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Seasonal profile and level of CD4+ lymphocytes in the occurrence of cryptosporidiosis and cystoisosporidiosis in HIV/AIDS patients in the Triângulo Mineiro region, Brazil

Perfil sazonal e nível de linfócitos CD4+ na ocorrência de criptosporidiose e cistoisosporidiose em pacientes HIV/AIDS na região do Triângulo Mineiro, Brasil

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ABSTRACT

Patients with AIDS are particularly susceptible to infection with intestinal coccidia. In this study the prevalence of infections with Cryptosporidium *sp and* Cystoisospora belli *were evaluated among HIV/AIDS patients in the Triângulo Mineiro region, Brazil. Between July 1993 and June 2003 faecal samples from 359 patients were collected and stained by a modified Ziehl-Neelsen method, resulting in 19.7% of positivity for coccidian (8.6% with* Cryptosporidium *sp, 10.3% with* Cystoisospora belli *and 0.8% with both coccidian). Patients with diarrhoea and T CD4+ lymphocyte levels ≤ 200 cells/mm³ presented higher frequency of these protozoans, demonstrating the opportunistic profile of these infections and its relationship with the immunological status of the individual. It was not possible to determine the influence of HAART, since only 8.5% of the patients positive for coccidian received this therapy regularly. Parasitism by* Cryptosporidium *sp was more frequent between December and February and thus was characterised by a seasonal pattern of infection, which was not observed with* Cystoisospora belli.

Key-words: Cryptosporidium *sp.* Cystoisospora belli. Isospora belli. *HIV/AIDS.*

RESUMO

Pacientes com AIDS são particularmente susceptíveis a infecção por coccídios intestinais e nesse estudo foi avaliada a freqüência de Cryptosporidium *sp. e* Cystoisospora belli *entre pacientes HIV/AIDS na região do Triângulo Mineiro, Brasil. No período de julho de 1993 a junho de 2003, amostras de fezes de 359 pacientes foram submetidas à coloração pelo método de Ziehl-Neelsen modificado, sendo detectada a presença de coccídios em 19,7% destas (8,6% de* Cryptosporidium *sp, 10,3% de* Cystoisospora belli *e 0,8% de ambos coccídios). Pacientes com diarréia e níveis de linfócitos T CD4+ ≤ 200 células/mm³ apresentaram maior frequência destes protozoários, demonstrando o perfil oportunista destas infecções e a relação com o status imunológico do indivíduo. Não foi possível determinar a influência da HARRT, pois apenas 8,5% dos pacientes positivos para coccídios fazriam uso regular desta terapia. Parasitismo por* Cryptosporidium *sp foi mais freqüente no período compreendido de dezembro a fevereiro caracterizando padrão sazonal desta infecção, fato não observado com* Cystoisospora belli.

Palavras-chaves: Cryptosporidium *sp.* Cystoisospora belli. Isospora belli. *HIV. AIDS.*

A high prevalence of gastrointestinal infections has been observed in AIDS patients since the first cases were described, especially those caused by opportunistic parasites. The rapid dissemination of human immunodefiency virus (HIV) has increased considerably the incidence of these entomopathogens, especially *Cryptosporidium* sp and *Cystoisospora belli*, which are responsible for potentially severe diarrhoeic profiles with morbidity directly related to the degree of immunodepression[20 24]. The clinical profile may be presented as a self-limiting infection, acute or chronic diarrhoeic syndromes, with debilitating diarrhoea

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frequently accompanied by weight loss, dehydration, abdominal pain and poor absorption syndrome[1 7 11]. The chronic nature of these infections contributes to the increase in morbidity and mortality in these patients. Cryptosporidiosis may be associated with gastrointestinal syndrome and respiratory infections and recurrences are common. Infection rates vary from 0.6 - 85% for *Cryptosporidium*[13] and 0.2 - 20% for C*ystoisospora belli*[18].

In Brazil, the prevalence of cryptosporidiosis in HIV/AIDS patients varies between 6.4-9.1% while for cystoisosporidiosis the rate is 4.4-18.0%[1 2 8 19 21]. Cystoisoporidiosis is estimated to be the second most important gastrointestinal protozoan disease in AIDS patients, exceeded only by cryptosporidiosis[13].

