Asymptomatic and Symptomatic Cryptosporidiosis: Their Acute Effect on Weight Gain in Peruvian Children

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This study investigated whether a child's first infection with Cryptosporidium parvum had an acute effect on weight gain. Specifically, the authors compared monthly rates of weight gain between C. parvum-infected and noninfected children. Over a 2-year period (1989-1991), a cohort of Peruvian children aged 0-3 months at recruitment were followed twice weekly for assessment of daily diarrheal status, weekly for C. parvum stool examinations, and monthly for anthropometric measurements. Data on 207 children permitted the authors to examine the effect of C. parvum infection on weight gain. During the 2-year study period, 45% (94/207) of the children became infected with C. parvum for the first time. Weight gain intervals in 57 of the 94 infected children met criteria for analysis. Of these, 63 percent (36/57) were asymptomatic (i.e., had no diarrhea). On average, children with symptomatic cryptosporidiosis gained (i.e., grew) 342 g less (95% confidence interval 167-517) during the first month of infection than did children without diarrhea who were not yet infected. The effect of asymptomatic cryptosporidiosis was less severe: On average, children with asymptomatic infection gained 162 g less (95% confidence interval 27-297) during the first month of infection than did children without diarrhea who were not yet infected. Symptomatic cryptosporidiosis retarded weight gain more than did asymptomatic cryptosporidiosis, but the latter was twice as common. Since asymptomatic cryptosporidiosis is more prevalent, it may have more of an overall adverse effect on child growth in the community than symptomatic cryptosporidiosis. Am J Epidemiol 1997;145:156-63.

Cryptosporidium parvum is a well recognized cause of diarrhea worldwide (1, 2). In developed countries, cryptosporidiosis is self-limiting and occurs most commonly among children in day care centers (1), travelers (3), and people who work with animals (4). Recently, in the United States the parasite has been associated with waterborne outbreaks of diarrhea (5). In immunocompromised individuals, particularly those with acquired immunodeficiency syndrome (AIDS), cryptosporidiosis is not self-limiting and may cause voluminous, persistent diarrhea (1). Since no effective therapy is available to date (6, 7), AIDS-related cryptosporidiosis is associated with increased mortality (1). In developing countries, cryptosporidiosis occurs predominantly among children. Infection occurs early in life, and diarrhea is the most common symptom (8-10). Household water sources, domesticated animals, and intrafamilial spread play an important role in C. parvum transmission (11, 12). Recently, C. parvum has been associated with malnutrition. Cross-sectional studies in children hospitalized for diarrhea have found cryptosporidiosis to be more prevalent among malnourished children than in well nourished children (13-15). In a Thai orphanage, children infected with C. parvum exhibited a significantly lower mean weight-for-height score than did noninfected controls (16). These studies, however, did not explore whether malnutrition was a result of C. parvum infection or whether the infection was a result of malnutrition.

In this study, we followed a birth cohort of Peruvian children longitudinally to determine whether a child's first infection with C. parvum had an adverse effect on
weight gain during the first month of infection. We also investigated whether poor nutritional status, as assessed by anthropometric measures, was a risk factor for C. parvum infection. Since stool examinations for C. parvum were conducted independently of assessments of diarrheal status, we were able to distinguish between the effects of symptomatic cryptosporidiosis and asymptomatic cryptosporidiosis on weight gain.

MATERIALS AND METHODS

Study settings

The study took place in Pampas de San Juan de Miraflores, a peri-urban shantytown ("pueblo joven") in Lima, Peru, comprising approximately 35,000 individuals. Households in this community are relatively homogeneous in terms of socioeconomic status (17), and one fourth of the population is 5 years of age or younger (18). While infections with geohelminths are rare in this community, children are frequently infected with Giardia lamblia (19, 20).

Admission to the study

Women in their last trimester of pregnancy were randomly selected from the censused community and were asked to participate in the study. From September to December of 1989, 80 children aged 0–3 months were recruited. Between January 1990 and June of 1991, approximately 10 newborns were recruited each month.

The study was approved by the Committee on Human Research of the Johns Hopkins University School of Public Health.

