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TOXOPLASMOSIS SEROLOGIC TESTS IN BRAZILIAN INDIANS (KREN-AKORORE) OF RECENT CONTACT WITH CIVILIZED MAN

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SUMMARY

Immunofluorescence and hemagglutination toxoplasmosis tests were done in serum samples from 70 Brazilian Indians, belonging to a group of 81 Kren-Akorore Indians recently moved to the Xingu National Park. These had first contact with civilized men about 2 years ago. Positive tests were seen in 88.6% of the samples, with titers of 1:4,000 or higher in most cases. The fluorescent test for IgM antibodies was positive in 58.6% of the samples, but such results could be demonstrated as non-specific and due to rheumatoid factors in sera. According to the serologic patterns we have recently described in acquired toxoplasmosis [4,6], most cases could be included under pattern II, transitional between pattern I — Acute Disease, and pattern III — Ancient Infections. It seems that these Indians have been infected very recently, as if exposed to some condition representing a high infection risk.

INTRODUCTION

Technical developments observed in serological tests for toxoplasmosis, as the use of specific anti-IgG and anti-IgM conjugates in the fluorescent antibody test or the preservation of sensitized cells for the hemagglutination test, resulted in new possibilities for seroepidemiological studies of this infection. With the help of such tests, quantitative evaluations of antibodies to different parasitic antigenic components can be done and antibodies identified according to immunoglobulin classes. In this way, different serologic patterns can be recognized, which are characteristic of successive stages of the infection [4,6]. Since practical for routine purposes, these tests are adequate for surveys, thus permitting to obtain information on epidemiological aspects such as prevalence and incidence of the infection.

Epidemiological studies of toxoplasmosis are of special interest in primitive human groups in view of their peculiar conditions of habitat, nourishment, customs and habits.

The present publication refers to a serologic survey in a Central Brazil Indian tribe, the Kren-Akorore, who until recently lived in the forests near the Peixoto de Azevedo River, completely isolated from civilized men. They were discovered during an aereal exploratory recognition of the area where a new road between the cities of Cuiabá and Santarem (BR 163) was to be built. First contacts with the Indians were then tried, which finally occurred in February, 1973. Two years later, when the road was opened for traffic, the Kren-Akorore were transferred to the Xingu National Park and at this time all the 81 members of the group were submitted to a through clinical examination, and blood samples collected.

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MATERIAL AND METHODS

Serum samples — Venous blood was collected from 70 Indians aged 4 to 40 years. Serum samples were kept at -20°, thawed and then heated at 56° for 30 minutes before testing.

Fluorescent toxoplasma antibody tests — Were performed as described [5], with specific anti-IgG and anti-IgM conjugates (Hyland Division, Travenol Laboratories, Costa Mesa, U.S.A.) respectively for the fluorescent IgG (FA-IgG) and the fluorescent IgM (FA-IgM) tests.

Hemagglutination tests (HA) — Were done as described, with a freeze-dried, preserved reagent, prepared by sensitizing human tanned red cells with extracts of **Toxoplasma gondii** [5].

Tests for rheumatoid factors — The latex slide test (RA test) was done according to the recommended procedure with a reagent produced by Laboratories Merieux (Lilly, France). For tube tests, 0,81 μ latex (Dow Chemical Co., USA) was sensitized with heat aggregated gamma-globulin, according to SINGER & PLOTZ [9]. Serum samples were tested in doubling dilutions, from 1:20 on.

Serum absorption with polimerized IgG — IgG immunosorbents were prepared by polimerizing human IgG, as described by AVRAMEAS & TERNYNCK [1]. To serum samples diluted at 1:8 in saline solution, about 30 mg of the polimer were added, the mixture left at room temperature for 60 minutes under a constant slow agitation. Polimer was removed by centrifugation and supernatants used as absorbed samples.

Fluorescent plasmodium antibody tests — **Plasmodium vivax** was used as antigen and tests carried out as described by SULZER et al. [10], with sera diluted at 1:50 and 1:250 and from then on in doubling dilutions to 1:2,000.

RESULTS

Positive anti-toxoplasma tests were seen for sera of 62 from 70 individuals (88.6%), and showed titers between 1:16 and 1:16,000 for the FA-IgG test and between 1:64 and 1:32,000 for the HA test, as shown in Table I. In sera from 41 Indians (58.6%) a positive FA-IgM test was observed, with titers ranging from 1:16 to 1:4,000 (Table I).

TABLE I

Anti-toxoplasma tests in serum samples of 70 Kren-Akorore Indians

Test results	FA-IgG	HA	FA-IgM
Non-reactive	8	8	29
Reactive to 1:16	0	0	7
1:64	1	3	0
1:256	7	6	22
1:1,024	4	9	7
1:4,000	15	15	5
1:8,000	15	14	0
1:16,000	20	10	0
1:32,000	0	5	0
Total	70	70	70

FA-IgG — fluorescent toxoplasma IgG test
FA-IgM — fluorescent toxoplasma IgM test
HA — hemagglutination test

Since rheumatoid factors in serum can result in false positive toxoplasma FA-IgM tests [7], samples were submitted to latex RA-tests, which were positive in 62 from 70 samples. Latex tube tests indicated high titers for rheumatoid factors (1:160 or more) in most cases (Table II).