In this study the frequency and seasonal profile of cryptosporidiosis and cystosporidiosis were evaluated among HIV/AIDS patients attended at the Hospital Escola da Universidade Federal do Triângulo Mineiro (UFTM). Possible associations based on examination of clinical and laboratory data of the patients were also investigated.

MATERIAL AND METHODS

Data were examined from previous studies on *Cryptosporidium* sp and *Cystoisospora belli* in faecal samples (diarrhoeic or not) from HIV/AIDS patients attended in the outpatients clinic or infirmary of the UFTM Department of Infectious and Parasitic Diseases between July 1993 - June 2003. This institution provides the regional reference centre for treatment of HIV/AIDS patients in Uberaba and the surrounding region. The examinations were performed in the parasitology laboratory, where the samples were submitted to sedimentation by the formalin-ether (Ritchie's) method. Faecal smears were made form the sediment, stained by a modified Ziehl-Neelsen method and examined under the 100X objective of the light microscope to look for *Cryptosporidium* sp and *Cystoisospora belli*. Samples were considered to be negative when no oocysts were detected after observation of at least 100 microscopic fields.

Clinical data, quantification of T CD4$^+$ lymphocytes, confirmatory examination of HIV infection and use of antiretroviral therapy (HAART) were obtained from the participants.

RESULTS

In all 638 faecal samples were examined from 359 patients (1.7 samples/patient). The ages of members of the study group varied from 2 - 72 with a mean of 33.3 (± 10.42). Males comprised 241 (67.2%) of the participants and females 118 (32.8%).

Coccidian were detected in 71 (19.7%) of the patients, with 31 (8.6%) presenting *Cryptosporidium* sp alone, 37 (10.3%) infected with only *Cystoisospora belli* and 3 (0.8%) harbouring both coccidian. The mean age of patients positive for coccidian was 32.42 (± 8.62), 48 (67.6%) being male and 23 (38.4%) female.

Diarrhoeic syndrome as a clinical indication of coccidian in the faeces was reported in 252 (70.2%) of the cases. Of these, 54 (21.4%) were positive for coccidian, 22 (8.7%) harbouring *Cryptosporidium* sp, 29 (15.5%) *Cystoisospora belli* and 3 (1.2%) both coccidian. In the group of patients without diarrhoea, prevalence of coccidian was 15.8% (17/107), with 9 (8.4%) positive for *Cryptosporidium* sp and 8 (7.4%) for *Cystoisospora belli* (Table 1).

T CD4$^+$ lymphocytes were quantified in 64 (17.8%) of all patients and a mean value of 194.9 ± 215.3 cells/mm^3. Of these patients, 50 (78%) presented diarrhoea and 14 (21.8%) coccidian in the faeces, with 7 (50%) positive for *Cryptosporidium* sp, 6 (43%), with *Cystoisospora belli* and 1 (7%) with both coccidian (Table 2).

Eleven of the 14 (78.6%) patients who presented coccidian in the faeces had diarrhoea and a T CD4+ lymphocyte count equal to or less than 200 cells/mm^3 (Table 2). The mean T CD4$^+$ lymphocyte count in patients with *Cryptosporidium* sp was 82.3 ± 65.8 cells/mm^3 and in those infected with *Cystoisospora belli* 98.0 ± 151.2 cells/mm^3.

Table 1 - Frequency of intestinal coccidian in faecal samples of HIV/AIDS patients presenting diarrhoea or not.

Intestinal coccidian	With diarrhoea		Without diarrhoea	
	nº	%	nº	%
	252	70.2	107	29.8
Cryptosporidium sp	22	8.7	9	8.4
Cystoisospora belli	29	15.5	8	7.8
Both coccidian	3	1.2	0	0.0
Coccidian	54	21.4	17	15.8

Table 2 - Cryptosporidiosis, cystoisosporidiosis and diarrhoea in HIV/AIDS patients and their relationship with T CD4+ lymphocyte counts.

T CD4+ counts	Patients with coccidian						Patients without coccidian		
	with diarrhoea			without diarrhoea			with diarrhoea	without diarrhoea	Total
	Cryptosporidium sp	*Cystoisospora belli*	*both*	*Cryptosporidium* sp	*Cystoisospora belli*	*both*			
≤50	2	4	0	0	0	0	9	4	19
51 - 200	4	1	0	1	0	0	8	7	21
201 - 400	0	1	1	0	0	0	9	0	11
401 - 600	0	0	0	0	0	0	8	1	9
601 - 800	0	0	0	0	0	0	1	1	2
> 800	0	0	0	0	0	0	2	0	2
Total	6	6	1	1	0	0	37	13	64

Only 8.5% (6/71) of the patients who presented coccidian in the faeces were receiving HAART.

