

## CRYPTOSPORIDIOSIS IN CHILDREN FROM SOME HIGHLAND COSTA RICAN RURAL AND URBAN AREAS\*

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**Abstract.** This report summarizes both a prospective study of diarrhea in cohorts of rural children in their natural ecosystem, and a vertical study of diarrheic urban children attending a hospital emergency service. *Cryptosporidium* oocysts were found in feces of 4.3% of the cases, while all controls were negative. No infection occurred in the first year of life among rural infants, contrasting with a 3% infection rate in children under 1 year of age in the metropolitan area. This could be attributed to intense and exclusive breast-feeding for several months in the rural area while in the urban area many infants are not breast-fed at all, or are weaned prematurely. No infection was found in wholly breast-fed infants. Diarrhea associated with *Cryptosporidium* was watery and without inflammatory cells. Dehydration was common in urban children, but was rapidly corrected by oral rehydration therapy, or by intravenous fluid therapy in some cases. Infections clustered in the warmer, rainy and humid months of the year.

*Cryptosporidium* oocysts have been found in the stools of man, mammals, birds and reptiles,<sup>1-3</sup> and antibodies to the parasite have been detected in several vertebrates.<sup>4</sup> While more than 10 species of *Cryptosporidium* have been described, the striking similarity of its oocysts and other evolutive forms found in animals and man, and the lack of host specificity of animal and human strains, suggest the existence of one single species with a broad range of vertebrate hosts.<sup>5,6</sup> *Cryptosporidium* was found to cause severe diarrhea in an immunocompetent child,<sup>7</sup> and in immunosuppressed<sup>8,9</sup> and immunodeficient patients,<sup>10-12</sup> including persons with acquired immunodeficiency syndrome (AIDS).<sup>13</sup> *Cryptosporidium* can infect immunocompetent individuals, and there are increasing reports of its occurrence in the general population.<sup>14-18</sup> The present communication summarizes 12 months of prospective observation of a child population in rural Puriscal, Costa Rica, as well as a 9-month surveillance of a hospital-based child population in metropolitan Costa Rica.

### MATERIALS AND METHODS

The populations were of similar ethnic background and lived at an average altitude of 1,000

meters. One consisted of rural children who were studied prospectively in Puriscal (a cohort study), a very mountainous region of about 600 km<sup>2</sup> with 152 localities of very sparse rural families in transition from the traditional to the modern way of life.<sup>19</sup> Communication between dwellings and among localities is often difficult. The marked ruralism contrasts with a high degree of education and social development, a low incidence of diarrhea, and a good level of health. One stool specimen was collected from each of 95 infants and preschool children with acute diarrhea, and from each of 39 comparable children without diarrhea, from January through December of 1982. Diarrhea cases represented about 10% of all expected episodes in 1982, according to past experience with Puriscal children observed prospectively from birth.<sup>20</sup> Undoubtedly, cases were those of greater severity demanding more attention from attendants and the health center staff.

The other population consisted of children of the San José and Heredia metropolitan area, who were seen as outpatients at the National Children's Hospital. One stool specimen was collected from each of 183 infants and preschool children with acute diarrhea, and from each of 51 comparable children without diarrhea, from April through December of 1982. They belonged primarily to poor sectors, living in relatively crowded conditions. Cases represented a small portion of the total diarrheas observed in the emergency service and there was no special selection of the sample. Studies on the etiology of

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TABLE 1

Population of children from rural Puriscal and from the metropolitan area, with and without diarrhea, by age, examined for *Cryptosporidium*

Age group (months)	Rural				Metropolitan			
	With diarrhea		Without diarrhea		With diarrhea		Without diarrhea	
	No.	Mean age (months)	No.	Mean age (months)	No.	Mean age (months)	No.	Mean age (months)
0-5	25	3.0	9	2.9	91	2.6*	11	2.9
6-11	28	8.6	12	8.5	62	8.2	24	7.8
12-17	25	14.4	11	14.6	21	13.8	11	14.3
18-23	12	20.7	4	21.7	9	20.2	5	19.6
24-29	5	27.2	3	26.0	NC†	—	NC	—
Total	95	11.1	39	11.6	183	11.0	51	9.3

\* Six infants were less than 1 month of age.

