GIARDIA INTESTINALIS INFECTION AS A RISK FACTOR FOR CYCLOSPORA CAYETANENSIS AND CRYPTOSPORIDIUM INFECTIONS IN PERUVIAN CHILDREN

By

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A THESIS

Presented to the Department of Public Health and Preventive Medicine

and the Oregon Health & Science University

School of Medicine

in partial fulfillment of

the requirements for the degree of

Master of Public Health

May 2009

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Department of Public Health and Preventive Medicine

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Oregon Health & Science University

CERTIFICATE OF APPROVAL

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Acknowledgements

I thank my committee members, Kevin, Dongseok, and Vita for your enthusiasm for this project, your mentoring, and your willingness to form a new collaboration.

I thank the Infectious Disease Society of America and the N.L. Tartar Trust for supporting my research in Peru.

Thank you to the faculty and staff of Public Health and Preventive Medicine including (but not limited to) John Stull, Bill Lambert, Tree Triano, and Katie Riley for your guidance through the theoretical and real-world elements of conducting research.

Thank you to the outstanding public health workers of Asociacion Benefica PRISMA, especially Lilia Cabrera, for collecting these data and teaching me about life in Peru.

Thanks to my many MPH and MD/MPH classmates for sharing your ideas and spirits.

Many thanks to Dave and to my family for all of your help.

Research Question:

Do Peruvian children infected with *Giardia intestinalis* have a different risk of infection with *Cyclospora cayetanensis* or *Cryptosporidium*?

Specific Aims:

Giardia intestinalis is a common intestinal parasite that affects humans worldwide. Infection can be asymptomatic, or can cause acute, intermittent, or chronic diarrhea. Transmission of the parasite typically occurs via contaminated water and food, or directly person to person. Clinicians do not always treat *Giardia* infections in Peru with antimicrobial drugs because the infection is very common and frequently asymptomatic. Past studies have also recommended against treating all *Giardia* infections because of high rates of reinfection within a short time period (Gilman, RH. 1988). However, studies have not addressed whether infection with *Giardia* changes the risk of infection from other parasites. Using data from a cohort study of 477 Peruvian children, we will conduct a secondary data analysis to test the following hypotheses:

Hypothesis 1 Peruvian children with *Giardia* infection have a different time to *Cyclospora* infection than children who do not have *Giardia*.

Hypothesis 2 Peruvian children with *Giardia* infection have a different time to *Cryptosporidium* infection than children who do not have *Giardia*.

Our specific aims:

- Identify the burden of clinical symptoms including diarrhea associated with *Giardia* infection.
- Determine the frequency of treatment for *Giardia* infection.
- Perform univariate analyses associating *Giardia* and other risk factors such as age and water source with *Cyclospora* and *Cryptosporidium* infections.
- Build a multivariate model that identifies risk factors for *Cyclospora* and *Cryptosporidium* infections.

Abstract:

We conducted a prospective cohort study among 477 Peruvian children to assess the frequency of, and risk factors for, infections with *Giardia, Cryptosporidium* and *Cyclospora*. We also sought to determine if *Giardia* infections within 60 days of enrollment into the study affected the incidence of *Cyclospora* or *Cryptosporidium* infections. One-hundred sixty (33.5%) of children had *Giardia* infection within the first sixty days of observation, and 381 (79.5%) had at least one infection during the five-year study. The median episode length was less than seven days, however, ten percent of infections lasted longer than one year. Results from the Cox proportional hazards analysis showed that *Giardia* increased the child's risk for acquiring subsequent infections with *Crypsporidium* (HR 5.49, 95% CI 2.74 – 11.0) or *Cyclospora*, though risk differed by sanitation status for *Cyclospora* (with toilet HR 1.12 95% CI 0.69-1.80, without toilet HR 2.22 95% CI 1.68-2.94). Antiparasitic treatment of *Giardia* did not affect the risk for later infection with *Cyclospora* or *Cryptosporidium*. These findings

suggest that *Giardia* may predispose children to subsequent infections with *Cryptosporidium* or *Cyclospora*, however, further research into specific risk factors such as specific foods, their sources, and other socioeconomic risk factors may help elucidate the relationships between these parasites.

Introduction:

The enteric parasite *Giardia intestinalis* (syn. *G lamblia* and *G duodenalis)* causes an estimated 2.8 million infections per year (Ali & Hill, 2003). *Cyclospora cayetanensis* and *Cryptosporidium* species are also enteric protozoans that cause diarrheal disease worldwide (Karanja, Gatei, & Wamae, 2007; Savioli, Smith, & Thompson, 2006). These parasites are common causes of infection among children in Peru (Bern et al., 2002; Gilman et al., 1988). *Giardia* and *Cryptosporidium* are transmitted through contaminated food and water, or directly person-to-person (Vidal et al., 1991; Yoder JS. Beach MJ. Centers for Disease Control and Prevention (CDC), 2007). *Cyclospora* is not transmitted person to person and is most frequently associated with foodborne transmission (Herwaldt, 2000).

