

Cryptosporidiosis in Colombian children with HIV/AIDS infection

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SUMMARY

Introduction: Enteric cryptosporidiosis in children with HIV/AIDS is an important cause of morbidity and mortality.

Objective: To determine the prevalence of *Cryptosporidium* spp. in feces, via the Ziehl Neelsen modified technique, among Colombian children with HIV/AIDS and to determine possible associations.

Methodology: Prevalence study in 131 children with HIV/AIDS at the Hospital Universitario del Valle in Cali, Colombia. We evaluated clinical records, laboratory results, environmental factors, and socio-demographic variables. Statistical analysis included the estimation of prevalence of infection in children and the corresponding 95% confidence interval; estimation of other descriptive conditions of interest and the association analyses by multiple logistic regression.

Results: In this group of children with a mean age of 57 months, we found a prevalence of infection of 29%, more frequently among male children and among those with vertical HIV transmission. The infection was also associated with abdominal pain, having pets inside the house and C stage for HIV, with $\geq 100,000$ copies/ml of viral load and CD4 percentage $\geq 25\%$. Association analysis showed a larger risk of *Cryptosporidium* infection with older age, and among patients not living in Cali, with more severe status of HIV disease, previous hospitalizations, and dried oral mucosa. Factors found finally associated were older age, chronic undernutrition, living in day-care institutions and having previous hospitalizations.

Conclusion: Almost a third of these children patients had *Cryptosporidium* infection, and it was found associated with age, previous hospitalizations, chronic undernutrition and living in day-care institutions.

Keywords: *Cryptosporidium* spp; Prevalence; HIV/AIDS infection; Children.

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Cryptosporidiosis en niños colombianos con infección por VIH/SIDA

RESUMEN

Introducción: La criptosporidiosis entérica en niños infectados por VIH es una causa importante de enfermedad y muerte.

Objetivo: Determinar la prevalencia de *Cryptosporidium* spp. en heces por medio de la técnica de Ziehl Neelsen modificada, en niños colombianos con infección por VIH/SIDA y establecer posibles asociaciones.

Metodología: Estudio de prevalencia en 131 niños con infección por VIH/SIDA. Fueron consideradas variables clínicas, paraclínicas, ambientales y sociodemográficas. El análisis estadístico incluyó estimación de la prevalencia de infección en los niños y su correspondiente intervalo de confianza al 95%, la estimación de otras medidas descriptivas de interés y el análisis de asociación por regresión logística múltiple.

Resultados: En esta población de niños con una edad promedio de 57 meses se encontró una prevalencia de 29% de infección, predominio del sexo masculino y el modo de transmisión vertical en VIH. La infección se asoció además con dolor abdominal, tenencia de animales en el intradomicilio y a estadio C para VIH, con ≥ 100.000 copias/ml de carga viral y porcentaje de CD4 $\geq 25\%$. En el análisis de asociación se encontró mayor oportunidad de infección por *Cryptosporidium* a mayor edad, en los pacientes de fuera de Cali, con mayor severidad del estadio para VIH, con hospitalizaciones previas, y con mucosa oral seca. Los factores finalmente asociados fueron la mayor edad, la DNT crónica, la convivencia en guarderías

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y las hospitalizaciones previas.

Conclusión: Casi una tercera parte de los pacientes presentaron infección y esta se encontró asociada con la edad del niño, con hospitalizaciones previas, con convivencia en guarderías y DNT crónica.

Palabras clave: *Cryptosporidium* spp; Prevalencia; Infección por VIH/SIDA; Niños.

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Cryptosporidium has been reported for causing disease in humans since 1976. It is an important cause of diarrhea in children with and without HIV infection in developing countries, where the infection at early ages has been reportedly associated to stunted physical and cognitive growth¹. In our experience, prevalence of *Cryptosporidium* among immunocompetent children varies between 4% and 32.3%, while among immunocompromised children it reaches values between 42 and 51.4%^{2,3}. In particular, a study in Medellín, Colombia, among 103 patients, 2-74 years of age, found 39.8% prevalence for *Cryptosporidium* spp. This was the first study in Colombia, which achieved the identification of *C. hominis*, *C. parvum*, and *C. felis*⁴.

