

## EVALUATION OF SEROEPIDEMIOLOGICAL TOXOPLASMOSIS IN HIV / AIDS PATIENTS IN THE SOUTH OF BRAZIL

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### SUMMARY

Toxoplasmosis is considered one of the opportunistic infections for individuals with the Acquired Immunodeficiency Syndrome (AIDS), and is also a major cause of morbidity and mortality. The aim of this study was to evaluate the prevalence of neurotoxoplasmosis, ocular toxoplasmosis and antibodies for *Toxoplasma gondii* in HIV-positive patients attending the SAE (Specialized Assistance Service for HIV/AIDS), as well as to associate their serological profile with epidemiological and clinical data. A total of 250 patients participated in the study from December, 2009 to November, 2010. Serological analysis was performed using the indirect immunofluorescent technique; epidemiological data were gathered by a questionnaire, and clinical history was based on the analysis of medical charts. Prevalence of seropositivity was 80%, with history of neurotoxoplasmosis in 4.8% and of ocular toxoplasmosis in 1.6% of the patients. The Highly Active Antiretroviral Treatment (HAART) was not used by 32% of the patients, 18.4% of the patients had CD4+ T- lymphocyte count less than 200 cells/mm<sup>3</sup> and 96.8% of them were not aware of the modes of disease transmission. These findings led us to conclude that the study population is at high risk of clinical toxoplasmosis, because of both reactivation of infection in the seropositive patients who do not make a regular use of HAART, and primo-infection in seronegative patients worsened by an unawareness of the modes of infection reported in this study.

**KEYWORDS:** Toxoplasmosis; AIDS; *Toxoplasma gondii*; Neurotoxoplasmosis; Ocular toxoplasmosis.

### INTRODUCTION

Infection by *Toxoplasma gondii* has high prevalence among humans and animals. Although it is usually asymptomatic in immunocompetent people, the protozoan can cause severe disease in fetuses and in immunodepressed individuals<sup>30</sup>.

In immunocompetent people, after the development of an appropriate immune response, the acute infection becomes chronic, characterized by the presence of tissue cysts that remain dormant<sup>29</sup>. However, in immunodepressed individuals there may be a reactivation of the infection through the rupture of the cysts, causing severe pathology which can lead to death<sup>6</sup>.

Cerebral toxoplasmosis, in AIDS patients, is the most common opportunistic infection, appearing when the levels of CD4+ T-lymphocytes are under 200 cells/mm<sup>3</sup>, toxoplasmic retinochoroiditis is also one of the important secondary ocular inflammations in these patients<sup>15</sup>. Neurological lesions were found in up to 90% of the pathological examinations with AIDS<sup>30</sup>.

Even with the use of HAART (*Highly Active AntiRetroviral*

*Treatment*), neurotoxoplasmosis remains the prevalent cause of neurological disorders in HIV-positive patients, being particularly severe among those with higher immunosuppression and an absence of prophylaxis<sup>2</sup>.

Around 95% of patients with cerebral toxoplasmosis present detectable levels of IgG anti-*T. gondii* serum antibodies<sup>25</sup>. Thus, it is extremely important that serology for *Toxoplasma gondii* be performed, so that, with an early diagnosis, the person can receive the appropriate treatment in order to prevent the reactivation of the disease.

This work aimed to verify the prevalence of antibodies for *T. gondii*, neurotoxoplasmosis and ocular toxoplasmosis in HIV-positive patients in care of a Reference Service (SAE) in the south of Brazil, by correlating seropositivity with the epidemiological and clinical data of each patient.

### MATERIALS AND METHODS

A cross-sectional study was conducted, involving 250 HIV-positive patients who were under medical supervision at the Specialized Assistance Service for HIV/AIDS (SAE), linked to the Medicine College of the Federal University of Pelotas (UFPel) from December 2009 to

November 2010. The SAE is a reference center for the treatment of people infected with HIV in the region of Pelotas (31°46'19"S, 52°20'34"W), in the state of Rio Grande do Sul (Brazil).