Infections can be asymptomatic, or can cause acute, intermittent, or chronic diarrhea. In hyperendemic areas of Peru, 13-43% of infections with *Cryptosporidium* or *Cyclospora* are associated with diarrhea (Bern et al., 2002; Hollm-Delgado et al., 2008). The long-term effects of diarrhea during childhood can include growth stunting (Checkley, Epstein, Gilman, Cabrera, & Black, 2003) and decreased physical fitness (Guerrant et al., 1999).

Giardia infections have also been associated with decreased cognitive function later in childhood (Berkman, Lescano, Gilman, Lopez, & Black, 2002).

Giardia, *Cryptosporidium*, and *Cyclospora* can be endemic in the same communities (Karanja et al., 2007; Lanata et al., 1992; Lopez et al., 2003), and yet little is known about how infection with one organism affects the risk for infection with another. *Giardia* and *Cyclospora* preferentially infect the proximal small intestine, while *Cryptosporidium* may colonize the whole small intestine (Borowski, Clode, & Thompson, 2008; Ortega et al., 1997; Troeger et al., 2007). *Giardia* trophozoites can cause intestinal damage through apoptosis of intestinal epithelial cells and disruption of the epithelial barrier (Troeger et al., 2007), despite their extracellular location. Contrarily, *Cyclospora* and *Cryptosporidium* are intracellular parasites (Ortega et al., 1997; Borowski et al., 2008). Disruption to the integrity or health of enterocytes could alter the ability of another parasite to establish an infection or could cause different clinical symptoms. Our objective was to investigate whether *Giardia* infection altered risk for *Cryptosporidium* or *Cyclospora* infection among Peruvian children.

Methods:

Overview:

This is a secondary analysis of a cohort designed by investigators at Johns Hopkins University Bloomberg School of Public Health (JHUSPH) and the US Centers for Disease Control and Prevention (CDC). The study was approved by the Institutional Review Board of JHUSPH (Protocol H.22.05.03.08.A2), CDC, and the in-country partner

Asociacion Benefica PRISMA. Anonymous databases were used for this analysis and the protocol was approved by the OHSU IRB (4149).

The study site was the community of Pampas de San Juan de Miraflores, a peri-urban shantytown, or *pueblo joven*, near Lima, Peru. Six-hundred sixteen (616) children were enrolled into a longitudinal cohort study. Participants provided weekly stool samples for surveillance of enteric parasites for a period of up to five years. Guardians of the participants were asked daily about the child's gastrointestinal symptoms. Socioeconomic data were collected on a quarterly basis.

Recruitment:

Investigators enrolled children into two non-overlapping cohorts of children ages less than one year to twelve years using random selection of Pampas families. The cohort 'CS' enrolled children from 2001 to 2003. The cohort 'TM' enrolled from 2003 to 2005. Investigators limited study participation to one child per household. Children were not eligible if they had a diagnosis of severe malnutrition, or if they were participating in any other study involving treatment of or vaccination for parasitic disease. A parent or guardian provided written informed consent for each child.

Criteria for data used in the analyses:

Data were included in these analyses the participant met the following criteria:

- Participation in the study for at least six consecutive months.

- At least 25 stool weekly stool samples evaluated during their study participation.
- At least 80% non-missing symptom surveillance entries.
- Free from *Cyclospora* or *Cryptosporidium* at initial sample.

Variable Definitions:

We defined the time to *Cyclospora* or *Cryptosporidium* infection for each child as the time in days from the first stool sample submitted to the first sample that contained *Cyclospora* or *Cryptosporidium*, respectively. We defined symptomatic infections as those with diarrhea, vomiting, or abdominal pain reported within 7 days prior to the start of the infection, during the infection, or within 7 days after the infection. We defined diarrhea as three or more liquid or semi-liquid stools on the daily symptom survey. Children who did not have the outcome infection were censored at the time they left the study.

***Giardia* infection** - The primary predictor of interest was *Giardia intestinalis* infection within the first 60 days of study surveillance. We considered *Giardia* present if the child had any positive stool sample within 60 days of enrollment. We also classified the number of *Giardia* infections per child. The first day of an infection episode was the first day that the parasite was detected in stool. The episode ended when parasite was absent from three consecutive weekly stool samples, with the last date of the episode corresponding to the date of the last positive stool. Characteristics of *Giardia* infection

included association with diarrhea or vomiting within 7 days prior to the start of the episode until 7 days after the end.

Anti-parasitic treatment – We classified treated children as those who received albendazole, mebendazole, metronidazole, piperazine, or praziquantel within the first 60 days of enrollment.

Age and Season – We calculated age at enrollment as a continuous variable from the date of birth to the date of the first stool sample. We characterized season for this southern hemispheric region as Summer (January-March), Fall (April-June), Winter (July-September), and Spring (October-December).

Water and Sanitation – We categorized the child's water source at enrollment into one of three unranked categories. These included a faucet inside the house, a faucet outside of the house, and unimproved water. The unimproved water category included water from delivery trucks, gifted from neighbors, or from a communal water source (pilon). We categorized the sanitation variable into indoor plumbed toilet versus other, such as latrine, yard, and use of toilets outside of the house.

Data Collection:

At enrollment, study workers administered a questionnaire to the adult guardians to record household characteristics and socioeconomic status. We collected clinical gastrointestinal symptom information daily and collected a weekly stool sample.