† NC, not collected.

diarrhea have been under way in this population since 1976.<sup>21</sup> No significant differences were noted in mean age of children with and without diarrhea, or between urban and metropolitan residence (Table 1). Also, the mean age for each age group was very similar across the four population groups (Table 1), owing to the unbiased manner in which cases were included in the study.

Children were evaluated by physicians who used a precoded form and standard definitions to record information. Dehydration was expressed as percentage of body weight lost during the attack. Stools were collected in cardboard boxes or glass jars and were processed within 2 hours of evacuation. Hospital investigations were performed for rotavirus, *Campylobacter*, and other parasites with techniques described elsewhere.<sup>21</sup> In Puriscal, cases were studied for these agents and for classic pathogenic enterobacteriaceae.<sup>22</sup>

To detect *Cryptosporidium* oocysts, fecal smears on glass slides were dried at room temperature, fixed with absolute methyl alcohol for 3 min, and stained with Giemsa for 20-30 min. Oocysts diagnosed under immersion oil (1,000 $\times$ ) appear as ovoid structures of relatively uniform size (Fig. 1). In heavy infections, oocysts can be easily found under low magnification, and may appear as "empty holes." Oocysts stain faintly blue with reddish and purple corpuscles; they can be recognized in unstained feces concentrated by zinc sulphate flotation, and in feces suspended in polyvinyl alcohol-Schaudinn fixative. Diagnosis is easier in Giemsa-stained smears where they can be clearly differentiated from yeasts, cysts of *Enteromonas*, *Blastocystis*, and other structures and artifacts.

## RESULTS

In Giemsa-stained smears examined under immersion oil, twenty *Cryptosporidium* oocysts measured  $4.1 \pm 0.5$  by  $5 \pm 0.4$   $\mu$ m (mean  $\pm$  standard deviation). The range of variation was 3.6-4.6  $\mu$ m (small diameter) and 4.6-5.4  $\mu$ m (large diameter). Oocysts were found in feces of four diarrheic rural children (4.2% frequency) and in eight diarrheic urban children (4.4% frequency) (Table 2). Nine of the 12 were pure infections (see Table 4). Oocysts were not found in non-diarrheic control children of both populations. Other agents found in rural and urban diarrheic children were, respectively, rotaviruses (18% and 34.4%), *Campylobacter* (8.4 and 7.1%), *Shigella* (3.1% rural), *Giardia* (7.2 and 3.1%) and *Entamoeba histolytica* (4.3% rural). Controls were negative for these agents.

*Cryptosporidium* was not observed in children less than 1 year of age in the rural area; the prevalence rose from 4% in the 3rd semester of life to 20% in the 5th semester (Table 2). In contrast, 3% of children under 1 year of age were found infected in the urban area; infection increased from 2% in the 1st semester of life to 11% in the 4th semester. The mean age of *Cryptosporidium*-infected urban children was 9.7 months, and that of rural children 22.7 months, a marked age difference, contrasting with the similarity in age composition of both groups (Table 1). Ninety-three percent of the rural cohort infants breast-fed exclusively for at least 1 month and more than 80% remained at the breast at age 3 months. About 15% of urban infants do not breast-feed at all and weaning starts earlier than in the rural area.<sup>23</sup>

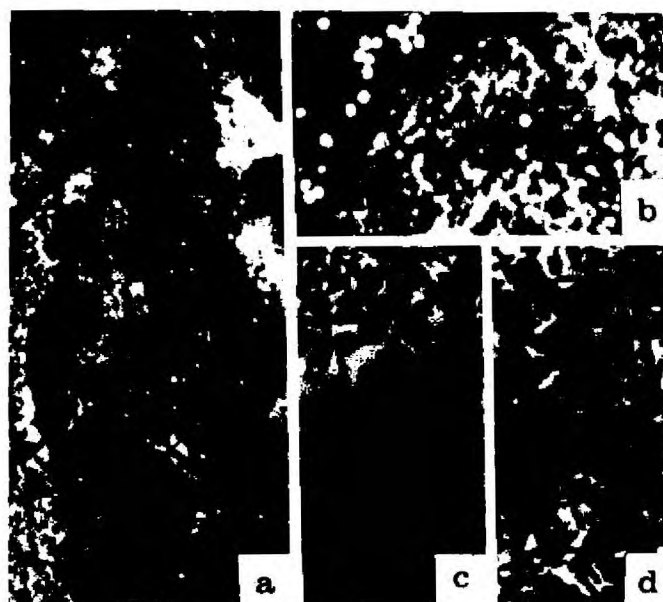


FIGURE 1. *Cryptosporidium* oocysts in feces of Costa Rican children. Note appearance as "empty holes" in a and b. Typical oval oocysts with distinct wall and internal corpuscles are shown in c and d. Giemsa stain. a,  $\times 100$ ; b,  $\times 400$ ; c and d, under immersion oil,  $\times 1,000$ .