Surveillance techniques

Children were followed longitudinally from September 1989 to November 1991. Anthropometric measurements were made monthly. Weight was determined with Salter scales and length with a locally made wooden length platform and sliding footboard. Stool specimens were collected weekly and examined for the presence of C. parvum. Direct and formalin ether concentrates were examined by light microscopy and were stained with both acid-fast and monoclonal antibody fluorescent-labeled stains (21). C. parvum oocysts were identified by two methods, light microscopy and ultraviolet fluorescent microscopy, as previously described (21). Specimens were labeled positive for C. parvum if at least one C. parvum oocyst was detected via either test. G. lamblia was detected as previously described (19, 20). Diarrhea surveillance took place twice weekly. The field workers obtained information from mothers or caretakers for the day of the interview and the three previous days, and for each day they inquired about the number of liquid or semiliquid stools the child had passed. Anthropometric measurements, stool sample collection, and diarrhea surveillance were conducted at different times by different field workers.

Definition of a growth interval

A growth interval was defined as two consecutive weight measurements separated by a time period of 30 ± 7 days. An interval was defined as C. parvum-positive if at least one stool sample containing C. parvum was recorded during that interval, and as diarrhea-positive if at least one day of diarrhea was recorded during that interval. A child had a day of diarrhea if three or more liquid or semiliquid stools were recorded for that day. A month of C. parvum infection was defined as a C. parvum-positive growth interval preceded by at least one C. parvum-negative day. All growth intervals were stratified into one of the following categories: C. parvum-negative and diarrhea-negative; C. parvum-positive and diarrhea-negative (i.e., asymptomatic cryptosporidiosis); C. parvum-positive and diarrhea-positive (i.e., symptomatic cryptosporidiosis); and C. parvum-negative and diarrhea-positive. An interval was defined as G. lamblia-positive if at least one positive G. lamblia stool was recorded during that interval.

Exclusion criteria

Not all growth intervals were included in the analysis, because of missing information. We defined a growth interval as non-evaluable and excluded it from the analysis if it was outside the range of 30 ± 7 days, had fewer than 2 weeks of stool specimens, or had fewer than 12 days of diarrhea surveillance. Given that the analysis sought to measure the effect of a child's first C. parvum infection on weight gain during the first month of infection, we restricted the analysis to growth intervals prior to and during the first month of infection.

Some children did not have an evaluable growth interval at the time of their first infection with C. parvum. We examined whether these children differed from children who had evaluable growth intervals at the time of their infection with regard to age at onset of infection, sex, prevalence of stunting prior to infection, and number of diarrhea episodes prior to infection.

Study sample

Initially, 253 mothers were asked to enter their children in the study. Thirteen mothers declined; one
child died of pneumonia early in the study; 22 children’s families migrated out of the community within 2 months of having been admitted to the study; four children were excluded from the analysis because all of their growth intervals met at least one exclusion criterion; and six children had a C. parvum-positive stool prior to their first growth interval. Thus, available data on 87 percent (207/239) of children and 77 percent (1,217/1,576) of growth intervals permitted us to analyze the effect of C. parvum on weight gain during the first month of infection.

Statistical analysis

Age at onset of first C. parvum infection and its association with diarrhea. The median age at onset of first infection and the lower bound of the 95 percent confidence interval were estimated using the Kaplan-Meier statistic (22). Children who were not infected with C. parvum and were lost to follow-up were right-censored at the time of their last stool examination. Age was categorized into one of the following 6-month age strata: 0–5, 6–11, 12–17, and 18–23 months. Incidence rates were estimated for each of the 6-month age strata. Relative risk estimation used the youngest age stratum as the reference group. To examine whether diarrhea was associated with a child’s first C. parvum infection, we estimated the relative risk of infection by 6-month age stratum and calculated a Mantel-Haenszel weighted relative risk.

Poor nutritional status as a risk factor for C. parvum infection. To determine whether malnutrition was a risk factor for C. parvum infection, we estimated the relative odds of C. parvum infection between malnourished and well-nourished children. We used the growth intervals to calculate these odds ratios. Given that the probability of finding C. parvum during one growth interval is low, the odds ratio is a good approximation of the relative risk. We defined a child as stunted during a particular growth interval if the child’s height-for-age Z score at the beginning of that growth interval was 2 standard deviations below the expected score; as wasted if the weight-for-height Z score at the beginning of that growth interval was 2 standard deviations below the expected score; and as underweight if the weight-for-age Z score at the beginning of that growth interval was 2 standard deviations below the expected score. (These are the US National Center for Health Statistics reference values.) Of the 207 children studied, 30 were stunted and seven were underweight in at least one of their growth intervals. Only three children exhibited wasting in at least one of their growth intervals. Of these children, none became infected with C. parvum while wasted.