The frequency distribution of coccidian per year of observation showed a higher occurrence of patients infected with *Cryptosporidium* sp during 1997 (23%) and with *Cystoisospora belli* in 2000 (18.8%) (Figure1). When seasonal distribution of the two coccidian in HIV/AIDS patients over a 10-year period was evaluated, seasonal distribution of *Cryptosporidium* sp was found to peak between December and February. A slight rise in *Cystoisospora belli* infections was seen from June - August (Figure 2).

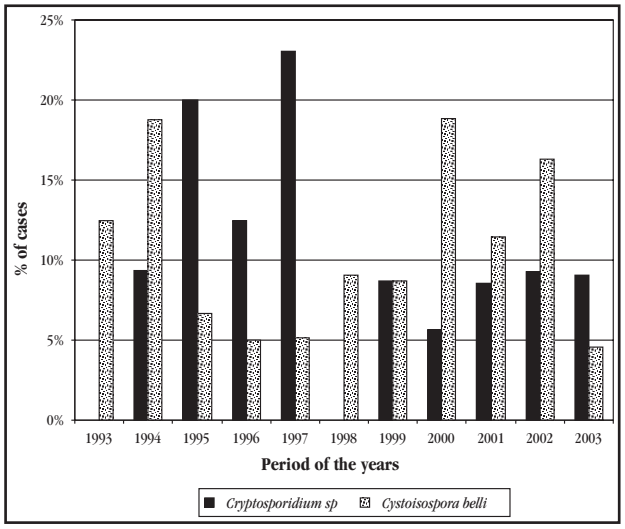

Figure 1 - Annual distribution of* Cryptosporidium *spp and Cystoi*sospora belli *in HIV/AIDS patients attended in at UFTM, Brazil, June 1993 - June 2003.

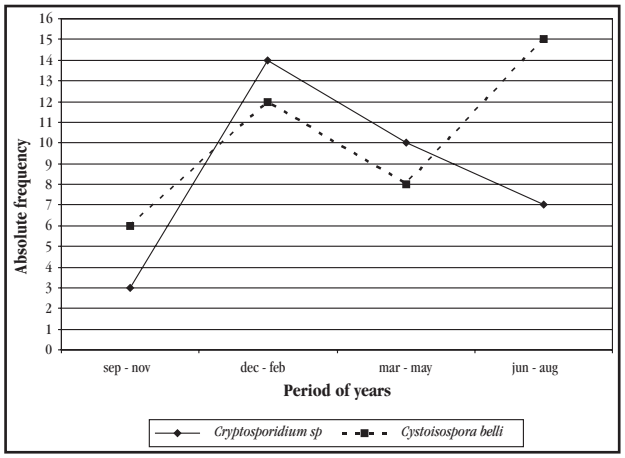

Figure 2 - Seasonal distribution of* Cryptosporidium *sp and* Cystoisospora belli *in HIV/AIDS patients attended at UFTM from January 1994 - December 2002.

DISCUSSION

Factors such as immunological status of the patient, use or not of highly active antiretroviral therapy (HAART), level of basic sanitation and climatic diversities of the different regions, as well as methodological differences between the various studies, have all been used to explain differences in prevalence values of these coccidioses in the literature.

The prevalence of *Cryptosporidium* sp in the Triângulo Mineiro (8.6%) only exceeded that reported in the Ribeirão Preto region (6.4%)[2]. It was lower than the values found for HIV/AIDS patients in the cities of Uberlândia (13%)[5], São Paulo (12.1%)[8], Santos (19.1%)[21] and Campinas (18%)[1]. In relation to *Cystoisospora belli*, the frequency (10.3%) was higher than that detected in São Paulo (5.7%)[8] or Ribeirão Preto (4.4%)[2], similar to rates in Rio de Janeiro (10.1%)[19] and Santos (9.9%)[21] and only lower than that in Campinas (18%)[1].

