Annals of Tropical Paediatrics (2001) 21, 313–318

Seven years' experience with *Cryptosporidium parvum* in Guinea-Bissau, West Africa

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(Accepted June 2001)

Summary In community-based studies conducted from 1991 to 1997 in Guinea-Bissau, West Africa, stool specimens from children aged less then 5 years with diarrhoea were routinely examined for enteric parasites. *Cryptosporidium parvum*, found in 7.7% of 4,922 samples, was the second most common parasite, exceeded only by *Giardia lamblia* which was found in 14.8% of the samples. The highest prevalence of cryptosporidium was found in children aged 6–11 months, whereas the prevalence of other enteric parasites increased with age. Cryptosporidiosis showed a marked seasonal variation, with peak prevalences found consistently at the beginning of or just before the rainy seasons, May through July. By contrast, no seasonality was found for the enteric parasites *Giardia lamblia* or *Entamoeba histolytica*. We conclude that *Cryptosporidium parvum* is an important pathogen in children with diarrhoea.

Introduction

The protozoan parasite *Cryptosporidium parvum* has in recent years been recognized as a common enteric pathogen in humans.[1] It has been described as an emerging infectious threat,[2] causing gastro-enteritis in otherwise healthy individuals, especially children,[3] with the potential to cause life-threatening disease in the immunocompromised host.

Most infections in immunocompetent hosts are self-limiting. However, infections in young children in developing countries have been associated with an acute and long-term effect on weight gain, severe persistent diarrhoea and excess mortality.[4–7] We therefore need to learn more about *Cryptosporidium* infections to improve treatment and control, especially in relation to children in developing countries.

Several descriptive and intervention studies of diarrhoea have been carried out in Bissau, West Africa.[8,9] As part of the continuous surveillance of intestinal parasites among children with diarrhoea, we examined all samples for *Cryptosporidium parvum*. Samples collected between 1991 and 1997 were used to examine the age pattern and seasonality of cryptosporidosis.

Material and methods

Study area and population

Faeces samples were collected from children less than 5 years of age living in the peri-urban

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ISSN 0272-4936 print/ISSN 1465-3281/online/01/040313-06
Carfax Publishing
DOI: 10.1080/07430170120093490

areas Bandim I, Bandim II, Belem and Mindera in the capital, Bissau. The area has a population of approximately 46,000, representing all major ethnic groups of Guinea-Bissau.

The houses are built in a modified traditional manner with clay bricks and concrete floors, and the roofs are usually of corrugated iron. Most houses are inhabited by several families who have access to a pit latrine and a well. Although there are standpipes in the area, they have insufficient capacity, especially at the end of the dry season. There is no sewerage system and no system for removing waste, although in recent years open drainage systems have been built in most parts of the area. Many households have domestic animals, including pigs, chicken and goats, that roam freely in the neighbourhood.

The climate in Bissau is dominated by two seasons: a dry season from December to May and a rainy season from June to November. The rainy season is characterized by daily showers, high temperatures (mean 29°C) and high humidity; temperatures in the dry season (mean 25°C) are slightly lower.

Faecal samples

This report summarizes data collected during a 7-year period from 1991 to 1997. Several epidemiological studies were carried out during this time, including studies of measles, varicella and treatment of diarrhoea. The study population was children less than 5 years of age[9,10] who represented random samples of the normal childhood population in the area. Because of the various study designs, sample size was not constant over the years. In 1995 particularly, a study on ORS efficacy resulted in a large number of samples.[10]

Children were followed weekly by home visits in the respective studies, but all children less than 3 years of age in the study area were visited at least once every 3 months. A faecal sample was collected from them when, according to the mother or person in charge, they had diarrhoea.

Microbiology

Stools were collected in plastic containers and kept in an insulated box with ice-packs until being processed within 15–18 hours. Examination for enteric parasites was made by direct microscopy of a wet mount and an iodine-stained wet mount. To demonstrate helminthic eggs, faeces were examined microscopically after sodium chloride flotation. Approximately 1 g of faeces was concentrated by the formol–ether technique and the iodine-stained sediment examined by microscopy for cysts of *Giardia lamblia* and *Entamoeba histolytica*. To demonstrate *Cryptosporidium* spp. oocysts, a thick smear was stained by the modified Ziehl–Neelsen technique.[11]

Results

From 3 January 1991 until 19 December 1997, 4,922 diarrhoea stool samples were collected and analysed for parasites. A total of 1,846 (37.5%) samples were positive for parasites. Table I shows the results; 377 (7.7%) were positive for *Cryptosporidium parvum*. Prevalences were similar for boys and girls, except that more female samples were positive for *Giardia lamblia* and *Entamoeba histolytica*.

The 4,922 samples were from 2,681 children, *Cryptosporidium parvum* parasites being demonstrated in 351 (13.1%). Figure 1 shows the age-specific prevalences of *Cryptosporidium parvum, Giardia lamblia, Entamoeba histolytica, Strongyloides stercoralis, Ancylostoma duodenale* and *Hymenolepsis nana*. The prevalences of all intestinal parasites, except *Cryptosporidium parvum*, increased with age. In contrast, the prevalence of cryptosporidium peaked in children aged 6–11 months and gradually decreased with age after 12 months. However, the prevalence in the 36–41 months age group diverged from this pattern. Seven of the positive samples in this age group were identified in 1991. Excluding the data collected in 1991, the prevalence in this age group was only 4.6%.