The odds ratio was fitted via a logistic regression model using generalized estimating equations with an unstructured correlation matrix (23). This model regressed the absence or presence of C. parvum infection during a growth interval on 1) stunting or underweight status at the beginning of that interval and 2) age at the beginning of that interval. Numbers of children were not sufficiently large to estimate the association between stunting status and symptomatic cryptosporidiosis. We report, however, the proportions of children with symptomatic cryptosporidiosis who were both stunted and nonstunted at the time of their infection with C. parvum.

Effect of first C. parvum infection on weight gain. To determine whether a child’s first C. parvum infection had an effect on weight gain, we compared the weight gain of infected children during the first month of infection with the monthly weight gain of children who had not yet been infected. We estimated the differences in weight gain between 1) C. parvum-positive and diarrhea-positive growth intervals (i.e., symptomatic infection) and C. parvum-negative and diarrhea-negative growth intervals and 2) C. parvum-positive and diarrhea-negative growth intervals (i.e., asymptomatic infection) and C. parvum-negative and diarrhea-negative growth intervals.

Model I: The growth rates of C. parvum-infected and noninfected children were compared in the 6-month age-stratified groups (0–5, 6–11, 12–17, and 18–23 months). A linear model regressed the weight measurement at the end of a growth interval on the following covariates: 1) weight at the beginning of the growth interval, 2) the presence or absence of C. parvum during that interval, 3) the presence or absence of diarrhea during that interval, 4) the interaction between C. parvum and diarrhea, 5) an indicator for stunting at the beginning of the growth interval, and 6) an indicator for the child’s sex. The purpose of the interaction term in the model was to distinguish between the effects of symptomatic and asymptomatic cryptosporidiosis on weight gain. This model estimated age-stratum-specific intercept and covariate terms (i.e., the model had a total of 28 parameters). A first-order autoregressive correlation structure was fitted to account for the serial correlation among measurements from the same child (24). The model’s parameters were estimated via maximum likelihood. A likelihood ratio test measured the statistical significance of estimating age-stratified effects of C. parvum on weight gain.

Model II: The net growth rates of C. parvum-infected and noninfected children were compared; i.e., we assumed that the effect of C. parvum on weight gain was the same for all age strata. A second linear model regressed the weight measurement at the end
of a growth interval on the following covariates: 1) weight at the beginning of the growth interval; 2) the presence or absence of *C. parvum* during that interval; 3) the presence or absence of diarrhea during that interval; 4) the interaction between *C. parvum* and diarrhea; 5) an indicator for stunting at the beginning of the growth interval; 6) an indicator for the child's sex; 7) the presence or absence of *G. lamblia* during that growth interval; and 8) three indicators created to adjust for the effects of seasonality. This model estimated age-stratum-specific terms for both the intercept and the first covariate term (term 1 above) but net covariate terms for the second through eighth terms (terms 2–8 above) (i.e., the model had a total of 17 parameters). A first-order autoregressive correlation structure was fitted to account for the serial correlation among measurements from the same child, and the model's parameters were estimated via maximum likelihood.

RESULTS

Description of the data

Forty-five percent (94/207) of the study children who met the criteria for analysis became infected with *C. parvum* for the first time. Sixty-one percent (57/94) of the children infected with *C. parvum* had an evaluable growth interval during their first month of infection. There were no significant differences between children who had an evaluable growth interval at the time of their infection and children who did not with regard to age at onset of infection (*p* = 0.80), sex (*p* = 0.90), stunting prior to infection (*p* = 0.92), and number of diarrhea episodes prior to infection (*p* = 0.87).

Of the 57 *C. parvum* infections included in the analysis, 63 percent (36/57) were asymptomatic (table 1). Forty percent (23/57) of the children with a *C. parvum*-positive growth interval also had *G. lamblia* during that interval.

