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OOCYST INGESTION AS AN IMPORTANT TRANSMISSION ROUTE OF *TOXOPLASMA GONDII* IN BRAZILIAN URBAN CHILDREN

V. C. C. Dattoli, R. V. Veiga*, S. S. Cunha†, L. Pontes-de-Carvalho‡, M. L. Barreto§, and N. M. Alcantara-Neves*||
Faculdade de Medicina da Universidade Federal do Acre, Rod. BR 364, km 4, 6637-Rio Branco-Acre, Brazil. *e-mail: neuzalcantara@gmail.com*

ABSTRACT: Toxoplasmosis is a cosmopolitan protozoan infection. Data regarding risk factors for the post-natal acquisition of *Toxoplasma gondii* infection in childhood are limited. We conducted a serological survey for *T. gondii* IgG antibodies and associated risk factors in 1,217 children 4–11-yr-old from Salvador, Brazil, using a commercial ELISA kit; antibodies were found in 17.5% of the children. Age (OR = 2.18; 95% CI: 1.50–3.17) and maternal schooling level (OR = 0.62; 95% CI: 0.42–0.92) were negatively associated with infection. A greater number of siblings (OR = 1.53; 95% CI: 1.12–2.09), cat at home (OR = 1.54; 95% CI: 1.06–2.24), house with non-treated piped water (OR = 2.54; 95% CI: 1.22–5.31), and the absence of a flush toilet at home (OR = 1.45; 95% CI: 1.04–2.01) were positively associated with *T. gondii* infection. Our data suggest that low socioeconomic levels and poor hygiene habits are important factors in favoring *T. gondii* infection.

Toxoplasma gondii infects most species of warm-blooded animals, and its prevalence in humans in Brazil is one of the highest worldwide (Dubey, 2009). As an example, 75% of blood donors from northeastern cities in Brazil were seropositive (Coelho et al., 2003). The ingestion of water or food contaminated with oocysts is considered the most important mode of transmission, at least in people of low socioeconomic status (Bahia-Oliveira et al., 2003). Most of the epidemiological information on toxoplasmosis is based on serological studies in adults, especially women of child bearing age (Pappas et al., 2009). There are many studies on post-natal infections in children (Dubey, 2009). In 1 study of 500 children 3–10-yr-old in Guatemala, antibodies were found in 37.8%; seroprevalence increased from 25% in 3-yr-old to 45% in 5-yr-old children (Jones et al., 2005). Souza et al. (1987) found marked differences in seropositivity among 608 school children from 2 neighborhoods, Bonsucesso (166 children) and Jacarepaguá (442 children) and 2 age groups (6–8-yr-old and 12–14-yr-old) within the city of Rio de Janeiro, Brazil. Overall, 68.4% of 608 children were seropositive (IFA, 1:16), but none had IgM antibodies; 36.5% of 81 children 6–8-yr-old and 70.4% of 81 children 12–14-yr-old in Bonsucesso, and 64% of 222 children 6–8-yr-old, and 84.5% of 220 children 12–14-yr-old in Jacarepaguá had anti–*T. gondii* antibodies. Results indicate a very high rate of post-natal transmission of *T. gondii* during childhood. Recently, using the same test (IFA, cut-off 1:16) Lopes et al. (2008) found that 46.4% of 4–11-yr-old children from Paraná, Brazil had *T. gondii* antibodies. Frenkel et al. (1995) found that 72 of 571 (12.6%) children in Panama City seroconverted between 1 and 6 yr of age.

In the present study, we determined *T. gondii* infection seroprevalence and its risk factors in children living in Salvador, a large urban center in northeastern Brazil.

MATERIALS AND METHODS

Study design

This study was conducted in the city of Salvador, northeastern Brazil, with a population of over 2.5 million. The children (n = 1,445) were born between 1994 and 2001 and enrolled in a cohort recruited from 1997 and 2003 for evaluating the impact of a sanitation program on the incidence of childhood diarrhea (Barreto et al., 2007). Demographic and social data were collected using a validated questionnaire. In 2005, these children were resurveyed to collect data on asthma symptoms and risk factors for asthma; in addition, information regarding *T. gondii* and social demographic data were obtained. Informed written consent was obtained from the children's parents or guardians, and ethical approval was granted by the Instituto de Saúde Coletiva at the Universidade Federal da Bahia and the National Commission on Ethics in Research (CONEP), Brazil. Among the original 1,445 children included in the 2005 survey, 1,217 with complete data set were included in the present study.

