Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. 82(4): 475-482, out./dez. 1987

EPIDEMIOLOGICAL ASPECTS OF TOXOPLASMOSIS IN SCHOOLCHILDREN RESIDING IN LOCALITIES WITH URBAN OR RURAL CHARACTERISTICS WITHIN THE CITY OF RIO DE JANEIRO, BRAZIL

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Immunofluorescence tests (IF) for toxoplasmosis were performed on a total of 608 schoolchildren in elementary and junior high grades. 166 being in the Bonsucesso district (an urban region of Rio de Janeiro) and 442 children from locations within the lowlands of Jacarepaguá (with rural characteristics). All the IF-IgM were nonreactive, whilst 416 schoolchildren (68.4%) were IF-IgG serum-reactive (⩾1:16). The percentages of serum-reactives in Jacarepaguá were significantly higher than in Bonsucesso, both as regards the total number of schoolchildren ($p < 0.001$), as also when subdivided according to the age-grades from six to eight years ($p < 0.001$) or from twelve to fourteen ($p < 0.05$). Both in Jacarepaguá and in Bonsucesso, the prevalence of reactions in the 12 to 14 year age-grade was significantly greater than in the 6 to 8 year age-grade ($p < 0.001$ in both cases). Expressively larger prevalences of serum reactions were found in Jacarepaguá among schoolchildren who preferred eating raw or undercooked meat, as well as among those having cats as pets; this occurred equally in the 6 to 8 year and in the 12 to 14 year age-grades. In Bonsucesso, the only significant difference was in the 6 to 8 year age-grades that had cats as pets. Thus, it has been verified that the risk of infection is greater and more precocious in localities with rural characteristics than in urban regions.

Key words: toxoplasmosis – transmission – seroepidemiology

Toxoplasma gondii Nicolle & Manceaux, 1909 is cosmopolitanly distributed, capable of infecting a great number of species of animals, including man (Apt et al., 1973; Frenkel, 1985). It is estimated that from 25 to 50% of the world's population bas been exposed to infection by this protozoan (Lobel & Kagan, 1978).

Human transmission of so-called acquired toxoplasmosis is generally connected with eating raw or undercooked meat, containing the parasite's cysts (Jacobs et al., 1960; Desmonts et al., 1965; Coutinho et al., 1982b). On other hand, it has been verified – after the studies by Hutchinson et al., 1970; Frenkel et al., 1970; Hutchinson et al., 1971; Work, 1971; Deane et al., 1971 – that the domestic cat and other felidae may complete a gametogonic sexual cycle in the epithelial cells of the mucous membranc of the small intestine, culminating in the discharging of immature oocysts together with the felines' feces. On the ground, the oocysts sporulate and generally become mature and infectious within 3 to 4 days (Frenkel, 1972). Contacts with cats, or the taking of water and/ or raw foods (greenstuffs, fruits, etc.) contaminated by feces containing oocysts, are considered important forms for transmitting toxoplasmosis to persons and animals (Dubey et al., 1970; Wallace, 1973; Fayer & Frenkel, 1979; Coutinho et al., 1982a).

Several factors have been described as capable of influencing the prevalence of persons who are serum-reactive or show skin test hypersensitivity to *T. gondii*. Under appropriate conditions, humidity and temperature favor the evolution of oocysts on the ground and consequently the transmission of toxoplasmosis to various mammls, including man (Ruiz et al., 1973; Ruiz & Frankel, 1977; Frenkel & Ruiz, 1981; Coutinho et al., 1982a). It also possibilitates the cat-rodent transmission cycle (Deane et al., 1971; Wallace et al., 1972; Coutinho et al., 1982a) that appears to be important in nature. The occupations of persons and their eating habits as regards the ingestion of raw or undercooked meat (the presence of viable cysts) may favor transmission of the parasite (Kimball et al., 1960; Desmonts et al., 1965; Coutinho et al., 1982b).

