Toxoplasma antibody and stool parasites in public school children, Rolândia, Paraná, Brazil

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Abstract The occurrence of toxoplasmosis and enteroparasitosis was studied in 434 children from elementary schools in the rural and urban areas of Rolândia, Paraná State, Brazil. Sera and fecal samples from all the students were submitted to IFA for Toxoplasma gondii and coproparasitological tests, respectively. The children were tested by Amsler grid and 72 of them were examined for the presence of lesions compatible with ocular toxoplasmosis. Some variables were tested but none showed increased risk for toxoplasmosis. The distribution according to sex and age and also same other variables are presented and discussed. Correlations between Amsler’s grid test, toxoplasma RIFI, occurrence of eyes lesions and enteroparasitosis are also considered.


Resumo Realizou-se exames de imunofluorescência indireta para detecção de anticorpos IgG anti-Toxoplasma gondii e testes com a tela de Amsler em 434 alunos da primeira à quarta série de escolas da rede municipal de Rolândia, PR. Destes, 72 foram examinados para detecção de lesões sugestivas de toxoplasmose em fundo de olho. Estes mesmos alunos e outras 191 crianças de creches tiveram amostras de fezes examinadas para determinar a prevalência de enteroparasitas. A distribuição dos achados, segundo sexo e faixa etária, bem como variáveis que podem influenciar na infecção por toxoplasma e enteroparasitoses são apresentados e discutidos. Correlações entre resultados da aplicação do teste com a tela de Amsler, sorologia positiva para toxoplasmose, lesões de fundo de olho e ocorrência de enteroparasitas são analisadas.


Toxoplasma gondii (Nicole & Manceaux, 1909), Apicomplexa, an obligatory intracellular protozoan, is the agent of human and animal toxoplasmosis and is distributed world-wide. The prevalence of T. gondii may vary from region to region, in accordance with social, cultural and climatic factors.¹⁵

Felines, the definitive hosts, eliminate oocysts in their feces that contaminate the environment.⁸ ¹⁷ In soil, oocysts pass through a sporulation process thus becoming infectious to human and animals.¹⁸

Human beings may acquire T. gondii infections typically through water ingestion and contaminated food, or by direct contact with soil containing sporulated oocysts, as well as ingestion of raw or partially cooked meat containing tissue cysts.¹⁸ ²³

Toxoplasmic infections in Man are usually asymptomatic, however serious symptoms or even death may occur in the congenital form or in immunocompromised individuals. In its congenital form, the parasite infects the mother and then the fetus which may develop fatal lesions. The child may have a normal delivery, but later present the effects of infection.² ¹⁵

Within the above mentioned possibilities for transmission, ocular toxoplasmosis may have a congenital or acquired origin. Studies performed in southern Brazil have revealed a high frequency of occurrence of
fundoscopic lesions from acquired toxoplasmosis\textsuperscript{11}. Silveira\textsuperscript{18}, studying a rural area of Erechim, Rio Grande do Sul, observed that 17.7% of studied patients showed fundoscopic lesions of toxoplasmic origin.

The main purpose of this work was to study the prevalence of T. gondii, and associated factors linked to T. gondii infection, including intestinal parasite, as well as to evaluate visual and ocular alterations in elementary school children in Rolândia, State of Paraná, Brazil.

MATERIAL AND METHODS

Elementary schools in Rolândia, PR, are attended by 2589 students, 2300 from urban areas and 289 from the rural region. Serologic tests for T. gondii, coproparasitologic tests and Amsler chart\textsuperscript{2} were applied to a sample of 276 students from urban areas and 158 from rural regions were performed between October and December, 1998. The size of the sample was determined by the Epi-Info 6 software, selecting pupils at random. At this time, the school children and/or their parents answered a questionnaire concerning epidemiological aspects of toxoplasmosis and enteric parasites: such as the practice of washing hands before meals, ingestion of non-pasteurized milk, raw meat or vegetables, whether their home has a garden, contact with dogs and cats, type of water supply and barefoot walking habits.

Serum levels of IgG class antibodies anti-T. gondii were determined in sera by indirect immunofluorescence antibody Test (IFA), according to standard techniques described by Camargo\textsuperscript{3}. Commercial human conjugate anti-IgG (Sigma Chemical, FITC) was used, considering a 1:16 dilution as a positive reaction. Positive and negative serum controls were included on each plate.

Fecal samples were brought from student’s home in appropriate plastic bottles, and labeled. They were immediately sent to a laboratory and stored in a refrigerator until processed, following the procedures of Faust et al\textsuperscript{7} and Hoffman et al\textsuperscript{13} to identify helminthes and protozoans.

