HYPERENDEMIC CRYPTOSPORIDIUM AND GIARDIA IN HOUSEHOLDS LACKING MUNICIPAL SEWER AND WATER ON THE UNITED STATES-MEXICO BORDER

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Abstract. This study identified differences in the epidemiology of Giardia and Cryptosporidium infection for low-income populations residing on the United States-Mexico border. Participation included 77 households in three communities lacking adequate municipal water and sewage services. The household was the unit of analysis and sampling was from household biosolid waste from newly installed composting toilets. The proportion of households positive for Giardia and Cryptosporidium was high, 82% and 70%, respectively, and this was in contrast to the few households (14%) reporting at least one individual with diarrhea symptoms. This finding indicated that most of the participant families were chronically infected but asymptomatic. In the multivariate analysis, there was a statistically significant protective effect for Cryptosporidium in those households that purified drinking water but not for Giardia. Those households with children less than five years of age were 1.3 times at risk for Giardia infection. Our findings highlighted differences in the transmission mode of these two pathogens and underscore the need for interventions addressing hygiene, water supply, and sanitation.

INTRODUCTION

Cryptosporidium parvum, the causal agent of cryptosporidiosis, and Giardia lamblia, the causal agent of giardiasis, are two widespread protozoan parasites. Both are human pathogens and have been responsible for both epidemics as well as endemic levels of intestinal diseases.1,2 In Milwaukee, Wisconsin, more than 400,000 people were infected with C. parvum after watershed contamination.3 On the United States-Mexico border, a C. parvum prevalence study (n = 105) reported that children less than 13 years old had a prevalence rate of 70.2%.4 Both C. parvum and G. lamblia transmission is fecal-oral and occurs in developed countries usually by contaminated drinking or recreational water. In developing countries where poor sanitation exists, transmission may also be by person-to-person and from fecally contaminated food.

Disinfection of water by chlorination often does not provide adequate protection, especially for Giardia, due to low free chlorous residuals and limited contact time.5,6 Boiling water for one minute, treating with iodine for 20 minutes, or filtration (1 μm cut-off) are the best methods for eliminating both viable C. parvum oocysts and G. lamblia cysts.6 However, these methods are not practical for municipal water supplies.

Cryptosporidium parvum oocysts and G. lamblia cysts (infectious stage) may remain viable for several months, especially under moist conditions.7 Certain wild and domestic animals, may also serve as hosts for these protozoa. Worldwide, it is estimated that 0.6–4.3% of humans are infected with Cryptosporidium and 1.5–20% with Giardia.8,9

Children attending day care centers are at higher risk for these infections. Studies investigating children in developed countries who attend day care centers noted the following risk factors: large family size, time in day care, toddlers in the class room, low socioeconomic status (SES), and young age.10–12 Children attending day-care centers may acquire and spread these agents to family members.

In a previous study, we analyzed ecologic sanitary toilets for their ability to reduce fecal coliforms in the same study area.13 These findings indicated that due to the hot, dry climate of the Chihuahuan desert, composting by biodegradation was not effective due to a rapid desiccation. As a result, after six months treatment of biowaste, only 36% of composting toilets produced United States Environmental Protection Agency (USEPA) Class A compost based on fecal coliform counts (< 1,000 most probable number fecal coliforms/gram).

This study examined the prevalence and risk factors for C. parvum and G. lamblia in three peri-urban settlements on the United States-Mexico border where living conditions are representative of developing countries. These communities lacked adequate municipal water and sewage services and household crowding was common. In this study, the household was the unit of analysis and sampling was from household biosolid waste from newly installed self-contained composting toilets.

MATERIALS AND METHODS

The study site consisted of three communities, Nueva Galeana, Plutarco Elias Calles, and Felipe Angeles, located peripheral to Ciudad Juárez, Chihuahua, Mexico. These communities lacked municipal sanitation services and most did not have water piped to their homes. Study participants in all three communities were of low SES, with an average yearly income of ~ US $3,300. Study variables included drinking water treatment, household size, diarrhea, children less than five years of age, and day care center attendance. The University of Texas, El Paso, Institutional Review Board approval for human subjects was obtained to administer a questionnaire for household information and to sample household toilets.