When occurrence of diarrhoeic syndrome was evaluated as an indicator of the presence of these coccidian within the study population, a higher positivity was observed in patients with diarrhoea (21.4%), than in non-diarrhoeic individuals (15.8%). Similar results were reported by Rigo & Franco (2000), who found higher rates of both cryptosporidiosis (18.3%) and cystoisosporidiosis (6.6%) in HIV patients with diarrhoea than in non-diarrhoeic individuals (1.6%). According to these authors, this data illustrates the risk of overestimating the frequency of *Cryptosporidium* sp and *Cystoisospora belli* infection rates when only patients with diarrhoea are included in the study population.

In immunocompromised patients a T CD4+ lymphocyte level above 200 cells/mm^3 is considered to be a limiting factor for opportunistic infections. Despite T CD4+ lymphocyte levels being quantified in only 17.8% of patients, the opportunistic character of intestinal coccidial infections could be perceived in 78.6% of the patients which presented coccidian in the faeces showing T CD4+ lymphocyte counts $\leq$ 200 cells/mm^3. This fact was closely related to the cases of cryptosporidiosis, corroborating results already reported in the literature[3]. This association was also detected in cases of cystoisosporiadosis, although the high standard deviation in the T CD4$^+$ lymphocyte counts observed in patients with *Cystoisospora belli*, demonstrated that this coccidiann suffers less from the host immune response than *Cryptosporidium*.

According to the literature, the numbers of opportunistic infections in HIV/AIDS patients[13] has diminished considerably since the introduction of HAART in 1996, including cases of cryptosporidiosis. However, in this study it was impossible to determine the influence of this therapy on infections with *Cryptosporidium* sp and *Cystoisospora belli*, since only 8.5% of patients which presented coccidian regularly received HAART.

In the Triângulo Mineiro region, as well as other parts of the world[16 17 23] a seasonal pattern was noted for *Cryptosporidium* sp, with a higher occurrence during the hottest months of the year (January-March). Although frequency of cystoisosporidiosis cases was somewhat higher in the months of June - August, it was not possible to show seasonal behaviour of infection by this coccidian in the studied area, and this remains to be established elsewhere[4].

The survey of *Cryptosporidium* sp and *Cystoisospora belli* in HIV/AIDS patients attended the UFTM, from June 1993 - June 2003, showed that these parasites occur at a similar frequency in the studied population and that are closely linked to the presence of diarrhoeic syndrome (particularly in cryptosporidiosis), with a drop in T CD4$^+$ lymphocyte counts. However, the influence of

T CD4+ lymphocytes in infection with *Cystoisospora belli* needs to be better evaluated due to the reduced number of samples screened in the present study and to the possible recurrence of cases after treatment. These factors may be related to the immune response of the patient or even the presence of extraintestinal cysts, which are less susceptible to treatment[10 15].

