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Temporal and spatial epidemiology of enteric parasitic infections in a pediatric cohort of children living in the peri-urban areas of Lima, Peru

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Bachelor of Arts
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2011

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A thesis submitted to the Faculty of the
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Abstract

Temporal and spatial epidemiology of enteric parasitic infections in a pediatric cohort of children living in the peri-urban areas of Lima, Peru

By Alice E. Arcury-Quandt

Parasitic infection is an important cause of diarrhea in children in low-income countries. In these children, diarrhea is a common source of morbidity and mortality. Knowing spatio-temporal patterns of infection allows investigators to understand the transmission dynamics of these parasites, leading to more efficient preventative interventions. This is a study of *Giardia, Cryptosporidium, Cyclospora,* and microsporidia infection in a longitudinal cohort of children in a peri-urban shantytown of Lima, Peru. Weekly stool samples were collected from 465 children over a five-year period. These samples were microscopically analyzed for infections. The temporal and spatial distributions of infections were statistically analyzed for clustering with scan statistics. Seasonal patterns of infection were also qualitatively described. *Cyclospora* displayed consistent, annual seasonality, though infections with the other evaluated pathogens showed different clusters over time. The spatial analysis identified two regions with high prevalence of infection. Adding time to the spatial analysis confirmed these areas of high prevalence, showing that they were consistently infected over time. Studying multiple parasites allowed for the identification of these areas. No analysis showed consistent infection over the entire study period in these areas for a single parasite, but comparing the results for these areas for all parasites showed that there was a cluster of at least one parasite in these regions in each warm season during the study. This analysis shows the benefits of examining an endemic community to understand the natural occurrence of infection and demonstrate the differential patterns of infection among these parasites. These findings can help elucidate infection risk factor heterogeneity and for the development of better strategies for infection control and prevention.

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Chapter 1: Review of the Literature

Diarrhea accounts for a large portion of childhood morbidity and mortality in low-income countries. In the 1990s, mortality due to diarrhea in children under five years of age decreased from an estimated 13.6 or 5.6 deaths per 1000 per year to 4.9 per 1000 per year. Despite this decrease, it still accounted for 21% of all deaths in children less than five years of age. The proportion of children in this age group that survived their diarrhea in this time period had a higher frequency of morbidity associated with diarrhea than previously observed with associated morbidities increased in this time period (1).

The morbidities associated with recurring, severe childhood diarrhea often have lasting effects. Diarrhea within the first two years of life was linked to long-term cognitive deficits in a Brazilian shantytown (2). Malnutrition can lead to these cognitive deficits. It also can lead to lifelong physical disability and premature death. Diarrhea has been found to be a primary risk factor for malnutrition, and infections have a complicated interaction with malnutrition (3). Linear growth retardation has been linked to early childhood diarrhea. A cohort study of Peruvian children found that these children were, on average, 2.5 cm shorter than the World Health Organization (WHO) standard. A diarrheal prevalence of 2.3% in the first 2 years of life explained up to a quarter of this difference, and the association between diarrhea and amount of growth stunting was proportional. Those with greater prevalence of diarrhea had more severe growth stunting. Further follow-up showed that diarrhea in the first 6 months of life likely led to permanent height deficits, while the effects of diarrhea later than 6 months were likely transient (4).

Wealth is a strong link to diarrheal prevalence and overall health. A study of the spatial distribution of intestinal parasites among residents of an agricultural settlement in Brazil found that disease clustering occurred in a location that was poorer and had greater crowding than the surrounding area (5). In an impoverished settlement in Peru, subtle differences in wealth were linked to two parasitic infections. Higher wealth was protective against *Giardia* infection, persistent (longer than 2 weeks) *Giardia* infection, and microsporidia infection (6). In Thailand, greater wealth was associated with lower amplitude in seasonal diarrhea (7).

Diarrhea can be prevented. Improvements in access to clean water and improved sanitation can reduce infections that cause diarrhea. Individual hygiene practices can also contribute to health. In a Peruvian shantytown, water storage and access to sewage were linked with height deficits and diarrhea. Children living in households with the worst quality water source, storage, and sanitation were shorter overall and had 54% more diarrheal episodes. Those living in households with small storage containers had 28% more diarrheal episodes. Lack of sewage was linked with growth deficits. Among those with access to a better water source, those with small storage containers and no sewage were much shorter than those with better water storage and sewage (8). High levels of waterborne parasites were found in wastewater in Tunisia, suggesting that contact with untreated wastewater can lead to infection (9). Individual hygiene practices can prevent diarrhea. Handwashing is a major target, as fecal contamination is a likely route of infection for diarrheal pathogens. In a Peruvian shantytown, very little handwashing was observed, and very little water was used when washing hands (10). As with diarrhea, individual hygiene practices can be linked to wealth. Children with more educated

parents and higher socioeconomic status (SES) were more likely to wash their hands before meals (11).

Parasitic infection is a cause of a large proportion of diarrhea in children in low-income countries. A study found a high proportion of parasitic infection among adults hospitalized in Chennai, India. A fifth, the largest proportion of any infectious agent, of the adults had *Cryptosporidium* infection. *Cryptosporidium* was also detected in 81% of patients with two or more co-infections. One patient was also found to be infected with *Cyclospora* (12). In Northern Iran, 9.1% of patients with diarrhea were infected by parasites. Of these, 4.1% were infected with *Giardia*. All other parasitic infections had fairly low prevalence (13). In a study of children under 13 years of age in Tehran, Iran 0.78% of 124,366 children referred to a hospital were infected with parasites. Most of these infections were protozoan, though there were some cases of intestinal worms. The most common infection was *Giardia* (14). A study among schoolchildren in Northern Iran showed that a third of children were infected with at least one parasite. The most common were *Blastocystis hominis* and *Giardia lamblia* (11). In a case-control of infant diarrheal disease in Madagascar, 36.5% of patients with diarrhea were infected with intestinal parasites, the most common type of pathogen in the study. Odds of infection with *Entamoeba histolytica*, *Trichomonas intestinalis*, and *Giardia lamblia* were much higher among the cases, though other parasitic pathogens were detected as well (15). In preschool children in a slum in India, 17.5% were infected with parasites. *Ascaris lumbricoides* and *Giardia lamblia* were the most common infections with respective prevalences of 68.1% and 32.9% among infected children (16).

This study focuses on four prevalent infections in a shantytown of Lima, Peru: giardiasis, cryptosporidiosis, cyclosporiasis, and microsporidiosis. It will discuss their seasonal patterns and analyze their clustering within space, time, and space-time.