The present work evaluates the prevalence of elementary and junior high students that are serum-reactive to *Toxoplasma*. It has been verified that in localities of rural characteristics, the risk of infection among schoolchildren was greater and more precocious than in urban locations. In this connection, both the presence of cats as pets and the eating of raw or undercooked meat appeared to have been influential factors.

Received March 9, 1987.
Accepted May 12, 1987.

MATERIAL AND METHODS

In order to detect the anti-*Toxoplasma* antibodies, blood samples of 608 schoolchindren in Rio de Janeiro were taken in heparinized capillary tubes (Inlab-São Paulo, Brazil). After centrifugation (centrifuge for micro-hematocrit, Danon/IEC Division) the plasma was diluted in a phosphate buffer solution (PBS), pH 7.2, starting at 1:16 and sucessively, at a rate of 4 to 1:4096, in accordance with the technique described by Coutinho et al. (1983).

The antigen used for the immunofluorescence tests (IF) was a sample of *Toxoplasma* isolated from a human congenital case and maintained by a series of passages in mice.

The fluorescent sets (human anti-IgM and anti-IgG) were manufactured by Biolab-Mérieux, having diluted in PBS-Evan's Blue at the moment of using.

An optical microscope (Carl Zeiss) with incidental illumination by mercury-vapor lamp (HBO-200, Oram) and a BG-12 filter was used to read the indirect immunofluorescent reactions.

166 schoolchindren, out of the 608 student that were examined, went to the Municipal School in Bonsucesso, an urbanized suburb of Rio de Janeiro. The remaining 442 schoolchildren were from three Municipal Schools, in the localities of Pau da Fome, Rio Pequeno and Camorim, within the lowlands of Jacarepaguá. These localities still reveal rural characteristics, such as the raising of domestic animals (cats, dogs, pigs, goats, chickens, cattle, etc.); unpaved roads; inadequate water supply and sewerage systems. The constant presence of a great number of household cats, according to the local residents, was to limit the rodent population.

The ages of the schoolchildren ranged from six through fourteen years. Preference was given to study only the limited six to eight and twelve to fourteen year-old children groups so that possible differences in the prevalences of serum-reactive schoolchildren, related to the greater or shorter period of living in the localities, be more clearly revealed. Thus, 166 schoolchildren in Bonsucesso and 442 in Jacarepaguá were studied, Their distribution according to sexes and age-grades are shown in Tables I and II.

It was possible to obtain conclusive information from 113 schoolchildren in Bonsucesso and 202 students in Jacarepaguá in order to investigate the presence of household cats and to determine the eating of raw or undercooked meat.

Test χ^2 was used for the statistical analysis of the IF-IgG results, the differences having been considered significant when $p < 0.05$.

RESULTS

All the 608 IF-IgM for toxoplasmosis were non-reactive up to a 1:16 dilution.

Table I shows the results of the IF-IgG distributed according to schoolchildren's sexes in the two studied localities. Statistical analysis demonstrates that there were no significant differences as regards the sexes in the results of the IF-IgG's (Table I). This datum permitted that the schoolchildren be grouped thereafter irrespective of sexes. Thus, it was verified that the prevalence of serum-reactive schoolchildren in Jacarepaguá (IF-IgG = 16-256 or $\geqslant$ 1024) was significantly greater than that found in Bonsucesso ($p < 0.001$).

When the schoolchildren were subdivided according to the age-grades (Table II), statistical analysis of the IF-IgG results demonstrated that: a) equally in Bonsucesso and in Jacarepaguá the prevalence of serum-reactives in the 12 to 14 year-old group was significantly greater ($p < 0.001$ and $p < 0.001$, respectively) than in the 6 to 8 year age-grade; the number of serum-reactives in Jacarepaguá was stastitically higher ($p < 0.001$) than in Bonsucesso in the 6 to 8 year-old group, there having been a greater prevalence of high antibody titers in the IF-IgG (> 1024) of this age-grade in Jacarepaguá ($p < 0.01$) as compared to Bonsucesso (13.1% x 4.7% – Table II); b) with reference to the 12 to 14 year-old schoolchildren, the results of the IF-IgG tests between those living in Jacarepaguá and those from Bonsucesso showed smaller – although still statistically significant ($p < 0.05$) – differences. However, the percentages of higher titers ($\geqslant$ 1024) were similar in the two regions (6.8% x 6.2% – Table II).

