In: The interaction of parasitic diseases and nutrition.
Chagas, C. & Keusch, T. (ed), Scripta Varia No. 61,
Pontificia Academia Scientiarvm, pp. 169-184, 1986.

CRYPTOSPORIDIUM DIARRHEA IN COSTA RICAN CHILDREN

LEONARDO MATA
Health Research Institute (INISA), University of Costa Rica, and
Department of Population Sciences, Harvard School of Public Health

and

ROSARIO ACHI
Health Research Institute (INISA), University of Costa Rica

Introduction

Coccidian parasites of the genus *Cryptosporidium* cause acute diarrhea in many vertebrates, including man. Recent reviews on the subject [1-5] were stimulated by demonstration of a chronic, debilitating and generally fatal diarrhea in immunodeficient and immunosuppressed individuals, and in persons with acquired immunodeficiency syndrome (AIDS) [6-16]. Additional interest arose after finding that cryptosporidiosis is not rare among immunocompetent or otherwise healthy children and adults, who suffer from acute diarrheal disease in industrialized and less developed countries [17, 18].

The first *Cryptosporidium* species (*C. muris*) was described by Tyzzer in 1907 [19], who found the parasite in gastric glands of the domestic mouse. Tyzzer described oocysts measuring 5-6×7 μm, with 4 sporozoites of about 12-14 μm after excystation [19, 20]. He attempted transmission of the coccidium to the white rat, without success. Later, Tyzzer described another species, *C. parvum,* with considerably smaller oocysts measuring 3.0-3.3 × 4.0-4.5 μm; excisted sporozoites measured 5.5-6.0 μm [21]. This species was found in the small intestine of the laboratory mouse, rabbit and chicken [33]. *Cryptosporidium* is currently placed in Api-

complexa, Sporozoea, Coccidia, Eucoccidiida, Eimeriina, and Cryptosporidiidae [22].

Many years after description of these species, additional "species" were named according to the vertebrate hosts in which they were found [22]. Most authors regard these species unjustified for several reasons. Oocysts found in different vertebrates are of similar size and morphology as those of *C. parvum* [23]. Infection and cross-infection occurs with oocysts of *C. parvum*-like strains in several vertebrate species and in man. Antibodies to one particular strain of *Cryptosporidium* have been detected in sera from diverse vertebrate hosts [24]. On the basis of this information, one single species was proposed [24], in analogy with *Toxoplasma,* although one expert proposed one species for each of the four groups of vertebrates harboring parasites [25].

Cryptosporidium parasites attach to the surface of human intestinal epithelial cells of jejunum, ileum and colon [26-27]. Cells of other epithelia may also be infected, for instance, respiratory tract and gall bladder, of immunodeficient individuals [28-30]. Infection in immunodeficient or immunosuppressed persons is characterized by acute watery diarrhea which may evolve into a chronic, emaciating and often fatal disease [9-11]. The parasite causes serious complications in immunosuppressed patients or in persons with terminal degenerative disease. Finally, *Cryptosporidium* induces a serious and lethal disease in a considerable proportion of patients with AIDS [13-16].

In immunocompetent individuals, cryptosporidiosis is a self-limited acute watery diarrhea of short duration, without serious epithelial damage or involvement of organs other than the intestine [17, 18]. The first case in an immunocompetent individual was described in 1976 [31]. Years later, the parasite was found in an important proportion of immunocompetent children presenting diarrhea in Australia [17] and Costa Rica [18]. The parasite has been found in humans in Rwanda, Bangladesh, Venezuela, Brazil, Peru and Liberia [see 5, 32-34]. Industrial nations reporting the parasite, in addition to Australia, are Finland, Canada, the United States and the United Kingdom [5].

Cryptosporidium is highly pathogenic for man and most infections are clinical. In fact, the number of carriers is small or negligible [5, 18, 35]. Infection in children and adults occurs readily by exposure to a contaminated source, as judged from observation in the community and accidental infections of personnel handling domestic animals [1-5]. Cryp-

tosporidiosis is less common in breast-fed than in weaned infants [18]. Infants and preschool children are more easily infected than adults [5, 18].

Cryptosporidium diarrhea in Costa Rica (located 10 degrees North of the Equator) occurs almost exclusively during the rainy season, from April through September [18, 35], a warm and humid period. The same seems to be the case in Bangladesh, where infections are more common during the rainy months, about the same time of the year [32]. The reports from Australia and Venezuela showed a high rate of *Cryptosporidium* during the warm months [17, 34]. No seasonal information is available for countries that have uniform temperature throughout the year.

The present report summarizes four years of observation of preschool children with acute diarrhea in Costa Rica. Most children were studied at the National Children's Hospital, the main child referral center, in San Jose, the capital city. Other children were studied at the Field Station of INISA, in Puriscal, a rural region in the Southern Intermountain Valley. The Field Station serves as a base for prospective field studies of mothers and children.

