Cryptosporidium infection in infancy as a cause of malnutrition: a community study from Guinea-Bissau, West Africa\textsuperscript{1–3}

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ABSTRACT  Cryptosporidium parvum causes persistent diarrhea in young children in developing countries. To determine the interaction between nutritional status and cryptosporidiosis, an open cohort of 1064 children younger than 3 y of age was followed for 1441 child-years by weekly diarrhea recall visits. A total of 5072 weight and 4264 height measurements were made. There were no tendencies of low weight (\( P = 0.38 \)) or height (\( P = 0.16 \)) in children who acquired cryptosporidiosis. Cryptosporidiosis in infancy was accompanied by an estimated weight loss of 392 g (95% CI: 247, 538 g) in boys and 294 g (95% CI: 109, 479 g) in girls, corresponding to 3.7% and 2.9% of mean weight, respectively, at 2 y of age. No significant catch-up growth covered for this loss in weight. A similar effect in linear growth was shown (\( P = 0.02 \)). Although it has been suggested that the effect of infections on nutrition is usually transient because of catch-up growth, the present study suggests that cryptosporidiosis in infancy has a permanent effect on growth. \textit{Am J Clin Nutr} 1997;65: 149–52.

KEY WORDS  Weight, height, malnutrition, Cryptosporidium, diarrhea, children, Guinea-Bissau

INTRODUCTION

The protozoan Cryptosporidium parvum has in recent years been recognized as a cause of severe diarrhea in immunodeficient patients (1). It also causes diarrhea in otherwise healthy persons, particularly in children in developing countries in whom it is a major cause of persistent diarrhea (2). Several studies have found significant associations between cryptosporidiosis and low weight-for-age (3–9). However, these studies did not determine whether low weight predisposed to cryptosporidiosis, or if the parasite was a cause of diarrhea-associated malnutrition.

In Guinea-Bissau, cryptosporidiosis is a frequent cause of diarrhea, particularly in infants and very young children (10). Cryptosporidiosis is associated with excess mortality in children who have the infection in infancy, and this excess mortality persists into the second year of life (11). The purpose of the present study was to analyze the interaction between nutritional status and cryptosporidiosis in a cohort of children younger than 3 y of age from Guinea-Bissau.

SUBJECTS AND METHODS

Field work

This study was conducted as part of a 3-y prospective community-based diarrhea surveillance of a peri-urban district, Bandim II, in the capital of Guinea-Bissau, West Africa. All children born after June 1, 1984, residing in a random sample of 301 houses, were included in the study, which started in April 1987 and lasted until April 1990. Children born in or moving into these houses were also included. Children who moved within the area were followed up from their new houses. The present paper reports only data of children younger than 3 y of age. The study was approved by the Ministry of Public Health in Guinea-Bissau as well as the Danish Central Scientific Ethical Committee.

The children were visited weekly by fieldworkers who collected information on episodes of diarrhea (mother’s definition) during the previous week. If a child had diarrhea, a stool sample was collected later the same day, if possible. A sequence of days with diarrhea was regarded as one episode of diarrhea, provided that it was separated from a previous episode by \( \geqslant 2 \) d.

Anthropometric measurements were taken at intervals of \( \sim 3 \) mo. Each child was weighed to the nearest 0.1 kg with a portable Salter-type spring scale while they were nude or dressed in light clothing. Length (or height in children older than \( \sim 2 \) y) was measured with a wooden measuring board.

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Microbiological methods

Stools were collected in plastic containers and kept in an insulated box with ice packs until they were processed within 15–18 h. Approximately 1 g of feces was concentrated by using a formol-ether technique (12), and a smear was stained by the modified Ziehl-Neelsen technique and examined microscopically (13). An episode of diarrhea was considered to be Cryptosporidium-positive if the parasite was found in at least one stool sample from the episode.

Statistical methods

In the assessment of nutritional status it is a common practice to use SD scores (Z scores) based on the National Center for Health Statistics (NCHS) reference data (14). However, in our study there was a marked variation in weight-for-age Z scores by child age. This pattern, typical of African children (15), made it impossible to compare Z scores between children in different age groups. We therefore analyzed the data using a generalized, linear mixed-model tailored for the analysis of repeated measurements (16). Thus, it was possible to make full use of the available data and to account for the growth of the individual children in the estimation of the regression coefficients and their variances.

