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Neospora caninum and *Toxoplasma gondii* serodiagnosis in human immunodeficiency virus carriers

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ABSTRACT

Introduction: *Neospora caninum* and *Toxoplasma gondii* belong to the Sarcocystidae family, and both have one definitive and various intermediary hosts. Owing to their weak immune systems, immunocompromised persons might be prone to opportunistic infections. The aim of this study was to investigate the presence of anti-*N. caninum* and anti-*T. gondii* antibodies in immunocompromised individuals. **Methods:** This cross-sectional study investigated the rates of *N. caninum* and *T. gondii*, as assessed using immunofluorescent antibody reaction (IFAT) with 1:50 and 1:16 dilution, respectively, in patients with human immunodeficiency virus (HIV). **Results:** The seropositivity for *N. caninum* was 26.1% (81/310) in Mato Grosso do Sul and 31.2% (10/32) in Paraná and for *T. gondii* was 76.8% (238/310) in Mato Grosso do Sul and 68.7% (22/32) in Paraná. **Conclusions:** There is evidence of anti-*N caninum* and anti-*T. gondii* antibodies in patients with HIV. Other aspects of *T. gondii*, which is a zoonosis, and *N. caninum*, which might affect immunodeficient individuals, need to be evaluated and reported.

Keywords: Serology. *Toxoplasma gondii*. *Neospora caninum*. HIV.

INTRODUCTION

Since 2009[1], there have been reports of opportunistic protozoan infections in immunocompromised patients. Owing to their inability to control parasite replication, clinical disease can occur, resulting in morbidity and mortality.

Following the first description of acquired immune deficiency syndrome (AIDS) in the 1980s, it has become one of the most researched diseases owing to its pandemic nature and severity. It is characterized by immune system suppression, primarily the immunity mediated by T cells; the decline of cluster of differentiation 4 *(*CD4) lymphocytes results in susceptibility to opportunistic infections, secondary neoplasms, and neurological diseases[2].

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Neospora caninum and *Toxoplasma gondii* are intracellular protozoans belonging to the Sarcocystidae family that can infect domestic and wild animals. Both are parasites that cause cyst formation and have in their definitive host cycle a carnivorous animal and a wide variety of species as their intermediary hosts[3].

Because it is a zoonosis, *T. gondii* is considered an important parasite in both human and veterinary medicine. The felid, which is an important host in the *T. gondii* lifecycle, is responsible for parasite dissemination, by introducing the oocyst in the environment. The intermediary host acquires toxoplasmosis through contaminated food and water[4].

Neospora caninum was identified in 1984 by Bjerkas et al.[5], before which time it was confused with *T. gondii.* When infected with *N. caninum*, some canids (dogs, dingoes, and coyotes) can become a definitive host and develop neurological problems. *N. caninum* is also known to cause reproductive problems, such as abortions and stillbirths, in production animals (bovines, caprines, and ovines)[6].

Toxoplasmosis is disseminated in various parts of the world and affects both humans and animals, with at least two billion infected people. In humans, the possibility of acquiring the disease is higher with age; its frequency varies depending on the population and geographical location and is lower

in colder locations[7]. The symptoms in immunocompetent humans are usually not apparent. However, symptoms of headaches, paralysis, and mental deterioration can present in immunocompromised patients, such as carriers of human immunodeficiency virus (HIV); these clinical symptoms are also common to various other opportunistic parasites[8].

Different methods are available to diagnose *N. caninum* and *T. gondii*, including direct methods, such as histopathological, immunohistochemical, or polymerase chain reaction (PCR) procedures, as well as indirect methods, such as enzyme-linked immunosorbent assay (ELISA), agglutination test, and immunofluorescent antibody reaction (IFAT) procedures. The latter is the technique of reference for *N. caninum*[9] [10].

The presence of anti-*N. caninum* antibodies in humans was detected in 1999 based on the analysis of 1,029 samples from the California blood bank[11]. Although 69 samples were positive on the IFAT test, 50 samples did not have *T. gondii* antibodies. In the same year, Peterson et al.[12] analyzed samples from 76 women with a history of abortion or intrauterine fetal death in Denmark, without detection of anti-*N. caninum* antibodies. However, the authors did not discard the possibility of a parasite infection and proposed that patients with neurological problems of unknown causes and immunocompromised patients should be examined.