Giardiasis

Giardiasis is caused by intestinal infection by the flagellated protozoan *Giardia lamblia*. Infected persons may remain asymptomatic or have acute diarrhea, sometimes accompanied by other gastrointestinal symptoms (e.g., abdominal cramps, bloating). Infection occurs by the ingestion of cysts. The incubation period is typically a week long, and the disease is communicable throughout the duration of the infection, usually several months long. The infection is usually self-limiting, though immunodeficient persons may have more severe infection (17).

Giardiasis follows the fecal-oral transmission cycle. Those infected may re-infect themselves; they may transfer cysts to objects in their environment, creating fomites. Cysts can also persist in water, so water source contamination can lead to infection. There is also a risk for zoonotic transmission (17).

Recent genetic studies have demonstrated that there are a variety of assemblages, or types, of *G. lamblia*. Certain assemblages are more likely to infect humans and others animals. Monitoring these assemblages can allow for more in depth study of transmission patterns. For example, varieties of assemblage B are more likely to be due to anthroponotic transmission than varieties of assemblage A, which may have anthroponotic or zoonotic transmission (18). However, animal studies must be conducted

to determine the true risk of zoonotic transmission (19). Molecular studies have been used successfully to determine likely transmission routes (19, 20).

Giardiasis is prevalent in children in low-income countries. It is one of the most common enteric pathogens and intestinal parasites(11, 14-16). In a population-based study in Nigeria, children 6 to 17 years of age had an infection rate of 40% (20). Saharawi children aged 6 to 12 were found to have a high rate as well (29%) (21). In a Brazilian sub-standard settlement, the prevalence among children was 18% (22).

There are a variety of risk factors that contribute to disease spread. Water scarcity and contamination are leading causes. If no municipal water is accessible, people in squatter settlements in Brazil often turn to wells and springs, most of which are contaminated. The odds of giardiasis among those who use wells are twice the odds among people using municipal water, and the odds among those who use spring water are nearly three times those of people using municipal water (22). Access to sewage is also a key factor. Those without access to municipal sewage have far greater risk of infection (8, 22). Handwashing and other personal hygiene tasks prevent giardiasis (22). Those with higher education and SES may be more likely to perform handwashing (11). Water scarcity can also contribute to likelihood of handwashing and the quality of handwashing (8).

Cryptosporidiosis

Cryptodsporidiosis is an infection caused by the either the parasite *Cryptosporidium hominis* or *Cryptosporidium parvum*. As with giardiasis, many people have asymptomatic infection. The predominant symptom is watery diarrhea, which may

be accompanied by abdominal cramps. Vomiting and anorexia may precede diarrhea in children, but these symptoms are much less likely in adults. Symptoms generally resolve themselves, but there is far greater risk of complication among immunocompromised patients. As with giardiasis, infection is caused by the ingestion of mature cysts. The incubation period is usually a week long, and infectious cysts may be excreted for weeks after the resolution of symptoms. Cysts may persist in a moist environment for months (17).

Cryptosporidiosis follows the same fecal-oral transmission cycle as giardiasis. Anthroponotic transmission is a risk, as are fomites and contaminated water, and there is a zoonotic risk (17). Molecular studies have demonstrated athropogenic and zoonotic transmission cycles within a single community (23).

Cryptosporidium is an emerging pathogen that disproportionately affects children and people with HIV (24). Its presence in all countries is becoming more recognized (24, 25). In two studies of hospitalized children with diarrhea, cryptosporidiosis was detected in at least 20% of the participants (12, 26). In another, children under two had the highest prevalence of cryptosporidiosis (20).

Similar prevention practices and risk factors exist for cryptosporidiosis and giardiasis. Certain aspects of *Cryptosporidium spp*. may contribute to its continued presence and threat. Cysts are particularly resilient to sanitation efforts (24). The disease is debilitating and life threatening in AIDS patients. In one instance, 86.5% of HIV patients with diarrhea had the parasite, and 90% of patients with low CD4+ counts were infected (27). Negative outcomes are highly associated with children and HIV patients, and there are few treatments (24, 26).

Cyclosporiasis

Cyclosporiasis is caused by the parasite *Cyclospora cayetanensis*. Symptoms include diarrhea, abdominal cramps, and low-grade fever. Asymptomatic infection is commonly seen in adults and older children in endemic areas. As with cryptosporidiosis and giardiasis, infection is usually self-limiting, and those with HIV may have far worse symptoms. Infection is caused by ingestion of mature cysts (28).

The transmission cycle is not typically fecal-oral. The excreted organism is an immature cyst, which is not immediately infectious. Cysts must spend seven to fifteen days in the environment to become infectious. The spread of the disease is not clear, but infection is associated with drinking untreated water in endemic countries where it exhibits marked seasonality. The zoonotic risk remains unclear (28).

Cyclospora is fairly recently discovered and was initially mistaken for *Cryptosporidium* (28). As such, studies have been undertaken to compare their epidemiology and risk factors. In Peru, it was found that incidence of cryptosporidiosis peaked at 1 year of age and declined to one-seventh the incidence at ages five to 9. Cyclosporiasis, however, had constant incidence, but the frequency of associated diarrhea declined as age increased. Cryptosporidiosis was more associated with sanitation-based risk factors, and cyclosporiasis was more associated with animal ownership (29).

Microsporidiosis

Microsporidiosis is caused by a variety of sporulating intracellular parasites in the phylum microsporidia. These may cause a variety of symptoms, including conjunctivitis,

genitourinary infections, and disseminated infections. Two species, *Enterocytozoon bienusi* and *Encephalitozoon instestinalis*, cause diarrhea and are the most common species. Infection is caused by ingesting mature spores, and spores are mature when excreted. Spores can persist in the environment for a long time (30). As with the other infections, there appears to be some zoonotic risk associated with microsporidiosis. Domestic dogs and cats in Iran were frequently infected, suggesting that household pets could serve as a reservoir for the pathogens (31).

Microsporidiosis was rare in humans until the AIDS epidemic, and the focus of its study is largely centered on AIDS patients worldwide. Prevalence has decreased with the advent of highly active antiretroviral therapy for HIV (32). However, recent studies have found it in water sources, leading to concerns of community-based infection, particularly among those without access to safe water (30, 33). A 10-year study found that a primary risk factor for microsporidiosis was youth, regardless of HIV status (34).