A mixed model generalizes the standard linear model as follows:

$$Y_i = X_i \beta + Z_i b_i + \epsilon_i$$

where $Y_i = (Y_{i,1}, \ldots, Y_{i,n})$ is a vector representing the $n$ measurements for the $i$th child. The term $X_i \beta$ represents the fixed effects (or population effect), with $X_i$ being the covariates for the $i$th child and $\beta$ is an unknown vector of fixed-effects parameters. $Z_i b_i$ represents the random effects for the $i$th child, with $Z_i$ being the covariates and $b_i$ the unobserved individual random effects, a vector assumed to be multivariate, normally distributed, with a mean of 0 and covariance matrix $D_i$, possibly dependent on the covariate for the $i$th child. Finally, $\epsilon_i$ models the measurement errors, which are assumed to be independent, normally distributed with mean 0 and covariance $\sigma^2 I$. Maximum likelihood methods were used for estimation, and hypothesis testing was done by using log likelihood ratio tests.

Because the effect of Cryptosporidium infection was likely to depend on age and sex, we estimated separate parameters for each sex and for children aged 0–11 mo and 12–35 mo at infection, respectively. Based on an evaluation of model fit and parallel residual plots (17), we decided to model the age dependence of growth by a piecewise linear function for each sex (knots at age 3 mo, 6 mo, 1 y, and 2 y). Because both growth and incidence of cryptosporidiosis (11) exhibit a marked seasonality, it was important to make an effective adjustment for seasonal effects. This was achieved by superimposed sine and cosine terms with period 1 y and 0.5 y on the intercept and slope for infants and 1–2-yr-old children. The random effect was modeled by a piecewise linear curve with knots at ages 1 and 2 y, thus having a random intercept, and piecewise linear random slope for infants and 1- and 2-yr-old children. The MIXED procedure of the SAS software package (SAS Institute, Cary, NC) was used for the regression analysis, and the ANTHRO program (Centers for Disease Control and Prevention, Atlanta) was used for computing weight-for-age Z scores (detailed statistical annex available from the authors).

RESULTS

The study included 1064 children followed for 1441 child-years; 433 children were present at the start of the study, 462 were included as newborns, and 169 entered the study by moving to one of the selected houses. A total of 102 children died and 310 moved away before 3 y of age or the end of the study. A total of 5072 weight (mean: 4.8 kg per child; range: 1–18) and 4264 height measurements (mean: 0.4 kg per child; range: 0–13) were taken. A tabulation of the pooled weight-for-age data showed that the children had a maximum mean weight-for-age Z score (WAZ) of 0.3 at 2 mo of age followed by a marked decline during infancy to a minimum of −1.6 at 12 mo of age. In the second and third years of life the mean WAZ was −1.5 and −1.2, respectively.

Of 236 children who had cryptosporidiosis during the study, 213 (90.3%) had at least one weight measurement before the infection and 181 (76.7%) had one or more measurements after Cryptosporidium infection. One hundred eight children acquired the infection before 1 y of age. There was no indication that children who experienced cryptosporidiosis had a lower weight or height in the period from 90 d to the time that the infection was detected. Preinfectious estimates were −71 g (95% CI: −252, 109 g) for infant boys and 154 g (95% CI: −56, 364 g) for infant girls. For children who had the infection after 1 y of age the estimates were −88 g (95% CI: −251, 76 g) for boys and 56 g (95% CI: −127, 239 g) for girls. These estimates were not significantly different from 0 ($P = 0.38$). Similar estimates were obtained for height. Among infants, the estimates were 6.6 mm (95% CI: 1.1, 12.0 mm) for boys and 2.1 mm (95% CI: −4.6, 8.7 mm) for girls, and for older children were 0.4 mm (95% CI: −4.7, 5.4 mm for boys) and −2.4 mm (95% CI: −7.9, 3.0 mm for girls). Again, the null hypothesis could not be rejected ($P = 0.16$).

Cryptosporidium infection had a significant effect ($P < 0.001$) on subsequent growth controlled for sex, age, season, and age at infection. The weight loss was largest for children contracting the infection before 1 y of age: in boys −392 g (95% CI: −538, −247 g) and in girls −294 g (95% CI: −479, −109 g). These estimates corresponded to 3.7% and 2.9% of the population mean for boys and girls, respectively, at 2 y of age. For children contracting the infection after infancy the effects were −136 g (95% CI: −292, 21 g) for boys and −16 g (95% CI: −178, 146 g) for girls. In a further analysis, we estimated separate effects in the acute phase (0–29 d after evidence of the parasite), during convalescence (30–179 d), and at follow-up (after 180 d) (Table 1). The time-dependent model was not statistically different from the model above, assuming no fade out of the effect of infection.

