Cryptosporidiosis in Adults in Lusaka, Zambia, and Its Relationship to Oocyst Contamination of Drinking Water

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In Lusaka, where human immunodeficiency virus seroprevalence in young adults is ~25%, four townships were studied to establish the prevalence of persistent diarrhea in adults and the etiologic importance of cryptosporidiosis in adults with persistent diarrhea. Cryptosporidium parvum oocyst contamination of urban water supplies was measured and the results used to categorize these populations into high or low exposure. In total, 506 adults were reported as having had diarrhea in the 2 weeks prior to the survey; 101 of these episodes were persistent. Adults with persistent diarrhea in the high-exposure areas were more likely to have cryptosporidiosis (odds ratio, 5.14; 95% confidence interval, 1.57–17.2; risk ratio, 1.83; 95% confidence interval, 1.04–3.21; P = .003) although overall prevalence of persistent diarrhea was not greater in these areas. This association was not confounded by animal exposure, travel, or boiling water. Within these urban populations, water contamination with C. parvum was a major influence on the prevalence of infection.

Cryptosporidium parvum is one of the world’s dominant enteropathogens [1], responsible for diarrhea in children, travelers, and immunocompromised patients, particularly those with human immunodeficiency virus (HIV) infection [2, 3], most of whom are in sub-Saharan Africa. In addition, large urban waterborne outbreaks have brought the organism to the attention of the public [4]. However, uncertainty exists as to the role of water in transmission of endemic or sporadic infection [5], as opposed to recognizable outbreaks, which are usually waterborne or foodborne [6].

There is good evidence that transmission can occur by direct contact with animals [7]. Direct contact from person to person can also disseminate infection, as in child care center outbreaks. Newman et al. [8] described secondary cases of cryptosporidiosis in the households of 31% of the index cases in a poor urban area in Brazil, suggesting either that direct contact may transmit infection or that there was shared exposure to another environmental source [9].

HIV seroprevalence in Lusaka in the age range 16–60 years is 22%–30% [10], so these populations are especially vulnerable to diarrhea due to C. parvum [11]. With the hypothesis that water quality is the major variable determining the prevalence of cryptosporidiosis, we studied oocyst contamination in municipal and other water sources and related this to the prevalence of cryptosporidial diarrhea in adults.

Study Setting and Methods

We identified four townships within Lusaka and studied them over the course of one rainy season (November 1995 through March 1996). Water was sampled from five sources (figure 1) that serve these populations. Community surveys were conducted early in the rainy season (November and December) to establish the prevalence of persistent diarrhea in these populations, and cases of persistent diarrhea were then studied in some detail to estimate the proportion of such illnesses attributable to cryptosporidiosis.

Water sampling. On each occasion, 100 L was filtered through a DPPPY Micro-Wynd filter (Cuno Europe, Tachbrook Park, Warrick, UK), the filter was opened, and the fibers were teased apart in 5 L of 0.01% Tween 80 in filtered, purified water. The filtrate was concentrated to 10 mL by centrifugation, and 15 smears were made from this concentrated filtrate. The intensity of oocyst contamination of each source was quantified by counting the proportion of these 15 smears that were positive for oocysts. Oocysts were identified for counting by use of the modified Ziehl-Neelsen (ZN) stain and confirmed by use of immunofluorescence with a monoclonal antibody (FICR120; Shield Diagnostics, Dundee, UK).

The Kafue pipeline was sampled three times within 1 month at the outset of the study and the results were consistent: Scores of 5, 4, and 3 of 15 were obtained. It was not possible to sample from one source sufficiently to measure the coefficient of variation of the assay, but largely consistent results were obtained from other sources over these 4 months.

Community survey of diarrhea prevalence in four townships.

Teams of interviewers recruited from local communities surveyed predefined and mapped sectors of the township, systematically...
cases of persistent diarrhea, the primary relationship was between cryptosporidiosis and water exposure. The relationship has been expressed as risk ratio (RR) or odds ratio (OR) and 95% confidence intervals (95% CIs). Population-attributable risk fraction (PAF) was calculated by use of a standard equation.