Spatial and seasonal aspects of diarrheal disease

Knowing the spatial and seasonal patterns of infections is essential for routine surveillance and interventions. SES, particularly low SES, tends to be tied to geographic areas. Because of the close association among SES, water supply, and diarrhea, diarrhea and diarrheal infections would likely follow these spatial patterns. Some of these associations have been described in endemic areas. For example, studies have examined the spatial patterns of diarrhea, particularly diarrheal mortality (7, 35, 36). Diarrhea, and in some instances parasitic infection, has been shown to follow seasonal patterns (7, 29,

35, 37). In non-endemic settings, pathogen specific spatial studies have been conducted in outbreak situations (38, 39).

Knowing where and when an infection is likely to occur is critical for successful interventions and resource allocation. While an area may be impoverished, it may not be uniformly so, and small differences in wealth can lead to large differences in health. Because of this, a uniform intervention may not be appropriate. Studying spatial patterns of disease can enable more targeted interventions, which may lead to better outcomes. Different diseases require different resources for treatment and intervention. If any temporal pattern exists, resource acquisition, allocation, and distribution can be more effectively timed.

This study is a spatio-temporal analysis of infection by four enteric parasites in a peri-urban shantytown near Lima, Peru. It seeks to describe clustering of infection among children in space and time over a five-year period. It is different from previous studies in several ways. It is a population-based study in an endemic area. Many spatial studies focus on outbreaks in non-endemic areas. Few studies have had a follow-up period of this length with weekly data collection. This allows for better temporal resolution and the ability to track pattern from year to year. Most studies in endemic areas have focused on diarrhea instead of on pathogens that cause diarrhea. However, different pathogens may necessitate different interventions, so finding different spatial patterns may allow better allocation of resources. Finally, those studies that have examined spatial patterns of pathogens in endemic areas have rarely looked at more than one or two. Studying four of the most common enteric parasitic pathogens together

allows us to compare infection patterns among them. Because the modes of transmission are not fully characterized in a community setting for two of these pathogens, it may help our understanding of these pathogens.

Chapter 2: Manuscript

Title: Temporal and spatial epidemiology of enteric parasitic infections in a pediatric cohort of children living in the peri-urban areas of Lima, Peru

Abstract

Parasitic infection is an important cause of diarrhea in children in low-income countries. In these children, diarrhea is a common source of morbidity and mortality. Knowing spatio-temporal patterns of infection allows investigators to understand the transmission dynamics of these parasites, leading to more efficient preventative interventions. This is a study of *Giardia, Cryptosporidium, Cyclospora,* and microsporidia infection in a longitudinal cohort of children in a peri-urban shantytown of Lima, Peru. Weekly stool samples were collected from 465 children over a five-year period. These samples were microscopically analyzed for infections. The temporal and spatial distributions of infections were statistically analyzed for clustering with scan statistics. Seasonal patterns of infection were also qualitatively described. *Cyclospora* displayed consistent, annual seasonality, though infections with the other evaluated pathogens showed different clusters over time. The spatial analysis identified two regions with high prevalence of infection. Adding time to the spatial analysis confirmed these areas of high prevalence, showing that they were consistently infected over time. Studying multiple parasites allowed for the identification of these areas. No analysis showed consistent infection over the entire study period in these areas for a single parasite, but comparing the results for these areas for all parasites showed that there was a cluster of at least one parasite in these regions in each warm season during the study. This analysis shows the benefits of examining an endemic community to understand the natural occurrence of infection and demonstrate the differential patterns of infection among these parasites. These findings can help elucidate infection risk factor heterogeneity and for the development of better strategies for infection control and prevention.

Introduction

Diarrhea accounts for a large portion of childhood morbidity and mortality in low-income countries, accounting for 21% of all deaths of children less than five years of age in the 1990s (1). The associated morbidities have been reported to have lifelong effects and include cognitive deficits (2) and growth stunting (4).

Parasitic infections are a major cause of diarrheal illness in children in low-income countries. A study among schoolchildren in Northern Iran showed that a third of children were infected with at least one parasite. The most common were *Blastocystis hominis* and *Giardia lamblia* (11). In a case-control of infant diarrheal disease in Madagascar, 36.5% of patients with diarrhea were infected with intestinal parasites, the most common type of pathogen in the study. Odds of infection with *Entamoeba histolytica*, *Trichomonas intestinalis*, and *Giardia lamblia* were much higher among the cases, though other parasitic pathogens were detected as well (15). In preschool children in a slum in India, 17.5% were infected with parasites. *Ascaris lumbricoides* and *Giardia lamblia* were the most common infections with respective prevalences of 68.1% and 32.9% among infected children (16).

Knowing the spatial and seasonal patterns of parasitic infection is essential for routine surveillance and interventions. These infections are preventable, and such analyses allow for better interventions and focusing of resources. This study is a spatio-temporal analysis of *Giardia, Cryptosporidium, Cyclospora,* and microsporidia infection in a peri-urban shantytown near Lima, Peru. It seeks to describe clustering of infection among children in space and time over a five-year period. A variety of findings on diarrhea and health have been documented in this community, including hygiene

practices (10), effects of water sources and storage (8), growth stunting (4), and effects of wealth on infections (6). However, the spatial distribution of infection of parasites in this endemic community has not yet been described. This analysis will provide additional information for understanding other factors that can be associated with transmission.

While spatio-temporal analyses regarding diarrheal disease have been conducted, this study is different in a variety of ways. Some studies in endemic areas have examined the spatial patterns of diarrhea, particularly diarrheal mortality (7, 35, 36). Diarrhea, and in some instances parasitic infection, has been shown to follow seasonal patterns (7, 29, 35, 37). In non-endemic settings, pathogen specific spatial studies have been conducted in outbreak investigations (38, 39). However, few, if any, long-term, multi-parasite analyses have been conducted in an endemic population. This is presents a new opportunity to track patterns of infection over time.

Methods

Study Area

Las Pampas de San Juan de Miraflores is a peri-urban shantytown approximately 25 km south of the city center of Lima, Peru. It was established in the late 1970s and now has a stable population of roughly 40,000 people. It is considered a low-income area, though there remain disparities in socioeconomic status within the population. The more established residents have better quality housing (e.g., houses with concrete floors and foundations, connections to city water and sewage). Recent settlers have very basic houses or shelters, usually made of thatched reed mats, corrugated tin, and cardboard or plastic. They are more likely to experience water scarcity or have access to less safe water, and typically lack sewer connections.

Study Sample

This analysis uses data collected from two consecutive longitudinal pediatric studies conducted between December 1, 2001 and June 30, 2006 in Las Pampas. For these studies, a census was conducted of the entire shantytown, and households with children under the age of 12 were enrolled until the target sample size was reached (6).

