Intestinal and Extraintestinal Cryptosporidiosis in AIDS Patients

R. López-Vélez[1]*, R. Tarazona[3], A. Garcia Camacho[1], E. Gomez-Mampaso[1], A. Guerrero[1], V. Moreira[2], R. Villanueva[3]

In a prospective study in AIDS patients with chronic diarrhea, the overall prevalence of intestinal cryptosporidiosis was 15.6 % (43/275). The prevalence was higher in homosexual patients (33.3 %) than in intravenous drug abusers (10.6 %) (p < 0.001). Extraintestinal infection was present in 30 % (13/43) of the patients with known intestinal cryptosporidiosis. Eight of the 13 (61.5 %) patients with extraintestinal cryptosporidiosis had Cryptosporidium in the bile and 7 of 13 (16.28 %) had it in the sputum. Of the seven patients with Cryptosporidium in the sputum, four had respiratory symptoms and an abnormal chest radiograph, although another pulmonary pathogen was isolated simultaneously. Two other patients from whom Cryptosporidium was the sole respiratory pathogen isolated had no respiratory symptoms and normal chest radiographs. The seventh patient had pulmonary symptoms, interstitial infiltrate on chest radiograph and excessive activity on a pulmonary Gallium scan; Cryptosporidium was the only organism detected in induced sputum and bronchoalveolar lavage specimens. The mean CD4+ lymphocyte count in patients with extraintestinal cryptosporidiosis was 55 cells/mm^3.

Cryptosporidium is a gastrointestinal coccidian parasite that produces severe diarrhea in immunocompromised individuals, mainly in those suffering from AIDS (1). It has been estimated that of patients with AIDS and diarrhea, 10 to 30 % in the developed countries and 30 to 50 % in the developing world are infected with Cryptosporidium spp. (2, 3). Cryptosporidium has been implicated in the etiology of acalculous cholecystitis, sclerosing cholangitis (4) and pancreatitis (5) in HIV-infected patients. The diagnosis of cryptosporidiosis is made from biopsy of the biliary tract or biliary aspiration. Oocysts have also been found in respiratory tract samples (6), but their relation to lung disease is still under debate, since in most cases they are associated with other respiratory pathogens.

The purpose of our study was to determine the prevalence of intestinal cryptosporidiosis in outpatients with AIDS and chronic diarrhea in Madrid and to establish the presence and charac-

teristics of biliary and pulmonary cryptosporidiosis.

Patients and Methods

A total of 275 AIDS patients with chronic diarrhea were studied between 1991 and 1992 at the Ramon y Cajal Hospital, one of the referral centers for AIDS patients in Madrid, Spain. Diagnosis of HIV infection was established by EIA and Western blotting techniques. The study design included a prospective case description with case controls. Patients for the study were selected if clinical features suggested gastrointestinal disease. Chronic diarrhea was defined as three or more loose stools per day for three or more weeks. Weight loss was defined as a loss of more than 10 % of initial body weight. Fever was defined as a body temperature over 38°C for at least three days. Clinical and epidemiological aspects of gastrointestinal infection were evaluated with the aid of a questionnaire.

Thirteen patients with clinical features suggestive of biliary disease presented right upper quadrant pain and abnormal liver function tests (alkaline phosphatase, serum aspartate, alanine aminotransferase and bilirubin). Abdominal ultrasound, computed tomography (CT) and endoscopic retrograde cholangiopancreatography (ERCP) were performed. The ERCP technique allowed us to see the structures of the ductal system, collect the bile, obtain

[1] Department of Clinical Microbiology & Infectious Diseases and [2] Department of Gastroenterology, Hospital Ramon y Cajal, Apartado 31057, 28034 Madrid, Spain.
[3] Preventive Medicine & Public Health, Facultad de Medicina, Universidad Complutense, Madrid, Spain.

endoscopic biopsy samples, and perform endoscopic papillotomy to provide symptomatic relief. All patients with intestinal cryptosporidiosis were studied for pulmonary involvement: symptomatology was recorded, three sputum samples were obtained and chest radiographs were taken to check for the presence of morphological lesions. Some patients were subjected to sputum induction, bronchoscopy, bronchoalveolar lavage (BAL) and transbronchial biopsy. Routine tests for other pulmonary pathogens (e.g. bacteria, mycobacteria, fungi, cytomegalovirus, Pneumocystis carinii) were also performed.

Detection of Cryptosporidium Oocysts. Three fecal samples were taken every other day from each patient. Stool specimens were concentrated according to the sucrose flotation method, and fecal smears were prepared with concentrated material (7). Three different staining techniques were used: a modified Kinyoun, phenol auramine, and an indirect immunofluorescent method using monoclonal antibodies (Merifluor-Cryptosporidium; Meridian Diagnostics, USA). Bile and respiratory samples were examined for Cryptosporidium spp. after staining as described above.