After obtaining the Amsler chart and serological results, four groups of 18 students were selected at random, and submitted to examination for fundoscopic lesions at a specialized clinic in Rolândia, using a Zeiss split lamp 100/16, and binocular ophthalmoscopy\textsuperscript{19}. The groups were organized as follows: Group A - Serum-positive, with alterations on Amsler chart; Group B - Serum-positive, without alterations on Amsler chart; Group C - Serum-negative, with alterations on Amsler chart and Group D - Serum-negative, without alterations on Amsler chart.

The odds ratio was calculated to establish associations between the variables studied and statistical significance level was determined when the 95% confidence interval did not include one. The associations between the groups was tested using the Chi-square test with statistical significance considered when \(p < 0.05\). The correlation coefficient was determined as described by Ulon & Marder\textsuperscript{21}.

RESULTS

Of 343 serum samples analyzed, 42.4% were positive for T. gondii. In 276 students from urban schools, 116 (42%) displayed positive serology. In 158 students from rural schools, 68 (43%) were positive by IFA. There was no difference in the prevalence rates of T. gondii antibodies between urban and rural students (\(x^2 = 0.04 \ p = 0.83\)).

The distribution of reactive individuals and the results of coproparasitologic tests according to each school are presented in Table 1. Statistical treatment revealed no differences in the prevalence of T. gondii antibodies between urban or rural schools. (\(x^2 = 36.9 \ p < 0.01\)).

Correlation studies between anti-T. gondii antibodies and the prevalence of enteroparasitosis, only urban areas showed significant differences between schools (\(x^2 = 15.3 \ p = 0.01\)). The same did not occur in rural areas. The most frequent titer was 256 (23.3% in urban areas and 35.4% in rural areas) (Table 2).

There was no substantial sex bias in either urban or rural areas. In urban areas, 50 (44.2%) of boys and 66 (40.5%) of girls (\(x^2 = 0.39 \ p = 0.53\)) were seropositive. In rural areas 32 (43.2%) of boys and 36 (42.9%) of girls (\(x^2 = 0.00 \ p = 0.96\)) were seropositive.

When children were divided into age groups of 6 to 7, 8 to 9 and 10 years old or over, it was shown that of 276, 116 (42%) were seropositive for T. gondii antibodies in urban schools, 27 (36%) were positive in the 6 to 7 years old group; 50 (43.5%) in the 8 to 9 years old group and 39 (45.4%) in those over 10 years. In rural schools, out of 158, 68 (43%) were seropositive, with 8 (23.5%), 32 (47.8%) and 28 (49.1%) in the respective age groups (urban area \(x^2 = 1.61 \ p = 0.04\) and rural area \(x^2 = 6.75 \ p = 0.03\)). Therefore, only children from the rural area exhibited a difference in the distribution of T. gondii antibody according to age (Table 3).

From the Amsler chart application, of 434 tested students, 151 (34.8%) revealed visual alterations, 120 (79.5%) from urban areas and 31 (20.5%) from rural schools (OR = 3.15 1.95 < OR > 5.12 \(x^2 = 25.21 \ p < 0.01\)).

In the 120 urban students that displayed Amsler chart changes, 56 (48.3%) showed a positive serology for T. gondii and, of the 156 that did not reveal visual changes in chart application, 60 (51.7%) presented positive serology. From 31 rural students who presented Amsler chart alteration 12 (17.6%) were seroreactive and from 127 students without visual alterations, 56...
(82.4%) were seropositive (urban area $x^2 = 1.87$ p = 0.17 and rural area $x^2 = 0.29$ p = 0.58). There was no correlation between results of Amsler chart examination and serology for *T. gondii* (urban area p = 0.47 and rural area p = 0.27) (Table 4). Upon fundoscopy, none of the students revealed any form of lesion.

Coproparasitologic tests showed that, from 434 students, 228 (52.5%) had enteric parasites, 152 (55.1%) from urban and 76 (48.1%) from rural schools ($x^2 = 1.96$ p = 0.16). There was a higher prevalence of protozoans than of helminthes. *Giardia lamblia* was present in 149 (34.33%) students, whereas other protozoans (*Endolimax nana*, *Entamoeba coli* and *Entamoeba histolytica*) were detected in 111 (25.58%) students. Nematodes (*Ascaris lumbricoides*, *Ancylostoma* sp, *Trichuris trichiura* and *Enterobius vermicularis*) were found in 57 (13.13) students. The only cestode found was *Hymenolepis nana* in 11 (2.53%) students.

**DISCUSSION**

Considering the ages of the individuals studied, we conclude that the prevalence of 42.2% seropositive titers for *T. gondii* is very high when compared to results from other authors in Southern Brazil, Sudan or Mexico\(^{(12,15)}\).
Although, Garcia\textsuperscript{4} found similar results (43.8\%) in a neighboring town when evaluating the same age groups.