All the patients signed the Statement of Free and Clarified Consent after an explanation about the research would entail.

To verify the determining factors in the risk of transmission of the disease, the patients answered a questionnaire about sex, age group, level of education, residence in rural area, knowledge about the disease, ignorance about ways of transmission of the disease, living with cats, presence of cats in the peridomicile, the habit of handling raw meat, the habit of eating raw vegetables, raw/undercooked meat and homemade sausages, the habit of drinking unpasteurized milk and untreated water, having meals in restaurants, not regularly washing hands before meals, direct contact of hands with soil and blood transfusion history.

The data of cerebral toxoplasmosis, ocular toxoplasmosis, levels of CD4+ T- lymphocytes, CD4/CD8, viral load quantification and antiretroviral therapy use were obtained using the patients' medical records.

The blood used for the study was collected by venipuncture in the Clinical Analysis Laboratory at the Medicine College, and then centrifuged, with the serum being stored at -20°C in Ependorff tubes, and all properly identified. The serological analysis was performed at the Parasitology Laboratory of the Biology Institute of UFPel.

The technique used to detect the antibodies IgG and IgM for *T. gondii* was Indirect Immunofluorescence Assay (IFAT) – (WAMA® Diagnostica), according to the manufacturer's instructions, and the samples were considered reactive when they were diluted from 1:32 on.

The sample size was calculated based on the number of HIV-positive patients being followed at the SAE, with a prevalence expectation of 50%, 5% error and confidence level of 95%, reaching a minimum sample size of 249 patients to be evaluated. The number of samples analyzed was 250.

The calculation of the sample size and the analysis of the results were assessed using Epi-Info version 6.04 and the Chi-square test was also performed, differences were considered statistically significant when  $p < 0.05$ . The value of OR (Odds Ratio) was determined with a confidence interval of 95%. As for risk factors, only those which presented  $p \leq 0.25$  in the bivariate analysis were included in the multivariate model performed by the program Statistix 9.0.

## ETHICAL CONSIDERATIONS

The Project was submitted to the Committee for Ethics in Research with human beings of the Medicine College of UFPel, and was approved and registered under number 121/07.

## RESULTS

No IgM antibodies for *T. gondii* were found among the HIV-positive patients in the study.

Seroprevalence was 80% (200/250) (95% CI; 74.7 - 84.6) concerning

IgG antibodies. The titers ranged from 32 to 512, the one with highest frequency being 64 (46.5%).

For the assessed women and men, seropositivity was 78.1% and 81.7%, respectively, with no statistically significant difference between sexes. The age of the patients ranged from 14 to 73 years old (average = 38.6).

It was found that patients were uninformed about the disease, because even among those who said they knew about it (70.4%), the lack of information about the main ways of transmission was high (95.5%). The patients who reported having knowledge about forms of transmission were asked about these forms, and the correct associations mentioned were: ingestion of raw or undercooked meat, contaminated vegetables and fruit and contact with cat feces.

Table 1 shows a significant association ( $p < 0.05$ ) or not between the risk factors and seropositivity for *T. gondii* of the patients in the bivariate analysis. For the multivariate model, this association is shown in Table 2.

Regarding the levels of CD4+ T- lymphocytes, based on the medical records of the patients, they ranged between 1 and 1575 cells/mm<sup>3</sup>, with an average of 405.07 cells/mm<sup>3</sup>. Among patients with positive serology for *T. gondii*, the variation was between 12 and 1482 cells/mm<sup>3</sup>, with an average of 414.11 cells/mm<sup>3</sup>, and 15% of them had CD4+ T- lymphocyte levels of < 200 cells/mm<sup>3</sup>.

Table 3 shows frequency of seropositivity related to the levels of CD4+ T- lymphocytes, CD4/CD8, viral load and antiretroviral use.