To identify confounding, variables indicating the potential confounders listed above were included in Mantel-Haenszel analysis and a logistic regression model of persistent diarrhea. Significance of association of univariate categorical variables was tested by \( \chi^2 \) or Fisher’s exact tests by use of Epi Info 6 (WHO, Geneva). Multivariate analysis was performed by use of STATA 4.0 (Stata, College Station, TX). The association between infection and risk was stratified for period (i.e., whether the sample was collected before or after 31 December 1995) as early or late rainy season.

Results

Water contamination with *C. parvum*. Oocysts of *C. parvum* were identified in five of the six water sources examined (table 1). On the basis of these results, each study population was categorized as having low (Chawama, Chipata, part of George served by new Japanese International Cooperation Agency borehole) or high (Misisi, the rest of George zone) exposure to *C. parvum* in their principal water source.

Community survey prevalence of diarrhea. Interviews were completed by 2258 households; 32 refused and 204 had no responsible adult present despite three visits. Of 6702 adults (3326 women and 3376 men) in the population surveyed, 506 (7.4%) were reported as having had diarrhea in the 2 weeks prior to the survey, and 101 of these (20%) were persistent illnesses.

Persistent diarrhea was not more prevalent in high- than in low-exposure areas and was not related to boiling water or keeping animals. However, boiling water did protect against acute and persistent diarrhea combined (OR, 0.6; 95% CI, 0.4–0.89). Only 75 persons were reported to go fishing, so no statements could be made about its influence on probability of

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**Table 1.** Cryptosporidial contamination of Lusaka water sources 1995–1996.

<table>
<thead>
<tr>
<th>Source</th>
<th>No. of samples</th>
<th>Contamination rate</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chipata supply</td>
<td>3</td>
<td>1.7</td>
<td>Low</td>
</tr>
<tr>
<td>Chawama borehole</td>
<td>3</td>
<td>1.0</td>
<td>Low</td>
</tr>
<tr>
<td>Misisi quarry</td>
<td>4</td>
<td>7.2</td>
<td>High</td>
</tr>
<tr>
<td>George zone (LWSC)</td>
<td>2</td>
<td>8.0</td>
<td>High</td>
</tr>
<tr>
<td>George JICA borehole</td>
<td>4</td>
<td>0</td>
<td>Low</td>
</tr>
<tr>
<td>Kafue main river pipeline</td>
<td>5</td>
<td>3.8</td>
<td>—</td>
</tr>
</tbody>
</table>

NOTE. Contamination rates shown refer to mean proportion of positive results of 15 slides prepared from filtrate from each sample of 100 L of water. On basis of contamination rates, residential areas were classified as high or low exposure. None of populations studied were served directly by Kafue main supply pipe. As water contamination assay used was crude, only 2 categories of exposure were used. LWSC, Lusaka Water and Sewerage Company; JICA, Japanese International Cooperation Agency.
persistent diarrhea. Persistent diarrhea was less common in men under the age of 25 years (OR, 0.3; 95% CI, 0.14–0.64; \( P = .002 \)) and more common in persons living in poor-quality housing (OR, 2.0; 95% CI, 1.4–3.0; \( P = .001 \)). Other indicators of hygiene or socioeconomic status were not related to the risk of persistent diarrhea.

**Proportion of persistent diarrhea attributable to cryptosporidiosis.** One hundred ninety-two adults with persistent diarrhea were investigated in the Urban Health Centers. One hundred sixty-one provided stool samples (the majority, 89 patients, gave 3 samples); of these, 36 had been identified in the community survey and the remainder were self-referred. Patients with persistent diarrhea attending the clinics were less impoverished than were patients identified during the survey (data not shown).