Data Collection

Field workers visited the participants at their homes and collected weekly stool samples. Specimens were kept in coolers with icepacks and transported to the laboratory every afternoon. Samples were concentrated using the ethyl acetate method. The concentrated specimens were microscopically analyzed for ova and parasites, which can detect *Giardia* cysts. Stained smears were prepared for the detection of *Cryptosporidium*, *Cyclospora*, and microsporidia. Acid fast staining was used to detect oocysts of both *Cryptosporidium* and *Cyclospora* using light microscopy at 100 and 400x magnifications. Confirmation of *Cyclospora* was made with UV epifluorescent microscopy at 400x, which allows for the observation of characteristic fluorescence. The Weber-modified trichrome stain was used to stain microsporidia and detected through slide diagnosis at 1000x magnification using light microscopy (6).

Global Position System (GPS) coordinates of each house were collected and verified three times during the study. All readings were taken using a Garmin GPSMAP 76S (Garmin International, Olathe, KS).

Data Analysis

All data were collected in printed forms and transcribed into electronic databases using FoxPro 2.6 (Microsoft Corporation, Redmon, WA). Integrity of the transcription

was verified through double data entry. Descriptive statistics were determined using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). Spatio-temporal patterns of parasitic infection were analyzed using SaTScan version 9.1.1 (40). The data were analyzed for purely spatial clusters, purely temporal clusters, and clusters in space and time. For purely spatial analysis, the program imposes circles of varying radii centered on each house on the map to identify clusters with higher than expected rates of infections. Observation time is ignored and we focus on clusters within the spatial pattern of all observations. Purely temporal clusters include all points (ignoring location) and examine the number of observations for windows of varying lengths. For the temporal analysis, data were analyzed by week. Clusters in space and time combine these two methods and consider potential clusters of events defined within cylinders extending spatial circles of varying radii through time intervals of varying length. The most likely clusters were identified using Monte Carlo simulation comparing the observed most likely clusters with those resulting from 999 simulated datasets under a null hypothesis of no space or time clustering to generate a p-value. A p-value of less than 0.05 was considered significant. Clusters were mapped using ArcMap version 10 (Environmental Systems Research, Inc., Redlands, CA, USA).

The data collection procedures were approved by the institutional review boards of Asociacion Benefica Prisma, University of Georgia, The Johns Hopkins University, and the Centers for Disease Control. The data analysis procedure was approved by Emory University.

Results

Population

663 children were initially recruited for this study. Inclusion in this analysis required at least three months participation in the study, at least 85% compliance with stool sample collection, and recorded household GPS coordinates. 198 children were excluded due to these criteria. 465 children were included in this analysis (48% male). The mean age at entry was 2.05 years (SD: 2.17, Range: 0-11). Each child participated for a mean of 963.39 days (SD: 428.42, Range: 179-1561). 53,001 stool samples were analyzed (Mean: 130.36, SD: 58.10, Range: 20-306).

Time Clustering

The number of weekly stool samples with positive test for each parasite was determined (Figure 1). *Giardia* was the most prevalent infection, but no seasonal patterns were evident. The others showed some clustering in time. *Cryptosporidium* show some temporal clustering, but there is latent year-round infection. *Cyclospora* had a much more defined seasonality, with most infections happening in 30-week windows following weeks 1, 57, 106, 156, and 216. These time periods corresponds to summer in this area. Microsporidia is the least prevalent, and most infections appeared to be grouped in time, though they do not follow any apparent seasonal patterns.

The temporal analysis using Monte Carlo simulations in SaTScan showed that each pathogen had a significant cluster in time (Table 1). Each corresponds with the period of highest prevalence shown in the charts, represented as a shaded area.

Infection Clustering

Clusters were detected for each pathogen in purely spatial analysis (Table 2). *Giardia* was found to have 18 significant clusters, *Cryptosporidium* 1, *Cyclospora* 4, and microsporidia 1. Maps representing the distributions are presented in Figure 2. The

largest cluster of *Giardia* is found in the easternmost section. There are groups of clusters in the area densely populated with participants just north of the large cluster. However, there are clusters in the northwestern and southwestern portions of the area as well. The cluster of *Cryptosporidium* encompasses much of the northeastern portion of the population. The clusters of *Cyclospora* are all in the northern area and mostly in the eastern area. The microsporidia cluster is in the far southern region.

Adding time to the analysis changed the patterns of infections somewhat (Table 3, Figure 3). Each pathogen was found to exhibit significant spatio-temporal clusters. *Giardia* had 15, *Cryptosporidium* had 3, *Cyclospora* had 2, and microsporidia had 3. Most clusters for each pathogen started in the warm season (December-May). Unlike the purely spatial analysis, each had at least one multi-house cluster and one single-house cluster. For *Giardia*, the largest significant cluster was in the easternmost portion of the area and included the densest area of participants. Almost all of the rest of the significant clusters were in the southernmost portion. Many significant clusters that began close to the same time were located near each other in space. All of the *Cryptosporidium* clusters were in the easternmost portion of the area. The multi-house clusters were in the northeast. The multi-house cluster was in the eastern part of the study area, but the single-house cluster was in the western portion. The larger multi-house microsporidia cluster was in the eastern area, while the other two clusters were in the southern part.

Discussion

Significant temporal, spatial, and spatio-temporal clustering was detected for all parasites. *Cyclospora* was the only infection that appeared to have annual seasonality.

Overall, most significant clusters occurred in the eastern and southern parts of the community, though different patterns were evident for each pathogen.

Time Clustering

The prevalence and seasonal patterns found correspond with what is known of these parasites. They are generally prevalent in the warmer months, and *Giardia* is prevalent year round. *Cyclospora* is known to have a definite seasonality (29).

The temporal clusters identified by SaTScan show the periods of highest prevalence. However, the study design may explain the temporal cluster of *Giardia* infection. The data were collected in two studies, and the second study began near the time of the end of this cluster, so this particular cluster may not be accurately identified. Because age, SES, and other demographic factors are closely linked to *Giardia* infection, the change in the study population could have led to this change. It is unlikely that the entire community experienced a decrease in *Giardia* prevalence at this point.

Spatial Analysis

The spatial analyses reported two types of clusters: single-house and multi-house. Single-house clusters were found when SaTScan considered a spatial window of radius zero. Multi-house clusters were found when the radius considered was greater than zero. However, the statistical test for both considers the number of positive and negative samples in the window compared to the number of positive and negative samples outside that window. Those with higher relative rates of infection were determined to be clusters.