Study personnel at Universidad Peruano Cayetano Heredia (UPCH) microscopically tested all stool specimens for ova and parasites, including *Giardia*. *Cryptosporidium* and *Cyclospora* were detected by acid fast stain, and microsporidia species by Weber-modified chromotrope stain. Data entry discrepancies were corrected using source documents. Data was further cleaned to verify logical discrepancies of the dataset.

Statistical Analysis:

Univariate Analyses:

We imported all collected interview and lab data into a Microsoft Access (Microsoft Corp., Redmond, WA) database to SAS 9.1 for Windows (SAS Institute, Cary, NC). We investigated the range and distribution of each categorical predictor by plotting Kaplan-Meier survival curves. We confirmed the proportionality assumption by testing for interactions with the observation time variable and by performing Kolmogorov-type supremum tests for each variable. We tested candidate covariates for equality across strata using the Wilcoxon log-rank for categorical variables (PROC LIFETEST) and using univariate Cox proportional hazards regression for continuous variables (PROC PHREG).

Multivariate Modeling:

We included all candidate covariates in an initial multivariate model and conducted backward elimination by removing variables with p-value greater than 0.05. We tested variables for interaction with *Giardia* infection. We used a p-value of less than 0.05 for significance in all analyses.

Results:

Demographics:

The CS and TM cohorts enrolled 616 children, however there were 477 children (51.1% girls) who met the criteria for our analysis. We excluded data from two children because they had *Cyclospora* infections on the first recorded stool sample. The median age at enrollment of the evaluable children was 22.6 months (10 days to 12 years). We captured 1306 child-years in surveillance with a median surveillance time of 2.6 years per child (0.6 years to 4.5 years).

Table 1: Characteristics by Enrollment Project (N=477)

Variable	Project CS (N=210)	Project TM (N=267)	P-value
Male sex	95 (45.4%)	139 (52.1%)	0.14
Mean Age	34.8 months	23.4 months	<0.001
Enrollment Season			<0.001
Summer	48 (22.9%)	29 (10.9%)	
Fall	1 (0.5%)	47 (17.6%)	
Winter	3 (1.4%)	25 (9.4%)	
Spring	158 (75.2%)	166 (62.2%)	
Indoor toilet in home	138 (65.7%)	177 (66.3%)	0.89
Water Source			0.84
Other Water	71 (33.85)	87 (32.6%)	
Indoor Water	121 (57.6%)	153 (57.3%)	
Outdoor Water	18 (8.6%)	27 (10.1%)	
***Giardia* infection within 60 days**	120 (57.1%)	197 (73.8%)	<0.001
Received antiparasitic treatment	24 (11.4%)	35 (13.1%)	0.58
***Cyclospora* infection**	114 (54.3%)	69 (25.8%)	<0.001
***Cryptosporidium* infection**	59 (28.1%)	84 (31.5%)	0.30

Stool Samples:

There were 54900 stool samples collected from the included children. *Giardia intestinalis* was the third most commonly identified parasitic organism (13% of samples, Table 2). Parasites infrequently detected included *Enterobius vermicularis* (n=55), *Strongyloides stercolis* (n=35), *Diphyllobothrium pacificum* (n=23), *Acylostoma*

duodenale (n=10), *Isospora belli* (n=6), *Hymenolepsis diminuta* (n=1), *Entamoeba histolytica* (n=0), Taenia spp (n=0).

Table 2: Parasites Detected in Stool Samples

Pathogen	Count of Positive Samples	Percent of Samples Positive
Entamoeba coli	11225	20.45
Endolimax nana	9398	17.12
Giardia intestinalis	7124	12.98
Chilomastix mesnili	3048	5.55
Hymenolepsis nana	1062	1.93
Trichuris trichuria	452	0.82
Cyclospora cayetanensis	387	0.70
Iodamoeba bütschlii	217	0.40
Ascaris lumbricoides	187	0.34
Cryptosporidium spp.	184	0.34
Blastocystis hominis	128	0.23
microsporidia	98	0.18

