

THE RELEVANCE OF LABORATORY DIAGNOSIS OF HUMAN CRYPTOSPORIDIOSIS AND OTHER COCCIDIA

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SUMMARY

Human infection by *Cryptosporidium* spp and other coccidia are due to opportunist non-host specific microorganisms. In HIV seropositive patients, the gastrointestinal symptoms accompanying such infections may be serious and prolonged and may include nausea, low-grade fever, abdominal cramps, anorexia and watery diarrhoea. We studied 188 stool samples from 111 patients (84 men and 27 women) with diarrhoea. A modified Ziehl-Nielsen technique for the detection of *Cryptosporidium* spp and *Isospora belli* was employed. The mean age of the patients was 31 years. *Cryptosporidium* spp was seen in 18% (n=20) of the patients, 90% (n=18) of whom were HIV seropositive. *Isospora belli* was recorded only from HIV seropositive patients (5.4% of all the patients studied and 6.5% of those who were HIV seropositive). These data confirm the good results obtained with this technique for the identification of *Cryptosporidium* spp and other coccidia and also reaffirm the clinical importance of correctly diagnosing the cause of diarrhoea, particularly in HIV seropositive patients.

KEYWORDS: *Cryptosporidium* spp; *Isospora belli*; Diarrhoea; AIDS; Staining.

INTRODUCTION

Cryptosporidium spp and *Isospora belli* have recently been recognized as enteric pathogens. In the last 10 years, *Cryptosporidium* spp has been recognized as one of the most common causes of acute and self-limited gastroenteritis induced by a protozoan parasite in immunocompetent people. In the immunocompromised, cryptosporidiosis is a common and life-threatening condition causing profuse diarrhoea accompanied by severe dehydration, malabsorption and wasting^{17,18}. *Isospora belli* is an opportunistic protozoan pathogen in patients with AIDS^{12,19}. The resulting infection is characterized by chronic watery diarrhoea and weight loss and is indistinguishable from that caused by related coccidia genus *Cryptosporidium*⁵.

The purpose of this investigation was to establish the frequency of cryptosporidiosis and isosporidiosis in

patients with chronic diarrhoea. The clinical relevance of this diagnosis is discussed.

MATERIAL AND METHODS

From July 1991 to May 1993, we examined 188 stool samples from 111 patients with diarrhoea (84 males and 27 females, with a mean age of 31 years (range: 2 months to 76 years)) treated at the University Hospital of the State University of Campinas (UNICAMP). Ninety-two of the 111 patients (83%) were HIV seropositive.

All fecal samples were studied using a modified Ziehl-Nielsen procedure for the detection of *Cryptosporidium* spp and *Isospora belli*^{11,13}. The diagnosis of enteric cryptosporidiosis and isosporidiosis was based on the identification of the acid-fast oocyst form of these protozoa which differs from morphologically similar but

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non-acid fast yeasts *Cryptosporidium* spp and *Isospora belli* both stain bright red against a green background. *Isospora belli* oocysts are distinguished from other coccidian oocysts by the size, shape and numbers of sporocysts. Thus, this method can identify both coccidia at the same time.

RESULTS

Cryptosporidium spp was identified in 20 patients (18% of the total) and in 29 stool samples (15.4% of the total). Eighteen of these 20 patients (90%) were HIV seropositive. The frequency of cryptosporidiosis among all the HIV seropositive patients (m=92), was 19.6%.

Isospora belli was observed only in HIV seropositive patients and occurred in 5.4% of all the patients and in 5.8% of the samples. Among HIV seropositive patients, the frequency of *I. belli* was 6.5%. None of these patients was positive for *Cryptosporidium* spp.

DISCUSSION

Diarrhoea is a prominent feature of AIDS and is present in 50-60% of the patients in the United States and in more than 95% of patients from Haiti and Africa^{5,14}.

Isospora belli and *Cryptosporidium* spp are known causes of enteric disease in man and both are capable of causing chronic disease and malabsorption in individuals with defective immunity^{2,10}. Furthermore, these microorganisms are the most commonly identified causes of chronic diarrhoea in AIDS patients¹⁸.

