



Frequency of *Toxocara* spp. antibodies in umbilical cords of newborns attended at the University Hospital in Southern Brazil and factors associated with infection



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ABSTRACT

Toxocariasis is a neglected and geographically widespread parasitic disease. The detection of specific antibodies associated with this disease is required to confirm its clinical diagnosis and to aid in prevention. Although helminth infection during pregnancy can promote foetal immune responses with long-term effects, specific information regarding the risk of *Toxocara* spp. infection to the human foetus during pregnancy is lacking. Therefore, the present study aimed to investigate the frequency of antibodies against *Toxocara* spp. in umbilical cord serum samples to determine the neonatal risk factors associated with *Toxocara* spp. infection.

A cross-sectional study of the frequency of specific antibodies against *Toxocara* spp. was performed on umbilical cord samples of 280 neonates. A cord blood sample was obtained from each newborn after parturition, and serum samples were examined by enzyme-linked immunoassay. Epidemiological data were obtained through a questionnaire regarding obstetric history (abortion history, premature birth history, and pregnancy and birth numbers), general aspects (animal contact and diet) and socio-economic factors. The frequency of anti-*Toxocara* spp. IgG antibodies in the umbilical cords of neonates was 20% in serum pre-adsorbed with *Ascaris* spp. antigen. Family income and dog ownership were considered risk factors associated with infection. No association was found between reproductive disorders and *Toxocara* seropositivity. The 20% frequency rate of anti-*Toxocara* spp. IgG antibodies in sera from umbilical cords of newborns can be related to IgG binding at the maternal-foetal interface, requiring greater care during pregnancy. Anti-*Toxocara* IgM and IgE antibodies were not found in umbilical cord serum samples, indicating that no vertical transmission of these parasites occurred in this population. Studies regarding antibodies against *Toxocara* spp. in umbilical cord sera are important for determining neonatal exposure to these parasites.

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1. Introduction

Helminth infection during pregnancy can promote foetal immune responses with long-term effects. Thus, determining the

immunological tolerance to ward helminths and identifying cross-reactive allergens due to structural and immunological similarities are important (Mpairweet al., 2014). Previous epidemiological studies have associated helminth infection during gestation with low birth weight and neonatal mortality (Imhoff-Kunsch and Briggs, 2012).

Human toxocariasis, which is a parasitic zoonosis with worldwide distribution, is among the most prevalent helminth infections (CDC, 2009). However, the impact of this infection on human health is severely underestimated. The aetiological agent most commonly

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associated with this parasitic infection is the nematode *Toxocara canis*, an intestinal parasite of dogs (Smith et al., 2009; Moreira et al., 2014).

In Brazil, studies in children revealed high *Toxocara* spp. seroprevalence (IgG), with rates varying from 15.5% (Cassenote et al., 2014) to 50.6% (Schoenardie et al., 2013). Furthermore, few studies involving adults recorded rates below 8.7% (Negri et al., 2013), and a population of pregnant women in China had a rate of 9.19% (Cong et al., 2014).

Nonetheless, Santos et al. (2015) registered a seroprevalence rate of 6.4% for pregnant women in Brazil, revealing a risk factor for infection contact with dogs, which is the most closely associated risk factor for toxocariasis in children (Carvalho and Rocha, 2011).

Vertical transmission of *T. canis* and *T. cati* in the definitive hosts (dogs and cats) (Schnieder et al., 2011) and *T. canis* in paratenic hosts (sheep and mice) demonstrates the migratory ability and the tropism of the larvae of this parasite during pregnancy or lactation, reinforcing the importance of performing studies in this area in humans (Anderson, 1996; Aguiar et al., 2015; Telmo et al., 2015).

However, the only record of congenital infection in humans occurred in a premature infant with retinopathy presenting characteristic lesions with the presence of structures compatible with the morphology of the larvae of *Toxocara* spp. The premature infant presented blood eosinophilia and negative IgG levels against *Toxocara* spp.; however, the mother presented seropositivity for *Toxocara* spp., confirming vertical transmission (Maffrand et al., 2006). The severe consequence of human congenital toxocariasis may be related to ocular toxocariasis or cerebral toxocariasis causing neurodegenerative disorders (Fan et al., 2015).

