Cryptosporidium sp. in Children Suffering from Acute Diarrhea at Uberlândia City, State of Minas Gerais, Brazil

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This study's objective was to search for Cryptosporidium *sp. in diarrheic feces from children aged zero to 12 years and cared for at medical units within Universidade Federal de Uberlândia or at a private practice in Uberlândia, State of Minas Gerais, Brazil, from September 1992 to August 1993. Three fecal samples preserved in 10% formalin, were collected from 94 children. Oocyst concentration was performed through Ritchie's (modified) method and staining of fecal smears for each sample (total of 1128 slides) was done by the "Safranin/Methylene Blue" and the "Kinyoun (modified)" techniques. The Hoffmann, Pons & Janer method was also employed to look for other enteroparasites. From 94 children, 4.26% excreted fecal* Cryptosporidium *oocysts. The infection seemed to vary according to age: 5.08% of patients aged zero to two years old; 33.33% of those aging eight to ten years (P>0.05).* Cryptosporidium *appeared in November, December and March, during the rainy season. 20.21% of the children harbored at least one enteroparasite different from* Cryptosporidium*, mainly* Giardia intestinalis *(12.77%). From* Cryptosporidium *infected patients, two had only this kind, another harbored* Giardia intestinalis*; the last one hosted* Strongyloides stercoralis.

Key words: *Cryptosporidium* sp. - cryptosporidiosis in children - intestinal parasites

Cryptosporidiosis is a parasitic zoonosis caused by protozoa of the genus *Cryptosporidium*, studied mostly in veterinary medicine, and only recently becoming a theme of growing importance to human pathologists. The World Health Organization (WHO 1980) has shown that from 30% to 40% of the papers dealing with the etiology of acute diarrhea previous to 1980 could not establish its identity, and since that year *Cryptosporidium* began to be included in the list of diarrhea-producing parasites. Within fecal smears its oocysts are not easily identifiable without the use of special concentration techniques such as the ones developed by Sheather (1923) or by Ritchie (1948), associated with staining techniques, such as Ziehl-Neelsen's, modified (Henrichsen & Pohlenz 1981), the Safranin/Methylene Blue (Baxby et al. 1984) and the Auramin/Fuchsin (Casemore et al. 1984) techniques, among others.

Cryptosporidiosis has an ample geographic distribution (Cordell & Addiss 1994), with a higher frequency in developing countries (Reinthaler 1989, Molbak et al. 1990, Rahman et al. 1990), but also with references of outbreaks in developed nations (Jokipii et al. 1983, Alpert et al. 1986, Garcia-Rodriguez et al. 1990, Assadamongkol et al. 1992).

The finding of this protozoon in Brazilian children has been reported in the Northern Region (Loureiro et al. 1989), Northeast Region (Weikel et al. 1985, Alves et al. 1989, Silva et al. 1994) and Southeast Region (Mangini et al. 1992, Lomazi et al. 1993).

Because of the lack of data about this subject concerning the city of Uberlândia (located in the State of Minas Gerais over the main crossroads linking Brazil along the axles North-South Belém and São Paulo cities and West-East-Porto Velho and Vitória cities) this study's major goal was to look for *Cryptosporidium* oocysts in diarrheic feces of children aged from zero to 12 years and cared for in urban public and private medical diagnose centres.

MATERIALS AND METHODS

Fecal sample collections - In plastic vials containing 10% formalin, were collected three fecal samples from each of 94 children suffering from acute diarrhea and cared for in one of the following four diagnose centres, the first three of which belong to the Universidade Federal de Uberlândia (UFU): (1) the Sickroom of UFU's Hospital; (2)

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the Central and Peripheral Polyclinic of the same Hospital; (3) the First Aid Clinic of the same Hospital; (4) a private pediatric practice. The collection period was from September 1992 to August 1993.

Methods for detection of Cryptosporidium *oocysts and other enteric parasites* - The Ritchie (1948) concentration technique (modified), associated to the staining methods of Safranin/Methylene Blue (Baxby et al. 1984) and Kinyoun's modified (Moura & Oliveira 1985) were employed; four slides for each sample, two for each staining method; the total number of examined slides was 1128. For the detection of other enteric parasites the Hoffmann et al. (1934) method was used.

Statistical analysis - The data were analyzed through the χ^2 test.

RESULTS

The patients distributed by the medical institutions which cared for them - From the 94 diarrheic children, 25 (26.60%) were from the UFU's Hospital Sickroom, 15 (15.96%) from the same Hospital's First Aid Clinic, 35 (37.27%) from its Polyclinic centres and 19 (20.21%) from one private pediatric practice.