In 2002, Magalhães et al.[13] analyzed blood samples from three groups, including a group of patients with HIV, in Bahia and found positive serological reactions in all groups. In Minas Gerais (MG) in 2006, positive samples were found for HIV-positive patients with neurological problems, newborns, and individuals considered to be healthy, with the group of HIV patients having the highest percentage, at 38%[14].

There is no evidence that *N. caninum* infection is zoonotic[15]; however, these reports of seropositivity in humans do not allow us to discard the possibility that the parasitic infection affects human beings[11] [16]. Humans might become infected with *N. caninum* in the same way as with *T. gondii*, by the accidental ingestion of oocysts found in the feces of the definitive host, the canid, and also by the consumption of badly cooked or raw meat containing cysts from the intermediary hosts[17].

In Brazil, serological studies of neosporosis and toxoplasmosis in human beings are scarce; this is also true in Mato Grosso do Sul (MS), where reports of anti-*T. gondii* antibodies are limited to university students and newborns in Campo Grande, the population from Pantanal, and the indigenous population of the Miranda county in the State of MS, which is the most recently reported study[18] [19] [20] [21] [22].

Considering the close interaction between people and seropositive animals via animal management and the cooking and ingestion of raw or badly cooked meat as well as the impaired immunity of immunocompromised individuals, such as HIV carriers and patients with neoplasia, a serological study of immunocompromised patients is the first step to evaluate the possibility of a *N. caninum* infection in humans in MS. Therefore, owing to its importance and the shortage of information regarding these parasites in humans in the States of Mato Grosso do Sul and Paraná (PR), the aim of this study was to investigate the presence of anti-*N. caninum* and anti-*T. gondii* antibodies in immunocompromised individuals using the IFAT method.

METHODS

A cross-sectional study involving 310 HIV-positive patients in two reference centers in Campo Grande-MS was conducted between November 2009 and June 2011 [Research Ethics Committee, *Comitê de Ética em Pesquisa/Universidade Federal de Mato Grosso do Sul* (CEP/UFMS) 1941/11]. In the State of PR, 32 HIV-positive patients, people who have agreed to be part of the survey, were selected from the serology lab of the *Erasto Gaertner* Hospital to be tested using chemiluminescence (*Jonhson &d Jonhson do Brasil Ltda*, Brazil) between August 2012 and August 2013 [Research Ethics Committee *Comitê de Ética em Pesquisa/Pontificia Universidade Católica do Paraná* (CEP/PUCPR) 5017/11].The sample sizes differed between MS and PR because of sample availability and the granted access to data for the researchers. The patients provided consent to participate and signed the Terms of Consent.

The cephalic vein was accessed for sample collection by a healthcare professional; 10mL samples were collected from either the right or left cephalic vein, equally divided in tubes without anticoagulant, and duly identified. After collection, the tubes were rested at room temperature and protected from light for clot retraction. Then, the tubes were centrifuged for blood serum, which was stored in microtubes and kept at -20°C until the serological exams.

Immunofluorescent antibody reaction for the detection of anti-*Neospora caninum* and *Toxoplasma gondii* antibodies

Serum samples were tested for immunoglobulin G (IgG) anti-*N. caninum* (NC-1 strain) and *T. gondii* (RH strain) using the IFAT method. Serum samples were tested at an initial dilution of 1:50 and 1:16 in phosphate buffered saline (PBS) solution (pH 7.2) for *N. caninum* and *T. gondii*, respectively, and the positives were diluted to an endpoint titer(i.e., the test was conducted again with an increased dilution until the fluorescence ceased to be present over the whole parasite). The IFAT technique and the slides for IFAT were prepared according to the method by Locatelli-Dittrich[23]. Bovine serum samples, knowingly positive or negative, were used as controls for all slides. The fluorescent conjugate human anti-IgG or bovine anti-IgG were used with a dilution of 1:100 (conjugate with fluorescein isothiocyanate [SIGMA©]). The slides were observed using a microscope equipped for fluorescence (epi-illumination system), with an objective of 40×. Fluorescent reactions around the entire periphery of the parasite were considered positive. In negative reactions, the parasites on the slide did not present fluorescence, or the fluorescence was located only on one end, characterized as *polar coloration* or *apical reaction*. Samples with total tachyzoite peripheral fluorescence were considered positive[24].

Statistical analysis

The results of the serological exams were statistically analyzed using the χ^2 test using Medcalc statistical package

and level of significance of p>0.05. Demographic data such as sex, ethnicity, education, and income as well as some of the risk factors such as surgery, blood transfusion, tattoos, and presence of other diseases were also analyzed within and between the surveyed parasites using χ^2 tests.