The significant spatial clusters represent people or areas with frequent infection, though those infections are not temporally linked. Knowing these areas is important. People in the same area may be infected at different times, but that area could still have

frequent infection and could be missed by the spatio-temporal analysis. The method of diagnosis used in this study may not consistently detect low-level parasite load, so those with long-term infection may be detected with the spatial analysis and not the spatio-temporal analysis. This is particularly important for the single-house clusters found in the spatial analysis. A child may have a low-level infection for a long period of time or may be re-infected throughout the study, but the positive samples may not be close enough in time to be identified with the spatio-temporal analysis.

The spatio-temporal clusters represent areas or people with infections that are linked by time. This could indicate a group of people being infected at the same time due to a seasonal increase in prevalence. It may also indicate an area where there is transmission between individuals. A cluster of a single house over time may indicate an infection of especially long duration or multiple infections with the same organism in a short period of time. Many of the single-house clusters found in the spatial analysis were identified in this analysis, suggesting that this is likely the case.

Both the spatial and spatio-temporal analyses found the largest clusters in the eastern part of the study area. This area tends to have the most recent immigrants, poorest people, most crowding, and worst access to water and sanitation. Finding clusters of infection in this area is consistent with what we know of this community (6, 8, 10) and of diarrheal disease and infection worldwide (7, 22). Including time in the analysis shows that, while a specific parasite does not show temporal patterns in this region, there is evidence of significant clusters of diarrheal infection in that area in each warm season.

There were many single-house clusters of *Giardia* and two multi-house clusters of *Microsporidia* located in the southern tip of the community, and several household clusters of *Giardia* and *Cyclospora* were found in the northwest corner of the community. These may represent other disease foci.

This study illustrates the effect of scale on analyses. An analysis of the metropolitan area of Lima or the department that contains this community and Lima might identify this community as having higher rates of poverty, diarrhea, and parasitic infection than the surrounding areas. As such, resources may be allocated and interventions pursued for the community as a whole. However, this community-level analysis shows heterogeneity of infection rates within the community, and a heterogeneous approach to resource allocation and intervention may be appropriate. This is consistent with recent analyses showing that local conditions must be considered when doing spatial analysis (36).

This study should be considered in light of its weaknesses. The stool sample detection method used may have low sensitivity in cases of low parasite load in stools. However, taking weekly samples increased the likelihood of finding each case for long-term infection. The spatial analysis did not allow for geographically overlapping clusters. Allowing for this may have enabled detection of seasonally recurring clusters but was impractical for this analysis.

Few similar studies exist. The duration and breadth of this study is unique among studies in endemic areas. Previous spatial analyses have focused on single parasites, diarrhea and diarrheal mortality, or on outbreak studies in non-endemic settings. Studying four parasites shows location-specific consistent, annual infection by different

parasites, but at different spatial scales, which may have been missed by single parasite analyses. Studying infection can determine if different interventions are needed in different areas. It can also show more of the burden of infection in a community than diarrhea alone because asymptomatic infection is common in endemic settings.

This study shows that spatial and temporal patterns of infection differ by pathogen and that infection is not evenly distributed among residents in this community. Further study is needed to determine the relationship between these infections clusters and other risk factors for diarrhea, as well as the relationship between infection and diarrhea. Based on these analyses, targeted interventions should be implemented to evaluate their efficacy compared to current methods.

Tables and Figures

Table 1. Most likely temporal cluster for each pathogen

Pathogen	Time Frame in weeks (calendar time)	Number of Stool Samples	Number of Positive Stool Samples	p-value
Giardia	1-94 (Dec. 2001-Nov. 2003)	13345	2369	0.001
Cryptosporidium	231-235 (June 2006)	407	14	0.001
Cyclospora	171-179 (March 2005)	2771	77	0.001
Microsporidia	37-47 (Aug. –Oct. 2002)	1810	30	0.001

Table 2. Most likely spatial clusters for each pathogen

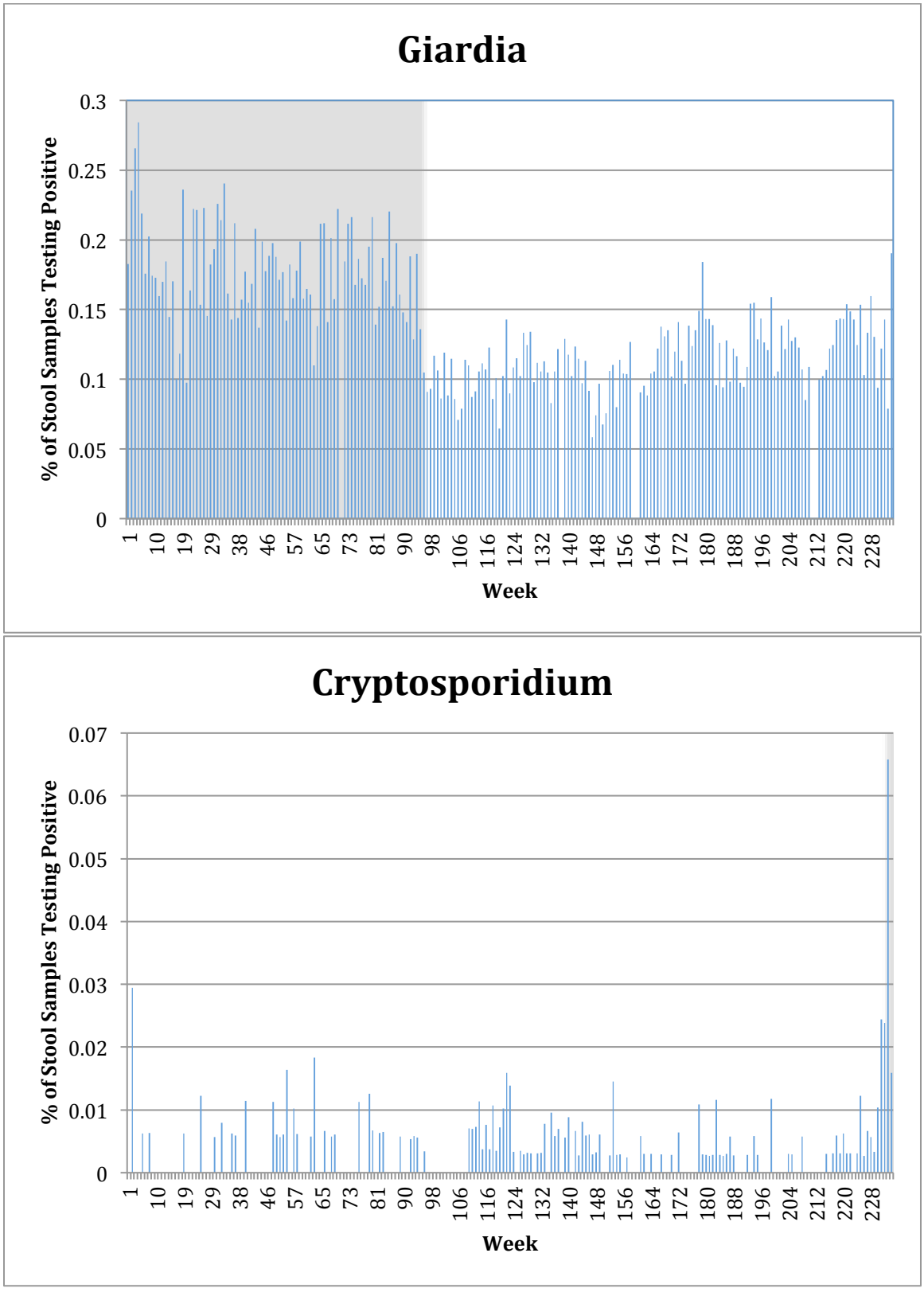
Pathogen	Number of Houses	Number of Stool Samples	Number of Positive Stool Samples	p-value
Giardia	140	18042	3230	<0.001
	1	183	126	<0.001
	6	943	320	<0.001
	1	182	113	<0.001
	1	67	61	<0.001
	1	197	99	<0.001
	1	117	73	<0.001
	1	78	58	<0.001
	1	178	84	<0.001
	1	88	55	<0.001
	1	106	56	<0.001
	1	120	56	<0.001
	1	111	46	<0.001
	2	218	70	<0.001
	1	114	42	<0.001
	1	55	25	<0.001
	1	95	31	0.003
	1	108	31	0.045
Cryptosporidium	217	21788	107	<0.001
Cyclospora	3	258	12	<0.001
	1	126	8	0.006
	4	245	10	0.021
	1	22	4	0.021
Microsporidia	1	71	4	0.010