TABLE I. Isolation of enteric parasites from 4,922 stool samples collected in conjunction with a diarrhoeal episode.

Organism	Proportion (%)	Male (%)	Female (%)	Relative risk (95% CI)
Chilomastix mesnili	2.1 (104/4904)	1.9 (52/2690)	2.3 (52/2214)	0.82 (0.56–1.20)
Cryptosporidium spp	7.7 (377/4922)	7.7 (209/2699)	7.6 (168/2223)	1.02 (0.84–1.25)
Endolimax nana	0.7 (34/4903)	0.5 (13/2690)	0.9 (21/2213)	0.51 (0.26–1.01)
Entamoeba coli	5.1 (249/4901)	5.2 (140/2688)	4.9 (109/2213)	1.06 (0.83–1.35)
Entamoeba hartmanni	2.8 (136/4904)	3.0 (80/2690)	2.5 (56/2214)	1.18 (0.84–1.65)
*Entamoeba histolytica**	1.9 (65/3463)	1.4 (28/1934)	2.4 (37/1529)	0.60 (0.37–0.97)
Entamoeba histolytica†	6.9 (339/4905)	7.1 (191/2690)	6.7 (148/2215)	1.06 (0.86–1.31)
Entamoeba histolytica‡	7.0 (345/4922)	7.2 (194/2699)	6.8 (151/2223)	1.06 (0.86–1.30)
*Giardia lamblia**	4.2 (206/4906)	3.6 (96/2691)	5.0 (110/2215)	0.72 (0.55–0.94)
Giardia lamblia†	14.7 (721/4906)	13.5 (364/269)1	16.1 (357/2215)	0.84 (0.73–0.96)
Giardia lamblia‡	14.8 (725/4903)	13.6 (365/2690)	16.3 (360/2213)	0.83 (0.73–0.95)
Trichomonas hominis	1.0 (49/4904)	0.9 (25/2690)	1.1 (24/2214)	0.86 (0.49–1.50)
Ancylostoma duodenale	1.8 (89/4906)	1.7 (47/2691)	1.9 (42/2215)	0.92 (0.61–1.39)
Hymenolepsis nana	1.6 (80/4906)	1.5 (41/2691)	1.8 (39/2215)	0.87 (0.56–1.34)
Strongyloides stercoralis	0.7 (33/4905)	0.7 (19/2690)	0.6 (14/2215)	1.12 (0.56–2.22)

*Trophozoites; † cysts; ‡ trophozoites and cysts (total no. of infections with either one or both).

The prevalence of *Cryptosporidium parvum* by month is shown in Fig. 2, together with the monthly rainfall. The peak prevalences (7–35%) were closely related to the rainy season (June–November). It will be seen that there was a sudden increase at the beginning of or just before the rainy season in May and June. The peak was followed by a gradual decrease during the following months. Practically no or very few cases of cryptosporidosis were found during the other half of the year. This pattern was not related to the amount of rain or the duration of the rainy season.

We did not observe seasonal variations for the other enteric parasites *Giardia lamblia* and *Entamoeba histolytica* (data not shown).

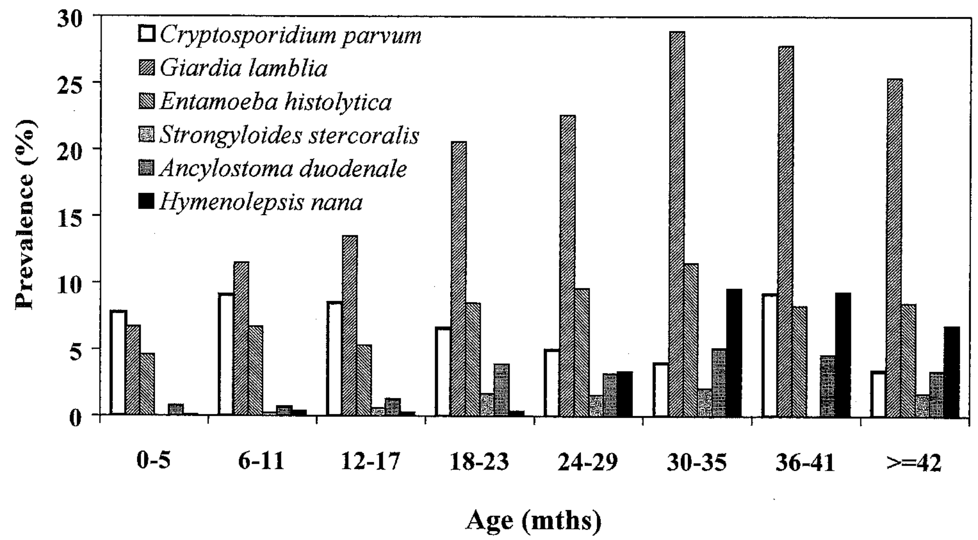

FIG. 1. Age-specific prevalences of *Cryptosporidium parvum, Giardia lamblia, Entamoeba histolytica, Strongyloides stercoralis, Ancylostoma duodenale* and *Hymenolepsis nana* in children aged 0–5 years in Bissau, 1991 to 1997.