Sex and ages - 57 (60.64%) were male and 37 (39.36%) were female children, 59 of the children had two years or less, 14 of them fell in the category from above two years to four, 8 in the group above four up to six, 7 in the class above six up to eigth, 3 above eigth up to ten and 3 above ten and up to twelve.

Signs and symptoms observed in those 94 acutely diarrhea children - Diarrhea (100%), fever (60.64%), vomiting (46.81%), abdominal pain (27.66%), dehydration (24.47%), abdominal inflammation (6.38%) and undernourishment (27.66%).

Cryptosporidium *infection* - From the 94 studied children in four (4.26%) were found fecal oocysts of this genus in the three examined samples, by both of two staining methods. According to the medical centres of diagnosis, two of the above four cases came from UFU's Hospital Sickroom and the other two from the private pediatric practice ($P>0.05$). Concerning the patient's age, three children (5.08%) were in the group aged from zero to two years and one (33.33%) in the above eight and up to ten group ($P>0.05$). All the observed children were from urban origin and only one of them lived in an apartment building; all the others dwelled in houses, of which only one was not linked to the city's water pipeline network, obtaining water from a private artesian well. Only one of the houses did not harbor one pet dog. The two *Cryptosporidium* infected children cared for in the private pediatric practice studied in private primary schools; the other two cared for by the UFU's medical services were not registered in any school or nursery.

Other enteric parasites - From the 94 children fecal samples, 20.21% were found infected with other parasitic organisms, such as: *Giardia intestinalis* (12.77%), *Entamoeba coli* (3.19%), *Ascaris lumbricoides* (2.13%), *Hymenolepis nana* (2.13%), *Strongyloides stercoralis* (2.13%), *Trichuris trichiura* (1.06%) and *Endolimax nana* (1.06%).

Table presents the distribution of children affected with acute diarrhea according to the criteria: medical centres where feces were sampled, months when infections were detected, patient's sex, age, colour of skin, clinical aspects and associated enteroparasites along the period from September 1992 to August 1993.

DISCUSSION

This is the first approach to cryptosporidiosis in children within Uberlândia community. Even if it is inserted in a developing country where higher level of infection (21.31%) has been found at Recife, State of Pernambuco (Alves et al. 1989), the infection percentage 4.26% registered in this paper approaches those of develop nations (Jokipii et al.1983, Hunt et al. 1984, Garcia-

TABLE

Presentation of four cryptosporidiosis cases (4.26%) from 94 diarrheic children in Uberlândia, MG, Brazil

Case no.	Medical centres	Month/ year	Sex	Age	Symptoms	Concomitant parasites
53	S	11/92	M	3m	Fever, vomition, abdominal pain, undernourishment dehydration	Negative
63	PP	12/92	F	9y 6m	Eutrophic, abdominal pain	Negative
64	PP	12/92	M	1y 10m	Eutrophic, vomition, abdominal pain	*Giardia intestinalis*
75	S	03/93	M	1y 5m	Eutrophic, fever, vomition, abdominal pain	*Strongyloides stercoralis*

M = male; F = female; m = month; y = year; S = sickroom; PP = private practice.

Rodriguez et al. 1990) a result that may be due to the circumstance of the observations having been made in one of this country's more developed part (Southeast Region).

When only hospital cared children are included in the analysis the infection percentage grows to 8%, a figure similar to the ones reported by Sarabia-Arce et al. (1990) in Peru (10%) and by Moodley et al. (1991) in Africa (9%).

Notwithstanding the fact that, when different papers on cryptosporidiosis are compared, levels of infection may change within the age group from zero to three years, this was the age group carrying the highest percent level of infection in the present study. It can be seen that this was also the age group containing the largest number of children with acute diarrhea. When this age group and their infected patients becomes the basis for determination of percent infection, this index grows from 4.26% to 5.08%, even if the difference between age groups does not reach statistical significance by the χ^2 test.

In Brazil, indexes similar to the above ones were found: 5.2% in Belém (Pará) by Loureiro et al. (1989) and 6.4% in Recife (Pernambuco) by Silva et al. (1994). But Loureiro et al. (1989) restricted their observations to children aged from one to two years and Silva et al. (1994) observed only patients from two to 12 months of age.

In the present study one of the infected children belonged to the age group of eight to ten years, what brings the index of infection of the group to 33.33%, a figure that must be looked at with care, since it may be the consequence of sampling deficiency and also having in mind that the percentual of children with a diarrhea clinic picture tends to decrease with age increase.

The employ of a cyst concentration technique must be emphasized, since such elements were scarce, one per three observed microscope fields. So it is supposed that without the use of such an aid, the level of infection could have been underestimated. In what the sating techniques are concerned, the infection rate they disclosed was the same, meaning that they detected the oocysts of *Cryptosporidium* in every single one of the three slides from the three samples from infected patients.

