and contact with dogs or cats.

To address these aims, we conducted a cross-sectional study on *Toxocara* spp. seroprevalence in and administered a questionnaire to young pregnant women (2030 years old) in 311 patients from the antenatal clinic of the University of Brasília Hospital. Patients resided in several administrative regions of Federal District, and the majority (90%) lived in very low income suburbs. The sample size was determined using Epi-Info version 6.0 software, based on an expected prevalence of 30% for anti-*T. canis*-positive immunoglobulin G (IgG), to evaluate with a 95% degree of confidence and a tolerated error of 5%. The exclusion criterion was based on a recent infection report (last 2 years) for other helminths. For each patient, 3mL of peripheral blood was collected via venipuncture of the forearm. A questionnaire was administered and included age;

Corresponding author: Dra. Mariana Hecht.
e-mail: marianahecht@gmail.com
Received 31 March 2016
Accepted 25 July 2016

race; marital status; address; and history of intestinal parasites, previous pregnancy, and contact with domestic animals. Only patients who signed the consent form were included in the study. All procedures were carried out in compliance with Brazilian regulations and international guidelines.

Of the 311 tested samples, ELISA identified anti-*Toxocara* spp. antibodies in 23 pregnant women, corresponding to a specific IgG-positive prevalence of 7.2% (**Figure 1A**). The distribution of seropositivity of toxocariasis according to history of contact with cats or dogs is shown in **Figure 1B**. Note the incidence of toxocariasis is higher (56.5%) in those with a history of contact with dogs or cats than in those without previous contact with these animals (26.4%). No relationship was observed for presence of infection and occurrence of abortions, since positive and negative pregnant women reported similar rates of miscarriage (**Figure 1B**).

The prevalence of toxocariasis among adults in Brazil Federal District is unknown. Accordingly, the only comparison that can be made is to a study conducted at University of Brasília Hospital that investigated anti-*Toxocara* spp. IgG in children, showing a prevalence of 22%[6]. This contrasts with other studies showing no statistically significant difference in the prevalence anti-*Toxocara* spp. antibody positivity in adults and children from the same region[7]. Since IgG antibodies are produced after host exposure to a particular antigen, initial infections of childhood often persist into adulthood[8], thus the percentage of positive individuals should remain the same. To clarify this issue, a cohort investigation should be conducted, taking into account pregnant women and type of antibody.

Some hypotheses can explain the lower prevalence found in the current study. One may be related to the hemodynamic and immunologic changes that occur in pregnant women and may affect IgG antibody detection. During pregnancy, due to placental demand, there is a 30-50% increase in plasma volume[9]. In consequence, this hemodilution decreases the relative concentration of blood elements, including immunoglobulins. As antibody detection using ELISA consists of an antigen-antibody reaction, the decrease in plasma IgG concentration can lead to antibody titers below the minimum detectable level. In addition, physicochemical and biological changes occur with IgG during pregnancy, interfering with the formation of antigen-antibody complexes detectable by serological tests. These molecules, termed asymmetric IgG, are unable to form the insoluble aggregates detected by ELISA, although they maintain their ability to bind to an antigen[10]. Thus, patients with positive serology prior to pregnancy could show seroconversion during gestation.