Method of procedure

Populations. The urban and rural populations studied live at an average altitude of 1000 meters above sea level, and are of comparable ethnic background, predominantly Spanish, with varying mixtures of Amerindian and to a lesser extent, Black. The urban population was the largest, and consisted of children with acute diarrhea, who were brought to the outpatient and emergency services of the National Children's Hospital, from the metropolitan area. This encompasses the capital city, the neighboring city of Heredia and dozens of small towns (cantons and districts). The population belongs to the middle and low socioeconomic strata, and has an adequate level of health and education. All children studied were less than two years of age.

The rural population was from the districts and villages of the Puriscal region, of similar demographic, socioeconomic and education characteristics as the urban population. Children under three years of age were included, the great majority under two years. During the study period, Costa Rica had an average infant mortality of 19 per 1000, a diarrheal disease death rate of 6 per 100,000 and a life expectancy at

birth of 75 years. Medical care and preventive health services are free and widely available throughout the country [36].

A slightly lower income in the rural area is compensated by a lesser cost of food and lesser need for expensive clothing and housing. In the urban area, the rate of not breast-fed infants is larger (15%) than in the rural area (8%). Also, the rate of premature weaning is greater and the introduction of weaning foods earlier in the urban area than in Puriscal. The highest incidence and duration of breast-feeding in the rural population can be accounted for by several factors, the most prominent ones being induction in hospitals, and support to mothers early in lactation by the rural health service [37].

Clinical information. Urban children with diarrhea were included at random in the hospital, each morning, five days per week. In the rural area, all children known to have diarrhea were also included as part of a prospective study of mothers and children [37]. All children were examined by a physician, and data on clinical condition, nutritional status and other variables were collected in precoded forms. Rectal temperature was measured daily. Mothers or attendants accompanied children in the outpatient service. When severe cases required internment in the emergency service, mothers stayed with their children until recovery. Breast-feeding was encouraged to avoid interruption during illness and treatment. In weaned children, foods were withheld for a few hours, two to six, as clinical condition (anorexia, vomiting, etc.) allowed. Body weight and length were collected upon admission; post-rehydration weight was used to help estimate the degree of dehydration upon admission. Patients received oral rehydration solution by mouth whenever possible [38]; other routes were used when required. Participation in the study and collection of samples was by informed consent of the parent or guardian.

Laboratory studies. One fecal specimen was collected from each child, in sterile containers; smears were prepared at the bedside and were immediately fixed in methanol. During the first year of study [18], search for *Cryptosporidium* oocysts was after staining with slow Giemsa. All specimens of the first year were reexamined with the modified cold Ziehl-Neelsen staining (Kinyoun) [39]; results were identical as those with Giemsa [35]. Since the modified cold Kinyoun is simple and permits an easier and more rapid diagnosis of *Cryptosporidium* oocysts than Giemsa,

it was adopted thereafter. Smears were examined for oocysts with low and high power light microscopy, and oocysts were confirmed under immersion oil. All data in this report are based on acid-fast-stained specimens.

RESULTS

Frequency of Cryptosporidium infection. Fifteen hundred and fifteen children with acute diarrheal disease, all under three years of age, were studied from 1982 through 1985, Table 1. Of these, 1235 were from the urban area and 280 from Puriscal. A total 248 children without diarrhea, of comparable age and socioeconomic condition, were included in the study as controls. Of these, 159 were studied at the hospital for other reasons, and 89 were from the rural area. Since there was certain incompleteness of the data for some variables, different totals will necessarily appear in the following tables.

The overall frequency of *Cryptosporidium* oocysts in both populations was 4.9%, slightly more in urban (5.3%) than in rural children, Table 1. None of the controls were found shedding oocysts, despite the fact that

TABLE 1 - *Frequency of Cryptosporidium oocysts in stools of preschool children with and without diarrhea, Costa Rica,* 1982-1985.

Age, months	Rural *			Urban			Total		
	With		Without	With		Without	With		Without
	No.	+(%)	**	No.	+(%)	**	No.	+(%)	**
0-5	60	0	21	521	26(5.0)	79	581	26(4.5)	100
6-11	63	0	19	413	22(5.3)	51	476	22(4.6)	70
12-17	61	3(4.9)	22	221	13(5.9)	21	282	16(5.7)	43
18-23	54	4(7.4)	19	68	3(4.4)	7	122	7(5.7)	26
24-29	18	1(5.6)	6	12	1(8.3)	1	30	2(6.7)	7
30-35	24	2(8.3)	2	0	0	n.c.	24	2(8.3)	2
Total	280	10(3.6)	89	1235	65(5.3)	159	1515	75(4.9)	248

* Only 1982-1984.
** All controls were negative for oocysts.
n.c. No cases studied.

they were from the same populations and had been included at random throughout the study period, as were the cases. *Cryptosporidium* oocysts appeared as oval-shaped structures of strikingly homogeneous morphology, measuring, in Giemsa-stained preparations, 4.1 × 5 μm (Mean) with 0.5 × 0.4 μm (S.D.), as described previously [18]. Oocysts had a morphology and dimensions compatible with those described by Tyzzer for *C. parvum* [21]. An occasional specimen was found harboring a few larger oocysts (like *C. muris*) and was disregarded for this analysis. One case had only large oocysts and was also not included. These specimens will be eventually reexamined to determine if they are compatible with *C. muris*.