Cryptosporidiosis is frequently associated to persistent diarrhea disease (PDD) and undernutrition (UNT) in immunologically compromised individuals, like those with HIV/AIDS infection, in whom therapy has not been effective⁵. However, it is also a serious frequent cause of acute diarrhea disease (ADD) at all ages of immunologically healthy individuals⁶. Until the introduction of antiretroviral therapy (ART) between 1995 and 1996, it was a serious opportunistic infection, which complicated HIV/AIDS infection. In immunocompetent children, enteric cryptosporidiosis by *C. parvum* induces light and self-limited gastroenteritis, while in HIV-infected children it is severe, with dehydration (DHT), and is associated to massive loss of liquids⁶. Risk of infection is closely related to immune disorders, mainly low count of CD4+ cells, and the severity of the symptoms is associated to the number of oocysts⁷. Although the therapeutic treatment is yet unclear, the use of macrolides and thiazolidines is an alternative. Among children with HIV/AIDS infection, the prevalence of *Cryptosporidium* spp. varies between 8% in the United States⁷ and 73.6% in Uganda⁴, with a frequency of <1.0 per 100 individuals per year^{8,9}. In our

experience, the prevalence of *Cryptosporidium* spp. in feces via the modified Zielh Neelsen (ZN) technique, in immunocompetent children from the municipality of Piedecuesta in Santander was of 32.3% and only 4.0% in the city of Cali in Valle del Cauca. Among immunocompromised children with cancer at the Hospital Universitario de Santander in the city of Bucaramanga, in northeastern Colombia, the prevalence was 42%, while among children with HIV/AIDS infection Hospital Universitario del Valle in the city of Cali, in the southwest region of the country, it was 51.4% (95% confidence interval CI: 39.9-62.9)^{2,3}.

Little is known, however, about local risk factors associated to this common cause of morbidity and mortality among patients with immune disorders. Our general objective was to determine the prevalence of *Cryptosporidium* spp. in feces, via the modified ZN technique, among a group of Colombian children with HIV/AIDS infection under highly active antiretroviral therapy (HAART), and to evaluate possible association with socio-demographic, clinical, paraclinical, and environmental factors.

METHODOLOGY

A prevalence study was conducted among children below 15 years of age diagnosed with HIV/AIDS infection and attending the HIV/AIDS Pediatric Clinic at the «Hospital Universitario del Valle Evaristo García» (HUV) in Cali, Colombia between February and April, 2009. We determined the presence and risk factors for *Cryptosporidium* spp. infection without considering seasonal climate changes, although they are well known to influence presence of *Cryptosporidium* spp., especially after rainy seasons¹⁰ and in tropical regions, like in our study area¹³. The target population was children from the Colombian southwest and under treatment or observation. The study included both male and female children, <15 years of age, diagnosed with HIV/AIDS infection through positive ELISA test, positive confirming Western blot test, and positive viral load (these tests taken at least 15 days before or after taking feces to identify *Cryptosporidium* spp.). Also, to be included, children must not have taken antiparasitic medications like nitazoxanide, corticosteroids or antibiotics like azithromycin or paromomycin, 15 days prior to the determination of the *Cryptosporidium*

spp. in feces. We excluded children with other confirmed immunodeficiency and patients hospitalized because of diseases concomitant to HIV/AIDS infection, which place their lives at risk, like septic shock, severe hydro-electrolyte or acid-alkaline imbalances. Assuming an expected prevalence of 51%, with an estimation error of $\pm 5\%$, and an α error of 0.05, a total population from the HIV/AIDS Pediatric Clinic at HUV of 161 children with HIV/AIDS infection by December 31, 2008, and a 15% adjustment of losses of samples from the children, or of no response, the sample size was of 131 children.

The main exposures of interest were the viral load and the percentage of CD4 cells; while the evaluated socio-demographic variables were age, gender, background, HIV mode of transmission; and the clinical variables included the nutritional status (weight, height, global undernutrition, chronic undernutrition, acute undernutrition, overweight), gastrointestinal signs and symptoms (acute diarrhea, chronic diarrhea, vomit, abdominal pain, abdominal distension, enophthalmos, acidotic breathing, distal perfusion, weeping without tears, abdominal fold, dry oral mucosa), general symptoms (fever), stage of HIV/AIDS infection, hospitalizations; the paraclinical variables: viral load for HIV, percentage of CD4, and environmental variables: household pets, consumption of potable water, excreta disposal, overcrowding, stay in day-care center. The result variable was the identification of *Cryptosporidium* spp. in at least one of the three slides of the fresh feces samples collected, and processed via the modified ZN technique¹⁴ one to three days after interviewing each child in the study.

This work was approved by the Ethics Committee at Universidad del Valle and at the HUV in Cali, Colombia. The children's parents or legal guardians who accepted to participate signed a written informed consent and answered a structured questionnaire applied by the main researcher. After the survey, and during three consecutive days, a fresh collection of fecal matter was obtained labeled with non-sequential random numbers and handed over to one of the two microbiology bacteriologists who divided the sample on three slides. The initial diagnosis of enteric cryptosporidiosis by *Cryptosporidium* spp. was conducted on the fresh collection sample of fecal matter from which three extends were obtained through fixation and concentration, via the modified ZN technique by the two

microbiology bacteriologists. The plates were revised individually and separately by each of the two microbiology bacteriologists; and in case of discrepant results these were again checked to give a joint final concerted verdict.