In the present study, *Isospora belli* occurred in six out of 92 patients (6.5%) with AIDS. This frequency exceeds that observed in AIDS patients studied by the Centers for Disease Control in the USA (<0.2%) (SELIK, R.: personal communication) and, in Brazil, by WUHIH et al.²⁰ (1.3%) and by SAUDA et al.¹⁶ (3%), but is lower than that observed in Haiti (15%) over a one year period⁵.

Cryptosporidial infection of the immunocompetent host has been described in over 40 countries. However, the true prevalence of such infections is not known⁸. Prevalence rates obtained from large scale surveys of selected populations range from 0.6 to 20% for developed countries and from 4 to 20% for developing countries^{7,9,18}. In the USA, 3-4% of AIDS patients have cryptosporidiosis¹² while in Haiti and Africa, 50% of such individuals suffer from this disease^{5,14}. In recent studies, the parasite in question was detected in 16% of patients with AIDS and diarrhoea¹¹. In Brazil, some studies have been carried out and cryptosporidial infec-

tion have been detected in 21.4%²⁰, 19.1%¹⁶, 14.3%¹⁵ and 12.1%⁶ of HIV-infected patients with diarrhoea or gastrointestinal manifestations. Our work shows that the frequency of *Cryptosporidium* spp in Campinas, Southeast of Brazil, is around 20% which is three times higher than for isosporidiosis.

Cryptosporidiosis is a cosmopolitan disease widespread in animals⁴ and only since the early 1980's has it been recognized as a significant pathogen for humans. The detection of *Cryptosporidium* spp in AIDS patients³ heightened physicians' awareness of its pathogenic potential in both immunocompromised and immunocompetent individuals. Cryptosporidiosis is the only opportunistic infection of persons with AIDS for which there is no known therapy and most attempts at treating it have failed. Thus, in the absence of effective anticryptosporidial therapy, the prevention of infection is of great importance.

Isospora belli is most commonly found in tropical and subtropical regions, but its prevalence is unknown¹⁸ even though it has been recognized as a human pathogen since 1915. Unlike cryptosporidiosis, treatment with oral trimethopim-sulfamethoxazole for one week is effective, despite the high frequency of recurrence.

Our data confirm the good results obtained with the acid-fast staining method for the recovery and identification of *Cryptosporidium* spp and *Isospora belli*. They also reaffirm the clinical importance of a correct diagnosis for chronic diarrhoea, particularly in HIV seropositive patients.

RESUMO

Importância do diagnóstico laboratorial da criptosporidiose humana e outras coccidioses.

Infecção humana por *Cryptosporidium* spp e outros coccideos deve-se a microrganismos oportunistas hospedeiros não-específicos e, em pacientes HIV positivos, os sintomas gastro-intestinais podem ser sérios e prolongados incluindo náuseas, febre baixa, dores abdominais, anorexia e diarreia aquosa. Estudamos 188 amostras de fezes provenientes de 111 pacientes (84 homens e 27 mulheres, com idade média de 31 anos) com diarreia, atendidos no Hospital das Clínicas da Universidade Estadual de Campinas. Para detecção de *Cryptosporidium* spp e *Isospora belli* empregou-se a técnica de Ziehl-Nielsen modificada. Oitenta e três por cento dos pacientes (n=92) eram HIV positivos. *Cryptosporidium* spp foi observado em 18% de todos os pacientes, sendo que destes, noventa por cento eram HIV positivos. *Isospora belli* foi evidenciada somente em pacientes HIV positivos, com uma fre-

quência de 5.4% de todos os pacientes e de 6.5% considerando-se apenas os pacientes HIV positivos. Este estudo confirma os bons resultados obtidos com a técnica empregada para identificação de *Cryptosporidium* spp e outros coccídeos, além de ressaltar a importância da investigação etiológica apropriada em casos de diarreia prolongada, especialmente em pacientes imunossuprimidos.

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