Age distribution. The age distribution of the coccidium varied between urban and rural children. Urban children had similar rates of excretion of oocysts in all six-month age periods, Table 1. Rural children under one year were virtually free of infection; later, the frequency rate was similar for each six-month period, Table 1.

Breast-feeding and infection. More than 90% of all Puriscal infants are breast-fed from the time of birth [37]. Weaning in this rural area begins between two and five months, but more than 50% of infants remain at the breast at age nine months. No *Cryptosporidium* diarrheas were recorded during infancy in the rural population. Shedding of oocysts by urban infants was not uncommon, and infections were detected since the first three months of life, especially among weaned infants, Table 2.

TABLE 2 - *Diarrhea associated with Cryptosporidium according to feeding regime at the time of examination, National Children's Hospital, Costa Rica*, 1983-1985.

Age, months	Number of children	Breast-fed		Weaned	
		No.	+(%)	No.	+(%)
0-2	214	89	2(2.2)	125	2 (1.6)
3-5	250	50	0	200	22(11.0)
6-8	189	37	0	152	8 (5.3)
9-11	161	31	0	130	6 (4.6)
12-23	282	39	0	243	16 (6.6)
Total	1096	246	2(0.8)	850	54 (6.3)

TABLE 3 - *Frequency (%) of Cryptosporidium oocysts by month, preschool children, Costa Rica*, 1982-1985.

Month	With diarrhea No.	With diarrhea +(%)	Without diarrhea No.
January	151	0	18
February	167	4 (2.4)	21
March	128	5 (3.9)	29
April	127	11 (8.7)	19
May	176	10 (5.7)	15
June	94	19(20.2)	18
July	165	16 (9.7)	11
August	131	9 (6.9)	15
September	154	8 (5.2)	21
October	152	4 (2.6)	35
November *	96	0	6
December *	98	0	9
Total	1639	86 (5.2)	217

* Data for the period 1982-1984 only.

Only two breast-fed infants less than three months old were found excreting oocysts, for an overall prevalence of 0.8%. Cryptosporidiosis was common in weaned children, particularly after three months of age, and showed a high value of 11% in the second trimester of life; the overall frequency for weaned children was 6.3%.

Seasonal distribution. Cryptosporidiosis has a marked seasonal distribution in the Costa Rican populations studied. Table 3 shows cumulative monthly data for the four-year study period. Numbers are greater than in Table 1 because the month of occurrence of infection was available for all cases, while others were incomplete. No cases were found in January, November and December. These months are generally dryer and slightly colder than the rest. Cryptosporidiosis rose after February, to peak in the period June-August. The highest frequency was observed in June (20.2%). This month generally is the most calid and humid; the rainy season starts in May. Considering individual years, monthly frequencies of 10 to 20% at the peak of the season were common.

Clinical features. None of the children had first or second degree malnutrition (less than 75% weight/age) using the 50th percentile of the curve of the National Center for Health Statistics. There was no clinical evidence of immunodeficienty in any of the children. *Cryptosporidium* diarrhea generally was mild and short-lived (usually one to three days) in rural children. The hospital population represents a selection of moderate and severe cases, with diarrhea lasting one to four days. About 5% of these develop prolonged diarrhea of one to three weeks. The great majority of episodes were acute, with gruel-like or watery stools. These were devoid of macrophages, leukocytes and erythrocytes. Occult blood was found in 12% of 41 cases in whom it was investigated, Table 4.

The main signs and symptoms were, in addition to watery stools, vomiting, fever, and abdominal pain. Table 4 shows children in whom only one single pathogen was found after careful study of possible agents, as described elsewhere [40]. Vomiting was as common in cryptospor-

TABLE 4 - *Clinical features of specific diarrheal diseases among preschool children, Costa Rica*, 1983-1985.