Potential risk factors for *Toxoplasma gondii* infection

The legal guardian of each child filled out 2 questionnaires. Between 1997 and 2003, information on socioeconomic and environmental factors was collected as part of the previous study to evaluate the impact of a sanitation program on childhood diarrhea (Strina et al., 2003). In 2005, an International Study of Asthma and Allergy in Childhood (ISAAC) Phase II–based questionnaire (ISAAC, 1998) was implemented in the same group of children. Although this questionnaire is a standardized tool for asthma and allergies, it contains several questions regarding environmental characteristics, which were used in the current work to study risk factors for *T. gondii* infection.

Toxoplasma gondii infection was studied as outcome and the following variables collected between 1997 and 2003 were analyzed as potential risk factors for *T. gondii* infection, i.e., having treated piped water at home, living in a house that flooded during the rainy season, having a flush toilet, living in a house served by a paved road, the presence of open sewage near the house, and rubbish collection frequency. Variables from the 2005 survey were also investigated as potential risk factors, i.e., maternal schooling; meat consumption (how often the child has eaten meat, chicken, beef, and pork); vegetable intake (how often the child eats vegetables); presence of older siblings; number of siblings; whether the child attended nursery; presence of rodents, flies, dogs, and cats at home; having treated piped water at home; living in a house served by a paved road; and presence of a waste system. The sex and age of the children as collected in 2005 were treated as a priori confounders.

Serological detection of anti–*Toxoplasma gondii* IgG antibodies

Blood collection was performed in 2005. Serum levels of anti–*T. gondii* IgG antibodies were determined by indirect enzyme immunoassay (ELISA), following the directions for commercially available kits (Diamedix, Miami, Florida). The assay cut-off was determined by an index value obtained by the following ratio: absorbance of sample/absorbance of a calibrator (a solution containing human serum or plasma, antibodies weakly reactive for anti–*T. gondii* IgG and 0.1% sodium azide). A ratio value greater than 1.00 was considered positive.

Statistical analysis

Only children with complete data were included in the analysis. We first performed a bivariable analysis between each potential risk factor and

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* Instituto de Ciências da Saúde, Universidade Federal da Bahia, Av. Reitor Miquel Calmon, sem no. Canlea, 40110-100, Salvador, Bahia, Brazil.
† Centro de Ciências Sociais Aplicadas, Departamento de Ciência Administrativas, Ciências Sociais, Cidade Universitária 50670-901, Recife-Pernambuco, Brazil.
‡ Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz, Rua Waldemar Falcão, 123, Brotas, 40296-710, Salvador-Bahia, Brazil.
§ Instituto de Saúde Coletiva, Federal University of Bahia, Rua Basílio da Gama s/n, Canela, 40110-040 Salvador-Bahia, Brazil.
|| To whom correspondence should be addressed.
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TABLE I. Sociodemographic characteristics frequencies and associations with *Toxoplasma gondii* infection of 1,217 studied children. Abbreviations: OR, odds ratio, CI, confidence interval.

	Toxoplasma gondii infection 213 (17.5%)			
Variables	n	(%)	OR (95% C.I.)†	Final model OR (95% C.I.)‡
Sex				
Female (n = 564)	99	17.6	1	1
Male (n = 653)	114	17.5	0.99 (0.74; 1.34)	0.90 (0.66; 1.22)
Age group (years)				
<6 (n = 459)	63	13.7	1	1
6 to 7 (n = 419)	65	15.5	1.15 (0.79; 1.68)*	1.10 (0.74; 1.61)
≥8 (n = 339)	85	25.1	2.10 (1.47; 3.02)*	2.18 (1.50; 3.17)*
Mother's schooling				
Illiterate or primary school complete (n = 578)	129	22.3	1	1
Secondary education complete (n = 271)	40	14.8	0.60 (0.41; 0.89)*	0.73 (0.49; 1.09)
High school complete (n = 368)	44	12	0.47 (0.33; 0.69)*	0.62 (0.42; 0.92)*
Number of siblings				
<2 (n = 738)	106	14.4	1	1
≥2 (n = 479)	107	22.3	1.72 (1.27; 2.31)*	1.53 (1.12; 2.09)*
Older siblings				
No (n = 464)	69	14.9	1	—
Yes (n = 753)	144	19.1	1.35 (0.99; 1.85)	
Nursery attendance				
No (n = 1020)	168	16.5	1	—
Yes (n = 197)	45	22.8	1.50 (1.04; 2.18)*	
Beef intake§				
Rare (n = 67)	10	14.9	1	—
≥once weekly (n = 1150)	203	17.7	1.22 (0.61; 2.43)	
Vegetable intake‖				
≥once weekly (n = 956)	167	17.5	1	—
Rare (n = 261)	46	17.6	1.01 (0.71; 1.45)	