Composting toilets and sampling. From August 1999 to March 2000, approximately 300 dry sanitation toilets were provided to the three study communities by a local foundation. These toilets, termed SIRDOs (Sistema Integral de Reciclamiento de Desechos Orgánicos), are single-vault, solar composting systems designed by Grupo de Tecnologia Alternativa (Naucalpan, Mexico). The SIRDOs are self-contained, fiberglass and plastic structures that stand separate from a home and serve a single household (Figure 1). A previous study analyzed these ecologic systems for their ability to re-

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ducify with organisms. This assay does not use on organism-specific antibodies. This assay does not

4.50–490 nm excitation filter was used to examine samples. Cryptosporidium oocysts or Giardia cysts were semi-quantified by counting the mean number of cysts/oocysts in microscopic fields (mean of 5) under 1,000× amplification (oil immersion). The mean number was scored as high (> 5), low (1–5), or negative (0). For most statistical analyses, the high and low groups were combined and categorized as positive. Positive and negative controls for Giardia and Cryptosporidium were provided in the Merifluor kit.

Household crowding was measured by dividing the number of household members by the number of bedrooms in the home. Other household characteristics included whether drinking water was purified by adding bleach or by boiling and if household members had at least one episode of diarrhea in the previous two weeks. Diarrhea was defined as having more than three loose stools in a 24-hour period.

Statistical analysis. The unit of analysis was the household, so a positive result indicated that at least one member of the family was infected. All data were analyzed with SPSS software (SPSS Inc., Chicago, IL) statistical software, and chi-square, Fisher’s exact, and t-tests were used to assess statistical significance of differences of proportions and means. Due to the variation in the number of users per SIRDO, some analyses were adjusted for the number of household members resulting in weighted values to account for these differences. Logistic regression was used to identify the relationship of environmental and household factors with Cryptosporidium oocysts or Giardia cysts present in biosolid waste while controlling for the effect of other variables. Because of high household prevalence of both Giardia and Cryptosporidium in the study area, odds ratio estimates were converted to prevalence ratios (PRs). The fit of the model was evaluated using the Hosmer-Lemeshow goodness of fit test.

RESULTS

From the three study communities, data were available for 77 households. Table 1 indicates that households in the three communities were similar with a few exceptions. Nueva Galeana had a slightly larger mean household size as well as a slightly higher mean number of households with children less than five years of age and more children attending day care centers or kindergarten. Felipe Angeles had the least household crowding. Half the households in all communities were sampled between three and six months after SIRDOs were placed into service. To assess whether timing of sample collection affected the results, a subset of 48 households was selected and two samples were taken from each, one at three months and one at six months. No significant difference was observed for households positive for G. lamblia cysts (P = 0.931) or for C. parvum (P = 0.852) throughout this time period, indicating that both cysts and oocysts remained relatively stable over the 3–6-month time period. In only one instance was a positive result observed to have changed to a negative value at the six-month sampling. There were no observations of a negative value followed by a positive sample.

Sample collection and analyses. Sampling consisted of stirring the compost heap with a garden hoe and collecting biosolids from five separate sites within the heap. Biosolid samples (total 100–200 grams fresh weight) were placed in a plastic bag and mixed to homogeneity by massaging the bag as previously described. Samples were transported to the laboratory in an ice chest within two hours of collection, stored at 4°C, and analyzed for Giardia and Cryptosporidium within 24 hours. After analysis, samples were disinfected or autoclaved.