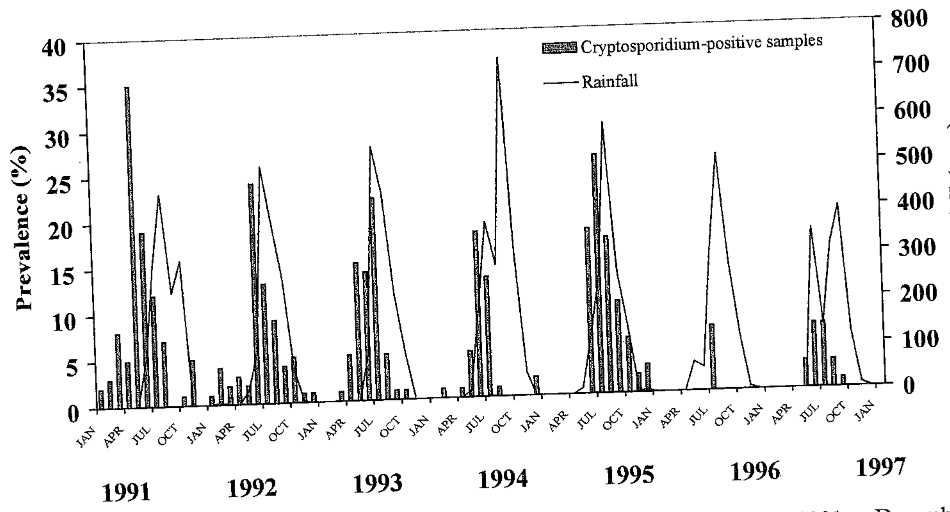

FIG. 2. Monthly prevalence of *Cryptosporidium parvum vs* monthly rainfall from January 1991 to December 1997.

Discussion

In the present study, unique because of its duration and the amount of data collected, we show that *Cryptosporidium parvum* is a common parasite in diarrhoeal stools. Only *Giardia lamblia* was found more frequently. This is consistent with other findings in the third world.[8,12]

The age distribution is unusual for an enteric parasite. The peak prevalence at 6–11 months of age might be related to a low infectious dose[13] compared with other enteric parasites as well as to decreased hygiene when children become more mobile, but other explanations are also possible. They include different modes of transmission, e.g. respiratory transmission[14] or different vehicles for faeco-oral transmission,[15,16] including water-borne transmission[17] and houseflies.[18,19]

Only a few samples were examined in children over 3 years of age, and the high prevalence among the 36–41 months age group (95% confidence interval, 4.5–15%) could be related to their small number or to some particular spread in 1991.The high prevalence at this age is not biologically plausible, and the overall pattern suggests a decrease in prevalence after 12 months of age.

This decrease is most probably related to the development of immunity.[20,21]

The age pattern indicates the possibility of inducing immunity and suggests the possibility of a vaccine against *Cryptosporidium parvum*.

The parasite exhibits a very marked seasonal variation, which we have now documented over 10 years.[6] A similar seasonality has been observed in other low-income societies.[22,23] The increase in childhood diarrhoea during the rainy season, observed in many developing countries, is likely to be related to an increase in the transmission of various pathogens, including *Cryptosporidium*, ETEC and in certain years also *V. cholera* and *Shigella*.[8]

The number of *Cryptosporidium*- and *Giardia lamblia*-positive samples has decreased during the last few years. This correlates with information on the total incidence of diarrhoea in the study area, which has decreased as well (unpublished data). The reason for this is unknown, but might be due to an extension of the standpipe as well as the sewerage system. Furthermore, following cholera epidemics in 1994 and again in 1996–1997, a number of hygiene measures were implemented which might have reduced overall transmission of enteric pathogens.

The limitations of the present study were twofold. First, the samples were not collected specifically for the study, and we did not use a uniform case definition for diarrhoea over the years. The classification was based mainly on the mother's perception, but studies have shown that the vast majority of mothers understand the concept of diarrhoea.[24] Second, there were no data on the prevalence of *Cryptosporidium parvum* in asymptomatic controls. However, previous studies from Bissau have shown that this parasite is associated with diarrhoeal disease.[8]

With these limitations, the present analyses show that *Cryptosporidium parvum* is an important pathogen in children with diarrhoea. Furthermore, the parasite has an age-specific prevalence pattern, which is unusual for enteric parasites, and exhibits a marked seasonality. Other studies suggest that infection with cryptosporidium is associated with malnutrition and mortality.[5–7,25] This might increase in future years owing to the rapid spread of HIV infection in the Third World, and the development of effective treatment and prevention should therefore be encouraged.

Acknowledgments

This study was supported by the Commission of The European Union (CT94–0311) and The Danish Medical Research Council (9701033). We thank Francisco Dias for providing laboratory facilities at the Laboratorio Nacional de Saúde Publica, Bissau.

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