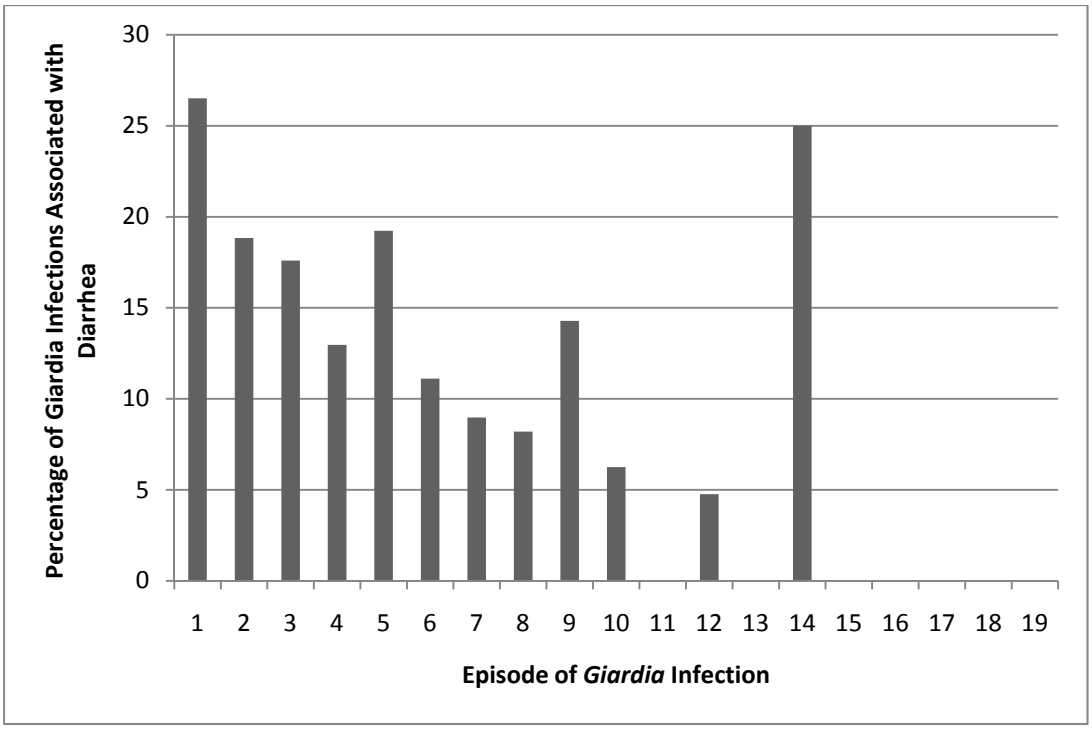
***Giardia* Epidemiology:**

Three-hundred eighty-one children (79.5%) had at least one episode of *Giardia intestinalis* infection. Of these, 292 (76.6%) had at least two episodes with the median of 106 days between the first and second episodes. However, 18 children had a *Giardia* recurrence within 21 to 30 days, just beyond the disease-free interval that defined the end of an episode. An additional 23 recurred within 31 to 40 days, and an additional 31 had a

recurrence between 41 to 50 days after their last positive sample. The maximum number of episodes was 19 in one child that was under surveillance for 4.5 years. The median number of episodes per child was two.

We examined the first episode of *Giardia* infection per child. Two-hundred-two (53.0%) of these first episodes lasted one week or less. An additional 67 (17.6%) were longer than one week but less than one month, 102 (26.8%) were longer than one month, and 10 (2.6%) were longer than one year. The longest episode lasted 793 days in a participant who entered the study at eight months of age. Diarrhea was present in 26.5% of the first *Giardia* infections and decreased in prevalence with subsequent *Giardia* infections (Figure 1). Nevertheless, it was the symptom most frequently associated with giardiasis.

Figure 1: Frequency of Diarrhea by *Giardia* Episode Number



Children who had a *Giardia* infection within the first 60 days of the study differed from uninfected children on many demographic indicators. Notably, they were older and less likely to have improved sanitation and water source (Table 3).

Table 3: Demographic Characteristics of Evaluable Participants and Infections with *Giardia.*

	No *Giardia* (N=317)	***Giardia* (N=160)**	**P-value**
Male sex	157 (49.5%)	77 (48.1%)	0.77
Mean age	23.3 months	40.1 months	<0.001
Enrollment Season			0.17
Summer	46 (14.5%)	31 (19.4%)	
Fall	28 (8.8%)	20 (12.5%)	
Winter	17 (5.4%)	11 (6.9%)	
Spring	226 (71.3%)	98 (61.3%)	
Indoor toilet in home	228 (71.9%)	87 (54.4%)	<0.001
Water Source			<0.001
Other Water	87 (27.4%)	71 (44.4%)	
Indoor Water	205 (64.7%)	69 (43.1%)	
Outdoor Water	25 (7.9%)	20 (12.5%)	
Received antiparasitic treatment	8 (2.5%)	51 (31.9%)	<0.001