Eleven grams of biosolid waste sample were mixed with 99 mL of water and blended in a Waring blender for 20 seconds after which an aliquot was taken for Giardia and Cryptosporidium analyses. A measured sample of 10 μL was used for each sample slide well. Samples with negative results were repeated one time to verify the negative result. The Merifluor Cryptosporidium/Giardia detection kit (Meridian Diagnostics, Inc, Cincinnati, OH) was used to quantify Giardia cysts and Cryptosporidium oocysts. This is a direct and highly specific immunofluorescence assay for C. parvum and G. lamblia based on organism-specific antibodies. This assay does not determine the viability of detected oocysts or cysts. A Zeiss axioskop fluorescence microscope (Thornwood, NY) with a 450–490 nm excitation filter was used to examine samples. Cryptosporidium oocysts or Giardia cysts were semi-quantified by counting the mean number of cysts/oocysts in microscopic fields (mean of 5) under 1,000× amplification (oil immersion). The mean number was scored as high (> 5), low (1–5), or negative (0). For most statistical analyses, the high and low groups were combined and categorized as positive. Positive and negative controls for Giardia and Cryptosporidium were provided in the Merifluor kit.
for *Giardia* (87.7%), while Nueva Galeana had the highest for *Cryptosporidium* (77.5%). Only 4.3% of all households had neither *Giardia* nor *Cryptosporidium*, while 65.0% had both.

In the weighted bivariate analysis, five study variables were statistically associated with the presence of *Giardia* and two with *Cryptosporidium* (Table 3). The first, size of household based on number of members, was statistically significant for *Giardia* but not for *Cryptosporidium*. Crowding likewise was statistically significant for *Giardia*, but not *Cryptosporidium*. Conversely, having children less than five years of age was significant for both *Giardia* and *Cryptosporidium*. Not purifying drinking water was a risk factor for *Cryptosporidium*, but not for *Giardia*. The variable children in day care centers was significant only for those households positive for *Giardia*, while diarrhea was not statistically significant for either pathogen.

In the multivariate analysis, there was a strong and statistically significant protective effect for *Cryptosporidium* in those households that purified their drinking water but not for *Giardia* (Table 4). Those households with children less than five years of age were 1.3 times more likely to test positive for *Giardia* and 1.1 for *Cryptosporidium*. An increase in the number of household members per room was statistically significant for *Giardia* with a PR = 0.9, indicating a small protective effect of increasing number of people per room. Although having a child in a day care center was statistically significant in the bivariate analysis, in the multivariate analysis it was not possible to calculate its effect due to small numbers. Having diarrhea was not significant for *Giardia* or for *Cryptosporidium* in the bivariate and multivariate analyses and was not included in the final model.

**DISCUSSION**

This study investigated the epidemiology of *Giardia* and *Cryptosporidium* infection in three, low-income populations on the United States-Mexico border. The household was used as the unit of analysis, which facilitated studying more than 400 individual residents of the participating households. This approach avoided the problem of obtaining stool samples from “healthy” individuals; rather the dry sanitation toilet for a particular household and its accumulated bio-waste in an enclosed vault was the source of biologic samples. Thus, biosolid waste samples represented a contribution from all members of the household, and the presence of *Giardia* cysts or *Cryptosporidium* oocysts indicated that at least one member of the household was infected. Detection was by immunofluorescence assay, which was direct and facilitated semi-quantitative counts of *C. parvum* oocysts and *G. lamblia* cysts.

The three communities included in the study were representative of discrete under-developed areas within Ciudad Juárez. Community criteria for inclusion included low income, a lack of municipal sanitation infrastructure, and limited water supply connections to the homes. Although these communities were located in different peri-urban areas of Ciudad Juárez, they were surprisingly similar in household size and high household positivity for both *Giardia* (81.6%) and *Cryptosporidium* (70.4%). Although these results cannot be generalized, they clearly indicate that living conditions in these communities should be improved to avoid such widespread parasitic infection.

Although the three communities were initially analyzed separately for demographic comparisons, combining data from all three communities increased sample size for a more precise analysis of study variables. The repeated measurements in the weighted analysis also effectively increased the sample size. Purification of drinking water was highly associated with and protective of households for the presence of *Cryptosporidium*. In these communities, the same relationships persisted in the multivariate analysis and suggested that