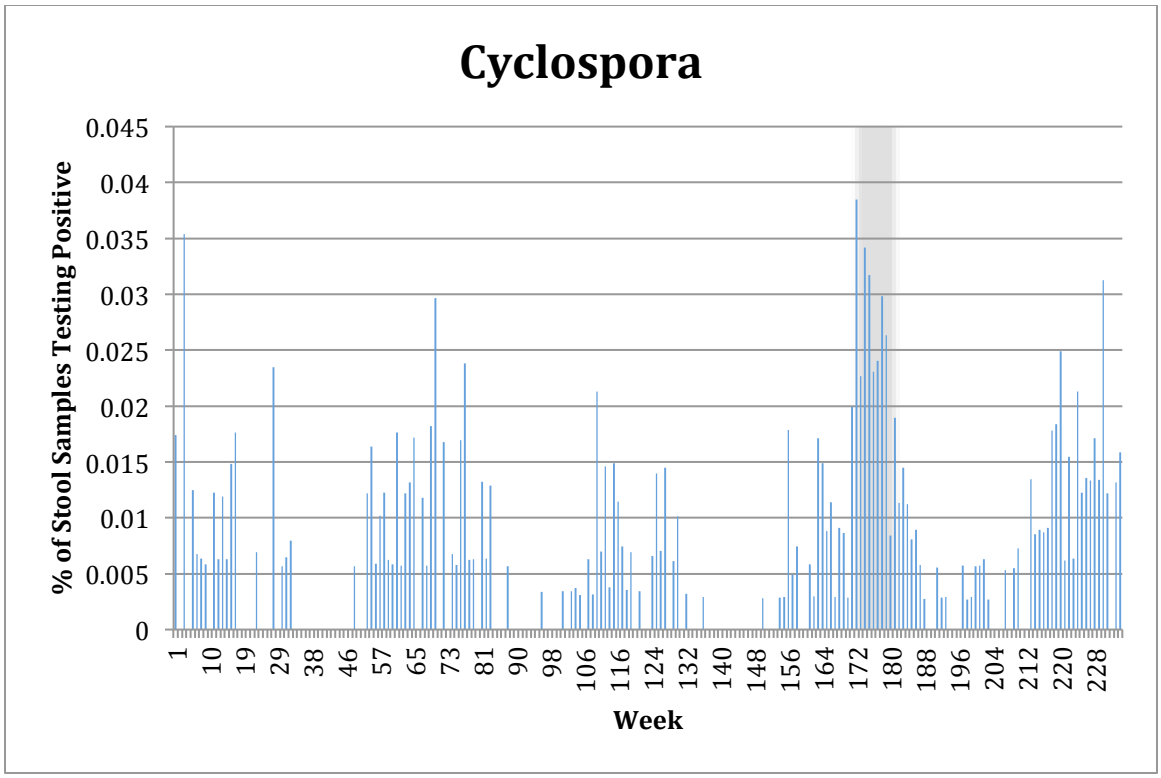
Table 3. Most likely spatio-temporal clusters for each pathogen