All cases of *Cryptosporidium* occurred in June–August in the rural area and in May–June in the urban area (Table 3), that is, during the warm, rainy and humid months locally referred as the "winter." However, climatic variations are minor throughout the year. No *Cryptosporidium* cases were discovered during September–March. September through November are very rainy but less warm than May–August, and December through April are generally cool and dry, a period locally referred as the "summer."

The diarrhea associated with *Cryptosporidium* ranged from mild to severe with numerous watery stools and dehydration (Table 4). No erythrocytes, macrophages, segmented leukocytes or other inflammatory cells were present in the stools. Vomiting, dehydration and fever were more common in hospital outpatients than in rural children. Three of the urban children were hyponatremic. Plasma values in mmol/liter ( $M \pm SE$ , range) in urban cases were, upon admission:  $Na^+ = 131.8 \pm 2.5$ , 125–138;  $K^+ = 4.0 \pm 0.76$ ,

TABLE 2  
Frequency of *Cryptosporidium* oocysts in feces of Costa Rican preschool children with and without diarrhea, 1982

Age (months)	Rural			Metropolitan		
	With diarrhea		Without diarrhea*	With diarrhea		Without diarrhea*
	No. children	No. (%) with <i>Cryptosporidium</i>		No. children	No. (%) with <i>Cryptosporidium</i>	
0–5	25	0	9	91	2 (2)	11
6–11	28	0	12	62	3 (5)	24
12–17	25	1 (4)	11	21	2 (9)	11
18–23	12	2 (17)	4	9	1 (11)	5
24–29	5	1 (20)	3	NC†	—	NC
Total	95	4 (4)	39	183	8 (4)	51

\* Controls were negative for *Cryptosporidium*.

† NC, not collected.

TABLE 3

*Seasonality of Cryptosporidium infection in Costa Rican preschool children with and without diarrhea, 1982*

Month	Rural			Metropolitan		
	With diarrhea		Without diarrhea*	With diarrhea		Without diarrhea*
	No. children	No. (%) with <i>Cryptosporidium</i>		No. children	No. (%) with <i>Cryptosporidium</i>	
January	2	0	NC†	NC	—	NC
February	12	0	NC	NC	—	NC
March	18	0	12	NC	—	NC
April	8	0	6	17	0	NC
May	9	0	4	30	2 (7)	5
June	6	1 (17)	1	24	6 (25)	7
July	8	1 (12)	2	20	0	6
August	12	2 (17)	2	NC	—	NC
September	4	0	2	29	0	10
October	6	0	6	33	0	12
November	6	0	2	9	0	4
December	4	0	2	21	0	7
Total	95	4 (4)	39	183	8 (4)	51

\* Controls were negative for *Cryptosporidium*.

† NC, not collected.

2.5–6.6; and osmolality  $275.5 \pm 3.1$ , 268–283. One rural child and five urban children were given oral glucose salt solution; two of these required gastroclisis and one intravenous fluid therapy for rehydration. Rehydration and prompt remission was observed within a few hours after administration of fluid therapy. There were no

fatalities. Two rural children, one of whom harbored *Trichuris*, developed recurrent diarrhea.