* $P < 0.05$.
† Odds ratio and 95% confidence interval from crude analysis.
‡ Adjusted for all variables which has kept the significance in the multivariable analyses, including sex and age as a priori confounders.
§ How often has the child eaten meat (chicken, beef, or pork)?
‖ How often has the child eaten vegetables?

outcome. Second, we built a multivariable model with standard logistic regression, including only variables that had been found to be significant with the bivariate analysis including sex and age as a priori confounders. Then, we assessed all non-significant variables once more by including them in the model one at a time. If the variable remained non-significant, it was removed from the analysis permanently. Each time a variable became significant, this variable was included in the model, and all remaining non-significant variables were assessed again, one at a time. We conducted these steps in the analysis until there were no variables left to assess. The final model was then adjusted with all variables found statistically significant in the bivariate analysis and which kept the statistical significance in the multivariate model. Although the final model was split in Tables I and II, socioeconomic and environmental variables were included in the same analysis to compound the final model. The association between outcome and risk factors was estimated with odds ratio and 95% confidence interval.

RESULTS

Socioeconomic and environmental risk factors for *T. gondii* infection

Anti–*T. gondii* IgG antibodies were present in 17.5% (213/1,217) of the children (Table I). The frequency of socioeconomic and environmental variables and their association with *T. gondii* infection in both bivariate and multivariate (final model) analyses have been shown in Tables I and II, respectively. The following study variables were associated with an increased risk of infection. Children with increased age and increased number of siblings were more likely to be infected. Children with cats at home were more likely to be infected. Children living in a house without piped water facilities, in both study periods (1997–2003 and 2005) were 2.5 times more likely to be infected than those with piped water in their houses in both periods. Children living in a house without a flush toilet were more likely to be infected. The level of schooling of the children's mothers was associated with a lower risk of infection. Children whose mothers had a higher level of schooling had less risk of being infected. Meat and vegetable intake showed no significant influence on *T. gondii* infection in this population. Nursery attendance, living in a house served by a paved road, the presence of open sewage nearby, and the frequency of rubbish collection were removed from the model due to a lack of significance in the multivariate analysis (data not shown).

DISCUSSION

The seroprevalence of anti–*T. gondii* IgG observed in this study was 17.5%; this is lower than the prevalence found by Rey and

TABLE II. Environmental characteristics frequencies and associations with *Toxoplasma gondii* infection of 1,217 studied children. Abbreviations: OR, odds ratio; CI, confidence interval.

	Toxoplasma gondii infection 213 (17.5%)			
Variables	n	%	OR (95% C.I.)†	Final model OR (95% C.I.)‡
Flies at home				
No or rare (n = 603)	101	16.7	1	—
Yes (n = 614)	112	18.2	1.11 (0.83; 1.49)	
Rodents at home				
No (n = 525)	85	16.2	1	—
Yes (n = 692)	128	18.5	1.18 (0.87; 1.59)	
Cat at home				
No (n = 1002)	163	16.3	1	1
Yes (n = 215)	50	23.3	1.56 (1.09; 2.23)*	1.54 (1.06; 2.24)*
Dog at home				
No (n = 735)	116	15.8	1	—
Yes (n = 482)	97	20.1	1.34 (1.00; 1.81)	
Paved road (1997–2003 and 2005)				
Both study periods (n = 335)	72	21.5	1	—
One study period (n = 395)	65	16.5	0.72 (0.50; 1.04)*	
Never (n = 487)	76	15.6	0.68 (0.47; 0.97)*	
Piped water (1997–2003 and 2005)				
Both study periods (n = 974)	151	15.5	1	1
One study period (n = 207)	49	23.7	1.69 (1.17; 2.43)*	1.38 (0.93; 2.05)
Never (n = 36)	13	36.1	3.08 (1.53; 6.22)*	2.54 (1.22; 5.31)*
Flush toilet				
Yes (n = 761)	106	13.9	1	1
No (n = 456)	107	23.5	1.89 (1.41; 2.55)*	1.45 (1.04; 2.01)*
Sewage system				
Yes (n = 1016)	179	17.6	1	—
No (n = 201)	34	16.9	0.95 (0.64; 1.42)	
Open sewage nearby				
No (n = 646)	93	14.4	1	—
Yes (n = 571)	120	21	1.58 (1.17; 2.13)*	
Rubbish collection				
≥once per week (n = 924)	149	16.1	1	—
<once per week (n = 293)	64	21.8	1.45 (1.05; 2.02)*	
Flooded house				
No (n = 842)	145	17.2	1	—
Yes (n = 375)	68	18.1	1.07 (0.78; 1.46)	