With reference to the 315 schoolchildren investigated as regards the presence of household cats and/or their habits of eating raw or undercooked meat, it was demonstrated that: a) of 113 student living in Bonsucesso, 22 (19.5%) did have cats, and 45 (39.8%) ate raw or undercooked meat; b) of 202 students living in Jacarepaguá, 74 (36.6% had cats and 66 (32.7%) ate raw or undercooked meat. The presence of cats in the homes, in Jacarepaguá, occurred with more significative frequency that in Bonsucesso ($p < 0.01$), whilst the eating of raw or undercooked meat was similar between the schoolchindren from both localities ($p < 0.1$). However, the fact should be stressed that it was not rare for several families in Jacarepaguá to slaughter animals (pigs, goats, chickens, game, etc.) for their own consumption.

TABLE I

Results of the indirect immunofluorescence tests (IF-IgG) for toxoplasmosis in schcoolchildren from Rio de Janeiro, according to their sex and the area where they lived

Area	Sex	Distribution of Titres (IF-IgG) SN*	16-256	⩾1024	Total of serum reactive (⩾16)	Total of tests (100%)
		No. (%)	No. (%)	No. (%)	No. (%)	
Bonsucesso	Masculine	38 (45,2)*	42 (50,0)	4 (4,8)	46 (54,8)	84
	Feminine	40 (48,8)	37 (45,1)	5 (6,1)	42 (51,2)	82
	Sub-total	78 (47,0)	79 (47,6)	9 (5,4)	88 (53,0)	166
Jacarepaguá	Masculine	51 (22,5)	152 (66,9)	24 (10,6)	176 (77,5)	227
	Feminine	63 (29,3)	132 (61,4)	20 (9,3)	152 (70,7)	215
	Sub-total	114 (25,8)	284 (64,3)	44 (9,9)	328 (74,2)	442
Total		192 (31,6)	363 (59,7)	53 (8,7)	416 (68,4)	608

* Serum negative at 1:16 dilution

Statistics analysis: Bonsucesso (sub-totals) x Jacarepaguá (sub-totals) = $p < 0.001$; Bonsucesso = masculine x feminine $p > 0.7$; Jacarepaguá = masculine x feminine $p > 0.3$.

TABLE II

Results of the indirect immunofluorescence tests (IF-IgG) for toxoplasmosis in schcoolchildren from Rio de Janeiro, according to the age grades and the area where they lived

Area	Age grade (years)	Distribution of Titres (IF-IgG) SN*	16-256	⩾ 1024	Total of serum reactive (⩾16)	Total of tests (100%)
		No. (%)	No. (%)	No. (%)	No. (%)	
Bonsucesso	6- 8	54 (63,5)	27 (31,8)	4 (4,7)	31 (36,5)	85
	12-14	24 (29,6)	52 (64,2)	5 (6,2)	57 (70,4)	81
	Sub-total	78 (47,0)	79 (47,6)	9 (5,4)	88 (53,0)	166
Jacarepaguá	6- 8	80 (36,0)	113 (50,9)	29 (13,1)	142 (64,0)	222
	12-14	34 (15,5)	171 (77,7)	15 (6,8)	186 (84,5)	220
	Sub-total	114 (25,8)	284 (64,3)	44 (9,9)	328 (74,2)	442
Total		192 (31,6)	363 (59,7)	53 (8,7)	416 (68,4)	608

* Serum negative at 1:16 dilution

Statistics analysis: Bonsucesso: age grade of 6-8 years x 12-14 years = $p < 0.001$; Jacarepaguá: age grade of 6-8 years x 12-14 years = $p < 0.001$; Age grade of 6-8 years: Bonsucesso x Jacarepaguá = $p < 0.001$; Age grade of 12-14 years: Bonsucesso x Jacarepaguá = $p < 0.05$.