There was an overall effect of cryptosporidiosis on linear growth ($P < 0.02$). The estimated effect was larger at follow-up of the infected infants, −4.7 mm (95% CI: −9.4, 0.0 mm) for boys and −8.3 mm (95% CI: −14.2, −2.5 mm) for girls, than for children infected after 1 y of age, −1.7 mm (95% CI: −6.3, 3.0 mm) for boys and −1.6 mm (95% CI: −6.7, 3.6 mm) for girls. Separate estimates in the acute phase, during convalescence, and at follow-up after 180 d are shown in Table 2. Among children infected before 1 y of age, there was a tendency toward increased impairment of linear growth at follow-up. The time-dependent model was, however, not significantly different from the model above, assuming no time-dependent effect of infection.
TABLE 1
Estimated effect of cryptosporidiosis on weight at follow-up of children with infection in infancy (<1 y of age) and postinfancy (1–2 y of age) in Guinea-Bissau, 1987–1990.

<table>
<thead>
<tr>
<th>Time after infection</th>
<th>Boys</th>
<th>Girls</th>
<th>Boys</th>
<th>Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–29 d</td>
<td>-446 (−615, -278)</td>
<td>-317 (−538, -97)</td>
<td>-292 (−492, -92)</td>
<td>-36 (−219, 147)</td>
</tr>
<tr>
<td>30–179 d</td>
<td>-341 (−513, -169)</td>
<td>-264 (−480, -48)</td>
<td>-21 (−200, 157)</td>
<td>6 (−193, 204)</td>
</tr>
<tr>
<td>≥ 180 d</td>
<td>-290 (−523, -57)</td>
<td>-313 (−590, -36)</td>
<td>-82 (−349, 185)</td>
<td>-55 (−353, 243)</td>
</tr>
<tr>
<td>Common estimate</td>
<td>-392 (−538, -247)</td>
<td>-294 (−479, -109)</td>
<td>-136 (−292, 21)</td>
<td>-16 (-178, 146)</td>
</tr>
</tbody>
</table>

From a mixed generalized linear regression based on 5072 weight measurements of 1064 children, controlled for sex, age, and season. Values are regression coefficients; 95% CIs in parentheses.

TABLE 2
Estimated effect of cryptosporidiosis on height at follow-up of children with infection in infancy (<1 y of age) and postinfancy (1–2 y of age) in Guinea-Bissau, 1987–1990.

<table>
<thead>
<tr>
<th>Time after infection</th>
<th>Boys</th>
<th>Girls</th>
<th>Boys</th>
<th>Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–29 d</td>
<td>-3.0 (−9.3, 3.3)</td>
<td>-4.5 (−13.6, 4.5)</td>
<td>-2.2 (−8.7, 4.4)</td>
<td>-0.1 (−6.8, 6.6)</td>
</tr>
<tr>
<td>30–179 d</td>
<td>-5.4 (−10.7, -0.1)</td>
<td>-9.4 (−15.9, -2.9)</td>
<td>-1.4 (−6.6, 3.8)</td>
<td>-2.7 (−8.7, 3.4)</td>
</tr>
<tr>
<td>≥ 180 d</td>
<td>-6.5 (−13.3, 0.2)</td>
<td>-10.7 (−18.9, -2.5)</td>
<td>-2.2 (−9.5, 5.1)</td>
<td>-3.2 (11.6, 5.3)</td>
</tr>
<tr>
<td>Common estimate</td>
<td>-4.7 (−9.4, 0.0)</td>
<td>-8.3 (−14.2, -2.5)</td>
<td>-1.7 (−6.3, 3.0)</td>
<td>-1.6 (−6.7, 3.6)</td>
</tr>
</tbody>
</table>

From a mixed generalized linear regression based on 4264 height measurements of 1064 children, controlled for sex, age, and season. Values are regression coefficients; 95% CIs in parentheses.