Students of urban or rural origin did not show significant differences. Authors studying the same region, have described that the toxoplastic infection risk is greater in rural populations\textsuperscript{10, 22}. However, there was no difference when considering the student's school origin; they exhibited a very similar distribution in urban or rural zones. The majority of antibody titers were less than 1,024 (64.7\% in urban or rural zones. The when considering the student’s school origin, they exhibited

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rural populations

such similarities suggest common risk

differences by IFA analysis for

recent rural origins and similar habits and culture.

There were differences according to age in antibody prevalence infection, which was directly proportional. The age-related antibody prevalence was similar to other authors\textsuperscript{5, 20}. The age results indicate that the infection is postnatal.

Other factors, such as habits of ingesting raw meat or vegetables; contact with dogs or cats or even having pets that frequent gardens; habit of washing hands before meals; origin of drinking water, disposal of feces and walking barefoot did not influence the serological results, in accordance with Garcia’s results\textsuperscript{5}. Camargo et al\textsuperscript{22} studying toxoplasmosis in urban areas of Minas Gerais State, observed a higher antibody prevalence in individuals that reported inadequate housing, periodic contact with cats, chickens and pigs. Although, feeding habits, contact with dogs, goats and rodents did not influence the seroprevalence.

There was a significant correlation between seroprevalence for \textit{T. gondii} with presence of enteroparasites in urban areas. The same was not observed in rural areas. Such results suggest that the transmission source for toxoplasmosis and enteroparasitosis were the same, occurring via soil, water and food. This is in agreement with the finding in studies by Etheredge & Frenkel\textsuperscript{22} that oocyst transmission is most common in Latin America.

The absence of a correlation between enteroparasites and toxoplasmosis in rural areas may be explained and children live in common space\textsuperscript{22}, the neighborhood inhabited by them is large, which would contribute to reduce the risk of acquiring \textit{T. gondii} via food contamination due to exposure from their surroundings.

Comparing urban and rural students with visual alterations, we observed a higher risk of these alterations in urban students. The Amser chart was described as a simple and rapid test for the evaluation of the macular visual field\textsuperscript{22}, although recent studies have demonstrated low sensitivity and specificity of the test\textsuperscript{1}, which may explain the absence of fundoscopic lesions even in students that presented Amser chart alterations. There was no correlation between \textit{T. gondii} serology and Amser chart findings.

Although the present study did not demonstrate Amser chart efficacy to detect fundoscopic lesions among school children, Garcia\textsuperscript{5}, from a trial using this method, was able to detect 9 patients with lesions suggestive of toxoplasmosis in 41 seropositive patients with chart alterations and all of these were more than 30 years old.

The ages studied showed a low prevalence for ocular toxoplasmosis, as demonstrated by Silveira\textsuperscript{10} in a study of ocular lesions suggestive of toxoplasmosis in rural populations of Erechim, RS, Brazil. In this study, with 1,042 patients of all ages, only one of the children presented fundoscopic lesions.

Further studies to evaluate the efficacy of the Amser chart in detecting ocular toxoplasmosis need to be performed.

Our work showed a high prevalence of anti-\textit{T. gondii} antibodies, in the early ages studied. The same occurred with enteric parasitosis. This suggested that urban populations in the studied areas had similar sanitary characteristics to the rural areas. These children have a potential risk of presenting ocular toxoplasmosis in the future. Hence, the importance for schools to monitor visual acuity cannot be overemphasized. Control measures and especially sanitary education should be provided in schools, which would hopefully reduce such high a prevalence of these infections.

| Table 4 - Association between IgG anti-\textit{T. gondii} IFA results and findings on application of Amsler chart among elementary school students from Rolândia, PR, Brazil, 1998. |
| Amser chart |
| IFA with alteration | n | % | no alteration | n | % | Total | n | % |
| Urban area |
| Positive | 56 | 48.3 | 60 | 51.7 | 116 | 42.0 |
| Negative | 64 | 40.0 | 96 | 60.0 | 160 | 58.0 |
| Subtotal | 120 | 43.5 | 156 | 56.5 | 276 | 100.0 |
| (\(x^2 = 1.87 \ p = 0.17\)) | | | (\(r = 0.32 \ p = 0.47\)) |
| Rural area |
| Positive | 12 | 17.6 | 56 | 82.4 | 68 | 43.0 |
| Negative | 19 | 21.1 | 71 | 78.9 | 90 | 56.0 |
| Subtotal | 31 | 19.6 | 127 | 80.4 | 158 | 100.0 |
| (\(x^2 = 0.29 \ p = 0.58\)) | | | (\(r = 0.47 \ p = 0.27\)) |
| Total | 151 | 34.8 | 283 | 65.2 | 434 | 100.0 |

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REFERENCES