Symptom or sign	*Cryptosporidium*		Rotavirus		*Campylobacter*	
	No.	+(%)	No.	+(%)	No.	+(%)
Vomiting	41	37(90)	300	268(89)	76	59(78)
Fever, C	28		214		53	
37.5-37.9		3(11)		15 (7)		5 (9)
38.0-38.4		5(18)		45(21)		13(24)
> 38.4		15(54)		87(41)		14(26)
Total		23(82)		147(69)		32(60)
Abdominal pain	37	21(57)	294	153(52)	74	36(49)
Dehydration, %	40		295		66	
< 5		30(75)		261(89)		54(82)
5-9		6(15)		29(10)		11(17)
> 9		4(10)		5 (2)		1 (1)
Total, 5+		10(25)		34(11)		12(18)
Convulsions	38	2 (5)	301	17 (6)	72	3 (4)
Occult blood	41	5(12)	301	32(11)	76	8(10)

idiosis as in rotavirus diarrhea. More cases of cryptosporidiosis had high fever and dehydration than did rotavirus and *Campylobacter* diarrheas. Abdominal pain was found in one half of the patients, as in other diarrheas. Weight loss wass as common as dehydration. However, no information on the natural course and impact of cryptosporidiosis on host nutrition can be provided, due to the prompt administration of fluid therapy. An effect on nutrition, however, was evident in some children, when individual growth charts were examined. Figure 1 shows the weight growth curve of a male child from Puriscal, who had adequate birth weight, and who was exclusively breast-fed for three months, and then completely weaned at age eight months. Growth faltering began around weaning time and the child failed to keep up in his growth track. When he was 19 months old, and after a spree of weight recovery, acute *Cryptosporidium*-associated diarrhea appeared after an attack of *Giardia* diarrhea. The episode lasted 25 days and was associated with pronounced weight loss, despite prompt and adequate oral fluid dehydration. Eventually, the child recovered to almost attain the weight he originally had at onset of infection. Information on the nutrition effect of *Cryptosporidium* diarrhea is under analysis.

Fluid therapy. At least 40 cases of cryptosporidiosis admitted to the hospital required fluid therapy. Oral rehydration therapy (ORT) is the choice in this hospital [38], and was applied as a single measure to 19 (47%) of the cases. Four additional patients required, in addition to ORT, either nasogastric rehydration (NGT) (two cases), intravenous fluid therapy (IVT) (one case), or both (one case). Due to the serious condition of 15 children, NGT had to be started at once, while two patients had to be given IVT upon admission. In the rural area, all cases were offered ORT, even though dehydration was not as common and severe as in urban children. Recuperation was successful. No deaths due to *Cryptosporidium*-associated diarrhea occurred during the study period.

Discussion

The homogeneity in morphology and size of oocysts excreted by Costa Rican children suggests that infections are by one single species, probably *Cryptosporidium parvum,* compatible with the description [21] and redescription [23] of Tyzzer's species. The appearance of *Crypto-*