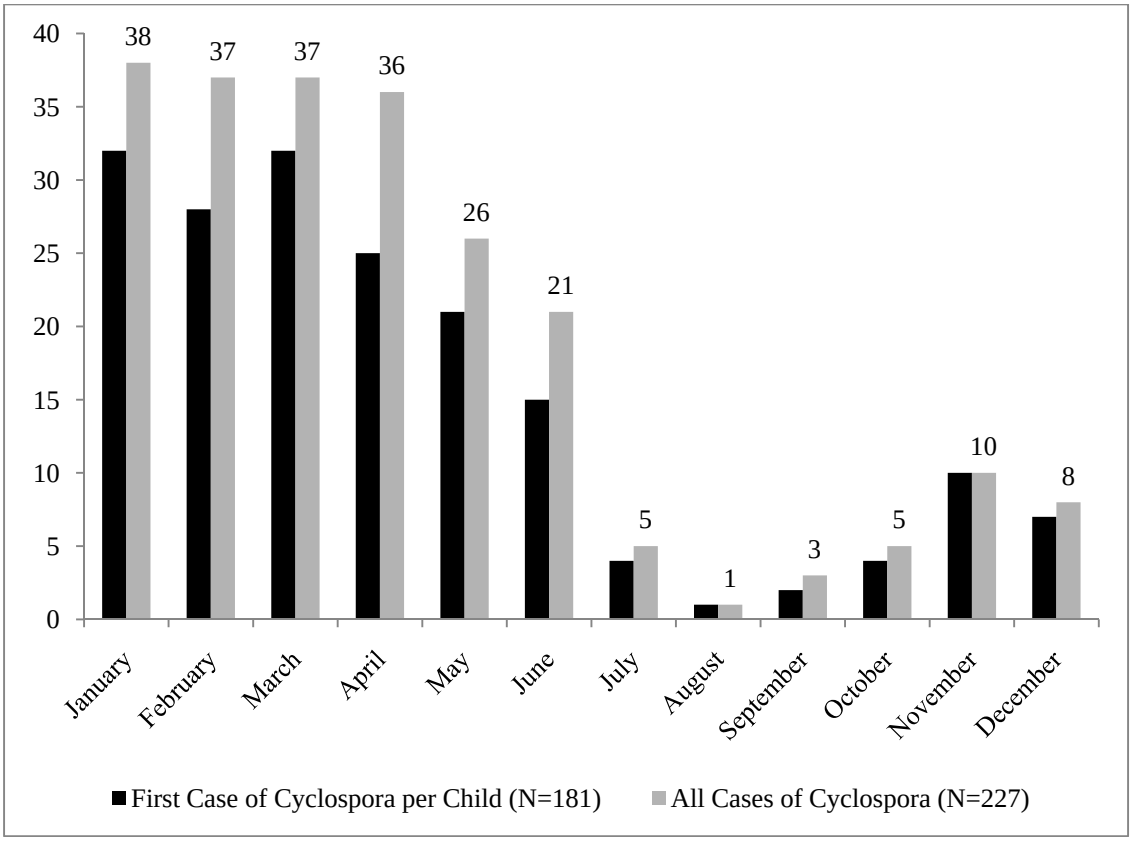
Cyclospora Epidemiology

One-hundred eighty-one children (37.9%) had at least one episode of *Cyclospora cayetanensis* infection. The median time to first infection was 431 days (range 7-1637 days). Forty-four children had a second infection. One-hundred thirty-one of the first episodes (70.8%) lasted less than one week (range less than 1 week to 18 weeks). *Cyclospora* infections displayed a marked seasonality with the highest incidence of cases in the summer and fall months of January through June (Figure 2). Very few cases were detected in July, August, September, and October, corresponding to winter and early spring. *Cyclospora* incidence did not change appreciably over the years of the study (Table 4). Incidence was highest among children aged two to five (Table 4).

Table 4: Annual Incidence of *Cyclospora* Infection by Age Category (Infections per 10,000 person-days)

Age in Years	2002	2003	2004	2005	2006
0 - 1	3.29	4.07	4.09	4.53	--
2 - 3	7.55	9.41	6.88	5.27	8.85
4 - 5	3.28	6.41	3.48	6.82	7.98
6 - 7	--	2.77	3.23	3.36	3.56
8 - 9	--	10.52	1.99	4.30	4.49
10 - 12	--	12.11	2.92	--	--

Figure 2: Cases of *Cyclospora* by Month



Giardia infection was associated with increased risk of *Cyclospora* infection with a univariate hazard ratio of 1.42 (95% CI 1.06-1.91). Younger age at enrollment, presence of toilet, and enrollment in the CS project were also associated with increased risk of *Cyclospora* infection (Table 5). Treatment with an antiparasitic agent did not affect risk for Cyclospora infection (HR 1.004, 95% CI 0.64-1.58). Sanitation status modified the effect of *Giardia* such that *Giardia* was only associated with *Cyclospora* in children without indoor plumbing (Figure 3). The parameter estimates for *Giardia* were similar in

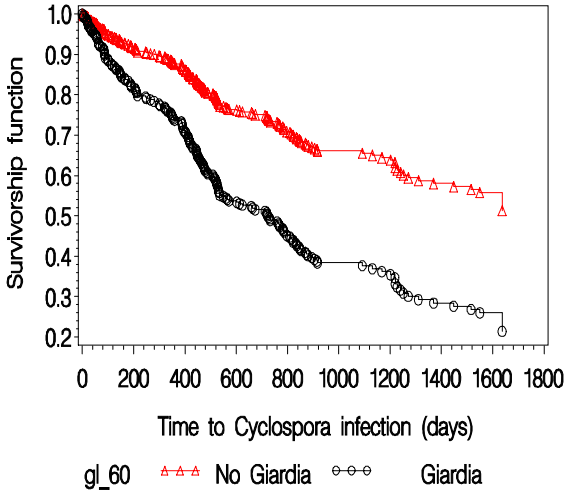
a model that included a multiplicative interaction between *Giardia* and sanitation status and a model stratified by sanitation status.