DISCUSSION

Studies from Jamaica (3), Gaza (4, 5), Peru (6), Thailand (7), Bangladesh (8), and Guatemala (9) found associations between low weight and infection with Cryptosporidium. However, because of the cross-sectional nature of these studies it has not been possible to conclude whether a poor state of nutrition predisposed to infection with Cryptosporidium, or whether the parasite, perhaps in association with other factors, was responsible for growth faltering. Nevertheless, it has been suggested that malnutrition is a determinant for cryptosporidiosis (3, 4, 7, 9).

However, in our data, children who acquired cryptosporidiosis had the same preinfectious weight and height as other children. In addition, preinfectious nutritional status was not significantly associated with the duration of diarrhea (data not shown). Furthermore, we showed that impaired cellular immune response as measured by CD4/CD8 status did not explain the incidence and severity of cryptosporidiosis in children from Guinea-Bissau (18).

In assessing the causal nature of the association between cryptosporidiosis and subsequent growth failure, possible sources of confounding and bias should be considered. Confounding due to socioeconomic and maternal factors is unlikely because the random effects in the model controlled for the characteristics of each child. However, mortality associated with cryptosporidiosis may have caused a selection bias. We reported previously a relative mortality rate of 2.9 (ie, children with cryptosporidiosis had a 2.9 times greater mortality than children without the infection; 95% CI: 1.7, 4.9) in infants with cryptosporidiosis (11). Five of the children in the present study died within 1 mo after infection, and follow-up weights were only available for two of these children. Thus, there may have been missing values because of hospitalizations and deaths and, consequently, conservative estimates. We conclude that Cryptosporidium diarrhea, as a precipitating event, has a marked and lasting effect on linear growth and weight, particularly when the infection is acquired in infancy. It should be emphasized that the present analyses do not formally compare the nutritional effect of cryptosporidiosis with that of diarrhea from other causes or all causes. A similar effect may be found for some other enteropathogens as well. It is, however, likely that Cryptosporidium may be a more important cause of diarrhea-associated malnutrition than most other diarrheal agents because it infects children at such a young age and because it causes persistent diarrhea in young children (2).

Though the model presented in Table 1 did not differ significantly from the more simplistic model with a time-independent effect of infection, it may be the biologically most plausible model. If so, there was a tendency for catch-up growth, which, however, was inadequate for infants. In boys who had...
the infection after 1 y of age, there was a transient effect, whereas girls at this age coped with the infection.

A decreased effect of *Cryptosporidium* on nutritional status in older children may be related to acquired partial immunity, which may reduce the severity of diarrhea. This is corroborated by several studies that report the highest prevalence of diarrheal *Cryptosporidiosis* in infants or very young children (1, 11, 19). In addition, it is likely that infections during the first year of life, when growth velocity is high, may affect further growth more so than infections in later childhood (20, 21). It is conceivable that some sort of “programming” occurs in intrauterine life and in infancy. Important factors in this programming are genetic background, nutritional support (including micronutrients), and infectious exposures. Later in childhood, when growth velocity decreases, children have—given adequate nutritional support—a better potential of catching-up to the programmed curve. In this interplay between genetic potential, nutritional intake, and infectious exposures, boys may be the more vulnerable sex because on average they have to build a larger muscle mass than girls, which may increase the risk of a negative micronutrient balance, eg, zinc deficit.

Our findings are in line with those of a smaller community study of infants from Guatemala City (9) that reported a marked deterioration of nutritional status during *Cryptosporidium*-associated diarrhea. The average fall in Z score during the first 6–11 d of illness was 0.064 SD/d (n = 11 children, P = 0.01).

Diarrhea is, in nonfamine situations, considered a major cause of malnutrition in developing countries, and the reduction of diarrhea-associated malnutrition is endorsed as an objective of the WHO program for the control of diarrheal diseases (22). The basis of this objective has been questioned (23, 24). A study from Bangladesh suggested that the effect of diarrhea on growth is transient because of catch-up growth (23). According to the view expressed, efforts to control diarrhea are therefore unlikely to improve children’s nutritional status in the long term. However, the present observations from Guinea-Bissau suggest that it is necessary to consider interactions between a particular diarrheal agent and host factors (eg, age and sex) when trying to understand the effect of diarrhea on nutritional status. The study adds to the growing evidence of the epidemiologic and clinical significance of *Cryptosporidium* (11, 25), and underscores the notion that if diarrhea-prevention programs are to reduce the incidence of mortality and prevent diarrhea-associated malnutrition, specific preventive or curative measures against *Cryptosporidiosis* need to be developed.

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REFERENCES