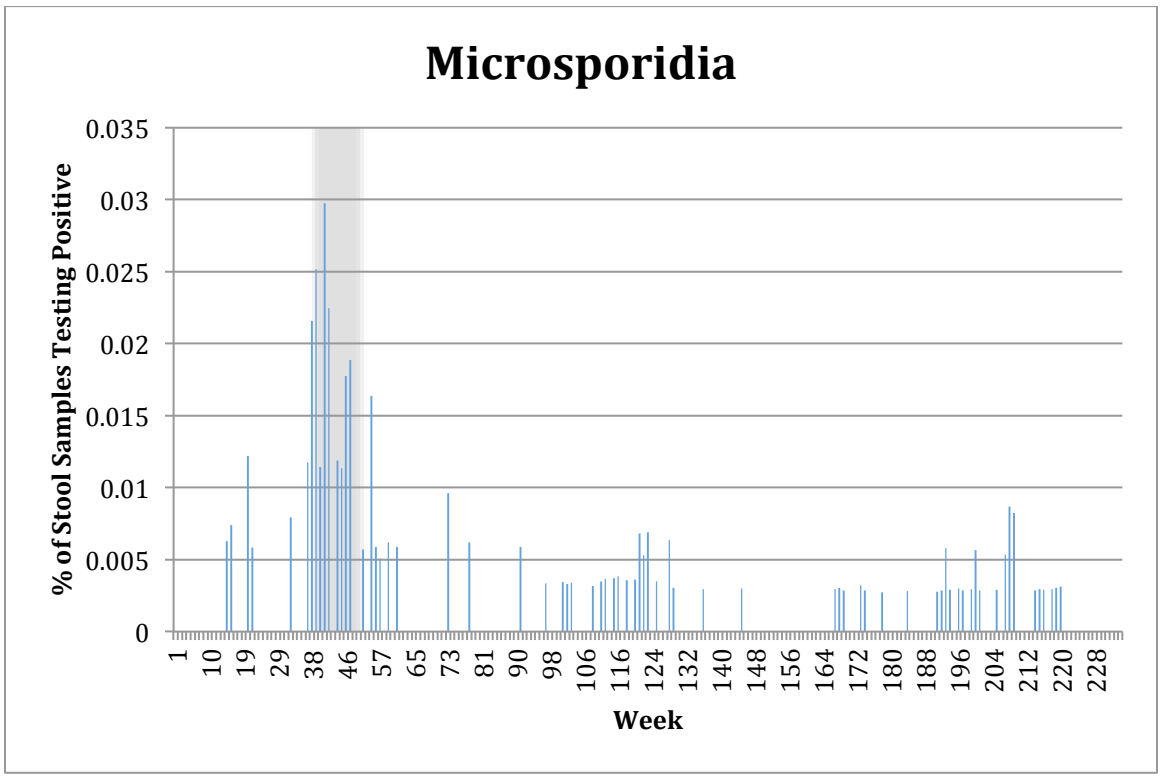
Pathogen	Time Frame in weeks (calendar time)	Number of Houses	Number of Stool Samples	Number of Positive Stool Samples	p-value
Giardia	1-95 (Dec. 2001-Nov.2003)	187	4860	1265	<0.001
	1-235 (Dec. 2001-June 2006)	1	176	128	<0.001
	1-235 (Dec. 2001-June 2006)	1	183	126	<0.001
	111-227 (Jan. 2003-March 2006)	1	104	88	<0.001
	143-235 (Aug. 2004-June 2006)	9	447	199	<0.001
	1-235 (Dec. 2001-June 2006)	1	182	113	<0.001
	4-96 (Jan. 2002-Nov. 2003)	1	67	61	<0.001
	114-198 (Feb. 2004-Sept. 2005)	1	62	56	<0.001
	165-215 Feb. 2005-Jan. 2006)	1	43	42	<0.001
	25-95 (May 2002-Nov. 2003)	1	49	44	<0.001
	4-113 (Jan. 2002-Feb. 2004)	1	80	57	<0.001
	87-137 (Sept. 2003-July 2004)	1	41	33	<0.001
	172-204 (March-Oct. 2005)	1	32	28	<0.001
	116-143 (Feb.-Aug. 2004)	1	22	22	<0.001
	214-228 (Jan.-April 2006)	3	28	21	<0.001
Crypto-sporidium	220-223 (Feb. 2006)	28	213	13	<0.001
	114-124 (Feb.-April 2004)	51	374	14	<0.001
	217-218 (Jan. 2006)	1	3	3	0.011
Cyclo-spora	171-185 (March-June 2005)	186	1992	69	<0.001
	154-158 (Nov. 2004)	1	4	4	0.001

Micro-sporidia	37-47 (Aug.-Oct. 2002)	122	439	22	<0.001
	14-42 (March-Sept. 2002)	9	135	11	<0.001
	214-216 (Jan. 2006)	1	3	3	0.002

Figure 1. Proportion of samples infected with each parasite







Data for weeks 211 and 212 are not shown due to sparse data collection in those weeks.
Shaded areas represent the most likely temporal cluster for each parasite.