The dominant protozoal enteropathogens were as follows: *C. parvum* (10%), *Isospora belli* (12%), and microsporidia (19%). The prevalence of *C. parvum* in persons with persistent diarrhea was higher in the high- (9/38) than in the low-exposure area (7/123) (OR, 5.14; 95% CI, 1.6–17.2; \( P = .003 \)). After stratification for period of the rainy season, the Mantel-Haenszel adjusted OR for high compared to low exposure increased to 11.63 (95% CI, 2.85–47.4; \( P < .001 \)). The crude RR was 1.83 (95% CI, 1.02–3.21), and by use of the crude RR, PAF was 0.25 (95% CI, 0.2–0.38). Stratification by whether the patient was drawn from the community survey or self-referred did not alter the OR, which remained at ~5.0 for both groups.

This association between cryptosporidiosis and water exposure was not altered after stratification for traveling outside Lusaka (n = 26) around the time of onset of illness or whether in the course of daily business the patient traveled outside the township and drank unboiled water. Cryptosporidiosis was not associated with housing quality, economic factors, education of the head of household, or hygiene practices. Boiling water always was only reported by 8 patients with persistent diarrhea and we could not therefore test its effect in protection against *C. parvum* infection. Of 16 patients with cryptosporidiosis, 15 were HIV-seropositive. Infection with *I. belli* and microsporidia was not related to high or low contamination of the water supply.

**Discussion**

These findings support the hypothesis that the prevalence of human infection with *C. parvum* in adults in these Lusaka townships is determined to at least some degree by the intensity of contamination of the water supply. Although only 16 cases of cryptosporidiosis were detected, there was a clear and significant relationship between water contamination and the prevalence of infection. This relationship was not confounded by other potential influences inasmuch as they can be measured. This does not mean that direct transmission from person to person is unimportant, rather that in ecological terms the distribution of this infection in human populations can be markedly influenced by exposure to the parasite in water.

It is possible that, as the ZN stain is not very sensitive, we have actually measured only the heavier infections. The community survey methodology relied on recall of cases of diarrhea in the 2 weeks preceding the interview, and there will probably have been underreporting. This would have influenced data from all of the townships equally. Only 36 of 101 patients with persistent diarrhea identified in the community attended for parasitologic investigation, and therefore the possibility of selection or ascertainment bias exists. We know that the self-referred patients were wealthier than the average resident, but this is unlikely to explain the association of infection and exposure to contaminated water.

The highest rates of water contamination were found in the areas on the periphery of George township and in Misisi, and we have categorized these residential areas as high-risk, while other areas have been labeled low-risk. This dichotomous classification may be simplistic, but we believe it to be appropriate given the crude assay for water contamination, which can distinguish high from low contamination but cannot be expected to quantify exposure precisely and continuously over a whole season. Misisi is supplied by an open quarry, easily contaminated by runoff water from the densely populated and insanitary settlement nearby. The infective dose of *C. parvum* is low (the mean infective dose for American volunteers was calculated at 132 oocysts [13]), so it is not surprising that such contamination should confer a risk of waterborne infection. However, it is not yet possible to define exactly the proportion of oocysts that would be viable or infective [14]. Cryptosporidiosis has been shown to be seasonal in other studies in other geographic locations, including West Africa [15] and Brazil [8], and in this study, adjustment for the period of the rainy season increased the crude OR from 5.14 to 11.63.

The PAF due to higher water exposure was estimated to be 25% (95% CI, 2%–38%) for cryptosporidiosis among patients with persistent diarrhea. This is a large fraction, but the PAF is premised on the assumption that the patients with persistent diarrhea who attended for investigation are representative of the whole population with persistent diarrhea. This assumption is not completely secure, as we know that those who attended for investigation of persistent diarrhea were somewhat biased compared with the whole population as surveyed. Nevertheless, this is probably a reasonable estimate.

Given that in these adult populations with high HIV seroprevalence, diarrheal disease is a major contributor to illness and death, provision of higher-quality water (of the quality of that of the better areas) to the areas with the most contaminated supply would be expected to have beneficial consequences for adult health. This hypothesis can now be tested directly.

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References