TABLE III

Results of the indirect immunofluorescence tests (IF-IgG) for toxoplasmosis in schoolchildren from Rio de Janeiro, according to the area where they lived, the age grades, and their habits of eating raw or undercooked meat

Area	Age grade (years)	Habit of eating raw or undercooked meat		Total of tests (100%)	No habit of eating raw or undercooked meat		Total of tests (100%)
		SN*	⩾16		SN*	⩾16	
		No. (%)	No. (%)		No. (%)	No. (%)	
Bonsucesso	6- 8	12 (66,7)	6 (33,3)	18	29 (74,4)	10 (25,6)	39
	12-14	8 (29,6)	19 (70,4)	27	4 (23,5)	13 (76,5)	17
Total		20 (44,4)	25 (55,6)	45	33 (58,9)	23 (41,1)	56
Jacarepaguá	6- 8	2 (10,0)	18 (90,0)	20	24 (45,3)	29 (54,7)	53
	12-14	5 (10,9)	41 (89,1)	46	10 (28,6)	25 (71,4)	35
Total		7 (10,6)	59 (89,4)	66	34 (38,6)	54 (61,4)	88

*Serum negative at 1:16 dilution

Statistics analysis: according to the habit of eating x no habit of eating raw or undercooked meat for each area, and each age grade

Bonsucesso (6- 8 years): $p > 0.5$ Jacarepaguá (6- 8 years): $p < 0.01$

(12-14 years): $P > 0.7$ (12-14 years): $p < 0.05$

TABLE IV

Results of the indirect immunofluorescence tests (IF-IgG) for toxoplasmosis in schoolchildren from Rio de Janeiro, according to the area where they lived, the age grades, and presence or absence of cats as pets

Area	Age grade (years)	Presence of cats		Total of tests (100%)	Absence of cats		Total of tests (100%)
		SN*	⩾16		SN*	⩾16	
		No. (%)	No. (%)		No. (%)	No. (%)	
Bonsucesso	6- 8	3 (37,5)	5 (62,5)	8	29 (74,4)	10 (25,6)	39
	12-14	4 (28,6)	10 (71,4)	14	4 (23,5)	13 (76,5)	17
Total		7 (31,8)	15 (68,2)	22	33 (58,9)	23 (41,1)	56
Jacarepaguá	6- 8	2 (7,4)	25 (92,6)	27	24 (45,3)	29 (54,7)	53
	12-14	5 (10,6)	42 (89,4)	47	10 (28,6)	25 (71,4)	35
Total		7 (9,5)	67 (90,5)	74	34 (38,6)	54 (61,4)	88

*Serum negative at 1:16 dilution

Statistics analysis: according to the presence x absence of cats as pets for each area, and each age grade

Bonsucesso (6- 8 years): $p < 0.05$ Jacarepaguá (6- 8 years): $p < 0.001$

(12-14 years): $P > 0.07$ (12-14 years): $p < 0.05$

TABLE V

Results of the indirect immunofluorescence tests (IF-IgG) for toxoplasmosis in schoolchildren from Rio de Janeiro, according to the area where they lived, the age grades, alimentary habits, and presence of cats as pets

Area	Age grade (years)	Habit of eating raw or undercooked meat and/or presence of cats		Total of tests (100%)	No habit of eating raw or undercooked meat and absence of cats		Total of tests (100%)
		SN*	⩾16		SN*	⩾16	
		No. (%)	No. (%)		No. (%)	No. (%)	
Bonsucesso	6- 8	13 (56,5)	10 (43,5)	23	29 (74,4)	10 (25,6)	39
	12-14	10 (29,4)	24 (70,6)	34	4 (23,5)	13 (76,5)	17
Total		23 (40,3)	34 (59,7)	57	33 (58,9)	23 (41,1)	56
Jacarepaguá	6- 8	4 (10,0)	36 (90,0)	40	24 (45,3)	29 (54,7)	53
	12-14	7 (9,5)	67 (90,5)	74	10 (28,6)	25 (71,4)	35
Total		11 (9,6)	103 (90,4)	114	34 (38,6)	54 (61,4)	88