Figure 2. Spatial distribution of parasitic infection

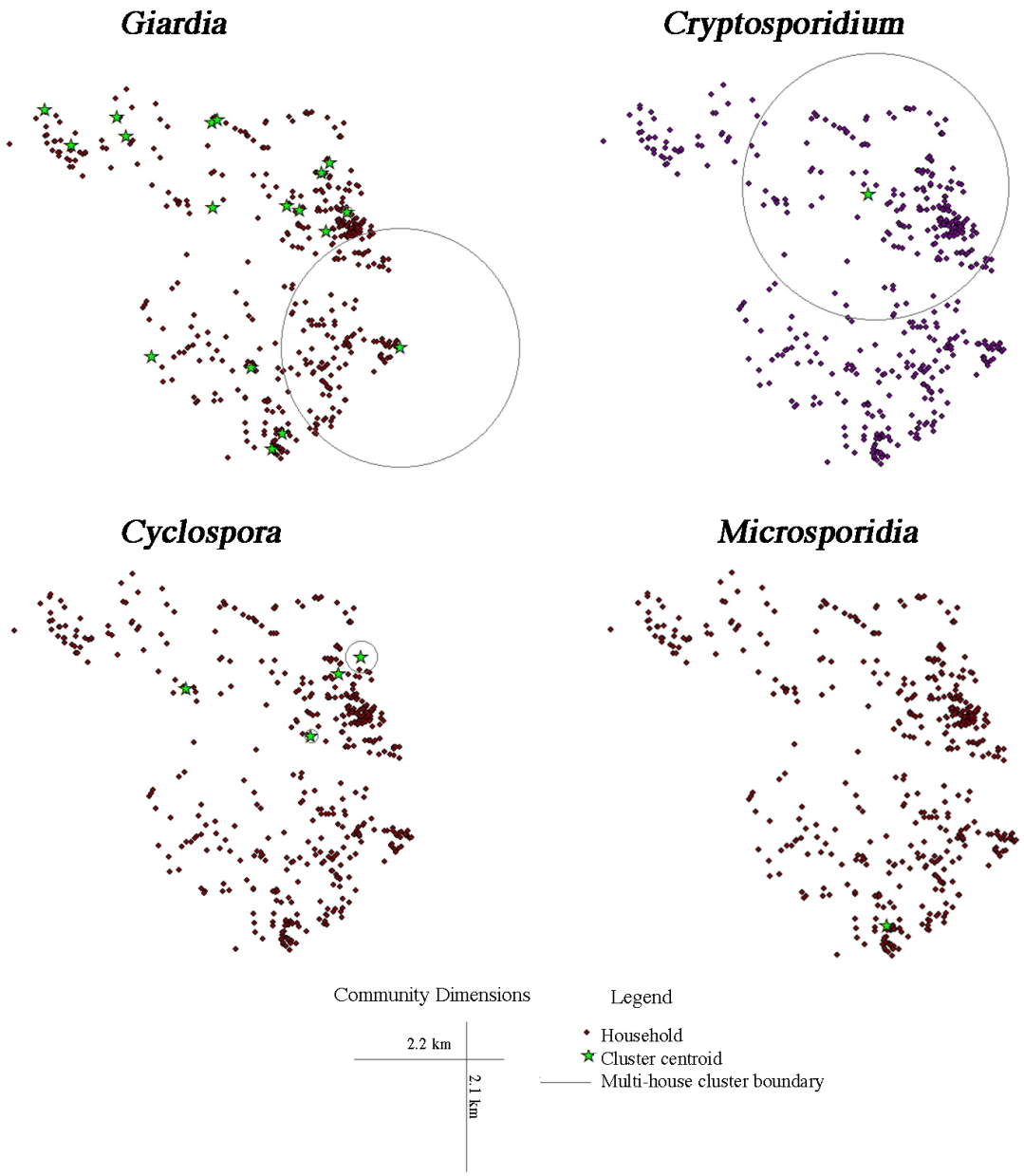
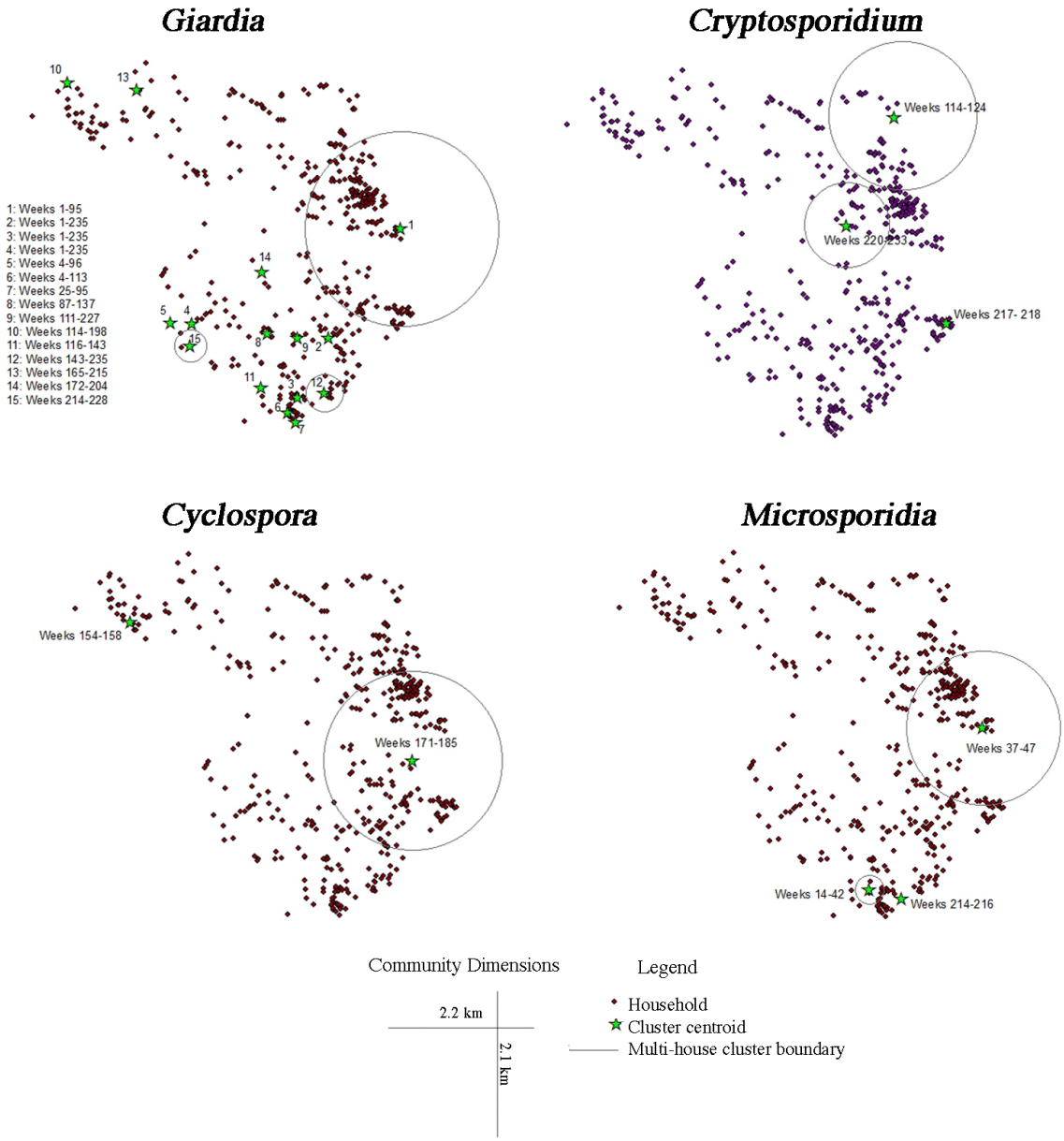


Figure 3. Spatio-temporal distribution of parasitic infection



Chapter 3: Public Health Implications

This study shows that parasitic infections tend to cluster in space and time in an endemic setting. Current intervention strategies tend to be uniform across a community. However, small differences within a community may mean that infection patterns are non-uniform. Knowing these patterns can lead to more effective targeting of interventions.

The less prevalent infections demonstrated temporal clustering if not complete seasonality. If this is a common situation in endemic areas, then adopting an outbreak approach when these infections are detected may aid in controlling these infections. Temporary, rapid, focused interventions could prevent further spread of less common infections and may provide benefits in terms of preventing infection by other, more common pathogens.

Further analysis of this dataset could show patterns and associations in this community that could be important for this community and other studies. Comparing patterns of infection to wealth, water source, and water storage may help explain these patterns, particularly the single household clusters that are not in or near the larger clusters. Detecting changes in infection patterns associated with changing one of these factors could also indicate other ways of targeting prevention. If an area has lower infection after changing to better water storage, areas with the common use of the older container may need intervention despite not having an evident cluster. The water container may not have caused the improvement in health, but may be an indicator of higher wealth.

Stratifying the dataset by age or time could provide useful information. There may be clusters of specific infections within specific age groups, so targeting those for prevention could be useful. Stratifying by time may allow for detecting repeating spatial patterns because this analysis did not allow for spatially overlapping clusters. This would give further evidence of seasonality and of infection foci.

If any of these analyses resulted in a targeted approach that effectively improved health, this strategy could be easily applied to other areas if the data are available. SaTScan is a free program with a guided user interface that allows for analyses to be run easily with easily interpretable results. This approach could allow investigators to identify visible, household indicators to help target their approach.

Few similar studies exist. The duration and breadth of this study is unique among studies in endemic areas. Previous spatial analyses have focused on single parasites, diarrhea and diarrheal mortality, or on outbreak studies in non-endemic settings. Studying four parasites shows location-specific consistent, annual infection by different parasites, which may have been missed by single parasite analyses. Studying infection can determine if different interventions are needed in different areas. It can also show more of the burden in a community than diarrhea because asymptomatic infection is common in endemic settings.

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