Table 5: Multivariate model for *Cyclospora* infection (N=477)

Variable	First *Cyclospora* infection		
	Hazard Ratio	**95% Confidence Interval**	**P**
Early *Giardia*			0.004
Toilet Present	1.12	0.69-1.80	
Toilet Absent	2.22	1.68-2.94	
Age in Months	0.992	0.986-0.999	0.031
Toilet Present	1.66	1.04 – 2.66	0.035
Project TM	0.56	0.41-0.78	<0.001
Interaction Term Sanitation * *Giardia*			0.042

Figure 3: Time to *Cyclospora* infection by *Giardia* status

Children without toilet



Children with toilet

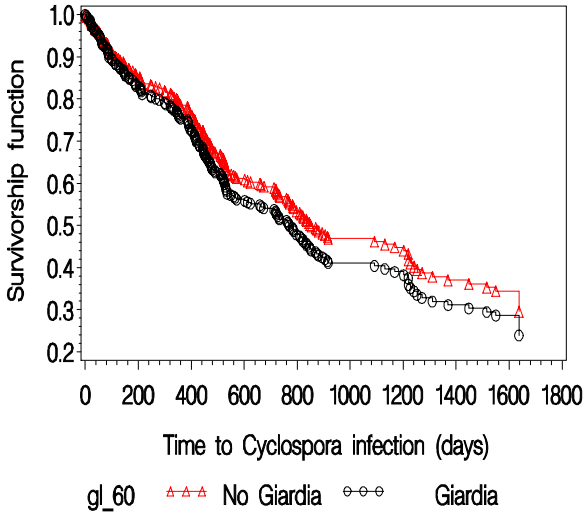


Figure 3: Composite survival curves for child age 3 years, enrolled in CS cohort, without and with toilet.

Symptomatic *Cyclospora* Infection

Diarrhea occurred in 57 out of 181 (31.5%) of the first *Cyclospora* infections per child and decreased to 3 out of 44 (6.8%) for the child's second episode. Abdominal pain or vomiting occurred with six and two cases of *Cyclospora* first and second infections respectively. Younger age was the only predictor of symptomatic *Cyclospora* infection (HR 0.985, p 0.02).

***Cryptosporidium* Epidemiology**

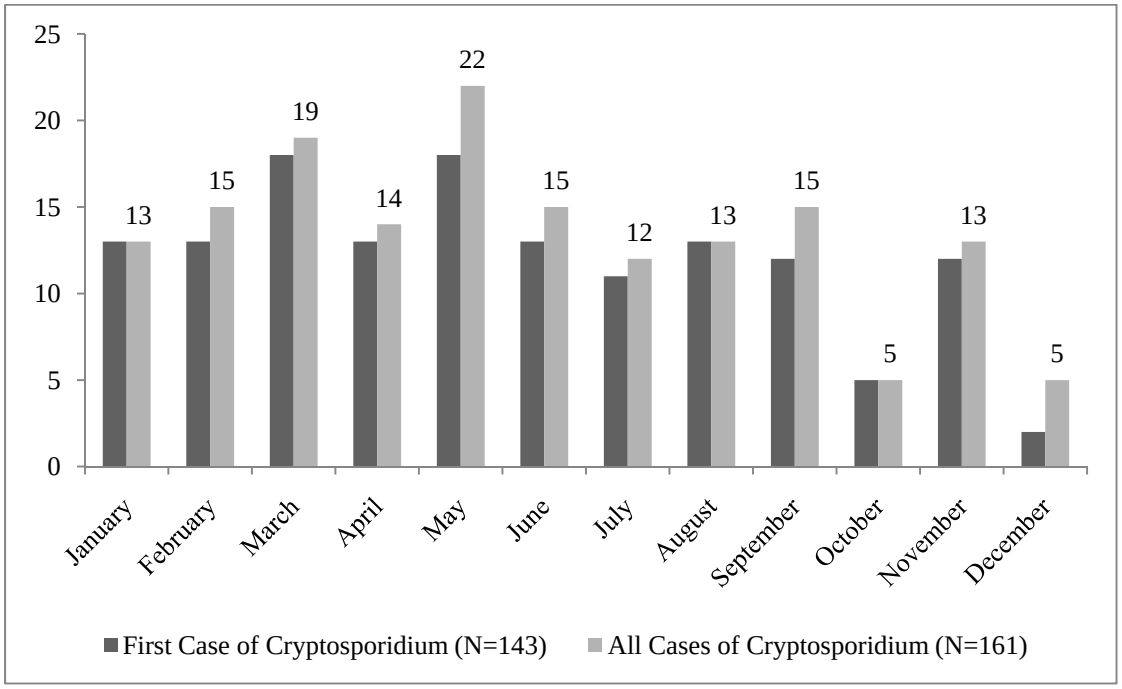
One-hundred forty-three children (30%) had at least one episode of *Cryptosporidium* infection. The median time to first infection was 387 days. Of these, 17 children (11.9%) had at least two episodes, and one child had three episodes. One-hundred nineteen (83.2%) of the first episodes lasted less than one week (range <1 week to five

weeks). Young children ages zero to three years had the highest incidence of *Cryptosporidium* infection (Table 6). Cryptosporidium incidence increased across age strata throughout the course of the study (Table 6). *Cryptosporidium* cases did not occur with the seasonality observed with *Cyclospora* (Figure 4).