<table>
<thead>
<tr>
<th>Age at onset of first <em>C. parvum</em> infection and its association with diarrhea</th>
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<tbody>
<tr>
<td>The estimated median age at onset of a first <em>C. parvum</em> infection was 16 months (lower 95 percent confidence limit, 13 months). Table 2 shows age-stratified incidence rates and the relative risk of infection with age.</td>
</tr>
<tr>
<td>The risk of symptomatic cryptosporidiosis increased with age (table 3). While children aged 0–5 months tended to have more asymptomatic infections, children aged 12–17 months and 18–23 months tended to have more symptomatic cryptosporidiosis. Overall, however, diarrhea was not significantly associated with <em>C. parvum</em> infection (Mantel-Haenzel relative risk = 1.27, 95 percent confidence interval (CI) 0.89–1.82).</td>
</tr>
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Poor nutritional status as a risk factor for *C. parvum* infection

Stunted children had 1.52 times greater odds (95 percent CI 0.82–2.82) of becoming infected with *C. parvum* than did nonstunted children, but this increase was not statistically significant. Underweight children did not have greater odds of becoming infected than children who were not underweight (odds ratio — 1.05, 95 percent CI 0.18–7.56). Of the 57 children with cryptosporidiosis, 14 percent (8/57) were stunted: 50 percent (4/8) of the stunted children and 35 percent

<table>
<thead>
<tr>
<th>TABLE 1. Descriptive information on the monthly growth intervals recorded as Cryptosporidium parvum-positive and diarrhea-positive in 207 Peruvian children, 1989–1991</th>
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</thead>
<tbody>
<tr>
<td><strong>Age group (months)</strong></td>
</tr>
<tr>
<td><strong>No.</strong></td>
</tr>
<tr>
<td>No. of children with an evaluable growth interval</td>
</tr>
<tr>
<td>No. of monthly intervals</td>
</tr>
<tr>
<td>Mean age (months)</td>
</tr>
<tr>
<td>Male:female ratio</td>
</tr>
<tr>
<td>No. of intervals with diarrhea</td>
</tr>
<tr>
<td>No. of intervals with <em>C. parvum</em> Asymptomatic <em>C. parvum</em> Symptomatic <em>C. parvum</em></td>
</tr>
<tr>
<td>Stunting at beginning of the growth interval</td>
</tr>
<tr>
<td>Symptomatic <em>C. parvum</em></td>
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</tbody>
</table>

TABLE 2. Age-stratified rate of first infection with Cryptosporidium parvum and relative risk of infection in 207 Peruvian children, 1989-1991

<table>
<thead>
<tr>
<th>Age group (months)</th>
<th>No. of Infections</th>
<th>No. of weekly stool examinations</th>
<th>RR*</th>
<th>95% CI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-5</td>
<td>19</td>
<td>4,191</td>
<td>1.00†</td>
<td></td>
</tr>
<tr>
<td>6-11</td>
<td>41</td>
<td>3,235</td>
<td>2.8</td>
<td>1.6-4.8</td>
</tr>
<tr>
<td>12-17</td>
<td>24</td>
<td>1,571</td>
<td>3.4</td>
<td>1.9-6.1</td>
</tr>
<tr>
<td>18-23</td>
<td>10</td>
<td>365</td>
<td>6.0</td>
<td>2.8-12.9</td>
</tr>
</tbody>
</table>

* RR, relative risk; CI, confidence interval.
† Reference category.


<table>
<thead>
<tr>
<th>Age group (months)</th>
<th>No. of Intervals</th>
<th>RR†</th>
<th>95% CI†</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-5</td>
<td>4/15</td>
<td>0.75</td>
<td>0.32-1.74</td>
</tr>
<tr>
<td>6-11</td>
<td>8/24</td>
<td>1.21</td>
<td>0.87-2.17</td>
</tr>
<tr>
<td>12-17</td>
<td>6/11</td>
<td>1.60</td>
<td>0.90-2.83</td>
</tr>
<tr>
<td>18-23</td>
<td>3/7</td>
<td>5.71</td>
<td>1.43-22.81</td>
</tr>
</tbody>
</table>

* C+D+ is the number of intervals that are C. parvum-positive and diarrhea-positive (i.e., symptomatic infection); C+ is the number of intervals that are C. parvum-positive; D+ is the number of intervals that are C. parvum-negative and diarrhea-positive; and C- is the number of intervals that are C. parvum-negative.
† RR, relative risk; CI, confidence interval.