The database was in EpiInfo 6.04 and statistical analysis was performed with Stata 10. To evaluate the possibility of typing errors, 10% of the records were checked and compared with the original forms and corrected accordingly. To describe the distribution of variables, exploratory analysis was performed of all the variables. For the continuous variables, we obtained plots (histograms, box and whisker plots, symmetry plots), which permitted describing their distribution, as well as the central tendency and dispersion measurements. An analysis of normality was done and, if needed, we developed data transformations for their normalization. For categorical variables, we obtained frequencies and proportions.

The HIV viral load variable was defined according to four categories (<400 copies/ml; 401 and 29,999 copies/ml; 30,000 and 99,999 copies/ml; and $\geq 100,000$ copies/ml), and the CD4 percentage was divided into three categories (<15%, 15 and 24%, and $\geq 25\%$).

The nutritional status of the children of the study was established by using reference tables from the United States National Center for Health and Statistics and the classifications by Gómez and Waterlow for global undernutrition (UNT) according to weight for age (1st degree or light = 10-24% deficit; 2nd degree or moderate = 25-39% deficit; and 3rd degree or severe = >40% deficit); chronic UNT according to height for age (1st degree or light = 5-9%; 2nd degree or moderate = 10-14%; and 3rd degree or severe $\geq 15\%$ deficit); and acute UNT according to weight for height (1st degree or light = 10-19%, 2nd degree or moderate = 20-29%, and 3rd degree or severe $\geq 30\%$ deficit). Overweight was defined as a >10% weight for height excess.

We estimated the proportion of children with infection with its corresponding 95% confidence interval (CI); and the descriptive measurements with their corresponding standard deviations and ranges. To evaluate infection risk factors, a univariate analysis was initially performed between each of the exposure variables of interest and the effect variable. We also explored the possible association among the exposure variables of greater interest (viral load and percentage

of CD4) and other co-variables and between infection by *Cryptosporidium* and the rest of the co-variables to evaluate confounding. Odds ratios (OR) were estimated with 95% CI, and statistical significance was evaluated with Fisher's exact test. For confounding and interaction evaluation, we performed multiple logistic regression analyses. To guide in obtaining the final multiple logistic regression model, we used a backward stepwise methodology with a probability of retirement of 0.10.

RESULTS

Between February and April of 2009, 161 children were eligible and 131 were finally included in the study. Some missing data was not available for some children with information of weight for height nutritional status (n=5), viral load (n=1), and CD4 percentage (n=8).

Among 38 children, at least one of corresponding three examined slides were found positive for *Cryptosporidium* spp. (prevalence of infection 29%; 95%CI: 21.2-36.8), and they are considered as cases for further analysis, while children with all three negative samples (n=93) are considered controls. Table 1 describes main characteristics of the study group. The average weight was 15.2 ± 7.7 kg (range of 4.1-45 kg); average height was 96 ± 22 cm (range of 54-165 cm); and 77.9% [CI 95% (70.8-85%)] of the children had been hospitalized at least once after HIV/AIDS diagnosis. The average viral load was $302,414 \pm 364,962$ copies/ml (range of 39-1'500,000 copies/ml) and the mean percentage of CD4 was $27\% \pm 13\%$ (range of 2%-74%).

Association analysis. We found higher odds of *Cryptosporidium* infection among older and male children among those not living in Cali, with acute UNT, in children presenting fever, dry oral mucosa, weeping without tears, with a more severe stage of disease, previous hospitalizations, and among those children staying in day-care centers. Conversely, lower odds of infection were found with global UNT, chronic UNT, higher viral load, and lower CD4 percentage (Table 2).

After controlling for potential confounders, the variables best explaining the presence of *Cryptosporidium* spp. were age, chronic UNT, stay in day-care centers, and prior hospitalizations (Table 3), even when the paraclinical variables, viral load and percentage of CD4, were also included in the analysis (Table 4).

DISCUSSION

Various studies have evaluated the occurrence of *Cryptosporidium* spp. in HIV-positive children. The study by Dankner *et al.*, included 3331 HIV positive children in 13 clinical trials before HAART with an average follow-up of 23.8 months and found 0.6 *Cryptosporidium* spp. infections per 100 child-years (CI95% 0.4-0.8)⁸. According to Tumwine *et al.*⁶, there is 44.4 times greater odds (CI95% 18.4-110.4 $p < 0.0001$) of presenting *Cryptosporidium* spp. in HIV-positive children as compared to HIV-negative children. In the current study, we found a 29.0% prevalence of *Cryptosporidium* in feces using the modified ZN technique, and our result is similar to the report by Amadi *et al.*¹⁵, which identified a 28.84% prevalence of *Cryptosporidium* spp. in 104 HIV-positive children from Zambia. Additionally, they found a diagnosis of persistent undernutrition-diarrhea syndrome, suggesting a high prevalence of intestinal infectious disease, and underpinning the importance of cryptosporidiosis as a contributing factor in the physiopathology of this syndrome.