RESULTS

The patients were mostly (54.5%) male, white, with a primary education, with an income between 1 and 3 minimum salaries (currently, the minimum salary in Brazil is of R$788,00), and born in the State of MS.

Anti-*Neospora caninum* antibodies were detected in 26.1% (81/310) of the samples with a 1:50 dilution from HIV-positive patients in MS; with a 1:400 dilution, only 1 patient was considered positive **(Table 1)**. In PR, 32 samples from HIV-positive patients were analyzed, and 10 individuals were found with anti-*N. caninum* antibodies (31.2%).

Anti-*T. gondii* antibodies were detected in 76.8% (238/310) of the samples with a 1:16 dilution from HIV-positive patients in MS; with a 1:2,048 dilution, only 4 patients were considered positive **(Table 2)**. Anti-*T. gondii* antibodies were detected in 68.7% (22/32) of the samples from HIV-positive patients in the State of PR.

Demographic analysis as sex and education was not significant with p> 0:05 and risk factors such as diseases, acupuncture, surgery also was not significant

Based on the χ^2 test **(Table 3)**, of the 310 samples from the HIV-positive patients, 81 (26.1%) were positive for *N. caninum*.

TABLE 1 - Presence of *Neospora caninum* in the serum of human immunodeficiency virus-positive patients in Mato Grosso do Sul.

	Neospora caninum				
	Number of samples	**IFAT (1:50) +/%**	**IFAT (1:100) +/%**	**IFAT (1:200) +/%**	**IFAT (1:400) +/%**
HIV	310	81/26.1	6/1.9	2/0.6	1/0.3

HIV: human immunodeficiency virus; **IFAT:** immunofluorescence assay; **+:** sample positive; **%:** percentage of sample positive.

HIV-positive patients who were not on dialysis treatment had a significantly higher probability of being infected with *N. caninum* (p<0.05).

Continuing the analysis **(Table 4)** in the same population, 238 (76.8%) positive samples (p < 0.0001) from the *T. gondii* serology were detected.

Coinfection was present **(Table 3)**, with 78 (25.2%) of the 238 *N. caninum*-positive samples also positive for *T. gondii* (p<0.0001).

DISCUSSION

In the State of MS (n = 310) and PR (n = 32), seropositivities for *N. caninum* were 26.1% and 31.2%, respectively, in HIV-positive individuals. Comparatively, the previously

TABLE 2 - Presence of *Toxoplasma gondii* in the serum of human immunodeficiency virus-positive patients in Mato Grosso do Sul.

	Toxoplasma gondii								
	Number of samples	**IFAT (1:16) +/%**	**IFAT (1:32) +/%**	**IFAT (1:64) +/%**	**IFAT (1:128) +/%**	**IFAT (1:256) +/%**	**IFAT (1:512) +/%**	**IFAT (1:1024) +/%**	**IFAT (1:2048) +/%**
HIV	310	238/76.8	141/45.5	128/41.3	113/36.5	75/24.2	17/5.5	8/2.6	4/1.3

HIV: human immunodeficiency virus; **IFAT:** immunofluorescence assay; **+:** sample positive; **%:** percentage of sample positive.

TABLE 3 - Analysis of the results of *Neospora caninum* samples using the χ^2test.

Risk factors or characteristics		*Neospora caninum*						**Chi-square**	**p-value**
		positive		**negative**		**total**			
		n	**%**	**n**	**%**	**n**	**%**		
HIV reactive		81	26.1	229	73.9	310	100.0	69.7	< 0.0001
Patients on dialysis	1	76	24.5	226	72.9	302	97.4	3.9	0.0495
	2	5	1.6	3	1.0	8	2.6		
Toxoplasma gondii	Positive	78	25.2	160	51.6	238	76.8	22.0	< 0.0001
	Negative	3	1.0	69	22.3	72	3.2		

HIV: human immunodeficiency virus; **IFAT:** immunofluorescence assay; **+:** sample positive; **%:** percentage of sample positive; **χ2:** the χ2 test using Medcalc statistical package and level of significance of p>0.05.

TABLE 4 - Analysis of the results of *Toxoplasma gondii* samples using the χ^2 test.

