

CRYPTOSPORIDIOSIS AMONG PATIENTS WITH ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS) IN THE COUNTY OF SÃO PAULO, BRAZIL

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S U M M A R Y

Stool samples of 157 patients with AIDS, living in the county of São Paulo, were submitted to several techniques in the search for *Cryptosporidium* sp.. Among the various techniques tested for slide preparation (direct smear, spontaneous sedimentation method, and formol-ether concentration), the latter, formol-ether concentration, offered the best results, clearly outdoing all the others. Nineteen samples out of 157 prepared by this technique, after dyeing by the Kinyoun method or by carbol fuchsin dimethyl sulfoxide, were found to be positive for *Cryptosporidium* sp..

KEY WORDS: *Cryptosporidium* sp.; Cryptosporidiosis; AIDS.

I N T R O D U C T I O N

Cryptosporidium sp. was first described by TIZZER, in 1907, who observed this coccidium in the gastric mucosa of asymptomatic mice. SLAVIN, in 1955, associated it to severe disease, when noticed its presence in turkeys with acute diarrhea¹³. Since that time, several publications mention the finding of *Cryptosporidium* sp. in different animal species, with varied clinical pictures^{5, 14}.

Since 1976, when the first human case of cryptosporidiosis was described, no more than seven cases had been reported in the literature until 1982¹¹.

With the emergence of AIDS, *Cryptosporidium* sp. came to be frequently considered as

the etiologic agent of prolonged and difficult to treat diarrhea in immunocompromised patients¹¹. More recently, this coccidium has been incriminated as the causative agent of severe diarrhea, usually auto-limited, in immunocompetent individuals^{4, 10}, particularly among groups exposed to infected animals¹².

In Brazil, the presence of *Cryptosporidium* sp. oocysts has been observed in the stool of patients with diarrhea episodes, with or without concomitant immunosuppressant process^{2, 3, 8, 15}. Nevertheless, reports on the frequency of *Cryptosporidium* sp. isolation on the stools of individuals with AIDS are scarce and this was the main goal of the present study.

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MATERIALS AND METHODS

The presence of *Cryptosporidium* sp. oocysts in diarrheic stool samples was studied. Such sample came from 157 AIDS patients, referred to the Enteroparasitosis Section of Adolfo Lutz Institute, from June to April 1987.

Aiming the testing of different methods for concentration and dyeing of *Cryptosporidium* sp. oocysts, the stool samples were submitted to various examination techniques. Direct stool smears, spontaneous sedimentation method (Lutz method), and formol-ether concentration were thus used. Sample staining techniques employed were lugol staining, Giemsa dye, carbol fuchsin dimethyl sulfoxide, and Kinyoun method.

All such techniques followed precepts stated in the literature^{1, 6, 9}, exception made for the formol-ether concentration which was slightly modified. For this technique, stool fractions were homogenized in 4 ml of 10% formaldehyde solution, pH 7.0; 2 ml of ethyl ether were added to the resulting solution, after gauze filtering and transference to a centrifugation test tube. After sealing with a rubber stopper, the test tube was stirred and submitted to centrifugation at 1,500 rpm for 5 minutes. The supernatant was discarded, smears were made on glass slides from the sediment which, after being dried and methanol fixed, were stained.

RESULTS

Cryptosporidium sp. oocysts were found through the modified formol-ether concentration method in 19 (12.1%) samples collected from 157 AIDS patients and submitted to several examination techniques.

Both the sedimentation and direct smear methods proved unable to demonstrate the presence of *Cryptosporidium* sp. on samples where formol-ether concentration method succeeded in demonstrating oocysts of the coccidium as, only in one case out of the 19 positive, such oocysts were elicited through those techniques.

DISCUSSION

The study of the frequency of opportunistic infections in AIDS patients secured great rele-

vance due to the growing number of the disease in the recent years. Therefore, research on safe, reproducible, and easy to perform techniques for the etiologic diagnosis of the many infections often associated to the nosologic picture of AIDS attains marked importance, since diagnosis and treatment of such infections are the only forms, presently known, to extent and improve life quality of these patients. Similarly, studies which seek to determine occurrence rates of the several agents implicated on the etiology of secondary infections in AIDS patients are justified.

Cryptosporidiosis, particularly in its intestinal form, is one of the most frequent opportunistic infections affecting AIDS patients^{5, 14}. Nevertheless, data are scarce on the affliction of AIDS patients by *Cryptosporidium* sp. in our country. According to COURA³, in the only Brazilian research involving a large number of patients, conducted in the county of Rio de Janeiro, oocysts of this coccidium were found in approximately 17% of AIDS patients with prolonged diarrhea.

Findings of this study, at a first glance, point to a lesser *Cryptosporidium* infection frequency among AIDS patients living in the county of São Paulo, on account of showing oocysts in no more than 12.1% of the stool samples examined. Nevertheless, it must be emphasized that differently from patients examined in Rio de Janeiro, not all São Paulo patients were diarrheic. Work recently performed in Zaire⁷, on AIDS suspected individuals with diarrhea episodes revealed 8% *Cryptosporidium* sp. infection rate.

It is still valuable to underscore the importance of using the formol-ether concentration technique, slightly modified from the GARCIA & col.⁶ method, before preparing the fecal smear to be stained. Stool samples prepared under different techniques — direct smear or spontaneous sedimentation — yielded results always less expressive than those of formol-ether concentration method, denoting this to be the method of choice for isolation of *Cryptosporidium* sp. oocysts.

RESUMO

Criptosporidiose em pacientes acometidos pela síndrome de imunodeficiência adquirida (AIDS), no município de São Paulo, Brasil

Amostras de fezes de 157 pacientes acometidos pela síndrome de imunodeficiência adquirida, residentes no município de São Paulo, foram submetidas a diversas técnicas para pesquisa de *Cryptosporidium* sp.. Entre as diversas técnicas testadas para o preparo das lâminas (esfregação direto, método de sedimentação espontânea e concentração pelo formol-éter) a concentração pelo formol-éter foi a que forneceu melhores resultados, superando nitidamente as demais. Nas 157 amostras preparadas por esta técnica, após coloração pelo método de Kinyoun ou pela fucsina carbólica dimetilsulfóxido, encontraram-se 19 (12,1%) positivas para oocistos de *Cryptosporidium* sp..

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Recebido para publicação em 22/3/1988.