Our prevalence triplicates that reported by Guarino *et al.*⁷, who identified the presence of *Cryptosporidium* spp. in 3 of 35 North American HIV-positive children with diarrhea (8.6% prevalence); and who unlike our study, identified *Cryptosporidium* spp. in 85% of feces samples via immunofluorescence and only in 15% with the modified ZN technique, which allows them to conclude that enteric cryptosporidiosis is a severe problem during advanced stages of HIV/AIDS infection. Likewise, our prevalence doubles that reported by Cegielski *et al.*⁵, of 13% in 3 of 23 HIV-positive children from Tanzania with chronic diarrhea who – unlike our study – identified *Cryptosporidium* spp. via Kinyoun stain and monoclonal antibodies through direct fluorescence; thus, for these authors, *Cryptosporidium* spp. was considered an important pathogen in children with chronic diarrhea and HIV/AIDS infection. Finally, our prevalence is low, compared to the results by Tumwine *et al.*⁶, in Uganda who reported 73.6% *Cryptosporidium* spp., unlike our study, via immunofluorescence and PCR in feces of 91 HIV-positive children with persistent diarrhea and without antiretroviral therapy, which (in part) for them could explain the very high prevalence of this parasite in this

Table 1
General characteristics of children with HIV/AIDS infection.
Hospital Universitario del Valle, Cali, Colombia 2008 (n=131)

	All (n=131)	Cases (n=38) <i>C. spp. +</i>	Controls (n=93) <i>C. spp. -</i>	p
Age (months)	57.0±41.3 (1-173)	68.3±41.6 (13-170)	52.4±40.4 (1-173)	0.08
Gender (n,%)				
Male	69 (52.7)	19 (50.0)	50 (53.8)	
Female	62 (47.3)	19 (50.0)	43 (47.2)	0.69
Place of origin (n,%)				
Cali	56 (42.7)	37 (39.8)	19 (50.0)	
Valle	40 (30.5)	31 (33.3)	9 (23.7)	
Other	35 (26.7)	25 (26.9)	10 (26.3)	0.28
Mode of transmission (n,%)				
Vertical	126 (96-18)	38 (100)	88 (94-62)	
Sexual	3 (2.3)	0	3 (3.2)	
Transfusion	2 (1.5)	0	2 (2.2)	0.36
Stage (n,%)				
A	34 (25.9)	9 (23.7)	25 (26.9)	
B	33 (25.2)	6 (15.8)	27 (29.0)	
C	64 (48.8)	23 (60.5)	41 (44.1)	
Signs and symptoms (n,%)				
Abdominal pain	19 (14.5)	5 (13.2)	14 (15.1)	0.77
Abdominal distension	16 (12.2)	3 (7.9)	13 (13.9)	0.33
Acute diarrhea	13 (9.9)	3 (7.9)	10 (10.7)	0.33
Fever	13 (9.9)	4 (10.5)	9 (9.7)	0.88
Vomit	9 (6.9)	0	9 (9.7)	0.11
Dry oral mucosa	4 (3.1)	2 (5.3)	2 (2.2)	0.34
Chronic diarrhea	3 (2.3)	0	3 (3.2)	0.33
Presence of tears	3 (2.3)	1 (2.6)	2 (2.2)	0.86
Enophthalmos	2 (1.5)	-	2 (2.2)	0.83
Distal malperfusion	1 (0.8)	-	1 (1.1)	0.88
Abdominal fold present	1 (0.8)	1 (2.6)	-	0.15
Acidotic breathing	1 (0.8)	1 (2.6)	-	0.15
Nutritional status				
According to weight for age (n,%)				
Eutrophic	35 (26.7)	10 (26.3)	25 (26.9)	
Light global UNT	49 (37.4)	18 (47.4)	31 (33.3)	
Moderate global UNT	41 (31.3)	9 (23.7)	32 (34.4)	
Severe global UNT	6 (4.6)	1 (2.6)	5 (5.9)	0.94

Table 1
General characteristics of children with HIV/AIDS infection.
Hospital Universitario del Valle, Cali, Colombia 2008 (n=131) (continuation)