* $P < 0.05$.
† Odds ratio and 95% confidence interval from crude analysis.
‡ Adjusted for all variables which has kept the significance in the multivariable analyses, including sex and age as a priori confounders.

Ramalho (1999), who reported 40.0% seroprevalence among schoolchildren from northeast Brazil aged 2–9-yr-old, as well as other reports in the literature for similar age groups (Etheredge and Frenkel, 1995; del Castillo and Herruzo, 1998). The lower prevalence found in this study may be attributed to differences in socioeconomic level and hygiene conditions between the study populations.

In our study, approximately 30% of the infected children were under 6 yr of age, suggesting that the transmission of this infection started during their early childhood (Mohan et al., 2002). The relatively gradual increase in seroprevalence associated with age lends further support to the characteristic finding that *T. gondii* infection is generally asymptomatic (Bóia et al., 2008).

The statistical significance of variables such as mother's level of schooling and number of siblings found in this study reinforces the contributing role of socioeconomic status in determining populations under high risk to be infected by this parasite, which preferentially affects people living in crowded conditions (Alvarado-Esquivel et al., 2008) and in households lacking basic facilities (Jones et al., 2001). The absence of treated piped water at home, for instance, showed a strong association with *T. gondii* infection; the odds of infection were greater when the absence of piped water was reported in both study periods (1997–2003 and 2005) than when it was reported in only 1 of the 2 periods. This finding supports previous reports that this parasite is water transmitted (Bahia-Oliveira et al., 2003; Dubey, 2004) and that it may cause waterborne outbreaks of toxoplasmosis (Bowie et al., 1997).

The presence of a cat at home was shown to be a risk factor for toxoplasmosis in our study, which is in agreement with previous studies that showed that the oocysts shed by this animal and ingested by human beings are likely an important route of infection (Cook et al., 2000; Alvarado-Esquivel et al., 2007).

In this work, the studied variables that showed significant associations with *T. gondii* infection, such as having a mother with a low level of schooling, having many siblings and the absence of piped water, are related to low socioeconomic status and consequently bad hygiene habits; they are directly (as absence of piped water) or indirectly (mother with low level of schooling) involved in the transmission of *T. gondii* infection. Variables related to low hygiene, as well as presence of cat at home found associated with *T. gondii*, indicate that oocyst ingestion is an important route for transmission of this parasite.

Although the current work did not evaluate the consumption of raw or undercooked meat, this route of infection has been more often reported in adult populations (Roghmann et al., 1999; Cook et al., 2000). These results are likely due to different habits between children and adults. Children in general have more contact with soil and eat less raw or undercooked meat compared with adults, although the presence of anti–*T. gondii* IgG in children from a Brazilian rural area has been associated with raw meat consumption (Souza et al., 1987). In contrast to what was expected, other variables associated with poverty and crowded living conditions, as well as the presence of animals potentially capable of spreading this infection, such as flies and rodents (Dubey, 2004), did not show any significant influence in the current study. These findings are in agreement with those of Galisteu et al. (2007), who did not find any association of these animals with toxoplasmosis in pregnant women.

The present study suggests that the presence of *T. gondii* infection in children from Salvador-Bahia, Brazil, is highly related to bad sanitation, crowded living conditions, the absence of treated water, and the presence of cats, suggesting the parasite to be mostly transmitted by oocyst ingestion. Simple improvements in education and basic sanitary conditions could help decrease childhood toxoplasmosis in this city and in other cities with populations living in similar environments.

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