The Safranin/Methylene Blue staining method is regarded as easier in handling besides being quicker and time saving, Rahman et al. (1990), Assadamongkol et al. (1992) and Bolbol (1992) have mentioned after concentration of cysts from faecal material, with out mentioning, though, any reason for such an option. Even observing the similarities of symptoms on the diarrhea syndrome, there has been diversity in the frequency of such symptoms between each individual of the 94 children. It can be observed that 27.66% of them manifested an under-nourishment picture, but only one of the *Cryptosporidium* infection coincided with such picture (Table). The χ^2 test did not show any significant difference between the undernourished and the rest of the children. Mangini et al. (1992), were also unable to detect such a difference based on the nutritional status, agreeing to Bogaerts et al. (1984), in Central Africa.

Looking at Table it can be seen that two patients had another parasitic infection concomitant with cryptosporidiosis. Informations from the reference-card of each of the four *Cryptosporidium* infected children show that none of them presented positive coproculture for intestinal bacterium processed from contemporary faecal samples; circumstantial evidence points out that in two of the diarrheic children *Cryptosporidium* was the only identified etiologic agent.

Notwithstanding the fact that this study covered only a year of observations, thus making it difficult to conclude adequately on season-depending characters of cryptosporidiosis in children, this infection was observed in November and December 1992 (three cases, 3.19%) at the full rainy season, and in March 1993 (one case, 1.09%), at the end of the rainy season. At the Northeast Region of Brazil, Weikel et al. (1985) conducted a study during two years and five months. All cryptosporidiosis cases were found from October to March, that is to say, from the end of the dry season through the wet season, up to its end.

Within the present study are included two *Cryptosporidium* infected children cared for at a private paediatric practice pertaining to a high standard of socio-economic status. That is an interesting finding since this kind of infection may be to prevail among the lowest layers of poor and uncared for communities three of the infected patients used at home pipeline water from a modern urban network, what doesn't preclude tap-water as a source of infection. Hayes et al. (1989), at the U.K., and Smith et al. (1989), in USA, reported cryptosporidiosis outbreaks transmitted through treated water; the presence of such organism's oocysts in water reservoir previous to treatment was shown by Ongerth and Stibbs (1987) and by Lechevallier et al. (1991). The use of water from an artesian well by one of the infected high-class patients, whose house was not connected to the urban sewage drain system, configures another pattern of exposition to oocysts and contamination risk.

Only one of the infected patients did not keep an animal at home. The presence of cryptosporidiosis in pet mammals is a known fact and their contact with man have been focused in cryptosporidiosis transmission (Lengerich et al. 1993).

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REFERENCES

Alpert G, Bell LM, Kirkpatrick CE, Budnick LD, Campos JM, Friedman HM, Plotkin SA 1986. Outbreak of cryptosporidiosis in a day-care center. *Pediatrics 77:* 152-157.

Alves JGB, Montenegro F, Regalato I 1989. Cryptosporidiose em crianças com desnutrição grave. *J Pediátr 65:* 220-222.

Assadamongkol K, Gracey M, Forbes D, Varanithya W 1992. *Cryptosporidium* in 100 Australian children. *Southeast Asean J Trop Med Public Health 23*: 132-137.

Baxby D, Blundell N, Hart CA 1984. The development and performance of a simple, sensitive method for the detection the *Cryptosporidium* oocysts in faeces. *J Hyg 92:* 317-323.

Bogaerts J, Lepage P, Rouvroy D, Vandepitte J 1984. *Cryptosporidium* spp., a frequent cause of diarrhea in Central Africa. *J Clin Microbiol 20:* 874-876.

Bolbol AS 1992. Cryptosporidiosis in young children suffering from diarrhoea in Riyadh, Saudi Arabia. *J Hyg Epidemiol Microbiol Immunol 36:* 396-400.

Casemore DP, Armstrong M, Jackson B 1984. Screening for *Cryptosporidium* in stools. *Lancet 31:* 734-735.

Cordell RL, Addiss DG 1994. Cryptosporidiosis in child care settings: a review of the literature and recommendations for prevention and control. *Pediat Infec Dis J 13:* 310-317.

García-Rodriguez JA, Martín-Sanchez AM, Blasco AC, Garcia Luís EJ 1990. The prevalence of *Cryptosporidium* species in children in day care centres and primary schools in Salamanca (Spain): an epidemiological study. *Eur J Epidemiol 6:* 432-435.

Hayes EB, Matte TD, O'brien TR, McKinley TW, Logsdon GS, Rose JB, Ungar BLP, Word DM, Pinsky PP, Cummings ML, Wilson MA, Long EG, Hurwitz ES, Juranek DD 1989. Large community outbreak of cryptosporidiosis due to contamination of a filtered public water supply. *New Engl J Med 25:* 1371-1376.