	All (n=131)	Cases (n=38) <i>C. spp. +</i>	Controls (n=93) <i>C. spp. -</i>	p
According to height for age (n,%)				
Eutrophic	48 (36.6)	16 (42.1)	32 (34.4)	
Light chronic UNT	26 (19.8)	9 (23.7)	17 (18.9)	
Moderate chronic UNT	40 (30.5)	11 (28.9)	29 (31.2)	
Severe chronic UNT	17 (12.98)	2 (5.26)	15 (16.13)	0.40
According to weight for height (n,%)				
	(n=126)	(n=36)	(n=90)	
Eutrophic	68 (53.97)	18 (50)	50 (55.6)	
Light acute UNT	26 (20.63)	10 (27.78)	16 (17.8)	
Moderate acute UNT	11 (8.73)	2 (5.56)	9 (10.0)	
Severe acute UNT	2 (1.59)	1 (2.78)	1 (1.1)	
Overweight	19 (15.08)	5 (13.89)	14 (15.6)	0.57
Prior hospitalizations (n,%)	102 (77.86)	33 (86.84)	69 (74.2)	0.11
Viral load (copies/ml) (n,%)				
	(n=130)	(n=38)	(n=92)	
<400	15 (11.54)	4 (10.53)	11 (11.9)	
≥400-<30,000	26 (20)	9 (23.68)	17 (18.5)	
≥30,000-<100,000	20 (15.38)	6 (15.79)	14 (15.2)	
≥100,000	69 (53.08)	19 (50)	50 (54.4)	0.81
%CD4 (n,%)				
	(n=123)	(n=37)	(n=86)	
<15	26 (21.14)	5 (13.51)	21 (24.4)	
≥15-<25	30 (24.39)	12 (32.43)	18 (20.9)	
≥25	67 (54.47)	20 (54.05)	47 (54.6)	0.17
Environmental variables (n,%)				
Household pets	59 (45.04)	14 (36.84)	45 (48.4)	0.22
Overcrowding	59 (45.04)	20 (52.63)	39 (41.9)	0.26
Stay in day-care center	55 (41.98)	19 (50)	36 (38.7)	0.23
W/out potable water	17 (12.98)	5 (13.16)	12 (12.9)	0.96
W/out excreta disposal	13 (9.98)	4 (10.53)	9 (9.7)	0.88

group of children with immune-compromise for HIV/AIDS infection.

Upon comparing the current prevalence of 29% with the 51% reported in 2006 by Siuffi *et al.*³, in the same population, we suggest this decrease in prevalence of infection may be due to several reasons, among others: different levels of precision due to different sample sizes; an over or under diagnosis by the bacteriologists in identifying *Cryptosporidium* spp. via the modified ZN technique; a spread use of HAART

therapy that now diminishes the morbidity and mortality of superimposed infections, and possible seasonal changes at the moment of identifying *Cryptosporidium* spp. in feces. Regarding the identification of *Cryptosporidium* spp. via the modified ZN technique, in this study from the same sample of fecal matter we obtained three slides, which along with the poor sensitivity of the test -even when taking the three samples at different times, and the short duration of oocysts excretion of *Cryptosporidium* spp.¹⁶, may

Table 2
Association between the presence of *Cryptosporidium* spp. and the socio-demographic, clinical, paraclinical, and environmental variables of children with HIV/AIDS infection.
Hospital Universitario del Valle, Cali, Colombia 2008

Socio-demographic variables	C. spp. +	C. spp. -	Odds Ratio	CI 95%	p
Age groups					
0-2 years (Infant)	8	34	1.00		
3-4 years (Pre-school)	9	26	1.47	[0.49-4.33]	0.48
5-11 years (School age)	19	30	2.69	[1.02-7.03]	0.04
12-14 years (Adolescents)	2	3	2.83	[0.40-19.87]	0.29
Gender					
Male	19	50	1.00		
Female	19	43	0.86	[0.40-1.83]	0.69
Place of origin					
Cali	19	37	1.00		
Valle	9	31	0.56	[0.22-1.42]	0.22
Other	10	25	0.77	[0.31-1.95]	0.59
Clinical Variables Nutritional status according to P/Age (Global UNT)					
Eutrophic	10	25	1.00		
UNT I (deficit $\geq 10\%$ -<25%)	18	31	1.45	[0.56-3.69]	0.43
UNT II (deficit $\geq 25\%$ -<40%)	9	32	0.70	[0.24-1.99]	0.50
UNT III (deficit $\geq 40\%$)	1	5	0.50	[0.05-4.83]	0.54
Nutritional status according to T/Age (Chronic UNT)					
Eutrophic	16	32	1.00		
UNT I (deficit $\geq 5\%$ -<10%)	9	17	1.05	[0.38-2.89]	0.91
UNT II (deficit $\geq 10\%$ -<15%)	11	29	0.75	[0.30-1.89]	0.55
UNT III (deficit $\geq 15\%$)	2	15	0.26	[0.05-1.31]	0.10
Nutritional status according to Weight/Height (Acute UNT or overweight)					
Eutrophic	18	50	1.00		
UNT I (deficit 10%-19%)	10	16	1.73	[0.66-4.51]	0.25
UNT II (deficit 20%-%)	2	9	0.61	[0.12-3.13]	0.56
UNT III (deficit $\geq 30\%$)	1	1	2.77	[0.16-46.77]	0.47
Overweight (excess $\geq 10\%$)	5	14	0.99	[0.31-3.14]	0.98
Signs and symptoms					
No		1			
Diarrhea	3	13	0.68	[0.17-2.64]	0.58
Fever	4	9	1.09	[0.31-3.80]	0.88
Abdominal pain	5	14	0.85	[0.28-2.56]	0.78