All but one *Cryptosporidium* infection occurred in children who either had not received human colostrum and milk, or who had been weaned several months before infection was demonstrated. One exception was recorded but

TABLE 4

*Clinical features of Cryptosporidium diarrhea in Costa Rican preschool children, 1982*

Case	Sex	Locality	Age (months)	Age of weaning (months)*	Duration of diarrhea (days)	Vomiting†	% dehydration	Fluid therapy‡	Fever (°C)	Hypotension‡	Other agents
<i>National Children's Hospital, San José</i>											
AGMA	M	Paso Ancho	1	NBF	10	—	5–7	IV	<37	+	—
YTA	M	Alajuelita	3	2	10	—	5–7	OR	38.2	—	—
JAAA	M	Cinco Esquinas	8	NBF	14	+	3	OR	39	—	—
CVO	F	Santo Dom. Heredia	8	2	3	+	3	GL	<37	—	Rotavirus
LCSR	M	San Pablo Heredia	10	NBF	10	+	3	OR	<37	—	—
EEV	F	Hatillo, S.J.	13§	1	15	+	5–7	OR	39.6	+	—
YMSC	M	San Anto. Belen	17§	NBF	19	+	5–7	OR	<37	+	Strongyloides
AGAL	M	Desamparados	18	8	23	+	3	GL	38.5	—	—
<i>INISA's Field Station, Puriscal</i>											
1092	F	Santiago	16	3	2	—	0	—	<37	—	—
0534	M	Santiago	23	NBF	20	—	0	—	<37	—	Trichuris
0574	F	Santiago	23	3	15	—	0	—	<37	—	—
0182	M	Grifo Alto	29	**	7	+	3	OR	+	—	—

\* NBF, not breast-fed.

† +, present; —, absent.

‡ OR, oral rehydration; GL, gastroclisis; IV, intravenous fluid.

§ Mild energy-protein malnutrition.

|| Unknown.

\*\* At the breast when seen but had received weaning foods since 1 month of age.

this child, who was found infected at age 29 months, had received weaning foods since 1 month of age. This child was receiving very small amounts of human milk after such a protracted weaning process. No infections occurred in children exclusively breast-fed.

#### DISCUSSION

The present systematic exploratory study suggests that *Cryptosporidium* is relatively common in small children in Costa Rica. The 4.3% rate in acute diarrheas from highland rural and urban areas of Costa Rica, compared with rates of other pathogens in the same population,<sup>21, 22</sup> places this parasite after rotavirus, enterotoxigenic *E. coli*, *Campylobacter* and *Shigella*. The pathogenic potential of *Cryptosporidium* seems high since no infections were found in non-diarrheic children in either the rural or urban setting. On the other hand, the absence of infection among rural infants effectively breast-fed during the first months of life<sup>19, 23</sup> contrasts with the precocity of infection in the urban area, where many infants are not breast-fed at all or are weaned at an early age.<sup>23</sup> In fact, all cases of *Cryptosporidium* were in infants who either did not receive colostrum or human milk, or who had been weaned several months prior to discovery of infection. The 1-month-old infected child had not received human milk at all. Furthermore, urban cases were more severe, and a previous study of a large series of neonates with dehydrating diarrhea of the same urban population showed a very low rate of breast-feeding.<sup>24</sup> Thus, breast-feeding may have accounted for the difference in severity and age distribution between the two populations.

All *Cryptosporidium* infections appeared in the warmer and humid months of the year in both settings, suggesting that environmental factors play a role in infection. *Cryptosporidium* readily infects immunocompetent individuals,<sup>14-18</sup> and the outcome of infection in children may be severe diarrhea with moderate to severe dehydration, although this is easily corrected by rehydration therapy. Infection could be serious in children with severe energy-protein malnutrition owing to their altered immune function, in analogy with observations in immunodeficient individuals,<sup>9-13</sup> but only two rural children had mild energy-protein malnutrition. The mechanism by which *Cryptosporidium* diarrhea develops is unknown. Since the parasite localizes on the surface

of ilean mucosal cells,<sup>2, 7-12, 16-18</sup> no serious permanent damage to the mucosa is expected in well-nourished children. However, *Cryptosporidium* diarrhea may evolve into chronic diarrhea and may induce malnutrition in young children, as has been shown to be the case with other specific diarrheas.<sup>19, 23, 25</sup>

#### ADDENDUM

Examination of children in the same highland rural and urban areas has continued, and 335 additional children with diarrhea were examined from January through August. The frequency of *Cryptosporidium* infection in this additional series was 2.4%. A marked seasonality was again observed and all infections were found in July-August, yielding a rate of 9.4%. The year 1983 has been unusual in that the rainy season has been the mildest in more than a decade, with rains starting about 1 month later than normal.

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