TABLE II

Rheumatoid factor latex test in serum samples from 70 Kren-Akorore Indians

Non-reactive	Reactive								
< 1:20	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	Total
8 (11.4%)	0	6 (12.9%)	3	4	15	16 (75.7%)	16	2	70 (100%)

TABLE III

Toxoplasma antibody tests and rheumatoid factor latex test in serum samples from 70 Kren-Akorore Indians

Indians	S	A	FA-IgG	FA-IgM	FA-IgM-ABS*	HA	LATEX	Indians	S	A	FA-IgG	FA-IgM	FA-IgM-ABS*	HA	LATEX
1	M	34	4,000*	256	16	8,000	1,280	36	M	4	0	0	0	0	40
2	F	32	4,000	0	0	4,000	320	37	M	14	8,000	1,000	64	4,000	640
3	F	16	16,000	0	0	16,000	0	38	F	20	256	0	0	128	640
4	F	14	0**	0	0	0	320	39	F	17	8,000	1,000	0	8,000	640
5	M	23	8,000	256	64	4,000	320	40	M	40	16,000	1,000	256	4,000	640
6	F	22	16,000	4,000	256	8,000	1,280	41	F	24	16,000	16	0	2,000	1,280
7	M	20	4,000	16	0	4,000	640	42	M	34	4,000	1,000	0	2,000	640
8	F	18	0	0	0	0	1,280	43	M	19	8,000	256	0	4,000	640
9	F	40	16,000	4,000	256	16,000	1,280	44	F	16	4,000	0	0	4,000	40
10	M	24	4,000	256	64	4,000	1,280	45	M	21	16,000	4,000	256	4,000	1,280
11	F	20	4,000	256	0	1,000	1,280	46	F	34	0	0	0	0	640
12	M	38	16,000	1,000	256	16,000	1,280	47	F	17	1,000	256	0	2,000	640
13	F	15	4,000	0	0	4,000	0	48	F	30	16,000	0	0	16,000	0
14	F	8	8,000	256	0	2,000	640	49	M	23	16,000	4,000	256	8,000	1,280
15	F	30	4,000	0	0	1,000	2,560	50	F	30	8,000	256	0	2,000	320
16	M	22	4,000	1,000	0	8,000	320	51	M	28	1,000	256	64	1,000	640
17	F	17	16,000	4,000	256	32,000	5,120	52	F	12	16,000	256	0	16,000	0
18	M	17	8,000	256	0	8,000	320	53	M	10	64	0	0	64	40
19	F	15	16,000	0	0	32,000	40	54	M	13	16,000	256	0	8,000	80
20	M	27	8,000	0	0	16,000	320	55	M	11	8,000	16	0	8,000	160
21	F	27	16,000	256	0	32,000	160	56	M	10	0	0	0	0	0
22	F	13	16,000	16	0	16,000	640	57	M	8	256	16	0	1,000	640
23	M	4	0	0	0	0	40	58	M	12	1,000	0	0	512	40
24	M	23	8,000	0	0	8,000	1,280	59	M	12	1,000	0	0	512	0
25	F	26	16,000	256	0	32,000	320	60	M	18	16,000	0	0	8,000	320
26	F	23	8,000	16	0	4,000	160	61	M	10	16,000	256	64	16,000	320
27	M	22	16,000	1,000	0	32,000	320	62	M	7	256	0	0	512	1,280
28	F	25	8,000	256	0	4,000	1,280	63	M	9	256	0	0	128	320
29	M	40	8,000	16	0	16,000	320	64	F	5	256	0	0	512	1,280
30	F	35	4,000	256	0	4,000	1,280	65	M	4	256	0	0	256	80
31	M	4	0	0	0	0	0	66	F	9	256	0	0	256	1,280
32	F	20	4,000	256	16	4,000	640	67	F	10	0	0	0	0	80
33	M	38	4,000	256	0	8,000	640	68	F	10	8,000	256	0	8,000	320
34	M	18	4,000	0	0	4,000	640	69	M	12	16,000	256	0	16,000	0
35	F	30	8,000	256	64	8,000	320	70	F	9	4,000	0	0	8,000	160

S = sex M = masculine F = feminine A = age
FA-IgG = fluorescent toxoplasma IgG antibody test
FA-IgM = fluorescent toxoplasma IgM antibody test
FA-IgM-ABS = fluorescent toxoplasma IgM antibody test after sorptions with polimerized IgG
HA = latex tube-test
* = titer
** = non-reactive

Specificity of the positive FA-IgM tests was investigated by testing samples after sorbing with polimerized IgG, as modified from a previous publication [7]. From 41 positive sera, negative FA-IgM tests resulted in 21 samples, with significant reductions in titers for the remaining sera. A second sorption resulted in test negativation for 5 more sera and a new titer reduction was seen for the remaining 9 samples. Results of the different tests as found for the 70 Indians are included in Table III.