Henricksen S, Pohlenz J 1981. Staining of Cryptosporidia by a modified Ziehl-Neelsen techninque. *Acta Vet Scand 22:* 594-596.

Hoffmann WA, Pons JA, Janer SL 1934. The sedimentation concentration method in schistosomiasis mansoni. *Puerto Rico J Pub Health 9:* 283-291.

Hunt DA, Shannon R, Palmer SR, Jephcott AE 1984. Cryptosporidiosis in an urban community. *Br Med J 289:* 814-816.

Jokipii L, Pohjola S, Jokipii AMM 1983. *Cryptosporidium:* a frequent finding in patients with gastrointestinal symptoms. *Lancet 13:* 358-361.

Lechevallier MW, Norton WD, Lee RG 1991. Occurrence of *Giardia* and *Cryptosporidium* spp. in surface water supplies. *Appl Environ Microbiol 57:* 2610-2616.

Lengerich EJ, Addiss DG, Marx JJ, Ungar BLP, Juranek DD 1993. Increased exposure to Cryptosporidia among dairy farmers in Wisconsin. *J Inf Dis 167:* 1252-1255.

Lomazi EA, Collares EF, Viccari MS, Castro AFP 1993. Identificação de agentes enteropatogênicos nas fezes de crianças com diarréia aguda no município de Paulínia-SP. *J Pediat 69:* 159-164.

Loureiro ECB, Linhares AC, Mata L 1989. Criptosporidiose em crianças de 1 a 2 anos de idade, com diarréia aguda em Belém, Pará, Brasil. *Mem Inst Oswaldo Cruz 84:* 117-122.

Mangini ACS, Dias RMDS, Grisi SJFE, Escobar AMU, Torres SMAGV, Zuba JPR, Quadros CMS, Chieffi PP 1992. Parasitismo por *Cryptosporidium* sp. em crianças com diarréia aguda. *Rev Inst Med Trop São Paulo 34:* 341-345.

Molbak K, Hojlyng N, Silva APJ, Jepsen S, Aaby P 1990. An epidemic outbreak of cryptosporidiosis: a prospective community study from Guinea Bissau. *Ped Inf Dis J 9:* 566-570.

Moodley D, Jackson TFHG, Gathiram V, Van Den Ende J 1991 *Cryptosporidium* infectious in children in Durban: seasonal variation, age distribution and disease status. *South Afr Med J 79:* 295-297.

Moura H, Oliveira LM 1985. *Cryptosporidium:* parasita de imunocomprometidos. *Rev Bras Pat Clin 21:* 198-202.

Ongerth JE, Stibbs HH 1987. Identification of *Cryptosporidium* oocysts in river water. *Appl Environ Microbiol 53:* 672-676.

Rahman M, Shahid NS, Rahman H, Sack DA, Rahman N, Hossain S 1990. Cryptosporidiosis: a cause of diarrhea in Bangladesh. *Am J Trop Med Hyg 42:* 127-130.

Reinthaler FF 1989. Epidemiology of cryptosporidiosis in children in tropical countries. *J Hyg Epidemiol Microbiol Immunol 33*: 505-513.

Ritchie LS 1948. An ether sedimentation technique for routine stool examination. *Bull N S Army Med Dep 8:*326-334.

Sarabia-Arce S, Salazar-Lindo E, Gilman RH, Naranjo J, Miranda E 1990. Case-control of *Cryptosporidium parvum* in Peruvian children hospitalized for diarrhea: possible association with malnutrition and nosocomial infection. *Pediatr Inf Dis J 9:* 627-631.

Sheather AL 1923. The detection of intestinal protozoa and mange parasites by floatation technique. *J Comp Pathol Ther 36:* 266-275.

Silva GAP, Magalhães M, Oliveira AMA, Melo VM 1994. Diarréia aguda do lactente associada ao *Cryptosporidium* sp. na cidade de Recife. *J Pediatr 70:* 44-47.

Smith HV, Patterson WJ, Hardie R, Greene LA, Benton C, Tulloch W, Gilmour RA, Girdwood RWA, Sharp JCM, Forber GI 1989. An outbreak of waterborne cryptosporidiosis caused by post-treatment contamination. *Epidem Inf 103*: 703-715.

Weikel CS, Johston LI, Souza M, Guerrant RL 1985. Cryptosporidiosis in Northeastern Brazil: association with sporadic diarrhea. *J Infect Dis 151:* 963-965.

WHO 1980. Parasites related diarrhea. *Bull WHO 58*: 819-830.