Risk factors or characteristics	*Toxoplasma gondii*						χ2	p-value
	positive		negative		total			
	n	%	n	%	n	%		
HIV reactive	238	76.8	72	23.2	310	100.0	87.8	< 0.0001

HIV: human immunodeficiency vírus; **χ2:** the χ2 test using Medcalc statistical package and level of significance of p>0.05.

reported rates of positivity for class IgG antibodies were 38% in 65 HIV-positive individuals in the State of MG, using the same serological test and dilution (1:50)[14] and 15% in individuals with HIV, 3.8% in healthy individuals, and 5% in women (with or without previous abortions) (n = 80 each) in Bahia, detected using IFAT[13]. Seropositivity of anti-*N. caninum* antibodies was detected in 7 serum samples (10.5%) from 67 rural workers on farms with serologically positive bovines and dogs in the State of Mato Grosso (MT), Brazil[24].

The seropositivities for *T. gondii* in the States of MS and PR in the present study were 76.8% and 68.7%, respectively, which are similar to those in the present literature. In adult humans in Brazil, the presence of anti-*T. gondii* antibodies varies from 30.34 to 97.1%[19] [25]. However, a higher seropositivity can be found in some Brazilian locations, including the Southwest of the State of MT (97.4%)[26]. The variability in Brazilian studies might be related to the different cutoff rates and lack of standardized serological techniques, among other factors.

The population of MS has habitual and socioeconomic characteristics that are considered predisposing factors for *T. gondii*[27].

In addition, analysis of seropositivity from reports of individuals with *T. gondii* indicated that the rates were similar to those found in Brazil[18] [25]. For inhabitants of rural areas, such as riverbank populations, seropositivityis reportedly 42.1%[21].

In university students participating in research in 2010, ELISA detected *T. gondii* in 39 of 100 participants, with a 95% confidence interval[18], and a hemagglutination test with a 1:16 dilution resulted in 44 of 145 individuals as positive[19]. In both studies, the presence of cats in the student's domicile or contact with these animals was positively correlated with seropositivity in the students.

In the indigenous Terena population of MS, 67 of 256 samples (26.7%) in 2014 were positive for *T. gondii*, as detected using the IFAT method with a 1:16 dilution[22]. Despite the different populations and difference in sample sizes between this study and the present study, the diagnostic method and dilution were the same; however, the percentage of seropositive samples in the present study was higher.

In other countries, no seropositivity for *N. caninum* was observed in a study in England, with 3,232 people from a serological vigilance program in 2000 and 518 agricultural workers from a population considered to have a high risk of zoonotic diseases, owing to direct contact with possible means of transmission, such as contaminated placenta, fetal fluids, or an environment contaminated with dog feces containing oocysts, who participated in a survey in 1995. Serological reactions were initially tested using ELISA and later using IFAT[17].

In Egypt in 2009[26], 8 (7.9%) and 52 (51.5%) of 101 samples from pregnant women at a private clinic in the City of Mansoura tested positive for *N. caninum* and *T. gondii*, respectively. Both parasites were found in 6 (5.9%) samples, and the positive result of *N. caninum* was confirmed using IFAT. Despite the number of samples and differing results, this study confirms the results of the present article, which showed the presence of anti-*N. caninum* antibodies in HIV-infected patients, who all presented with concurrent seropositivity for *T. gondii*.

In addition to HIV patients, Lobato et al.[14] also surveyed hemodialysis patients, with seropositivity in 6 of 53 individuals. Two of the positive samples also tested positive for *T. gondii*; however, these data were not significant for *T. gondii* seropositivity or seronegativity. In MS, *N. caninum* was also reported in a smaller sample of HIV carriers undergoing hemodialysis (p<0.05).

To the best of our knowledge, this is the first report of anti-*N. caninum* antibodies in immunocompromised humans in MS, resulting in a relatively high seropositivity of 26.1% when compared with the infection rate of immunocompetent individuals; therefore, this might be an opportunistic infection. Regarding the means of contagion, the presence of anti-*N. caninum* antibodies was found in different animals used for consumption, as well as in dogs, which are its definitive hosts, increasing the possibility of contagion. These initial data for *N. caninum* signal the need to determine if this disease is only an opportunistic disease in immunocompromised persons or if it is a zoonosis.

We believe that this is also the first report of seropositivity for *T. gondii* in immunodeficient persons, with a high seropositivity of 76.4%. The *T. gondii* parasite circulates in immunocompromised patients; because this is a public health issue, broader data about the epidemiology of these parasites is needed, especially in immunocompromised individuals.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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