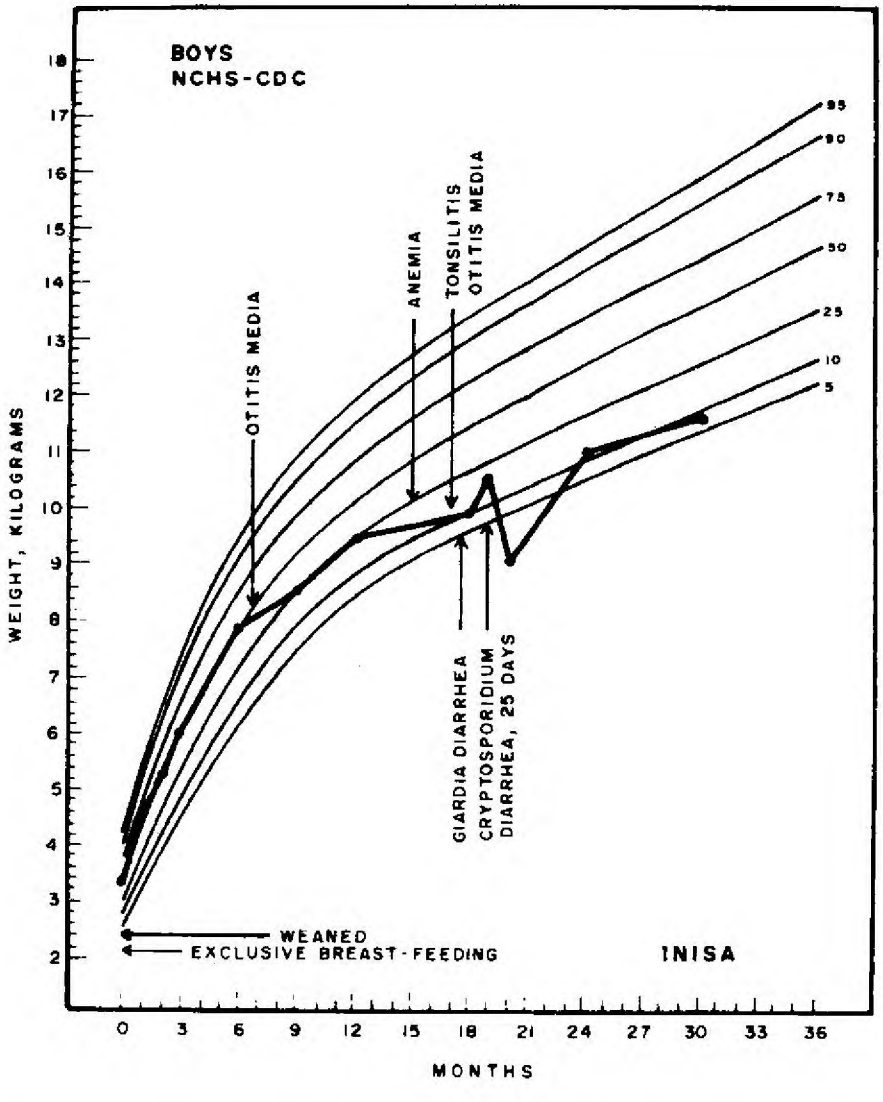

FIG. 1. Weight curve of a rural child from Puriscal, Costa Rica, in comparison with the reference curves of the National Center for Health Statistics. The child grew adequately up to 9 months of age at which point weight began faltering, to reach the 10th percentile. After an attack of *Giardia* diarrhea, and later one of *Cryptosporidium* diarrhea, the child became malnourished. After 25 days of illness, the child improved his weight, and several months later had attained the weight he had at onset of illness.

TABLE 5 - *Rehydration therapy administered to 40 children with diarrhea and dehydration associated with Cryptosporidium, National Children's Hospital, Costa Rica,* 1983-1985.

Therapy	Cases (%)
Oral rehydration therapy (ORT)	19(47)
Nasogastric rehydration therapy (NGT)	15(37)
Intravenous fluid therapy (IVT)	2 (5)
ORT + NGT	2 (5)
ORT + IVT	1 (2)
ORT + NGT + IVT	1 (2)

All treatment was given in the hospital, by health personnel supervised by pediatricians

sporidium with large oocysts in calves with different host range [41], however, suggests the possibility of additional species or strains, morphologically similar to *C. parvum* and *C. muris*. Several laboratory concentration and staining techniques have been used to diagnose these coccidian parasites [17, 39, 43-45], but the cold acidfast Kinyoun staining seems to be the best [39]. The technique does not reveal the internal morphology of oocysts, but no other structures are found in feces that can be mistaken for *Cryptosporidium* oocysts stained with these procedures. The strong bright red of the wall of oocysts permits rapid and easy identification under low power microscopic magnification. It is convenient to examine all positives with an alternative method, for instance, with Giemsa or auramine-rhodamine.

Reliance on visual examination of oocysts, however, is not satisfactory, and methods for demonstration of small amounts of antigen are urgently needed. This is particularly important to determine if healthy and asymptomatic carriers are more common than presently realized [1-5, 46], to investigate animal reservoirs, and to better understand the phenomenon of seasonality of the parasite [18, 35]. Experimental infections can be easily induced in mice [47], embryonated hen's eggs [48], and tissue culture [49], widening the opportunity to develop better diagnostic tools.

Unpublished observations in Costa Rica indicate that contact with domestic animals, in the rural area of Puriscal, does not seem to explain most

infections in rural children. It certainly does not seem to be important in urban centers devoid of cattle and other animals, but the possibility of rodents and certain insects needs investigation. A study of high-risk populations for AIDS in Costa Rica failed to reveal *Cryptosporidium* infection in male homosexuals who were free of diarrhea at the time of sampling [unpublished]. Thus, although person-to-person transmission seems logical [5], carriers are not generally detected in homes of Costa Rican children with cryptosporidiosis. Studies elsewhere have demonstrated that asymptomatic persons are reservoirs for humans, and that transmission occurs among homosexuals [see 5].

Cryptosporidium is an ubiquitous organism which was found wherever someone looked for it [1-5, 46]. Nevertheless, there is need for information on the geographical range of infection in humans, particularly as a function of socioeconomic conditions, age, nutritional status, personal hygiene and sanitation, and seasonal distribution. Due to the relatively low rate of infection found in most studies (about 5%), and the wide variability in monthly prevalence, as shown in Costa Rica, studies should extend for prolonged periods to be fruitful. Except for the studies in Costa Rica, Bangladesh and Brazil [18, 32, 33], there is no information on this question. There is no plausible explanation for the seasonal variation of the coccidium, and particularly for the virtual "disappearance" during certain months, in analogy with seasonality of rotavirus in temperate regions, or of *Shigella* in the tropics.

The apparent variation in rate of *Cryptosporidium* by age shown in this study might be related to feeding regime more than to age. In the urban area, the rate of non breast-fed and prematurely weaned infants is considerably large, while in the rural area the situation has significantly improved in the last 7 years [36]. The hospital population most likely represents a selection of severe diarrhea cases, in whom the frequency of artificial feeding and feeding problems is greater than in children observed in the rural population. The very low rate of cryptosporidiosis in breast-fed children, in contrast with high rates in weaned children, suggests protection derived from breast-feeding. Susceptibility of colostrum-fed calves to *Cryptosporidium* [50] does not equate with the situation in infants. Human colostrum and milk contain large amounts of secretory immunoglobulin A (sIgA) and other immune and resistance factors which are either very low or absent in cow's colostrum and milk. Furthermore, while antibodies in cow's colostrum are readily absorbed by the newborn calf, those in human colostrum and milk act *in situ*.