The diagnosis of this disease is based on the association of symptoms with epidemiological and laboratory data (specific serology, eosinophilia and total IgE). The detection of specific antibodies is important for confirming the clinical diagnosis and for treating different clinical forms of human toxocariasis (CDC, 2009).

Placental transfer of immunoglobulin class G (IgG) antibodies from the mother to the foetus is an important neonatal immune mechanism (Hashira et al., 2000). Studies have shown that the IgG concentration in umbilical cord serum is up to 15% higher than the concentration found in maternal serum. Furthermore, IgG levels in maternal serum may show a reduction of 60%–70% from the beginning of gestation (Hironaka and Casanova, 2003).

However, class M and E immunoglobulins are produced by the foetus at approximately 11 weeks gestation and have no placental transfer capacity. Thus, the detection of these immunoglobulins in umbilical cord serum suggests intrauterine infection (Holt and Jones, 2000).

In this context, specific information regarding the risk of foetal *Toxocara* spp. infection during pregnancy in humans is lacking (Taylor et al., 1996).

The aims of the study were to investigate the frequency of IgG, IgE, and IgM antibodies against *Toxocara* spp. in umbilical cords of neonates treated at the University Hospital Miguel Riet Correa Junior in the city of Rio Grande, Rio Grande do Sul, Brazil, and to determine associated risk factors.

2. Methods

2.1. Population study

A cross-sectional study of the frequency of specific antibodies against *Toxocara* spp. in the umbilical cords of 280 neonates treated at the University Hospital Miguel Riet Correa Junior in the city of Rio Grande, Rio Grande do Sul, Brazil. The study participants, pregnant women, signed an informed consent form (ICF), authorising the collection and analysis of infant cord blood as well as access to medical

records. Moreover, the participants agreed to answer an epidemiological questionnaire. The informed consent of a legal guardian was ordered for pregnant women under the age of 18 years. The Health Research Ethics Committee (CEPAS) of the Federal University of Rio Grande (FURG) (opinion 33/2011) approved this study.

2.2. Epidemiological data

A structured questionnaire was administered by two previously trained researchers after birth in the maternity ward at the University Hospital Miguel Riet Correa Junior. This questionnaire included questions regarding the mother's obstetric history (abortion history, premature birth history), general aspects (animal contact and diet) and socio-economic. The questionnaires were double-entered using the EpiData 3.1 program.

2.3. Medical records survey

Medical records of the pregnant women were surveyed for the eosinophilia level, laboratory diagnosis results for other infectious and parasitic diseases (i.e., acquired immune deficiency syndrome, hepatitis, and toxoplasmosis) and child birth weight.

2.4. Umbilical cord serum sample collection

An umbilical cord blood sample collected from each newborn after parturition at the Obstetrics Centre of HU/FURG. To obtain serum, umbilical cord blood samples were centrifuged at 2.500 rpm for 10 min, placed in Eppendorf® tubes and stored at –20 °C.

2.5. Excretory/secretory products of *T. canis* larvae

The antigens were produced in the Parasitology Laboratory, Interdisciplinary Area Biomedical Sciences (AICB), School of Medicine (FAMED), FURG.

2.6. Excretory-secretory antigen (TES) production

Toxocara canis eggs were collected from female adult parasites after treating young dogs with pyrantel pamoate (15 mg/kg). The eggs were then incubated in a 2% formalin solution at 28 °C and oxygenated for 30 days (Avila et al., 2012). Thereafter, the larvae derived from embryonated eggs were incubated in RPMI-1640 medium with antibiotics and antifungals at 37 °C in 5–8% CO₂ (Maizels et al., 1991) to obtain excretory-secretory antigens (TES) of the infective *T. canis* larvae (De Savigny et al., 1979). The determination of protein concentration was performed using the Bicinchoninic Acid Method (BCA) (Smith et al., 1985).

