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Missing the Mark? A Two Time Point Cohort Study Estimating Intestinal Parasite Prevalence in Informal Settlements in Lima, Peru

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Abstract

Objectives: The World Health Organization's (WHO) recommendations list Peru as potentially needing prevention of soil-transmitted helminthiasis (STH). Prevalence of STH varies regionally and remains understudied in the newest informal settlements of the capital city, Lima. The purpose of this study was to evaluate the need for Mass Drug Administration (MDA) of antiparasitic drugs in the newest informal settlements of Lima. The aim of this study was to estimate the season-specific prevalence of STH to determine if these prevalence estimates met the WHO threshold for MDA in 3 informal settlements. *Methods*: A 2 time point cohort study was conducted among a sample of 140 children aged 1 to 10 years living in 3 purposively sampled informal settlements of Lima, Peru. Children were asked to provide 2 stool samples that were analyzed with the spontaneous sedimentation in tube technique. The season-specific prevalence proportions of MDA-targeted STH were estimated using a hidden (latent) Markov modeling approach to adjust for repeated measurements over the 2 seasons and the imperfect validity of the screening tests. *Results*: The prevalence of MDA targeted STH was low at 2.2% (95% confidence interval = 0.3% to 6%) and 3.8% (95% confidence interval = 0.7% to 9.3%) among children sampled in the summer and winter months, respectively, when using the most conservative estimate of test sensitivity. These estimates were below the WHO threshold for MDA (20%). *Conclusions*: Empiric treatment for STH by organizations active in the newest informal settlements is not supported by the data and could contribute to unnecessary medication exposures and poor allocation of resources.

Keywords
parasite, Lima, soil transmitted helminths

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Introduction

Intestinal infections from soil-transmitted helminthiasis (STH) are linked to multiple health problems in children.[1-3] Evidence of this link has led the World Health Organization (WHO) to recommend Mass Drug Administration (MDA) campaigns, with a dose of albendazole or mebendazole, to eliminate the infections and/or reduce the worm burden in populations with more than 20% prevalence of STH infections.[4] WHO's MDA programs specifically target the roundworm, *Ascaris lumbricoides*; the whipworm, *Trichuris trichiura*; and the hookworms, *Necator americanus* and *Ancylostoma duodenale*, which are the most prevalent STH intestinal

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infections in South America.[5] However, recent evidence dictates caution regarding the utility of mass deworming programs, making their careful application even more important. A 2015 reanalysis of data from a widely cited study from Kenya revealed that while deworming did have an effect on worm infection and nutritional status, the originally reported effects on anemia and school attendance had been overstated.[6] Multiple groups engage in MDA programs with varying levels of planning. Though many governments have robust evidence-based programs, nongovernmental and faith-based organizations not sanctioned by the WHO or local governments also conduct deworming programs.[7] To comply with WHO recommendations regarding initiation of MDA programs, it is imperative that local prevalence data be available.

Five studies performed in orphanages, hospitals, and schools of Lima have reported the prevalence of STH in children.[8-12] These studies likely drew participants from the informal settlements but were not conducted directly in the areas of newest growth. Four of these prevalence estimates were below the 20% WHO MDA threshold.[4] The one study with a higher prevalence estimate was conducted in a semirural area.[10] Yet, to our knowledge, no studies have been performed in the newly settled communities of Lima, or *pueblos jovenes*, where community-level and individual-level risk factors for STH such as poor sanitation, education, and socioeconomic status are common.[13,14]

Lima's geographic location on the Pacific coast, with the Andes directly to its east, produces a unique climate with very little rainfall and desert conditions during summer, which peaks around January. During winter, which peaks in July and August, increased humidity leads to muddy conditions in the new informal settlements, where there are very few sidewalks or drainage systems, causing increased difficulty in maintaining sanitation. While some studies have shown seasonal differences in STH infection prevalences in a variety of geographic areas,[15,16] it is unknown if this is the case in Lima's new informal settlements.

The primary objective of this study was to estimate, among children living in 3 new informal settlements in Lima, the season-specific prevalence of infection with the species of roundworms, whipworms, and hookworms that the WHO targets in its MDA programs.