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REFERENCES

1. **Baraldi SR, Marques EGL, Dias RMDS. Occurrence de** *Cryptosporidium parvum* e *Isospora belli* na region de Campinas, SP. Revista Instituto Adolfo Lutz 58: 97-103, 1999.
2. Capuano DM, Okino MHT, Bettini MJCB. Frequency de *Cryptosporidium* sp e *Isospora belli* in patients soropositivos para o HIV na region de Ribeirão Preto, SP – Brazil Revista Instituto Adolfo Lutz 60: 11-15, 2001.
3. Carey CM, Lee H, Trevors JT. Biology, persistence and detection of *Cryptosporidium parvum* and *Cryptosporidium hominis* oocyst. Water Research 38: 818-862, 2004.
4. Cimerman S, Cimerman B. Isosporíase. Laes & Haes 104: 154-156, 1997.
5. Costa-Cruz JM, Ferreira MS, Rossin IR: Intestinal parasites in AIDS and +HIV patients in Uberlândia, Minas Gerais, Brazil. Memórias do Instituto Oswaldo Cruz 91: 685-686, 1996.
6. **De Carli GA. Parasitologia Clinical: Diagnóstico de Laboratory dos Coccidian e** Microsporídios Intestines. Cadernos Edipucrs. Série Farmácia: p.102-107, 2000.
7. **DeHovitz JA, Pape JW, Boncy M, Johnson Jr WD. Clinical manifestations and** therapy of *Isospora belli* with the adquired immunodeficiency syndrome. The New England Journal of Medicine 315: 87-90, 1986.
8. Dias RMDS, Pinto WP, Chieffi PP, Mangini ACS, Torres DMAGV, Del Bianco R, Ferrari L. Enteroparasitoses in patients acometidos pela syndrome de imunodeficiência adquirida (AIDS/SIDA). Revista Instituto Adolfo Lutz 48: 63-67, 1988.
9. Ferreira MS. Infections by protozoa in immunocompromised hosts. Memórias do Instituto Oswaldo Cruz 95: 159-162, 2000.
10. Frenkel JK, Silva MBO, Saldanha JC, Silva-Vergara ML, Corrêa D, Barata CH, Silva EL, Ramirez LE, Prata AR. Presence extra-intestinal de cistos unizóicos de *Isospora belli* in paciente com SIDA. Relato de caso. Revista da Sociedade Brasileira de Medicina Tropical 36: 409-412, 2003.
11. **Garlipp CR, Bottini PV, Teixeira ATLS. The relevance of laboratory diagnosis of** human cryptosporidiosis and other coccidian. Revista do Instituto de Medicina Tropical de São Paulo 37: 467-469, 1995.
12. **Guizelini E, Amato Neto V. Pesquisa de oocistos de** *Cryptosporidium* sp nas fezes diarrheic de aidéticos e de crianças e adultos imunocompetentes, in São Paulo. Revista do Hospital das Clínicas da Faculdade de Medicina de São Paulo 47: 150-152, 1992.
13. **Kosek M, Alcântara C, Lima AAM, Guerrant RL. Cryptosporidiosis: an update.** Lancet Infectious Diseases 1: 262-269, 2001.
14. Lamounier JA, Westin OF, Salles DSV. Infecção pelo Protozoário *Isospora belli:* Apresentação de Caso e Revisão Bibliográfica. Revista de Medicina Minas Gerais 2:113-115, 1992.
15. Lindsay DS, Dubey JP, Blagburn BL. Biology of *Isospora* spp. from humans, nonhumans primates, and domestic animals. Journal of Clinical Microbiology 10: 19-34, 1997.
16. Mangini ACS, Dias RMD, Grisi SJFE, Escobar AMU, Torres DMAGV, Zuba IPR, Quadros CMS, Chieffi PP. Parasitismo por *Cryptosporidium* sp. in crianças com diarrhoea aguda. Revista do Instituto de Medicina Tropical de São Paulo 34: 341-345, 1992.
17. **McLauchlin J, Amar C, Pedraza-Diaz S, Nichols GL. Molecular epidemiological** analysis of Cryptosporidium spp. in the United Kingdom: results of genotyping Cryptosporidium spp. in 1,705 fecal samples from humans and 105 fecal samples from livestock animals. Journal of Clinical Microbiology 38: 3984-3990, 2000.
18. Mirdha BR, Kabra SK, Samantray JC. Isosporiasis in children. Journal of the Indian Academy of Pediatrics 39: 941-944, 2002.
19. **Moura H, Fernandes O, Viola JPB, Silva SP, Passos RH, Lima DB. Enteric parasites** and HIV infection in AIDS patients in Rio de Janeiro, Brazil. Memórias do Instituto Oswaldo Cruz 84: 527-533, 1989.
20. **Rigo CR, Franco RMB. Comparação entre os métodos de Ziehl-Neelsen modified** e Acid-Fast-Trichrome para a pesquisa fecal de *Cryptosporidium parvum* e *Isospora belli*. Revista da Sociedade Brasileira de Medicina Tropical 35: 209-214, 2002.
21. Sauda FC, Zamarioli LA, Ebner Filho W, Mello LB. Prevalence of *Cryptosporidium* sp. and *Isospora belli* among AIDS patients attending Santos Reference Center for AIDS, São Paulo, Brazil. Journal Parasitology 79: 454-456, 1993.
22. **Souza LR, Rodrigues MA, Morceli J, Kemp R, Mendes RP. Cryptosporidiosis of the** biliary tract mimicking pancreatic cancer in an AIDS patient. Revista da Sociedade Brasileira de Medicina Tropical 37: 182-185, 2004.
23. Tzipori S, Ward H. Cryptosporidiosis: biology, pathogenesis and disease. Microbes and Infection 4: 1047-1058, 2002.
24. Whiteside ME, Barkin JS, May RG, Weiss SD, Fischl MA, MacLeod CL. Enteric coccidiosis among patients with the acquired immunodeficiency syndrome. American Journal Tropical Medicine and Hygiene 33: 1065-1072, 1984.