Table 2
Association between the presence of *Cryptosporidium* spp. and the socio-demographic, clinical, paraclinical, and environmental variables of children with HIV/AIDS infection.
Hospital Universitario del Valle, Cali, Colombia 2008 (continuation)

Socio-demographic variables	<i>C. spp.</i> +	<i>C. spp.</i> -	Odds Ratio	CI 95%	p
Abdominal distension	3	13	0.52	[0.14-1.96]	0.34
Dry oral mucosa	2	2	2.52	[0.34-18.63]	0.36
Weeping without tears	1	2	1.22	[0.10-13.97]	0.86
Vomit	0	9	0.21	[0.004-1.64]	0.11
Enophthalmus	0	2	0.78	[0.01-10.16]	0.83
Diminished distal perfusion	0	1	1.19	[0.01-23.49]	0.88
Abdominal fold	1	0	4.94	[0.24-294.87]	0.15
Acidotic breathing	1	0	4.94	[0.24-294.87]	0.15
Stage					
A	9	25	1.00		
B	6	27	0.61	[0.19-1.98]	0.41
C	23	41	1.55	[0.62-3.89]	0.34
Hospitalizations					
No		1			
Yes	33	69	2.29	[0.80-6.55]	0.12
Paraclinical variables					
Viral load (copies/ml)					
<400	4	11	1.00		
400-29,999	9	17	1.45	[0.35-5.90]	0.59
30000-99,999	6	14	1.17	[0.26-5.23]	0.82
≥100,000	19	50	1.04	[0.29-3.68]	0.94
Percentage CD4 (%)					
<15	5	21	1.00		
15-24	12	18	2.80	[0.82-9.46]	0.09
≥25	20	47	1.78	[0.59-5.40]	0.30
Environmental variables					
No		1			
Household pets	14	45	0.62	[0.28-1.34]	0.23
Potable water	33	81	0.97	[0.31-2.99]	0.96
Excreta disposal	34	84	0.91	[0.26-3.15]	0.88
Overcrowding	20	39	1.53	[0.72-3.28]	0.26
Stay in day care centers	19	36	1.58	[0.74-3.38]	0.23

explain the low prevalence we found in this study. To diminish the possible affectations in the validity of our study, perhaps due to information bias (poor classification), due to the number of samples to be evaluated (suggested minimum of two samples in

patients with immune compromise as in the case of children with HIV/AIDS infection), we decided to identify *Cryptosporidium* spp. in three slides from a feces sample from each child.

The modified ZN technique was used in this study

Table 3
Factors associated to *Cryptosporidium* spp. incidence according to the socio-demographic, clinical, and environmental variables of children with HIV/AIDS infection. Hospital Universitario del Valle, Cali, Colombia 2008 (N= 131)

Variable	Odds Ratio	CI 95%	p
Socio-demographic			
Age groups	1.86	[1.16-2.98]	0.01
Clinical			
Chronic UNT	0.67	[0.44-1.01]	0.05
Prior hospitalizations	2.74	[0.91-8.24]	0.07
Environmental			
Stay in day care centers	2.11	[0.92-4.85]	0.07

Table 4
Factors associated to *Cryptosporidium* spp. incidence according to the socio-demographic, clinical, and environmental variables of children with HIV/AIDS infection. Hospital Universitario del Valle, Cali, Colombia 2008 (N= 122)

Variable	Odds Ratio adjusted	CI 95%	p
Socio-demographic			
Age groups	1.79	[1.12-2.86]	0.01
Clinical			
Chronic UNT	0.63	[0.41-0.96]	0.03
Prior hospitalizations	2.62	[0.85-8.04]	0.09
Environmental			
Stay in day care centers	2.35	[1.00-5.52]	0.05

because of its high specificity (100%), although low sensitivity (37%-77%)¹⁶⁻¹⁹. The test is relatively unknown by most physicians²⁰ and many laboratories only analyze fecal samples for *Cryptosporidium* if requested by the clinician²¹. However, most health personnel believe cryptosporidiosis is a relatively rare disease, which is mainly caused by drinking water or exposure to swimming pools²².

The ZN diagnostic technique is troublesome because of the short period of oocyst excretion and the time-consuming analysis procedure. Nevertheless, rapid techniques like immunoassays (ELISA)¹⁰ or direct immunofluorescence have limited use in epidemiological and diagnostic studies²³. In consequence, and given that experimental data support that oocyst could be undetected by the ZN technique in 40-50% of individuals

with diarrhea, we concentrated the samples by sporulation²⁴ and, subsequently, identified the oocysts by light microscopy²⁵.