Statistical Analysis. Data was analyzed using a biostatistics program (Sigma, Horus hardware, Spain). Normally distributed continuous variables were compared using the Student's t-test, and discrete variables were compared using either the chi-square test or Fisher's exact test. Nonparametric statistical tests (Wilcoxon and Mann-Whitney tests) were performed for non-normally distributed variables (limited data and SD greater than mean value).

Results

The sex, age, risk group and symptoms of the patients with and without cryptosporidiosis are shown in Table 1. Eighty-nine percent of the 275 patients were males. The mean age of the patients was 33 ± 9 years; 27.3 % were homosexual, 61.4 %

intravenous drug abusers and 11.3 % sexual partners of an AIDS patient.

Intestinal cryptosporidiosis was diagnosed in 43 (15.6 %) of the 275 patients. Most of the individuals (58 %) infected with Cryptosporidium spp. were homosexual (p < 0.001). The prevalence of cryptosporidial infection was approximately 33 % among homosexual patients with chronic diarrhea, compared with about 11 % among intravenous drug abusers (p < 0.001).

Abdominal pain was more frequent in patients with cryptosporidial enteritis (p = 0.01), while fever was more frequent in the group not infected with Cryptosporidium (p < 0.001). No statistical differences in other clinical features were observed among Cryptosporidium infected and noninfected patients.

Extraintestinal cryptosporidiosis was detected in 13 of 43 (30.2 %) patients with known intestinal cryptosporidial infection (Table 2). Eight of 13 patients with acalculous cholecystitis (thickening of the gallbladder wall on CT) or sclerosing cholangitis (duct dilatation and stenosis of the bile ducts on ERCP) had biliary cryptosporidiosis.

Three patients had pulmonary and biliary cryptosporidiosis concomitantly. Cryptosporidium oocysts were detected in the respiratory secretions of seven patients with intestinal cryptosporidiosis. Four had symptoms of respiratory infection and an abnormal chest radiograph, but another possible pulmonary pathogen was isolated: Mycobacterium tuberculosis in two cases

Table 1: Age, risk group, symptoms and CD4+ lymphocyte count in 275 AIDS patients with chronic diarrhea with and without cryptosporidial infection.

	Patients with cryptosporidiosis (n = 43)	Patients without cryptosporidiosis (n = 232)	P value
Age (years)	38 ± 14	32 ± 7	NS
Risk group			
Homosexual	58.1 %	21.5 %	< 0.001
Intravenous drug abuser	41.9 %	65.1 %	
AIDS partner	0.0 %	13.4 %	
Symptoms			
Diarrhea	72.1 %	73.0 %	NS
Fever (> 38°C)	41.86 %	70.3 %	< 0.001
Weight loss	58.14 %	43.2 %	NS
Abdominal pain	58.14 %	32.4 %	0.01
Vomiting	0.0 %	3.0 %	NS
CD4+ count	81.4 ± 79.6	123.4 ± 121.3	< 0.01

NS: non-significant.

Table 2: Clinical data on extraintestinal cryptosporidiosis in AIDS patients with intestinal infection by *Cryptosporidium* spp.

No. of patients	13/43 (30.2 %)
Site of cryptosporidiosis[a]	
Pulmonary tract	7
Biliary tract	8
Mean CD4+ count	55/mm^3
Mortality[b]	9/11 (82 %)

[a] Three cases with concomitant isolation of *Cryptosporidium* spp. from the pulmonary and biliary tracts.
[b] Two patients did not attend follow-up. Mortality evaluated 3 months after diagnosis.

and *Mycobacterium avium* in the other two. Two patients did not present any symptoms: the chest X-ray was normal and no pathogen other than *Cryptosporidium* was found. One patient had pulmonary symptoms, interstitial infiltrates on the chest X-ray and excessive activity on the pulmonary gallium scan. *Cryptosporidium* spp. were the only organisms isolated from induced sputum and BAL from this patient.

Discussion

The results of this study show that cryptosporidiosis has a prevalence of 15.6 % among outpatients with AIDS and chronic diarrhea in Madrid. This percentage is similar to the rate in some European countries, where the prevalence of cryptosporidiosis ranges between 10 and 30 % (2). We have found that infection with *Cryptosporidium* spp. is more frequent in homosexual patients (33.3 %) than in intravenous drug abusers (11.2 %). The differences were statistically significant (p < 0.001), suggesting that homosexuality is a risk for cryptosporidial infection. Although several authors have proposed cryptosporidiosis as a sexually transmitted disease (2, 8, 9), factors such as socioeconomic status, other behaviour and dietary habits should be investigated before conclusions are made.