Human sIgA is active after passing through the intestine [51]. Also, studies in adults showed lower prevalences of cryptosporidiosis than in children [46]. One study, however, revealed a rate of 2.9%, almost as in children in the tropics [52]. Data on adult infection in the tropics is badly needed. Summarizing, age does not appear as important as feeding regime, as seen from the Costa Rican data.

The relative frequency of *Cryptosporidium* infection in immunocompetent children in Costa Rica, (the most prevalent after rotavirus, enterotoxigenic *Escherichia coli* and *Campylobacter jejuni*), calls for routine investigation of this coccidium. In otherwise healthy individuals, *Cryptosporidium* diarrhea is a non-invasive process, without inflammatory cells in stools, as previously reported [53]. The coccidium could be relatively more important in industrial than in less developed nations [52, 54]. One report from the United States indicates that it is the most frequent parasite found in routine serial examinations [53]. In addition to being a common pathogen in immunocompetent persons, *Cryptosporidium* is an opportunist in immunosuppressed and immunodeficient individuals, including AIDS patients [1-5]. The role of this coccidium in chronic diarrhea and in the genesis of severe malnutrition deserves consideration in poor countries.

ACKNOWLEDGMENT

The authors received support from University of Costa Rica and National Council of Scientific Research and Technology (Costa Rica), United Nations University (Tokyo), Rockefeller Foundation, Ford Foundation and UNICEF (New York), International Development Research Centre (Ottawa), and Harvard School of Public Health (Boston).

REFERENCES

[1] ANDERSON B.C., *Cryptosporidiosis: a review.* « J. Am. Vet. Med. Assoc. », *180*, 1455-7 (1982).

[2] ANGUS K.W., *Cryptosporidiosis in man, animals and birds: a review. 76*, 62-70 (1983).

[3] TZIPORI S., *Cryptosporidiosis in animals and humans.* « Microbiol. Rev. », *47*, 84-96 (1983).

[4] URBINA A., ROJAS J.C. and MATA L.J., *Criptosporidiosis: una nueva zoonosis.* « Adel. Microbiol. Enf. Infecc. », *3*, 159-181 (1984).

[5] NAVIN T.R., *Cryptosporidiosis in humans: review of recent epidemiologic studies.* « Eur. J. Epidemiol. », *1*, 77-83 (1985).

[6] LASSER K.H., LEWIN K.J. and RYNNING F.W., *Cryptosporidial enteritis in a patient with congenital hypogammaglobulinemia.* « Hum. Pathol. », *10*, 234-40 (1979).

[7] STEMMERMANN G.N., NAYASHI T., GLOBER G.A., OISHI N. and FRANKEL R.I., *Cryptosporidiosis, report of fatal case complicated by disseminated toxoplasmosis.* « Am. J. Med. », *69*, 637-42 (1980).

[8] WEINSTEIN L., EDELSTEIN S.M., MADARA J.L., FALCHUK K.R., MCMANUS B.M. and TRIER J.R., *Intestinal cryptosporidiosis complicated by disseminated cytomegalovirus infection.* « Gastroenterol. », *81*, 584-91 (1981).

[9] Centers for Disease Control (CDC). *Human cryptosporidiosis.* Alabama Vet. Pub. Hlth. Notes, pp. 75-6 (1982).

[10] MEISEL J.L., PERERA D.R., MELIGRO C. and RUBIN C.E., *Overwhelming watery diarrhoea associated with a* Cryptosporidium *in an immunosuppressed patient.* « Gastroenterol », *79*, 1156-76 (1976).

[11] WEISBURGER W.R., HUTCHEON D.F., YARDLEY J.H., ROCHE J.C., HILLIS W.D. and CHARACHE P., *Cryptosporidiosis in an immunosuppressed renal transplant recipient with IgA deficiency.* « Am. J. Clin. Pathol. », *72*, 473-8 (1979).

[12] MILLER R.A., HOLMBERG R.E. and CLAUSEN C.R., *Life-threatening diarrhea caused by* Cryptosporidium *in a child undergoing therapy for acute lymphocytic leukemia.* « J. Pediat. », *103*, 256-9 (1983).

[13] ANDREANI T., LE CHARPENTIER Y., BROUET J.C., LACHANCE J.R., MODIGLIANI R., GALIAN A., LIANCE M. and MESSING B., *Acquired immunodeficiency with intestinal cryptosporidiosis: possible transmission by Haitian whole blood.* « Lancet », *1*, 1187-91 (1983).

[14] CHIAMPI N., SUNDBERG R., KLOMPUS M.D. and WILSON A.J., *Cryptosporidial enteritis and* Pneumocystis *pneumonia in a homosexual man.* « Hum. Pathol. », *14*, 734-7 (1983).