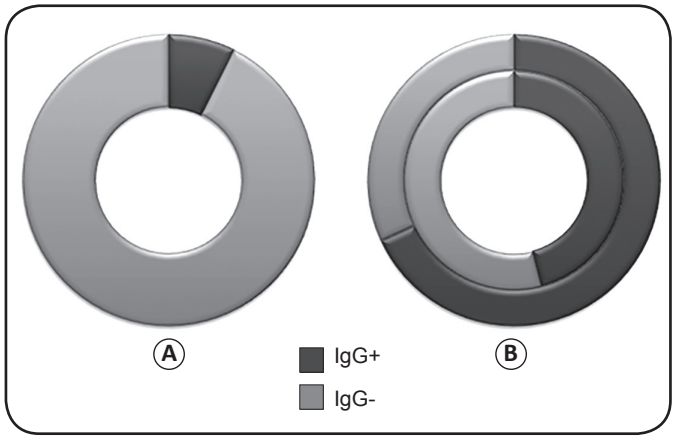

FIGURE 1. Prevalence of toxocariasis in pregnant women of Brasília, Brazil, and infection-related factors. **(A)** Anti-*Toxocara* spp. IgG-positive and -negative patients determined using an enzyme-linked immunosorbent assay to detect the presence of secretory-excretory antigens. **(B)** Outer circle: association of anti-*Toxocara* spp. IgG serology with canine or feline contact ($p < 0.01$.); inner circle: abortion ($p = 0.86$). **IgG:** immunoglobulin G.

Our data suggest no significant difference in abortion history between toxocariasis-positive and -negative groups. This finding, however, differs from other data in the literature. For instance, in a study including 52 postpartum women, 35.3% of those with positive anti-*Toxocara* spp. IgG had at least one abortion in previous pregnancies, while the frequency of abortion in those with negative serology was 8.6%[4]. Moreover, Gasanova[11] found a prevalence of abortion in 56% of *T. canis*-infected patients, as well as reproductive changes, reinforcing the tropism of *Toxocara* spp. to the genital tract.

Contact with domestic dogs or cats is considered a risk factor associated with *T. canis* or *T. cati* infection. The present study showed a higher prevalence of toxocariasis in pregnant women who had contact with dogs or cats. This finding is in agreement with other published data showing a strong association between contact with domestic animals and development of toxocariasis[3] [12]. Indeed, a recent article revealed that a history of owing a dog increases the rate of *T. canis* seropositivity almost 13-fold[13].

In conclusion, toxocariasis is one of the most important neglected diseases listed by the Centers for Disease Control, having significant morbidity in socioeconomically disadvantaged countries. Due to the lack of adequate surveillance programs, the true number of cases of toxocariasis is likely to be underestimated[2]. In endemic areas, poor sanitation habits and education, including poor hygiene and contact with animals, are keystones for disease establishment.

There are few reports of toxocariasis in pregnant women, and there is disagreement in the literature regarding whether toxocariasis is linked to a higher rate of abortions in pregnant women. However, diagnosis based on antibody detection may fail to identify the infection in pregnant women. Unfortunately, molecular tests and direct optical diagnosis also have several limitations[14]; accordingly, there is a need to develop new strategies to improve identification of *Toxocara* infections. Further investigation is needed to confirm or refute the suspicion that the prevalence of anti-*Toxocara* spp. IgG positivity among pregnant women is related to infertility and genitourinary changes.

Ethical considerations

The research protocol was approved by the Ethical Committee on Human Research of Brasília University (protocol number 060/2003).After collection, blood was centrifuged to obtain serum and was sent to the Tropical Medicine Institute of

São Paulo. Detection of anti-*Toxocara* spp. IgG was performed using ELISA, following previously standardized protocols[6]. Briefly, 96-well plates were sensitized with 0.130µg of TES antigen. Serum samples were pre-adsorbed with the SoAs antigen to avoid cross-reaction with *Ascaris* proteins and then incubated with *Toxocara* spp. antigens. The second antibody consisted of peroxidase-conjugated anti-human IgG. Optical densities were determined at 492nm. The cut-off absorbance value was defined as the mean absorbance for negative controls plus three standard deviations. Test and control serum assays were run in duplicate. Epi-info version 6.0 software was used for statistical analysis. The chi-squared test was employed to confirm the difference among groups. The level of significance was established at $\alpha = 0.05$.

Acknowledgments

We offer our thanks to *Hospital Universitário de Brasília*.

Conflicts of interest

The authors declare no conflicts of interest.

Financial Support

This study was funded by the Interdisciplinary Laboratory of Biosciences.

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