Risk factors

Age. Dankner *et al.*⁸ reported an age for acquiring cryptosporidiosis of 5.9 years. The average age of our patients was 4.8±3.4 years (range: 1 month-14.4 years); while in the Cegielski *et al.*, study it was 21 months⁵ (range 15-51 months), in the Tumwine *et al.* study⁶ it was 14±9 months, in the Guarino *et al.* paper⁷ it was 17 months (range 2-155 months), and in the study by Amadi *et al.*¹⁴, it was 18 months (range: 14-21 months). In the statistical model of our study, older children were at higher risk of *Cryptosporidium* spp. OR=1.79 [CI 95% 1.12-2.86] (p=0.01); according to data previously reported by us^{2,3}, we hypothesize this is at least in part

explained by this group of children being more exposed to parasite reservoirs

Gender. This study reports a slight predominance of the male gender over the female, at a ratio of 1.1, as described in studies by Dankner *et al.*⁸ and Tumwine *et al.*⁶, and unlike that observed by Cegielski *et al.*⁵ and Guarino *et al.*⁷; however, there are studies that do not manage to explain the gender differences regarding the presence of *Cryptosporidium* spp.¹²

Mode of transmission. This study revealed predominance of the vertical mode of transmission, as reported by Guarino *et al.*⁷, which according to Dankner *et al.*⁸, permits a better interpretation of the results, given that children infected through sexual contact later in life represent a population with a different duration of the infection. Our findings regarding mode of transmission are as expected, bearing in mind our own previous results^{2,3} and that over 90% of the cases of HIV/AIDS infection in children is because of vertical transmission, from the mother to the child, during child birth (intrauterine), during labor, at delivery (intrapartum), or postnatal through maternal breastfeeding.

Viral load and percentage of CD4. The study's final statistical model did not include the viral load or the percentage of CD4 as risk factors in children with HIV/AIDS infection to present *Cryptosporidium* spp.; data initially reported by us^{2,3}, and unlike that described by Tumwine *et al.*⁶, who state that children low CD4 percentage ($\leq 15\%$) have 6.4 times the opportunity [CI 95% 0.64-64.74 $p=0.114$] of presenting *Cryptosporidium* spp. than children with $CD4 > 15\%$. Likewise, our data suggest in the univariate analysis that as CD4 percentage increases, the chances for *Cryptosporidium* spp. infection are lower: among children with $\%CD4 \geq 15$ and < 25 : OR=2.8 [CI 95%: 0.82 - 9.46; $p=0.09$]; and in those with $\%CD4 \geq 25$: OR=1.78 [CI 95% 0.59-5.40; $p=0.30$]. This finding, apparently contradictory with the natural history of the disease, could be simply due to random effects or because our patients, unlike the children from Uganda⁶, received antiretroviral therapy, which could have favored increased percentage of CD4 and their better evolution. Our findings are paradoxical in that when our patients are included in the study, they presented in 53.1% of the cases viral loads over 100,000 copies/ml, meaning poor adherence to the HAART therapy. This does not agree with the results of CD4 percentage reported, which state the contrary.

This, we could probably interpret to the labile results of the viral load to the HAART therapy, especially bearing in mind that when the children were included in the study, the viral load used had been 15 days before or after taking the fecal matter sample. According to that reported by Dankner *et al.*⁸, in children with HIV infection and cryptosporidiosis, the mean %CD4 was 4%, while in our study this percentage was at $27 \pm 13\%$ (range of 2-74%); also, they reported the presence of 1.3 events per 100 individuals per year when the CD4 percentage is $< 15\%$ and 0.2 events per 100 individuals per year when the CD4 percentage is between 15-25% and $\geq 25\%$. In our case, over half of the children studied (54.47%), presented a CD4 percentage $\geq 25\%$.

Undernutrition. Upon comparing our results to that reported by Amadi *et al.*¹⁵, and Tumwine *et al.*⁶, who described severe acute undernutrition (UNT) in 22% of the cases, severe global UNT in 47%, and severe chronic UNT in 63%, our patients presented better nutritional conditions and we did not find nutritional status compromise of our patients in terms of severe UNT; only 1.6% of them presented severe acute UNT, 4.6% severe global UNT, and 13% severe chronic UNT, which permits us to conclude that the presence of *Cryptosporidium* spp. is associated to UNT. These data equally contradict that reported by Guarino *et al.*⁷ and Cegielski *et al.*⁵ In general, we could assume that the nutritional status of the children from the study is acceptable, especially if compared with our prior data in this same group of children, who in 2005 presented global UNT in 72% of the cases, chronic UNT in 67%, and acute UNT in 35%, this is very probably secondary to the efficacy and effectiveness of the HAART therapy, and to the follow-up and control program at the HIV/AIDS Pediatric Clinic at HUV, among others, which permit greater adherence to therapy and diminished morbidity and mortality in this group of children, which is outlined by their better nutritional status. The paradoxical protective effect found in our patients may be interpreted more than merely as chronic UNT, as being short stature for the age, whose causes are multifactorial and not only due to UNT; causes among which could be involved constitutional short stature, short family stature, constitutional retarded development, slow maturity, etc. In our particular case, and *per se*, because of the short stature reported in children due to the HIV/AIDS condition. It is also worth noting that

the tables of reference used for the analysis of the nutritional status of our children were those from the United States National Center for Health and Statistics, given that these are universally considered in most of the studies reviewed and also because we used them previously in other studies^{2,3}.