2.7. Somatic antigen of *Ascaris suum* (SoAs)

This antigen was produced from adult female *A. suum* acquired from a slaughterhouse in the city of Pelotas, Rio Grande do Sul, Brazil, following the methodology described by Souza et al. (2011). The determination of the protein concentration of this antigen was performed using BCA (Smith et al., 1985).

2.8. Serological analysis of umbilical cord serum samples by ELISA-TES for IgG detection with or without pre-adsorption with SoAs

To conduct research on IgG anti-*Toxocara* spp. antibodies, indirect ELISAs were performed using the TES (2 µg/mL) antigen in carbonate/bicarbonate buffer. The free binding sites were blocked with 5% casein in 0.05% PBS/Tween-20 (PBS-T), sera were diluted 1:50 in PBS/Tween and peroxidase conjugates and anti-human

Table 1

Positive serology (IgG) for *Toxocara* spp. in umbilical cord samples and sociodemographic factors of new borns attended at the University Hospital of Rio Grande –RS (n = 280).

Variable	Samples N (%)	Positivity N (%)	Prevalence ratio	95% CI	p value
Age					0.932
13–19	58 (20.7)	16 (27.6)	1	0.92–2.53	
20–24	82 (29.3)	17 (20.7)	1.05	0.63–1.74	
25–29	73 (26)	12 (16.4)	0.77	0.43–1.38	
30–34	41 (14.6)	06 (14.6)	0.69	0.32–1.52	
35 or more	26 (9.4)	05 (19.2)	0.95	0.41–2.18	
Family income (Minimum wage)					0.009
3 or more	78 (27.9)	13 (16.6)	1	0.44–1.37	
Up to 2	146 (52.1)	28 (19.9)	0.91	0.57–1.46	
≤ 1	56 (20.0)	15 (26.8)	1.5	0.87–2.44	
Domicile					0.435
Periphery	180 (64.3)	30 (16.6)	1.0		
Downtown	36 (12.8)	08 (22.2)	1.16	0.60–2.26	
BalnearyCassin	18 (6.4)	07 (38.9)	2.13	1.13–4.01	
Rural	29 (10.4)	08 (27.6)	1.48	0.78K2.83	
Another municipality	17 (6.1)	03 (17.6)	0.87	0.30–2.51	

(Chi-square test, p ≤ 0.05).

IgG (Fc specific) (1:7000) (Sigma Aldrich, San Diego, CA, USA). The chromogen used was orthophenylenediamine (OPD). Each serum sample was examined in duplicate at a 450 nm wavelength. The cutoff for specific IgGs in cord blood samples was calculated by the mean absorbance of the sera of newborns of mothers with a negative history of eosinophilia and without contact with dogs (Mendonça et al., 2013) plus two standard deviations. Serum samples pre-adsorbed with SoAs were subjected to intermittent agitation for one hour at 37 °C (Camargo et al., 1992).

2.9. Serological analysis of umbilical cord serum samples by ELISA-TES for IgM detection

The IgM assay for *T. canis* in serum samples from pregnant women and umbilical cord of newborns was performed by ELISA-TES, as described in the previous section. Was used conjugated anti-human IgM Fc-specific peroxidase conjugated—Sigma® in dilution 1: 6000. The same criteria to calculate the cut-off point was used.

2.10. Serological analysis of umbilical cord serum samples by ELISA-TES for IgE detection

The analysis of *T. canis* IgE antibodies in serum samples from pregnant women and from newborn umbilical cords was performed by ELISA-TES as described in the previous section. Was used conjugated anti-human IgE Fc-specific peroxidase conjugated—Sigma® in dilution 1: 5000. The same criteria to calculate the cut-off point was used.

2.11. Statistical analysis

To determine associations between seropositivity against *Toxocara* spp. and sociodemographic factors, obstetric history, diet and animal contact, we performed chi-square tests for categorical comparisons between variables. The prevalence ratio was calculated for each variable, and p < 0.05 was considered significant. Multivariate analysis was performed by logistic regression, followed by the construction of a multivariable hierarchical linear model that incorporated variables with p ≤ 0.20 in the crude analysis. All analyses were performed with SPSS and Epi Info 3.5.2 programs.