[15] CURRENT W.L., REESE N.C., ERNST J.V., BAILEY W.S., HEYMAN M.B. and WEINSTEIN W.M., *Human cryptosporidiosis in immunocompetent and immunodeficient persons. Studies on an outbreak and experimental transmission.* « N. Engl. J. Med. », *308*, 1252-7 (1983).

[16] MA P., VILLANUEVA T.G., KAUFMAN D. and GILLOOLEY J.F., *Respiratory cryptosporidiosis in the acquired immune deficiency syndrome. Use of modified cold Kinyoun and Hemacolor stains for rapid diagnoses.* « JAMA », *252*, 1298-301 (1984).

[17] TZYPORI S., SMITH M., BIRCH C., BARNES G. and BISHOP R., *Cryptosporidiosis in hospital patients with gastroenteritis.* « Am. J. Trop. Med. Hyg. », *32*, 931-933 (1983).

[18] MATA L., BOLANOS H., PIZARRO D. and VIVES M., *Cryptosporidiosis in children from some highland Costa Rican rural and urban areas.* « Am. J. Trop. Med. Hyg. », *33*, 24-9 (1984).

[19] TYZZER E.E., *A sporozoan found in the peptic glands of the common mouse.* « Proc. Soc. Exp. Biol. Med. », *5*, 12-13 (1907).

[20] TYZZER E.E., *An extracellular coccidium*, Cryptosporidium muris (Gen. et sp. nov.), *of the gastric glands of the common mouse.* « J. Med. Res. », *23*, 487-509 (1910).

[21] TYZZER E.E., Cryptosporidium parvum (sp. nov.), *a coccidium found in the small intestine of the common mouse.* « Archiv. fur Protistenkunde », *26*, 394-412 (1912).

[22] LEVINE N.D., CORLISS J.O., COS F.E.G., DEROUX G., GRAIN J., HONIGBERG B.M., LEEDALE G.F., LOEBLICH A.R., LOM J., LYNN D., MERINFELF E.G., PAGE F.C., POLJANSKI G., SPRAGUE V., VAVRA J. and WALLACE F.G., *A newly revised classification of the protozoa.* « J. Protozool. », *27*, 37-58 (1980).

[23] UPTON S.J. and CURRENT W.L., *The species of* Cryptosporidium (*Apicomplexa: Cryptosporidiidae*) *infecting mammals.* « J. Parasit. », *7*, 625-9 (1985).

[24] TZIPORI S., ANGUS K.W., CAMPBELL I. and GRAY E.W., Cryptosporidium: *evidence for a single-species genus.* « Infect. Immun. », *30*, 884-6 (1980).

[25] LEVINE N.D., *Taxonomy and review of the coccidian genus* Cryptosporidium (*Protozoa, Apicomplexa*). « J. Protozool. », *31*, 94-8 (1984).

[26] Clinicopathological Conference. *Immunodeficiency and cryptosporidiosis: demonstration at the Royal College of Physicians of London.* « Br. Med. J. », *281*, 1123-7 (1980).

[27] BIRD R.G. and SMITH M.D., *Cryptosporidiosis in man: parasite life cycle and fine structural pathology.* « J. Pathol. », *132*, 217-33 (1980).

[28] PITLIK S.D., FAINSTEIN V., RIOS A., GUARDA L., MANSELL P. and HERSH E., *Cryptosporidial cholecystitis.* « N. Engl. J. Med. », *308*, 967-9 (1983).

[29] KOCOSHIS S.A., CIBULL M.L., DADIS T.E., HINTON J.T., SEIP M. and BANWELL G., *Intestinal and pulmonary cryptosporidiosis in an infant with severe combined immunodeficiency.* « J. Pediat. Gastroenterol. », *3*, 149-57 (1984).

[30] MELE L., NADLER H., PAPPALARDO S., FORGACS P., SHEA J., JURTZ S. and MA P., Cryptosporidium: *an unusual respiratory tract isolate.* In: Proc. 83rd Ann. Meet. Am. Soc. Microbiol. Washington DC: Am. Soc. Microbiol., 1983.

[31] NIME F.A., BUREK J.D., PAGE D.L., HOLSCHER M.A. and YARDLEY J.H., *Acute enterocolitis in a human being infected with the protozoan* Cryptosporidium. « Gastroenterol », *70*, 592-8 (1971).

[32] SHAHID N.S., RAHMAN A.S.M., ANDERSON B.C., MATA L.J. and SANYAL S.C., *Cryptosporidiosis in Bangladesh.* « Lancet », *1*, 114-5 (1985).

[33] LOUREIRO E.C.B., LINHARES A. DA C. and MATA L., *Acute diarrhoea associated with* Cryptosporidium *sp. in Belem, Brazil.* « Rev. Bras. Microbiol. » in press (1986).

[34] PEREZ-SCHAEL I., BOHER Y., MATA L., PEREZ M. and TAPIA F.J., *Cryptosporidiosis in Venezuelan children with acute diarrhea.* « Am. J. Trop. Med. Hyg. », *34*, 721-2 (1985).