The medical charts showed that 3.6% of the patients (nine cases) developed neurotoxoplasmosis, 0.8% (two cases) neurotoxoplasmosis associated with ocular toxoplasmosis, 0.8% (two cases) ocular toxoplasmosis and 0.4% (one case) neurotoxoplasmosis associated with ischemia.

## DISCUSSION

The incidence of infection by *T. gondii* varies within communities, depending on dietary habits, contact with animals carrying the protozoan/parasite and climate conditions<sup>11</sup>.

The non-detection of IgM antibodies in patients throughout the study is indicative that primo-infection by *T. gondii* has not occurred. However, serological tests for IgM antibodies in people with HIV/AIDS are usually negative, since their low immunity response may lead them to produce antibodies at levels undetectable by these techniques<sup>3</sup>.

Seroprevalence of 80% found in this study was higher than that reported for immunocompetent populations in the same region (54.8% for pregnant women<sup>10</sup> and 57.5% for blood donors<sup>17</sup> in Pelotas-RS and 59.8% for pregnant women in Porto Alegre-RS<sup>28</sup>). Seroprevalence in immunosuppressed individuals was similar to that reported by other studies in Brazil: Pará (82.9%)<sup>13</sup> and Bahia (77.3%)<sup>23</sup>.

However, it was higher than the ones found in Norway (17.8%)<sup>12</sup>, Iran (49.75%)<sup>21</sup>, Malaysia (44.7%)<sup>22</sup> and Africa (31.3%)<sup>24</sup>. The variations in the prevalence of anti-*Toxoplasma* antibodies found in

**Table 1**  
Frequency of the possible risk factors and relation with seropositivity for *Toxoplasma gondii* in 250 HIV-positive patients in Pelotas, RS, 2009-2010

Variables	Freq. of risk factor	Serodiagnosis				p value
		Pos.		Neg.		
		n	%	n	%	
Direct hand contact with soil	46.8%	103	88	14	12	0.003*
School level < high school	81.2%	169	83.2	34	16.7	0.007*
Consumption of untreated water	39.2%	86	87.8	12	12.2	0.014*
Living with cats	60%	127	84.7	23	15.3	0.024*
Residence in rural area	4%	10	100	0	0	0.102
Consumption of homemade sausages	60.4%	125	82.8	26	17.2	0.174
Consumption of raw or undercooked meat	27.6%	58	84.1	11	15.9	0.322
Ignorance about the disease	29.6%	62	83.8	12	16.2	0.332
Presence of cat in the peridomicile	71.2%	145	81.5	33	18.5	0.364
Meals in restaurants	6.4%	12	75	4	25	0.402
Blood transfusion	24.4%	51	83.6	10	16.4	0.418
Ignorance about ways of transmission	96.8%	194	80.2	48	19.8	0.499
Consumption of raw vegetables	94%	188	80	47	20	0.608
Not washing hands before meals	2.4%	5	83.3	1	16.7	0.655
Age group > 30 years old	72%	145	80.5	35	19.4	0.725
Consumption of unpasteurized milk	24.4%	49	80.3	12	19.7	0.941
Handling of raw meat	86%	172	80	43	20	1.000

\*  $p < 0.05$

**Table 2**  
Association ( $p < 0.05$ ) of the factors of risk of infection by *Toxoplasma gondii* with the seropositivity in 250 HIV-positive patients, after the application of the multivariate model in Pelotas, RS, 2009-2010

Variables	Positive		Negative		OR	95% CI	p value
	n	%	n	%			
School level < High School							
Yes	169	83.2	34	16.7			
No	31	66	16	34	2.47	(1.20-5.08)	0.0140
Direct hand contact with soil							
Yes	103	88	14	12			
No	97	7.9	36	27.1	2.66	(1.34-5.26)	0.0049

OR, Odds ratio; CI, confidence interval.

different studies may be related to the risk of infection by the protozoan, reflecting the dietary habits and the sanitary conditions of the places where these studies were performed. It was found that having a low educational level (less than high school) is a risk factor for infection, as found in previous research done in Brazil<sup>16,18,28</sup>. The low level of instruction of the analyzed patients explains the lack of knowledge about the ways of transmission of toxoplasmosis, in agreement with another study conducted in the same city<sup>4</sup>. This makes it evident that improvements in the quality of education are needed, especially because the level of instruction lowers the exposure to risks, this is down to

the adoption of more appropriate measures of hygiene and nutrition by the subjects<sup>18,28</sup>.