Hospitalizations. In the study Cegielski *et al.*⁵, hospital stay was significantly longer in the group of children with cryptosporidiosis (average of 10 days; range of 2-18 days) than in children without this infection (average of 4 days; range of 1-17 days) ($p=0.03$). In our study, children with prior hospitalizations have 2.75 times greater chance [CI 95% 0.89-8.46 $p=0.07$] of having *Cryptosporidium* spp., which could be explained not necessarily by what we would have expected, from the potential severity of enteric cryptosporidiosis in these types of patients, but rather, by the presence of co-infections due to other pathogens or co-morbidity from other conditions having to do with these types of patients, which were not considered for this study. We do not report any case of mortality during the study; however, Amadi *et al.*¹⁵, describe 3.3 greater opportunity of dying [CI 95% 1.6-6.85 $p=0.001$] in children with *Cryptosporidium* spp. Again, the lack of deaths in our study may reflect the better nutritional status of the children included, better access to antiretroviral therapies and to the healthcare institution, and to adequate follow up within the HIV/AIDS Pediatric Clinic at HUV in Cali, Colombia, among others.

Signs and symptoms. For Cegielski *et al.*⁵ clinically, the distinctive findings were vomit and dehydration among children with acute diarrhea who had cryptosporidiosis; for us, fever [OR=1.09, (CI 95% 0.31-3.80) $p=0.88$], dry oral mucosa [OR=2.52, (CI 95% 0.34-18.63) $p=0.36$], and weeping without tears [OR=1.22, (CI 95% 0.10-13.97) $p=0.86$] were important findings, but they were, nevertheless, statistically insignificant. We only reported one child with dehydration, but Tumwine *et al.*⁶, describe 7.4% of their children with dehydration, and in their conclusions they even identify severe dehydration as a bad prognosis factor in children with HIV/AIDS infection and positive *Cryptosporidium* [OR 0.63 (CI 95% 0.15-2.67) $p=0.526$], as reported by Guarino *et al.*⁷, who associate such dehydration to the secretor component and to the persistence of diarrhea secondary to *Cryptosporidium*.

Stage. Our patients were predominantly stage C,

according to the classification from the United States Center for Disease Control (CDC), as that described by Guarino *et al.*⁷, but differing with their conclusions, because the evolution of our patients was satisfactory, while they not only found a clear association with the advanced stage of the HIV/AIDS infection but also with the rapid clinical and immunological compromise of their patients; suggesting for them that the severe disorder of the immune response is associated to colonization and subsequent clinical disease, which suggests them to propose that enteric cryptosporidiosis should be considered a key point in the progression of the HIV/AIDS infection.

Environmental. Cegielski *et al.*⁵ found no significant associations among household pets, crawling children, or walking children, or the type of or location of the bath or water. In our final statistical model, we found that children staying in day-care centers have 2.75 times greater opportunity [CI 95% 1.12-6.72 $p=0.02$] of having *Cryptosporidium* spp.; this is because of the possibility of transmission among children attending day-care centers as secondary cases and asymptomatic infections among intra-family contacts; therein, the importance of keeping children with diarrhea from attending these daycare centers while they are sick.

Our recommendations are aimed at identifying the taxonomic characteristics of the parasite in our environment through high-resolution molecular epidemiology, which will help researchers to better understand its transmission in children and, thus, once characterized, associate it to the clinical manifestations of the different *Cryptosporidium* spp. species and subtypes. Although the highly active antiretroviral therapy (HAART) has reduced the incidence of the disease in children with HIV/AIDS infection, affecting the viral load, as well as the parasite load of *Cryptosporidium* spp., future studies could be guided towards the development of effective therapeutic agents and drug therapies for human cryptosporidiosis, particularly in patients with advanced immune compromise due to HIV/AIDS infection; thereby, diminishing their morbidity and mortality.

In conclusion, the prevalence of Colombian children with HIV/AIDS infection under HAART therapy was of 29%, with the variables that best explained the presence of *Cryptosporidium* spp. included being older than two years of age, presenting chronic UNT with a deficit in height for age above 5%, staying in day-care

centers, and having been previously hospitalized.

Conflict of interests. The authors declare having no conflict of interests or conflicts with the institution where the research took place.

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