Correlation between the number of CD4+ cells and the presence of cryptosporidial infection has been reported: patients with a CD4+ count of < 180/mm^3 had persistent infection, while patients with counts of > 300/mm^3 had a self-limited infection (10). Our results show that the number of CD4+ cells is higher in AIDS patients with diarrhea and without intestinal cryptosporidiosis than in those infected by *Cryptosporidium* spp., particularly in patients with ex-

traintestinal cryptosporidiosis (CD4+ count of 55/mm^3). Moreover, mortality was higher in patients with extraintestinal cryptosporidiosis (probably because of severe immunosuppression) than in those with intestinal cryptosporidiosis only: 9 of 11 of patients with extraintestinal cryptosporidiosis died within three months after the diagnosis. The presence of *Cryptosporidium* spp. outside the gut suggests several possible ways of dissemination: direct local invasion from the gastrointestinal tract to contiguous organs, surface contamination due to shedding of large numbers of organisms, and hematogenus transmission.

Cryptosporidium has been involved in the production of acalculous cholecystitis and sclerosing cholangitis. Biliary tract involvement has been documented in up to 10 % of AIDS patients with intestinal cryptosporidiosis (11) and in almost 33 % of AIDS patients with cryptosporidial enteritis and symptoms of hepatobiliary disease (12, 13). A biliary reservoir of *Cryptosporidium* spp. may contribute to the chronicity of the infection and the inability to eradicate the organism (14). It is difficult to ascertain the prevalence of sclerosing cholangitis in our group of patients. Not all individuals had an ERCP and only those with intestinal cryptosporidiosis and hepatobiliary symptoms were studied for biliary infection. This could explain the very high prevalence (61.5 %, 8/13) of *Cryptosporidium* spp. in the bile (by ERCP technique) found in this study.

Occasionally, *Cryptosporidium* spp. have been found in the respiratory tract, colonizing the trachea (15, 16), the lungs (5, 17–27) and the nasal mucosa (25, 28). Most cases occur in AIDS patients (5, 17, 19, 21, 22, 26), although several cases in patients with hypogammaglobulinemia, severe combined immune deficiencies or hematological malignancies have been reported (18, 20, 22, 24, 29, 30). Likewise, a case of cryptosporidial laryngotracheitis in an immunocompetent infant has been reported (15) and *Cryptosporidium* has been found in sputum from an elderly man (31). The significance of pulmonary cryptosporidiosis in humans remains unclear; however, there is no doubt that life-threatening pulmonary cryptosporidiosis occurs in avian species. In most cases of human respiratory infection, *Cryptosporidium* spp. have been isolated from lung biopsy concomitantly with other possible pathogens such as cytomegalovirus, *Pneumocystis carinii* and *Mycobacterium avium* (5, 17, 18, 21, 22, 24–26). The presence of these other pathogens makes it more difficult to establish the role of *Cryptosporidium*

as a true pulmonary pathogen. Nevertheless, several cases in which it was the sole pathogen isolated suggest that *Cryptosporidium* may act as a true pathogen, infecting the respiratory tract (15, 20, 26).

In this study *Cryptosporidium* spp. were isolated from sputum in 7 of 43 (16.3 %) patients with intestinal cryptosporidiosis. Two of these patients had no pulmonary symptoms and a normal chest radiograph, indicating that in these cases *Cryptosporidium* spp. were just colonizing organisms. Four patients, all with pulmonary symptoms and abnormal chest radiograph, had another concomitant pulmonary microorganism: two of these patients were colonized by *Mycobacterium avium* and the other two by *Mycobacterium tuberculosis*. Consequently, we are unable to establish the pathogenic role of *Cryptosporidium*. The last of the seven patients presented with pulmonary symptoms (cough, dyspnea), abnormal chest radiograph (interstitial infiltrates) and excessive activity in a pulmonary gallium scan. *Cryptosporidium* spp. were the only microorganisms isolated from an induced sputum and bronchoalveolar lavage, suggesting that *Cryptosporidium* spp. could act as true pulmonary pathogens. However, further studies will be necessary to determine the potential pathogenic role of *Cryptosporidium* spp. in the respiratory tree.

We conclude that cryptosporidiosis is a frequent disease in Madrid among AIDS outpatients with chronic diarrhea, being most prevalent among homosexual men. Extraintestinal cryptosporidiosis, detected in one-third of the patients in the present study, is associated with a poor prognosis. The role of *Cryptosporidium* as a true pathogen in the respiratory tract, however, is unclear.

Acknowledgement

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