Methods

Study Design and Study Population

This is a cohort study with 2 assessment points performed in March 2014 (summer/fall) and in August 2014 (winter). A purposive sample of 3 informal settlements in Lima settled in the previous 3 years and that were still under active construction were selected with one each in the districts of Ventanilla (Community 1), San Juan de Miraflores (Community 2), and Villa Maria del Triunfo (Community 3). These 3 communities were the most recently established among those in which 2 of the coauthors had been working. The 3 communities were also selected to reflect both the sandy coastal and rocky mountain regions of Lima. The communities differed on the level of basic services, but all lacked formal running water, garbage disposal, electricity, and sewer systems. Using the networks available in each community (centrally located community meetings), we informed families of the opportunity for their children ages 1 through 10 years to participate during the first sampling period (March 2014). We gave all families the opportunity to participate in a second round of sampling (August 2014) irrespective of their participation in the first round. Anthropometric measurement and an interview questionnaire were completed at both time points.

Questionnaires

The interview included child-level information including age, gender, history of infection with intestinal parasites, and prior individual treatment for intestinal parasites as well as household-level information on history of immigration to the area, water source, water treatment strategies, human waste disposal strategies, and animals in the home. Human waste disposal was classified as either involving the transfer of excrement in buckets or basins or use of an outhouse or septic tank. Water source was defined as provisional or delivered by truck, where provisional water was defined as an informal network of PVC pipes with intermittent water flow. Delivery truck water came several times per week in a large tanker truck and was stored in plastic and concrete containers close to the dwellings.

Medical Examination

Children with positive stool samples were examined by the principal investigator for symptoms of STH and other detected intestinal pathogens.

Stool Samples

Each study subject was asked to provide 2 stool samples during each round. Samples were obtained 24 to 48 hours apart, in an attempt to increase the sensitivity of testing for helminth infections.[17] Each parent or guardian was given a wide mouthed jar with 10 cc of sterile water and sterile tongue depressor and instructed regarding appropriate collection technique.

Each sample was identified with a unique code and transported to a local laboratory in Lima (within 20 km) immediately following collection. The presence of STH and protozoa was assessed by direct microscopic examination, the spontaneous sedimentation in tube technique (SSTT)[18-20] and Baermann technique. The SSTT is a locally available technique that was developed in Peru by Machicado and colleagues, who have shown this technique to be noninferior to the Kato Katz technique for the detection of intestinal helminths, and superior for the detection of intestinal protozoa.[21]

Data Analysis

Children with a positive test result using any laboratory test on at least one stool sample were defined as being screen positive for that particular helminth or protozoa. Descriptive statistics of the population were performed using SAS 9.4 (Cary, NC). *Hymenolepsis nana* and *Taenia* species were excluded from the analyses of MDA-targeted STH because they require different treatment and are not targeted by standard MDA programs. *Enterobius vermicularis* was also excluded from our definition of clinically significant STH due to its limited clinical significance and the poor performance of the utilized tests to detect it.

The season-specific prevalence proportions of MDA targeted STH were estimated using a hidden (latent) Markov modeling (HMM) approach to adjust for repeated measurements over the 2 seasons and the imperfect validity of the screening tests.[22] To take the repeated measurements over season into account, dependence of the winter results on the summer ones was modeled through the use of Markov transition probabilities on the latent summer disease states. The imperfect nature of the screening tests were adjusted for using the formula of Lew and Levy.[23] Of the screens employed, the SSTT was assumed to be the most accurate for STH and was assumed to perform identically to the Kato-Katz for the detection of intestinal helminths based on published data.[21] In previous analyses, the single specimen sensitivity values for the Kato-Katz to detect *A lumbricoides, T trichiura*, and hookworm were found to be 96.9%, 91.4%, and 65.2%, respectively.[24] Taking the lowest of the 3 values, the sensitivity value for SSTT to detect any STH was fixed at 65%. This sensitivity value was chosen to minimize the chances of overestimating sensitivity. Since a positive event for STH was defined as at least one positive test for any of the 4 STH, true probability of detection of any single STH or combination of STH would necessarily be greater than 65%. The specificity value was fixed at 100%. These low sensitivity and perfect specificity values were deliberately set to maximize the adjusted true prevalence of STH in this population in order to avoid underestimating the need for MDA. The analysis was further adjusted for the fact that not all children provided 2 stool samples as requested, and that the sensitivity of the test increases with the number of stool analyzed while the specificity decreases. This was accounted for by calculating subject-specific sensitivity and specificity values based on an assumption of independence among within-subject stool samples at a specific assessment point (summer or winter). This resulted in the following formulas:

$$p(\sum_{j=1}^{n_{is}} x_{ijs} = 0 \mid D_{is}-) = p(T- \mid D-)^{n_{is}} \tag{1}$$

$$p(\sum_{j=1}^{n_{is}} x_{ijs} > 0 \mid D_{is}-) = 1-\left(1-p(T+ \mid D+)\right)^{n_{is}} \tag{2}$$

where $p\left(T-|D-\right)$ and $p(T+ \mid D+)$ are the single-sample test specificity and sensitivity, x_{ijs} indicates the observed test results for the *j*th stool sample of individual *i* at seasonal assessment *s*, D_{is} is the disease status for individual *i* as the *s* assessment, and n_{is} is the number of stool samples collected from the *i*th individual at season *s*. Binary, test-positive indicators from each assessment ($y_{is} = \left(\sum_{j=1}^{n_{is}} x_{ijs} > 0\right)$) were modeled as a function of these subject-specific sensitivities and specificities. For the results presented (where $p\left(T-|D-\right) = 1$ and $p\left(T+|D+\right) = 0.65$), this had the obvious effect of increasing the sensitivity for those who had 2 stool samples examined instead of 1 sample. In an alternative separate sensitivity analysis, we assumed complete dependence between the 2 samples and set the sensitivity for the both 1-sample and 2-sample patients at 0.65.

The HMMs was run in R using the R2jags package, which implements Bayesian Markov-chain Monte Carlo (MCMC) techniques.[25,26] The analyses presented herein involved 90,000 iterations from each of 3 independent chains after discarding an initial burn-in of 10,000. To reduce serial correlation, we thinned the combined 120,000 iterations by selecting every 50th, leaving 5400 total for summary calculations. We used 3 independent chains to evaluate convergence.

While one of the original aims of the study was to evaluate the relationship between STH prevalence and other covariates both at the individual and community levels, this was not possible due to the low number of STH detected. Household clustering was negligible and not considered further.

Table 1. Individual and household level characteristics of all children included in the analysis.

Characteristic		Summer Sample Only	Winter Sample Only	Summer and Winter Samples	All Sampled at Least Once
Individual-level characteristics		N = 63	N = 30	N = 47	N = 140
Age (years)	Mean (SD)	5.9 (2.9)[a]	5.7 (3.0)	5.1 (2.7)	5.6 (2.8)[a]
Gender	Number (%) male	40 (63.5%)	7 (24.1%)[a]	25 (53.2%)	72 (51.8%)[a]
Number of stool samples provided	Number (%) with 1 sample	30 (47.6%)	12 (40.0%)	NA	NA
	Number (%) with 2 samples	33 (52.4%)	18 (60.0%)	NA	NA
Received prophylaxis	Number (%) receiving prophylaxis	31 (49.2%)	11 (36.7%)	24 (51.1%)	71 (50.7%)
Household-level characteristics		N = 39	N = 16	N = 35	N = 90
Number of people living in the household	Median (IQR)	4 (3-5)	4 (3-5.5)	4 (4-5)[a]	4 (3-5)[a]
District of residence	Ventanilla	10 (25.6)	5 (31.3)	15 (42.9)	30 (33.3)
	SJM	18 (46.2)	8 (50.0)	14 (40.0)	40 (44.5)
	VMT	11 (28.2)	3 (18.2)	6 (17.1)	20 (22.2)
Animals or pets in house	Number with animals or pets (%)	28 (71.8)	13 (81.25)	31 (88.6)	72 (80.0)
Water source	Provisional	10 (25.6)	5 (31.25)	15 (44.1)	30 (33.7)
	Truck/barrel/cistern/pilon	29 (74.4)	11 (68.75)	19 (55.9)[a]	59 (66.3)[a]
Disposal of waste/excrement	Bucket/basin	17 (43.6)	5 (31.25)	13 (37.1)	35 (38.9)
	Household latrine	22 (56.4)	11 (68.75)	22 (62.9)	55 (61.1)
Water treatment	Not always treated	5 (12.8)	1 (6.25)	5 (14.3)	11 (12.2)
	Treated by boiling or bleach	34 (87.2)	15 (93.75)	30 (85.7)	79 (87.8)

Abbreviations: IQR, interquartile range; SJM, San Juan de Miraflores; VMT, Villa Maria del Triunfo.