3. Results

Of the 280 newborn umbilical cord serum samples analysed by ELISA-TES, specific anti-*Toxocara* spp. IgG antibody frequency

rates of 30.4% (85/280) for sera unadsorbed with *A. suum* and 20% (56/280) for previously adsorbed sera were determined.

Bivariate analysis of the sociodemographic characteristics of the study population indicated that the risk factor for *Toxocara* spp. infection was family income (Table 1). However, the analysis of epidemiological factors indicated that dogownership was a risk factor (Table 2).

Twenty-five percent of the positive samples were from women who reported previous abortions. However, no significant difference was observed between positive serology for *T. canis* in newborn umbilical cord serum and reproductive disorders and blood eosinophilia in the mothers (Table 3). Multivariate analysis identified the following variables as independent risk factors for *Toxocara* spp. infection: dog ownership (PR = 1.87, CI = 1.02–3.42; p = 0.04) and family income at or below the minimum wage (OR = 4.11, CI = 1.69–10.03; p ≤ 0.001).

No IgM and IgE was detected in umbilical cord serum by ELISA-TES.

4. Discussion

Although toxocariasis is distributed worldwide, the prevalence rates reported during pregnancy are scarce and can vary from 6.4 to 9% (Cong et al., 2014; Santos et al., 2015). However, this study is the first to evaluate the frequency of anti-*Toxocara* spp. IgG antibodies in umbilical cord. In studies conducted in Brazil or other developing countries, the sera must be pre-adsorbed with other helminth antigens, particularly with *Ascaris* spp., to prevent cross-reactions (Camargo et al., 1992). Thus, in our study, an approximately 10% reduction in the frequency of antibodies was observed after prior adsorption of sera with *A. suum*, confirming the importance of prior adsorption for reducing cross-reactivity. In this study, an frequency rate of 20% was observed in previously adsorbed sera from umbilical cords of newborns, a result similar to that found in a study with children in Brasília (Campos et al., 2003) and lower than that found in Pelotas (Schoenardie et al., 2013). However, the seroprevalence measured in the present study was higher than in studies of pregnant women in China and Brazil, which recorded seroprevalence rates of 9% (91/990) (Cong et al., 2014) and 6.4% (Santos et al., 2015), respectively.

The transplacental passage of IgG antibodies against *Toxocara* spp. occurs by a receiver (alkaline phosphatase) present in the foetal circulation that binds IgG at the maternal-foetal interface; consequently, immunoglobulin concentrations decrease in pregnant women, as observed when measuring the difference between maternal and newborn IgG levels in this study. This pas-

Table 2

Variable	Samples N (%)	Positivity N (%)	Prevalence Ratio	95% CI	p value
Dog ownership					0.02
No	134 (47.9)	20 (14.9)	1		
Yes	146 (52.1)	36 (24.7)	1.8	1.01–3.42	
Contact with dog faeces					0.937
No	231 (82.5)	46 (19.9)	1		
Yes	49 (17.5)	10 (20.4)	1.03	0.47–2.2	
Cat ownership					0.155
No	224 (80)	41 (18.3)	1		
Yes	56 (20)	15 (26.8)	1.4	0.87–2.44	
Onychophagia					0.089
No	196 (70)	35 (17.8)	1		
Yes	84 (30)	21 (25)	1.4	0.86–2.25	
Meat consumption raw and/or undercooked					0.132
No	182 (65)	40 (21.9)	1		
Yes	98 (35)	16 (16.3)	0.74	0.43–1.25	
Consumption of processed food (sausage)					0.174
No	30 (10.7)	4 (13.3)	1		
Yes	250 (89.3)	52 (20.8)	1.56	0.60–4.0	
Raw vegetable consumption					0.255
No	75 (26.8)	13 (17.3)	1		
Yes	205 (73.2)	43 (20.9)	0.82	0.47–1.44	
Contact with sand					0.419
No	193 (68.9)	38 (19.7)	1		
Yes	87 (31.1)	18 (20.7)	1.05	0.63–1.73	
Attends squares and/or parks					0.422
No	97 (34.6)	20 (20.6)	1		
Yes	183 (65.4)	36 (19.7)	0.95	0.58–1.55	

(Chi-square test, $p \leq 0.05$).**Table 3**

Relationship between seropositivity in umbilical cord serum and obstetric history and eosinophilia in their pregnant women attended at the University Hospital of Rio Grande –RS (n = 280).