*Serum negative at 1:16 dilution

Statistics analysis: according to the habit of eating meat and/or presence of cats x no habit of eating meat and absence of cats

Bonsucesso (6- 8 years): $p > 0.1$ Jacarepaguá (6- 8 years): $p < 0.001$

(12-14 years): $P > 0.5$ (12-14 years): $p < 0.02$

In Bonsucesso, as shown in Table III, there were no significant differences as regards the reactive of non-reactive IF-IgG tests of the age-grades, with respect to their food habits. However, in the case of Jacarepaguá – both in the 6 to 8 year-old and the 12 to 14 year-old groups of schoolchildren – a greater prevalence of serum-reactives was found among those who ate raw or undercooked meat, when compared to the children who did not. This difference, however, was more clearly defined in the 6 to 8 year-old group ($p < 0.01$) than in the 12 to 14 year-old group ($p < 0.05$).

According to Table IV, there was a greater prevalence of serum-reactives ($p < 0.05$) in the Bonsucesso 6 to 8 year-old group of children who had household cats, than in those who did not have. However, this difference did not occur in the 12 to 14 year-old group. In the case of Jacarepaguá, in both the 6 to 8 and the 12 to 14 year age-grades, a significant association ($p < 0.001$ and $p < 0.05$, respectively) was found between the presence or absence of cats and a greater or lesser prevalence of serum-reactives.

When analyzing the cobination of the presence of cats and the habit of eating raw or undercooked meat (Table V) in connection with the greater or lesser prevalence of serum-reactives, it was demonstrated that in Bonsucesso the diferences were not significant, whilst in Jacarepaguá such significance did occur, both in the 6 to 8 year-old age-grade ($p < 0.001$) as in the 12 to 14 year-old group ($p < 0.02$).

DISCUSSION

Infection by *T. gondii* occurs most frequently through the ingestion of the parasite's cysts, found in the musculature of animals (swine, cattle, etc.) and/or due to the ingestion of oocysts sporulating on the ground that have been discharged with the feces of cats or other felidae.

Generally, a large portion of the human population becomes infected by *T. gondii* during the first decade of life (Remington et al., 1970; Coutinho et al., 1972; Frenkel & Ruiz, 1980; Amendoeira & Coutinho, 1981), by means of one of the above mentioned mechanisms. After toxoplasmic infection a cel-

lular and humoral immune response is developed which is generally protective and tends to last during the person's entire life (Frenkel, 1985). Such immune response can be demonstrated by various methods, principally by serologic methods which are extremely useful in laboratory diagnosis and in populational surveys on toxoplasmosis (Camargo et al., 1977; Kagan, 1979). For such reasons and depending on a greater or lesser exposure to the risk of infection during the lifetime of persons, there is a tendency towards a gradual increase in the prevalences of persons serum-reactives to *Toxoplasma* (Frenkel & Ruiz, 1981; Frenkel, 1985), according to the aging process (accumulated data).

In surveys carried out on populational groups in the city of Rio de Janeiro, prevalences of 31.7% have been found in persons 0 to 5 years-old, 59.2% in the 6 to 10 year-old persons, 68.9% in the 11 to 15 year-old individuals and 71.4% in the 16 to 20 year-old persons (Coutinho et al., 1972), up to percentage rates that vary from 50-80% in difference adult populations, both in Rio de Janeiro and other Brazilians States (Walls & Kagan, 1967; Lamb & Feldman, 1968; Araújo, 1970; Baruzzi, 1970; Gomes et al., 1975; Leser et al., 1977; Coutinho et al., 1981).