Table 6: Annual Incidence of *Cryptosporidium* Infection by Age Category (Infections per 10,000 person-days)

Age in Years	2002	2003	2004	2005	2006
0 - 1	3.29	3.25	8.77	4.53	5.87
2 - 3	2.30	4.91	6.88	4.13	10.03
4 - 5	3.28	1.28	3.80	1.62	3.55
6 - 7	--	2.77	1.62	0.48	1.78
8 - 9	--	3.51	--	--	4.49
10 - 12	--	--	--	--	--

Figure 4: Cases of *Cryptosporidium* by month



Giardia infection was associated with *Cryptosporidium* infection when controlling for age (Table 7). Enrollment in the TM project was also a risk factor for *Cryptosporidium* infection (Table 7). The hazard ratios for *Giardia* among children age less than one year and one to three years were 2.6 (95% CI) and 1.6 (95% CI). *Giardia* was not associated with *Cryptosporidium* infection among older children (Table 8). Treatment within the first sixty days of enrollment with an antiparasitic agent was not associated with subsequent *Cryptosporidium* infection (HR 1.12, 95% confidence limits 0.68-1.83).

Table 7: Risk factors for *Cryptosporidium* infection (N=477)

Variable	First *Cryptosporidium* infection		
	Hazard Ratio	**95% Confidence Interval**	**P**
Early *Giardia*	5.49	2.74-11.03	<.001
Age in Months	0.986	0.974 – 0.997	0.016
Project Code TM	1.59	1.09 – 2.31	0.016
Interaction Term **Age * Giardia**			<0.001

Table 8: Association between *Giardia* and *Cryptosporidium* Infection by Age Group

Age Group	**N**	***Cryptosporidium* Cases**	**Hazard Ratio* (95% Confidence Interval)**	**P**
<1 year	125	48 (39.4%)	2.56 (1.14 – 5.75)	0.02
1-3 years	200	74 (37.0%)	1.55 (0.96 – 2.48)	0.07
3-5 years	97	14 (14.4%)	0.47 (0.16 – 1.35)	0.16
>5 years	55	5 (9.1%)	1.1 (0.18 - 6.58)	0.92

*Hazard ratio for *Cryptosporidium* in children with *Giardia*

Symptomatic *Cryptosporidium* Infection

Diarrhea occurred in 42 out of 143 (29.4%) of the first *Cryptosporidium* infections per child and decreased to 2 out of 17 (11.8%) for the child's second episode. Abdominal pain or vomiting occurred with four and one cases of *Cryptosporidium* first and second infections respectively. Younger age was the only predictor of symptomatic *Cryptosporidium* infection (HR 0.951, 95% CI 0.929 – 0.973, $p<0.001$).

Discussion:

We conducted a large prospective cohort study of Peruvian children and found that *Giardia* is a frequent parasitic gastrointestinal infection in this community, although most infections are asymptomatic. Importantly, our study documented that in some children, *Giardia* infection increased risk of subsequent infection with *Cyclospora* and *Cryptosporidium* infection.

We characterized the epidemiology of *Giardia intestinalis* infection in Peruvian children. We demonstrated a high period prevalence of Giardia infection within 60 days of study enrollment. Consistent with prior reports from this community (Hollm-Delgado et al., 2008), we found the majority of these infections to be asymptomatic and lasting less than one week. However, we also identified some children with infections lasting over two years. Unlike a previous report (Hollm-Delgado et al., 2008), we found that lack of toilet and indoor water was associated with *Giardia* infection. Though 31.9% of children with *Giardia* infection received antiparasitic treatment, this did not affect their risk for *Cyclospora* or *Cryptosporidium* infection, or reinfection with *Giardia*. This was previously observed and suggests that termination of a *Giardia* infection episode did not confer protection from subsequent *Giardia* infections (Gilman et al., 1988).

Despite treatment, the recurrence of *Giardia* was frequent in this study population. However, intermittent shedding of *Giardia* could not be fully assessed through weekly stool surveillance. We attempted to control for intermittent shedding by using a stringent episode definition of a *Giardia*-free interval of at least twenty-one days with at least three

negative stools to separate our episodes. However, many children recurred shortly after their twenty-one day interval. It is unclear where these 'recurrences' represent a separate infection episode, or intermittent shedding during one long infection episode. Genetic analysis of the *Giardia* could help determine if these apparent long infections are indeed one infection episode or consecutive infections due to the high prevalence of the parasite in this community.

The finding that *Giardia* infection is a risk factor for subsequent *Cyclospora* and *Cryptosporidium* infections may have multiple explanations. Damage to the intestinal epithelium by the *Giardia* protozoan may allow subsequent organisms to invade the epithelium and establish an infection. However, we identified an interval of over a year between the *Giardia* exposure and the median time to *Cryptosporidium* or *Cyclospora* infection. This indicates that *Cryptosporidium* and *Cyclospora* infections did not immediately follow *Giardia* infections in many children. Despite this, *Giardia* infection likely persisted beyond the 60-day interval in some children, so the time from *Giardia* to subsequent *Cryptosporidium* or *Cyclospora* infection may be shorter than the interval we reported.