[a] Data missing for one participant or household.

Ethics and Treatment

The study protocol was reviewed and approved by the institutional review board for human studies at both the University of Oklahoma Health Sciences Center (#3960) and the Comite de Ética del Hospital del Niño in Lima, Peru (CL-03/14). Written consent was provided by a parent or legal guardian for all study participants, and we obtained child assent for participants aged 7 or older. We provided appropriate treatment to any child found to have pathogenic parasitic infection at any time during the study. No children who had received treatment in the first round that would have affected MDA-targeted STH prevalence were tested in the second round. Participants did not receive incentives for participation, but all costs for treatment and testing were covered by the study.

Results

Study Population

Children from approximately half of the households in Communities 1 and 2 participated in the baseline recruitment, with a slightly smaller proportion participating at follow-up. The most common reason for nonparticipation was absence from the home at the time of recruitment. Community 3 was geographically smaller and contained fewer households, but in the first round of participation followed the pattern of Communities 1 and 2. However, in the second round of sample collection, many of the children had been moved out of the community temporarily due to intercommunity violence, further reducing the number participating from this community. Because the communities were new informal settlements, there was no census available, which prevented us from determining how many of the nonparticipating households had eligible children in each of the communities. Only one family in Community 1 actively declined to participate. A total of 140 children among the 164 who consented to participate in the study provided at least one stool sample during at least one of the assessments. Table 1 describes the sociodemographic and household-level characteristics of the 140 children who provided at least one stool sample at either or both visits. Sixty-three children provided at

Table 2. All Potentially Pathogenic Organisms Identified in Each Season.

	Number (%) of Children With at Least One Positive Sample	
Parasite Identified	Summer (n = 110)	Winter (n = 77)
Giardia lamblia	27 (24.6)	12 (15.6)
Entamoeba histolytica	0 (0)	3 (3.9)
Blastocystis hominis	31 (28.2)	24 (31.2)
Ascaris Lumbricoides	1 (0.9)	1 (1.3)
Enterobius vermicularis	0 (0)	2 (2.6)
Hymenolepsis nana	2 (1.8)	4 (5.2)
Trichuris trichiura	1 (0.9)	0 (0)
Necator americanus	0 (0)	0 (0)
Ancylostoma duodenale	0 (0)	0 (0)

Table 3. Estimated Prevalence of MDA-Targeted STH Adjusting for Sensitivity and Variable Sample Number in Each Season Using Hidden Markov Modeling.

Season	Estimated Prevalence	95% CI
Summer	2.2%	0.3% to 6%
Winter	3.8%	0.7% to 9.3%
Difference	1.5%	−2.6% to 7.4%

Abbreviations: MDA, Mass Drug Administration; STH, soil-transmitted helminthiasis; CI, confidence interval.

least one stool sample in the summer months only, 30 in the winter months only, and 47 at both visits. Including those sampled in both seasons, 2 stool samples were obtained from 65% (71/110) of total participants during the summer season and 56% (43/77) of total participants during the winter season.

There was a similar proportion of male and female participants and the average age was 5.6 years old. Approximately half of the participants had been previously treated or received prophylaxis for intestinal parasites.

Participating children were sampled from 30 households from the district of Ventanilla, 40 households from the district of San Juan de Miraflores, and 20 households from the district of Villa Maria del Triunfo, with a median household size of 4. Most households had at least one animal in the house (either domesticated pet or food animal such as chicken or guinea pig). Approximately a third of households obtained their water through a provisional system of PVC pipes in the community (all of these were in the Ventanilla community), and the rest had water delivered by truck. The overwhelming majority reported always treating their water by boiling, addition of bleach, or both. Slightly more than half of participants had a usable latrine either inside or near their house. The remainder deposited excrement into a bucket or basin and transferred it to nearby shared informal waste sites (a neighbor's latrine or common site).