Our data confirm that the percentages of serum-reactives increased in accordance with the age-grades of the schoolchildren in the two areas that were studied. It was also demonstrated that the division of age-grades, into 6 to 8 and 12 to 14 year-olds, was appropriate, having permitted the statistical identification of significant differences as regards prevalences of serum-reactives to *Toxoplasma* between these two age-grades, equally Bonsucesso as in Jacarepaguá.

In cases of prime infection by *T. gondii*, the immunofluorescence reaction (IF) may precociously show IgM antibodies that generally rapidly increase to titers over 1024. However, false negative results may occur in the IF-IgM tests, due to competition with high levels of IgG anti-*Toxoplasma* in the person's serum (Filice et al., 1980; Camargo et al., 1983).

The presence of acute toxoplasmosis within the 608 schoolchildren under study was not characterized since no cases of IF-IgM $\geqslant$ 16 were found. However, this possibility may not be discarded since titers of IF-IgG $\geqslant$ 1024 were observed. As mentioned above, such high IgG titers could originate false negative IF-IgM toxoplasmosis results.

It was verified that the schoolchildren's food habits (eating raw undercooked meat) and the presence of household cats probably were influential in transmitting *T. gondii*, particulary in the Jacarepaguá lowlands (areas with rural characteristics), where the percentagens of serum-reactive schoolchildren were greater than in those from Bonsucesso (an urban area of Rio de Janeiro).

Thus, according to the results obtained and statistically analyzed, the risks of *T. gondii* infection were greater and more precocious in Jacarepaguá than in Bonsucesso, since the 6 to 8 year-old schoolchildren of Jacarepaguá revealed significantly higher percentagens of serum-reactive tests (16-256 and $\geqslant$ 1:1024) than those of the same age-grade living in Bonsucesso.

The greater risk of infection for schoolchildren in the Jacarepaguá areas should be related to the larger quantity of household cats, as well as to the habit of eating meat from non-commercial sources (slaughtering of animals raised near homes), when compared to the schoolchildren from the Bonsucesso area, who had lesser quantities of domestic cats and usually ate meat from cold storage commercial sources.

RESUMO

Aspectos epidemiológicos da toxoplasmose em escolares residentes em localidades com características urbana e rural do Rio de Janeiro, Brasil – Foram feitos testes de imunofluorescência (IF) para toxoplasmose em 608 escolares do 1º grau, sendo 166 de Bonsucesso (área urbana do Rio de Janeiro) e 442 da baixada de Jacarepaguá (área com características rurais). Todas as IF-IgM foram não-reagentes, enquanto que 416 escolares (68,4%) foram soro-reagentes na IF-IgG ($\geqslant$ 1:16). As percentagens de soro-reagentes em Jacarepaguá foram significantemente superiores às de Bonsucesso, tanto em relação ao número total de escolares, como quando subdivididos segundo as faixas etárias de 6 a 8 anos ou de 12 a 14 anos. Tanto em Jacarepaguá quanto em Bonsucesso, a prevalência de reagentes no grupo etário de 12 a 14 anos foi significantemente maior do que no grupo de 6 a 8 anos de idade. Em Jacarepaguá encontraram-se prevalências significantemente maiores de soro-reagentes entre os escolares que referiam a ingestão de carne crua ou mal cozida, assim como entre o que possuiam gato; isto ocorreu tanto no grupo etário de 6 a 8 anos como no de 12 a 14 anos. Em Bonsucesso só houve diferença significante entre as crianças de 6 a 8 anos que possuiam gatos no domicílio. Verificou-se assim que em área com característica rural o risco de infecção era maior e mais precoce do que em área urbana.

Palavras-chave: toxoplasmose – transmissão – soroepidemiologia

ACKNOWLEDGEMENTS

Thanks are due to Edilson de Souza, Gentil Dutra and Ricardo Mineiro Rodrigues for technical assistance; Miss R. Pelegrino for typing the manuscript; and the Secretariat of Education from Rio de Janeiro Municipality.

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