**Table 1**

Household demographics and characteristics of the three study communities

<table>
<thead>
<tr>
<th>Community</th>
<th>Plutarco Elías No (%)</th>
<th>Felipe Ángeles No (%)</th>
<th>Nueva Galeana No (%)</th>
<th>Total No (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Household members</td>
<td>5.83(1.65)</td>
<td>5.88(2.57)</td>
<td>7.64(3.95)</td>
<td>6.57(3.10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Household crowding*</td>
<td>3.03(1.67)</td>
<td>2.07(1.63)</td>
<td>3.05(1.79)</td>
<td>2.75(1.74)</td>
<td>0.078</td>
</tr>
<tr>
<td>Children &lt;5 years old</td>
<td>0.82(0.89)</td>
<td>1.04(0.92)</td>
<td>1.18(1.29)</td>
<td>1.02(1.08)</td>
<td>0.016</td>
</tr>
<tr>
<td>Purify drinking water†</td>
<td>16(59.3)</td>
<td>8(44.4)</td>
<td>11(42.3)</td>
<td>71</td>
<td>0.417</td>
</tr>
<tr>
<td>Child in day care/kindergarten</td>
<td>3(11.1)</td>
<td>1(4.3)</td>
<td>7(25.9)</td>
<td>77</td>
<td>0.079</td>
</tr>
</tbody>
</table>

*Household members/no. of bedrooms.
†Used bleach or boiled water.
‡Loose stools ≥3 times/day during the past 2 weeks.

**Table 2**

Infected households with *Giardia* and *Cryptosporidium* weighted by number of household members*

<table>
<thead>
<tr>
<th>Communities</th>
<th>Plutarco Elías No (%)</th>
<th>Felipe Ángeles No (%)</th>
<th>Nueva Galeana No (%)</th>
<th>Total No (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Giardia</em> positive</td>
<td>116 (87.7)</td>
<td>89 (79.5)</td>
<td>140 (82.8)</td>
<td>345 (81.6)</td>
<td>0.774</td>
</tr>
<tr>
<td><em>Cryptosporidium</em> positive</td>
<td>93 (69.5)</td>
<td>74 (66.1)</td>
<td>131 (77.5)</td>
<td>298 (70.4)</td>
<td>0.034</td>
</tr>
</tbody>
</table>

*Values are the number of household members weighted by household size.
†One-way analysis of variance.
Table 3
Bivariate analysis of selected variable and positive households for *Giardia* and *Cryptosporidium* (all three communities combined)

<table>
<thead>
<tr>
<th>Variable</th>
<th><em>Giardia</em></th>
<th><em>Cryptosporidium</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Household members</td>
<td>6.3 (2.9)</td>
<td>6.6 (3.3)</td>
</tr>
<tr>
<td>Positive</td>
<td>7.7 (3.5)</td>
<td>6.4 (2.5)</td>
</tr>
<tr>
<td>Household members/rooms</td>
<td>&lt;0.001</td>
<td>0.240</td>
</tr>
<tr>
<td>Positive</td>
<td>2.9 (1.7)</td>
<td>3.1 (1.8)</td>
</tr>
<tr>
<td>Negative</td>
<td>4.1 (2.1)</td>
<td>3.3 (1.9)</td>
</tr>
<tr>
<td>Children &lt;5 years old</td>
<td>&lt;0.001</td>
<td>0.010</td>
</tr>
<tr>
<td>Positive</td>
<td>0.7 (0.5)</td>
<td>0.7 (0.5)</td>
</tr>
<tr>
<td>Negative</td>
<td>0.4 (0.5)</td>
<td>0.5 (0.5)</td>
</tr>
</tbody>
</table>

Table 4
Multivariate analysis of weighted variables by household members for *Giardia*- and *Cryptosporidium*-positive households

<table>
<thead>
<tr>
<th>Variable</th>
<th><em>Giardia</em></th>
<th><em>Cryptosporidium</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PR (95% CI)</td>
<td>PR (95% CI)</td>
</tr>
<tr>
<td>Purify water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.1 (1.0–1.2)</td>
<td>0.7 (0.6–0.8)</td>
</tr>
<tr>
<td>No</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Children &lt;5 years old</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.3 (1.2–1.3)</td>
<td>1.1 (1.0–1.2)</td>
</tr>
<tr>
<td>No</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Crowding‡</td>
<td>0.9 (0.9–1.0)</td>
<td>1.0 (0.9–1.0)</td>
</tr>
</tbody>
</table>

* Multivariate prevalence ratio (PR), unweighted Hosmer-Lemeshow test for *Giardia* = 7.29, *P* = 0.506; for *Cryptosporidium* = 4.392, *P* = 0.820. CI = confidence interval.