Direct manipulation of soil was the factor most associated with the infection, and it was also seen in immunocompetent groups (pregnant women) in some cities in Rio Grande do Sul<sup>4,26</sup>.

It was found that all the patients from rural areas were seropositive for *T. gondii*, which may be due to the higher likelihood of handling soil with bare hands, a factor associated with this infection.

**Table 3**

Frequency of 250 HIV-positive patients seropositive and seronegative for *Toxoplasma gondii*, related to the levels of CD4+, CD4/CD8, use of HAART and viral load in Pelotas, RS, 2009-2010

Variables	Frequency		Serodiagnosis			
			Positive		Negative	
	n	%	n	%	n	%
CD4 < 200	46	18.4	32	69.6	14	30.4
CD4 > 200	204	81.6	168	82.3	36	17.7
CD4/CD8 < 1	229	91.6	181	79	48	21
CD4/CD8 > 1	21	8.4	19	90.4	2	9.6
Detectable viral load (> 50)	126	50.4	98	77.7	28	22.3
Undetectable viral load (< 50)	124	49.6	102	82.2	22	17.8
Regular use of HAART	154	61.6	122	79.2	32	20.8
Irregular use of HAART	16	6.4	11	68.8	5	31.2
No use of HAART	80	32	67	83.7	13	16.3
Treatment-naive patients	56	70	46	82.1	10	17.9
Treatment dropouts	24	30	21	87.5	3	12.5

Also, it was discovered that 39.2% of the analyzed patients drank untreated water. However, through multivariate analysis, it was found that there was no association between this factor and infection by *T. gondii*, agreeing with other studies done in the southern region of Brazil<sup>4,18</sup>.

Living with cats as pets did not present risk of infection by for *T. gondii*, although this species is the main disseminator of the protozoa in the environment because it eliminates oocysts in its feces, and the former become infectious after a minimum of 24 hours<sup>11</sup>. However, this association is polemic, since in certain studies living with cats was considered a risk factor<sup>16,18,26</sup>, whereas in others it was not<sup>4,7</sup>. There are authors who claim that there are no impediments for immunosuppressed people and pregnant women to have cats<sup>4,9</sup>, as long as basic precaution measures are taken<sup>3</sup>.

Neurotoxoplasmosis presented low prevalence (4.8%), similar to those found by researchers in other countries like Mexico (5.1%)<sup>8</sup>, Norway (8%)<sup>12</sup> and Iran (10%)<sup>21</sup>.

Only 1.6% of the patients developed ocular toxoplasmosis, either alone or associated with neurotoxoplasmosis. In São Paulo<sup>19</sup>, this prevalence was 4.4%, and in Recife<sup>1</sup> it was 5.7%. Although the incidence of opportunistic infections has decreased since the introduction of HAART, ocular toxoplasmosis still occurs in patients with neurotoxoplasmosis<sup>31</sup>.

Literature on the subject shows that opportunistic infections/co-infections is one of the main factors for risk of death in patients affected by HIV/AIDS. In the relationship between these infections and the count of CD4+ T- lymphocytes, the latter remains as a short term predictor<sup>14</sup>.

In this study, the average count of the CD4+ T- lymphocyte levels in patients with neurotoxoplasmosis was 116 cells/mm<sup>3</sup>, similar to the one found in Recife<sup>1</sup> (139.8 cells/mm<sup>3</sup>). On the other hand, more severe degrees of immunosuppression were found in Mexico<sup>8</sup> (78 cells/mm<sup>3</sup>), Norway<sup>12</sup> (75 cells/mm<sup>3</sup>) and Iran<sup>21</sup> (66.4 cells/mm<sup>3</sup>). A significant

association between neurotoxoplasmosis and CD4+ T- lymphocyte levels below 100 cells/mm<sup>3</sup> was found, showing that patients with a low number of CD4+ cells present a higher risk of reactivation of the infection<sup>8</sup>.