Effect of first C. parvum infection on weight gain

Children with cryptosporidiosis, whether symptomatic or asymptomatic, gained less weight (i.e., grew less) during the first month of infection than did children who were not yet infected (figure 1). When stratified by age, children aged 6-11 months with symptomatic cryptosporidiosis had a net loss in weight during the first month of infection (figure 1).

Model I. Children with symptomatic infection gained 338 g less (95 percent CI 161-515) during the first month of infection than did children without diarrhea who were not yet infected (C. parvum-negative and diarrhea-negative children). When data were stratified by age, this effect was statistically significant for children aged 0-5 months and 6-11 months (figure 2). Children aged 0-5 months with C. parvum gained 530 g less (95 percent CI 134-927) during the first month of infection than did children who were C. parvum-negative and diarrhea-negative; children aged 6-11 months gained 407 g less (95 percent CI 124-689).

Children with asymptomatic infection gained 154 g less (95 percent CI 19-290) during the first month of infection than did C. parvum-negative and diarrhea-negative children. When data were stratified by age, this difference was significant for infected children in the 6- to 11-month age stratum (figure 3), who gained 199 g less (95 percent CI 0-401) during the first month of infection than did C. parvum-negative and diarrhea-negative children of the same age (figure 2).

(17/49) of the nonstunted children had symptomatic cryptosporidiosis.
Children with symptomatic cryptosporidiosis gained less weight than did children with asymptomatic cryptosporidiosis (figure 1). On average, children with symptomatic cryptosporidiosis gained 184 g less (95 percent CI -36 to 403) during the first month of infection than did children with asymptomatic cryptosporidiosis, but this effect was not statistically significant. When data were stratified by age, children aged 0–5 months with symptomatic cryptosporidiosis gained 427 g less (95 percent CI -34 to 888) than did children with asymptomatic cryptosporidiosis, and children aged 6–11 months with symptomatic cryptosporidiosis gained 208 g less (95 percent CI -134 to 549).

The likelihood ratio test did not find significant differences in the effect of C. parvum on growth by age stratum ($p = 0.29$).

Model II. We examined changes in the effect of C. parvum after adjusting for the potential effects of G. lamblia and seasonality on weight gain. This model assumed that the effect of C. parvum on weight gain was the same for all ages. After these adjustments, children with symptomatic cryptosporidiosis gained 342 g less (95 percent CI 167–517) in weight during the first month of infection than did C. parvum-negative and diarrhea-negative children. Children with asymptomatic cryptosporidiosis gained 162 g less (95 percent CI 27–297). While the adjusted effect of season-
our intervals were shorter (i.e., monthly) and greater in number. In addition, since stool surveillance for *C. parvum* for each child was conducted independently of diarrheal surveillance, we were able to separately examine the effects of symptomatic and asymptomatic infection on weight gain. Sixty-one percent (57/94) of the *C. parvum* infections were included in the monthly growth intervals analyzed. We found no differences in any of the characteristics we compared between children whose *C. parvum*-positive growth intervals were included in the analysis and children whose *C. parvum*-positive intervals were not included.

The mechanism by which *C. parvum* causes diarrhea remains unclear, although preliminary results suggest that *C. parvum* infection may alter the intestinal permeability of the gastrointestinal tract: AIDS patients who are infected with *C. parvum* demonstrate increased intestinal permeability in comparison with noninfected controls (R. L. Guerrant, University of Virginia School of Medicine, personal communication, 1995). Results of another study have suggested that AIDS-related cryptosporidiosis results in intestinal damage and malabsorption (32). It is possible that even asymptomatic *C. parvum* infection alters intestinal function enough to cause nutrient malabsorption. This could explain our finding that asymptomatic infections adversely affected growth.

Other parasites, such as *G. lambia* and *Ascaris lumbricoides*, alter the absorption of nutrients (33, 34) even when diarrhea is absent. In developing countries, there are high rates of asymptomatic infection by a variety of enteropathogens. Therefore, it is important to determine whether asymptomatic infections with agents other than *C. parvum* also cause growth faltering. In our study, children with symptomatic cryptosporidiosis gained less weight than did children with asymptomatic infection. However, asymptomatic cryptosporidiosis—which occurred nearly twice as often as symptomatic cryptosporidiosis—also had an adverse effect on weight gain. Thus, since asymptomatic cryptosporidiosis was more prevalent, it may have more of an overall adverse effect on child growth in the community than symptomatic cryptosporidiosis.

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