† Referent group.
‡ Change per number of persons per bedroom.

The number of household members/room is a combined and direct measure of both SES as well as close physical contact among household members. In many studies, crowding is a negative factor and correlates with increased risk. In contrast, this study showed a protective effect of crowding and positive households for *Giardia*. When crowding was adjusted for household size and the effect of other variables, the multivariate model indicated that crowding remained significantly inversely related to *Giardia* infection (PR = 0.9). Although this finding runs counter to evidence reported for most infectious diseases, this is a statistical finding, which may not reflect a causal association and requires further study. This finding may be accounted for by the following arguments. Having a larger household size consisting of several working-aged individuals may relate to increased household income and thus a higher standard of living. Another possible explanation for this finding is that working household members are likely to use a more modern flush toilet at their place of work, thus minimizing their contribution to the household toilet.

The fact that no association was found with cases of diarrhea requires some clarification. Diarrhea is usually underreported because of the social stigma associated with it. One study (Hill J, unpublished data) found that interviews noted significant reluctance to discuss diarrhea, presumably because of the shame associated with it. Conversely, assuming that diarrhea reporting was accurate and that most participants were chronically infected with *Giardia* and *Cryptosporidium*, they may be asymptomatic carriers.

There are several studies reporting that *Giardia* is pathogenic only to those individuals who have been clear of infection for a number of years or most of their lives.\(^{16,17}\) Although progress has advanced in this research recently, many questions remain unanswered as to the role of asymptomatic infection, immunity, and parasite transmission. The present understanding (specifically for *Giardia*) is that the immune response is intimately involved in prevention of organism attachment to the intestinal epithelium, clearing of infection once it is established, and the development of protective immunity.

*Giardia* and *Cryptosporidium* are two of many infectious agents affecting families living in substandard housing developments. This study confirmed the long-established understanding that clean water and proper biosolid waste disposal are important factors in preventing transmission of diseases. In addition to improvements in community water and sewer

...good quality drinking water was more important to prevent *Cryptosporidium* transmission.

In these communities, community health workers instructed households to add bleach to their water so that the usual method of purifying water is chemical rather than by boiling. This may be adequate for many microorganisms but not for *Giardia* cysts as noted in other studies.\(^5,6\) Our results showed two findings related to this issue: 1) the treatment of water with sodium hypochlorite was associated with a decreased presence of *Cryptosporidium* but not *Giardia*, and 2) the presence of children less than five years old was associated with an increased presence of *Giardia*. The latter suggests that *Giardia* exposure may also be the result of less hygienic practices in young children. Unfortunately, we did not collect data on hygienic practices in this study. The diverse effects of chlorination of domestic water for the presence of these two organisms merits further research.

Results from the bivariate analysis revealed that having a child less than five years of age in the household was more strongly associated with *Giardia* than with *Cryptosporidium* infection. Several explanations could account for young children as a risk factor for *Giardia* infection. Children are less careful hygienically and, to complicate matters, areas where the children played in these study sites were usually packed dirt yards with often noticeable piles of animal or human fecal matter. Some children attended day care centers where they may come in close contact with many children, some of which are diapered toddlers. However, in these communities day care centers were not widely used as family members often take care of young children. Thus, it is not surprising to discover that in this study the variable, day care, was less a potential risk factor as it has been reported in other studies in developed nations.\(^10–12\) Although significant for *Giardia* in the univariate analysis, day care was not included in the multivariate analysis due to the small number.
infrastructure, interventions should focus on hygienic behavior such as hand washing and water purification methods (e.g., boiling or iodine treatment rather than using only bleach) to minimize the spread of pathogens.

With the introduction of these new ecologic sanitary toilets, there is now a need for standards for disposal of the composted waste. Based on our findings, there is the potential that viable Giardia and Cryptosporidium cysts and oocysts are still present in the composted waste. For hygienic reasons, this waste should be disposed of by burying or bagging it for transporting to a landfill to prevent future human exposure. Currently, due to lack of standards, families are disposing of compost biowaste by either applying it on non-edible plants, bagging it for an extended treatment period, removing it by municipal waste disposal, or burying it.

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