An alternative to the hypothesis of a direct effect is that *Giardia*, *Cryptosporidium*, and *Cyclospora* may share a common risk factor for infection that we did not capture. Household animals have been implicated as a risk factor for *Cyclospora* (Bern et al., 2002) and *Giardia* (Traub, RJ. 2007) in previous studies. In the case of *Cyclospora*, an anthroponotic parasite, presence of animals may be markers for other sources of

infection. As for *Giardia,* the assemblages of *Giardia* detected in several domestic animals are of both of wide and narrow host specificity, thus the zoonotic potential specific assemblages still needs to be determined.

We identified *Giardia* infection, younger age, and presence of an indoor toilet as risk factors for *Cyclospora* infection. Prior studies have found the reverse association (Chacin-Bonilla, Barrios, & Sanchez, 2007) or lack of an association (Bern et al., 2002) between availability of flush toilet and *Cyclospora* infection. Pampas de San Juan is an underserved community, and is composed of migrant people who used this non-urbanized area to settle in the outskirts of the city of Lima. Thus, the overall classification of the community is of low socio economic status (SES). Indoor plumbing is expensive in this community, and may serve as a marker of higher SES within Pampas. Families with higher SES may be able to afford fresh produce, which has previously been identified as a risk factor for *Cyclospora* infection (Ortega, YR. 1997). Detailed studies on foods consumed, their sources and dietary variety may help to explain the transmission of cyclosporiasis.

We found that sanitation status was an effect modifier in the relationship between *Giardia* and *Cyclospora,* such that *Giardia* was a risk factor for *Cyclospora* infection among children who lacked an indoor toilet, but not among children with an indoor toilet. Children without an indoor toilet may have a different source of both *Giardia* and *Cyclospora* exposure. Additionally, lack of an indoor toilet may allow for re-infection with *Giardia,* which could alter susceptibility to *Cyclospora* infection.

We identified age at enrollment as an effect modifier for the relationship between *Giardia* infection and *Cryptosporidium* infection. *Giardia* was associated with *Cryptosporidium* among young children, but not among children enrolled at age three or older. In part, this is due to the infrequency of *Cryptosporidium* infection among children older than three years. This is consistent with previous studies finding that Peruvian children ages 5-12 years had a four-fold lower incidence of *Cryptosporidium* infection than children ages 4 years or younger (Bern et al., 2002).

The enrollment project predicted subsequent infection with *Cryptosporidium* and *Cyclospora* even after adjusting for age. The enrollment projects did not differ in their methodology, but only in their calendar years and age at enrollment. The increase in *Cryptosporidium* incidence in the later years of the study could account for part of this finding. Additionally, the TM cohort ended before many children reached the age of four or five. Incidence of *Cyclospora* was high among the four and five year-olds in the CS cohort. Thus, the children from the TM cohort may not have been observed long enough to document their *Cyclospora* infections.

Several limitations affect this study. First, enrollment occurred at a range of ages. Children may have had infections with *Giardia*, *Cryptosporidium*, or *Cyclospora* prior to their enrollment, and the symptoms and interactions between the parasites may changes depending on the infection episode. We attempted to mitigate this problem by controlling for age in the analysis and using a short period of *Giardia* exposure after enrollment. Second, although we attributed gastrointestinal symptoms to concurrent

Giardia, *Cryptosporidium*, or *Cyclospora* infection, there are many other pathogens that cause diarrhea, abdominal pain, and vomiting. For some of these pathogens, testing is possible and was not undertaken, but for others, testing was not available. However, rotavirus, a common cause of diarrheal disease in Peruvian children, has been found most frequently as a single agent rather than in mixed infections (Cama et al., 1999).

Giardia intestinalis infection is common and is associated with subsequent *Cryptosporidium* and *Cyclospora* infections in some Peruvian children. Treatment of *Giardia* with antiparasitic agents was not shown to reduce risk for subsequent *Cryptosporidium* or *Cyclospora* infections. The mechanism for the relationship between these parasites is unclear. Intestinal damage from *Giardia* infection may predispose to *Cryptosporidium* or *Cyclospora* infection. Alternatively, there may be a common risk factor such as a food or animal exposure. Further studies may investigate the timing of these parasitic infections using a matched case-control design. Additional investigation into the socioeconomic risk factors for *Cyclospora* and *Cryptosporidium* infections would broaden our understanding of the epidemiology of these emerging pathogens.

Appendix:

Table 1: Clinical Symptom Definitions

Symptom	Type	Answer Represents
Diarrhea	Numeric	Number of semi-liquid stools in past 24 hours
	Numeric	Number of liquid stools in past 24 hours
Vomiting	Yes/No	Has the child vomited in the past 24 hours?
Abdominal Pain	Yes/No	Has the child had abdominal pain in the past 24 hours?

Table 2: Candidate Covariates

Factor	When Assessed	Type
Age at Enrollment	Date at first stool sample.	Continuous
Season of Enrollment	Date at first stool sample.	Categorical - Summer, Fall, Winter, Spring
Gender	Survey at enrollment.	Categorical – Male, Female
Water Source	Household survey at enrollment.	Categorical – Indoor faucet, Outdoor faucet, Other
Sanitation	Household survey at enrollment.	Categorical – Indoor plumbed toilet, Other
Giardia Infection	Infection within 60 days of enrollment	Categorical – Present or Absent
Anti-parasitic Treatment	Treatment within 60 days of enrollment	Categorical – Present or Absent

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