Anti-plasmodium fluorescent tests were positive for all 70 Indians, with titers of 1:500 or more in 93% of the cases.

DISCUSSION

Prevalence of anti-toxoplasma antibodies (88.6%) in the Kren-Akorore Indians here studied was significantly higher than for Indians living in the Xingu National Park, of about 50%, as observed by BARUZZI et al. [3]. In our cases, even children 10 years old or less presented a high prevalence of the infection, since tests were positive in 11 from 16 children (68.8%). Antibody levels were also much higher for the Kren-Akorore than for the Xingu National Park Indians previously studied by BARUZZI et al [3]. For these, titers of 1:8,000 or more were observed in only 2.4% of cases, but occurred in more than 50% of the Kren-Akorore tribe.

Such high antibody levels, plus the observation of positive FA-IgM tests in most cases, could be highly suggestive of very recent toxoplasma infections in the Kren-Akorore group, even configuring and epidemic outburst of the disease. However, as we have referred [6], another very constant serologic characteristic usually seen in recently acquired toxoplasmosis is a marked dissociation between high FA-IgG titers and low HA titers. Thus, in 78% of 136 cases of recently acquired toxoplasmosis we have studied, differences of 16 times or more were found between such titers [6]. For the Kren-Akorore Indians, no significant differences were seen in most cases even when presenting a positive FA-IgM test. This observation and the high percentage of positive tests for rheumatoid factors in these sera led us to investigate the specificity of the FA-IgM positive results.

By treating sera with polimerized IgG, FA-IgM tests became negative or showed marked decrease in titers, thus indicating that the positive results were due to antiglobulin IgM antibodies.

High percentages of positive tests for rheumatoid factors are frequently observed in populations under a continued antigenic stimulus, especially of parasitic origin [2,8]. In our cases malaria could be such an stimulation since anti-plasmodium antibodies were found in every Indian and with high titers in most cases.

According to the serologic patterns previously defined [4,6], from the 62 individuals with positive tests, 25 showed pattern III, with low FA-IgG and HA titers and corresponding to ancient infections. The other 37 cases can be included in transition pattern II, as characterized by high FA-IgG titers, high HA titers and absence of IgM anti-toxoplasma antibodies.

In our experience, such transition pattern usually follows pattern I, which is to be found only in the first weeks or months of infection. Pattern II is then observed for longer periods, of several months, occasionally even for 2 or 3 years, changing then gradually to pattern III, which is characteristic of ancient infections.

Although no serological or clinical evidences of "acute toxoplasmosis" were found, observed data strongly suggest Kren-Akorore Indians were recently exposed to a high risk of toxoplasmosis, an elevated percentage of infection then resulting. From present serologic data, such exposure probably can be dated as having occurred just a few months before collection of serum samples. Serologic evolution of the group could perhaps bring more data to confirm this supposition.

RESUMO

Testes sorológicos para toxoplasmose em população indígena de recente contacto com o homem civilizado (índios Kren-Akorore)

Realizaram-se testes sorológicos de imunofluorescência e de hemaglutinação para a toxoplasmose em 70 índios Kren-Akorore, do Brasil Central, de um grupo de 81 recentemen-

te transferidos para o Parque Nacional do Xingú, cerca de 2 anos após o estabelecimento dos primeiros contactos com civilizados.

Verificou-se positividade dos testes de imunofluorescência anti-IgG e de hemaglutinação em 88,6% dos índios, com títulos na maioria de 1:4,000 ou mais. O teste de imunofluorescência anti-IgM foi positivo em 41 soros (58,6%), mas tornou-se negativo ou apresentou acentuada redução de tíulos após remoção de anticorpos antiglobulínicos dos soros, mostrando não se tratar de verdadeiros anticorpos IgM anti-toxoplasma. A presença de "fator reumatóide" nos soros, responsável pela positividade nos testes de imunofluorescência anti-IgM, foi confirmada pela elevada positividade do teste do látex sensibilizado com globulinas, em geral com títulos altos.

Os resultados dos testes para a toxoplasmose permitiram incluir a maioria dos casos no que chamamos de perfil sorológico II [4,6], ou de transição entre o perfil I, de infecção recente ou aguda, e o perfil III, de infecção antiga. Isto faz supor que o grupo de silvícolas foi exposto, recentemente, a alto risco de infecção, que atingiu então a maioria das pessoas, o que deve ter ocorrido apenas alguns meses antes da realização dos testes.

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