The HAART provides a significant improvement in the quality of life for HIV-positive people, preventing the weakening of their immune system and reducing the appearance of opportunistic infections<sup>20</sup>.

The antiretroviral treatment was not used regularly by 32% of patients in this study, which is in accordance with data reported in the study conducted in Recife<sup>29</sup>. A total of 9.2% (23/250) of patients had CD4+ T- lymphocyte count less than 200 cells/mm<sup>3</sup>, which means a high risk of reactivation of the disease (69.6% seropositives) or primo-infection (30.4% seronegatives). Antiretroviral therapy is indicated for HIV-positive asymptomatic patients who have CD4+ T- lymphocyte count between 200 and 350/mm<sup>3</sup>; and the closer the T- lymphocyte count is to 200 cells/mm<sup>3</sup>, the higher the risk of progression for AIDS and the establishment of opportunistic infections<sup>20</sup>. It is clear that some difficulties must be overcome, such as the non-compliance with the treatment and the correct use of medication aiming towards improving the quality of life and lifespan of these patients<sup>26</sup>.

The seronegatives for *T. gondii* have a higher level of schooling, better hygiene and food habits (Table 1), which could explain the lack of contact with the parasite. These patients had special attention regarding explanations about risks of infection and how to prevent it. All subjects attending the SAE have been serologically monitored for *T. gondii* and other important opportunistic infection causative agents, since a primo-infection associated with immunosuppression could cause them death<sup>30</sup>.

The results of this study highlight the importance of establishing primary prevention programs against *T. gondii* infection in the community, so that risks of primo-infection in seronegative individuals can be reduced, particularly in those with severe immunosuppression and without appropriate treatment. Moreover, all HIV positive patients should have the importance of a strict compliance with the medical prescription

explained to them, so that their immune system will be able to prevent opportunistic infections like the toxoplasmosis, which could be fatal.

## RESUMO

### Avaliação soroepidemiológica de toxoplasmose em pacientes HIV/SIDA no sul do Brasil

A toxoplasmose é considerada uma das infecções oportunistas em indivíduos com a Síndrome da Imunodeficiência Adquirida (SIDA), constituindo importante causa de morbidade e mortalidade. Este trabalho objetivou conhecer a prevalência de neurotoxoplasmose, toxoplasmose ocular e de anticorpos para *Toxoplasma gondii*, em pacientes HIV positivos em atendimento no SAE (Serviço de Assistência Especializada em HIV/AIDS) além de correlacionar seu perfil sorológico com dados epidemiológicos e clínicos. Foram analisados 250 pacientes no período de dezembro de 2009 a novembro de 2010. A análise sorológica foi realizada pela técnica de imunofluorescência indireta, os dados epidemiológicos foram obtidos pela aplicação de um questionário e o histórico clínico, pela análise dos prontuários. A prevalência de soropositividade foi de 80%, com histórico de neurotoxoplasmose em 4,8% e de toxoplasmose ocular em 1,6%. Observou-se que 32% dos pacientes não faziam uso regular de HAART, 18,4% apresentaram contagem de linfócitos T CD4+ inferior a 200 células/mm<sup>3</sup> e 96,8% desconheciam os modos de transmissão da doença. Conclui-se que o risco de toxoplasmose clínica grave é alto na população estudada, tanto por reativação da infecção nos soropositivos que não fazem uso regular do HAART, quanto por primoinfecção dos soronegativos, agravado pela desinformação sobre as formas de infecção constatada na pesquisa.

## ACKNOWLEDGMENTS

WAMA Diagnostica, for the donation of Indirect Immunofluorescence kits.

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Received: 8 February 2012

Accepted: 20 July 2012