[35] URBINA A., MATA L. and PIZARRO D., Cryptosporidium *en niños de Costa Rica: cuadro clínico, variación estacional y tratamiento.* « Acta Med. Costarricense », *27*, 191-8 (1984).

[36] MATA L., CARVAJAL J.J., GARCIA M.E., SAENZ P., ALLEN M.A., ARAYA J.R. and RODRIGUEZ M.E., *Promotion of breast-feeding, health and survival of infants through hospital and field interventions.* In: *Malnutrition Determinants and Consequences.* Western Hemisphere Nutrition Congress, Miami, 1983. New York: Alan R. Liss, Inc.; 123-38.

[37] MOHS E., *Infectious diseases and health in Costa Rica: the development of a new paradigm.* « Ped. Infect. Dis. », *1*, 212-6 (1982).

[38] NALIN D.R., LEVINE M.M., MATA L., DE CESPEDES C., VARGAS W., LIZANO C., LORIA A.R., SIMHON A. and MOHS E., *Oral rehydration and maintenance of children with rotavirus and bacterial diarrhoeas.* « Bull. Wld. Hlth. Org. », *57*, 453-9 (1979).

[39] MA P., *Laboratory diagnosis of coccidiosis.* In: Leive L., Schlessinger D., eds., *Microbiology 1984.* Washington DC: Am. Soc. Micriobiol., pp. 224-31 (1984).

[40] MATA L., SIMHON A., PADILLA R., GAMBOA M.M., VARAGAS G., HERNANDEZ F., MOHS E. and LIZANO C., *Diarrhea associated with rotaviruses, enterotoxigenic* Escherichia coli, Campylobacter, *and other agents in Costa Rican Children, 1976-1981.* « Am. J. Trop. Med. Hyg. », *32*, 146-53 (1983).

[41] ANDERSON B.C., *Abomasal cryptosporidiosis.* « Am. Con. Vet. Path., Dec. 1985.

[42] GARCIA L.S., BRUCKNER D.A., BREWER T.C. and SHIMIZU R.Y., *Techniques for the recovery and identification of* Cryptosporidium *oocysts from stool specimens.* « J. Clin. Microbiol. », *18*, 185-90 (1983).

[43] KAGERUKAP J., BRANDT J.R., TAELMAN H. and JONAS C., *Modified Koster staining method for the diagnosis of cryptosporidiosis.* « Ann. Soc. Belg. Med. Trop. », *64*, 171-5 (1984).

[44] ZIERDT W.S., *Concentration and identification of* Cryptosporidium *sp. by use of a parasite concentrator.* « J. Clin.. Microbiol. », *20*, 860-1 (1984).

[45] POHJOLA S., JOKIPII L. and JOKIPII A.M., *Dimethylsuophoxide-Ziehl-Neelsen staining technique for detection of cryptosporidial oocysts.* « Vet. Rec. », *116*, 442-3 (1985).

[46] NAVIN T.R. and JURANEK D.D., *Cryptosporidiosis: clinical, epidemiologic, and parasitologic review.* « Rev. Infect. Dis. », *6*, 313-27 (1984).

[47] ANDERSON B.C., *Moist heat inactivation of* Cryptosporidium *sp.* « Am. J. Pub. Health », *75*, 1433-4 (1985).

[48] CURRENT W.L., Cryptosporidium *and cryptosporidiosis of domestic animals and man.* Fourth International Symposium on Neonatal Diarrhea, Saskatoon, Canada. Vet. Rec. Infect. Dis., pp. 293-307 (1983).

[49] CURRENT W.L. and HAYNES T.B., *Complete development of* Cryptosporidium *in cell culture* « Science », *224*, 603-5 (1984).

[50] SNODGRASS D.R., STEWART J., TAYLOR J., KRAUTIL F.L. and SMITH M.L., *Diarrhoea in diary calves reduced by feeding colostrum from cows vaccinated with rotavirus.* « Res. Vet. Sci. », *32*, 703 (1982).

[51] HANSON L.A., CARLSSON B., AHLSTEDT S., SVANBORG C. and KAIJSER B., *Immune defense factors in human milk.* « Mod. Prob. Pediatr. », *15*, 63-72 (1975).

[52] HUNT D.A., SHANNON R., PALMER S.R. and JEPHCOTT A.E., *Cryptosporidiosis in an urban community.* « Br. Med. J. Clin. Res. », *289*, 814-6 (1984).

[53] BOLANOS H., SIMHON A. and MATA L., *Citología de excretas diarreicas asociadas a rotavirus,* Campylobacter *y* Cryptosporidium. « Acta Med. Cost. », *28*, 62-5 (1985).

[54] HOLLEY H.P. and DOVER C., Cryptosporidium: *a common cause of parasitic diarrhea in otherwise healthy individuals.* « J. Infect. Dis. », *153*, 365-8 (1986).