Variable	Samples N (%)	Positivity N (%)	Prevalence Ratio	95% CI	p value
Abortion					0.233
No	220 (78.6)	42 (18.5)	1		
Yes	60 (21.4)	14 (25.0)	1.22	0.71–2.08	
Reporting difficulty conceiving					0.259
No	248 (88.6)	52 (20.9)	1		
Yes	32 (11.4)	4 (12.5)	0.596	0.23–1.53	
History of premature parturition					0.347
No	240 (85.7)	49 (20.4)	1		
Yes	40 (14.3)	7 (17.5)	0.85	0.41–1.75	
History of low birth weight					0.347
No	240 (85.7)	49 (20.4)	1		
Yes	40 (14.3)	7 (17.5)	0.85	0.41–1.75	
Eosinophilia					0.380
No	262 (93.6)	53 (20.2)	1		
Yes	18 (6.4)	3 (16.6)	0.82	0.28–2.37	

(Chi-square test, $p \leq 0.05$).

sage of antibodies occurs until the foetus exhibits IgG levels similar to those of an adult (Hironaka and Casanova, 2003). Factors that may contribute to the prevalence of toxocariasis in populations of pregnant women remain underestimated.

Risk factors for *Toxocara* spp. infection in populations of pregnant women, such as dog contact and socio-economic characteristics, are associated with toxocariasis (Santos et al., 2015). Accordingly, in the present study, analysis of the epidemiological data indicated that dog ownership ($p = 0.04$) and income at or below the minimum wage ($p \leq 0.001$) are risk factors for *Toxocara* spp. infection in this population.

Reproductive disorders resulting from *Toxocara* spp. infection have been established in experimental studies; however, studies regarding reproductive implications of these infections in humans are scarce (Taylor et al., 1996; Santos et al., 2015). Taylor et al. (1996) observed a significant association between seropositivity for anti-*Toxocara* spp. IgG antibodies and the occurrence of abortions in the United States. However, a study conducted in Brazil that

evaluated positive serology for *Toxocara* spp. in pregnant women with previous abortions, premature birth and low birth weight did not find a significant difference between these variables (Santos et al., 2015).

Studies evaluating the presence of anti-*Ascaris* spp. IgM and IgE antibodies in umbilical cord serum suggest that this helminth infection during pregnancy can promote an immune response in the foetus and that the vertical transmission of these parasites may occur (Mpairwe et al., 2014). However, few studies have assessed the presence of anti-*Toxocara* spp. IgM and IgE antibodies in umbilical cord serum to evaluate vertical transmission.

IgM and IgE immunoglobulins have no placental transfer capacity; thus, the detection of these immunoglobulins in umbilical cord serum suggests intrauterine infection (Holt and Jones, 2000). In the present study, we investigated the detection of both of these immunoglobulins (anti-*Toxocara* spp. IgM and IgE). However, these immunoglobulins were not found in umbilical cord serum samples, indicating that no vertical transmission of these parasites occurred

in this population. This result was also found by Taylor et al. (1996), though the presence of anti-*Toxocara* spp. IgE was not investigated. The absence of a specific IgE in umbilical cord serum is justified by low total IgE levels detectable in the circulation (Guadalupe et al., 2009).

Thus, the search for antibodies against *Toxocara* spp. in umbilical cord is important for determining neonatal exposure to this parasite and for promoting greater care in the execution of serological tests and complementary analyses at birth.

5. Conclusions

This study is the first to assess the specific seropositivity for anti-*Toxocara* spp. IgG antibodies in umbilical cord serum samples. Although vertical transmission did not occur, as determined by antibody detection, the frequency rate indicates the exposure risk of the analysed population, justifying further studies in humans to detect vertical transmission of *Toxocara* spp. and the implications of this parasitosis in foetal development.

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