Prevalence of Parasitic Infections

Table 2 describes the type and number of potentially pathogenic intestinal parasites identified in each season. Overall, 11 species of intestinal parasites were detected in stool samples from the 140 participants sampled during the 2 visits. These included 2 pathogenic protozoa (*Giardia lamblia, Entamoeba histolytica*), 4 pathologic helminths (*Ascaris lumbricoides, Enterobius vermicularis, Hymenolepsis nana*, and *Trichuris trichiura*), and 4 non-pathogenic protozoa (*Entamoeba coli, Endolimax nana, Chilomastix mesnilii, Iodamoeba butschlii*). *Blastocystis hominis*, a protozoa that can be pathogenic in certain situations, was also isolated in approximately a third of those sampled, but was not associated with symptoms in any of the participants.

Primary Outcome of Interest: Population Prevalence of MDA-Targeted Helminths

Table 3 shows the estimated population prevalence of clinically significant STH after adjustment for imperfect test sensitivity and variable sample numbers. The season with the highest estimated prevalence was winter with 3.8% (0.7%, 9.3%), clearly showing the upper bound of the confidence limit falling well short of the 20% treatment recommendation threshold. In the sensitivity analysis run with complete dependence between the 2 samples, the highest estimated clinically significant STH prevalence (winter) remained well under the MDA threshold at 4.5% (0.9%, 11%).

Discussion

The communities included in this study did not meet the WHO recommended threshold of 20% for empiric preventive STH chemotherapy in children. Even with extensive adjustment conservatively accounting for imperfect sensitivity and multiple measurement and designed to minimize the possibility of underestimation of MDA-targeted STH, the upper limit of the 95% confidence interval for our estimates of prevalence of MDA-targeted STH fell well below the WHO recommended treatment threshold of 20%.

Despite the very low prevalence of STH, approximately half of the participants had been treated with antiparasitic drugs previously. Reports of treatment did not distinguish between those who had been treated for helminths or protozoa, but the participants' general impression was that they had been treated for worms. Detection of parasitic protozoa, which chemoprophylaxis strategies do not target, was considerably more common in this study's population, and there were several cases of *Hymenolepsis nana*, also not targeted by current MDA strategies.

Our study provides the only recent estimates of intestinal parasite prevalence in newly established informal settlements in Lima of which we are aware in either the Spanish or English literature. Because Lima differs from much of the rest of Peru in its climate and terrain, comparisons with other studies in and around Lima are more relevant than comparisons with the rest of the country. Studies conducted in the Lima area have generally found low prevalence comparable to ours. The Peruvian government, in a compilation of several other studies examining intestinal nematode prevalence, found low prevalences, in Lima, of *A lumbricoides* (6.2%), *T trichiura* (4.5%), and *N americanus/A duodenale* (1.4%).[12] Pajuelo found that, among patients in one Lima emergency department in 2010, prevalence of *A lumbricoides* was 6.5%.[9] Iannacone et al found low prevalence in school children of all target nematodes (*A lumbricoides*, 1.6%; *T trichura*, 0.5%; *N americanus/A duodenale*, 1.6%).[8] Iannacone et al found a higher prevalence of several organisms in 2 communities including *A lumbricoides* (15.3% and 35.5%), but this study included a more rural, wetland setting, which is markedly different from the geography of the rest of the city.[10] Many of these studies involved school or hospital populations or were conducted several years ago, and may not reflect Lima's rapidly changing environment. Additionally, our study adjusted for measurement error.

The major strength of our study was the method of data and sample collection. We canvassed 3 communities door to door and offered the families the chance to enroll their children. Moreover, we visited the communities at times of the week when the families were most likely to be home to increase participation. We collected samples each of the subsequent 4 mornings, again increasing the likelihood of sample delivery. By going directly into the community, we ensured that we reached our target population and reduced the chance that our sample was diluted by children from other communities who may have had different living situations. We chose 3 separate communities from 3 geographically distinct areas of Lima, and we collected samples during 2 distinct seasons. This decreased the possibility that our findings were the result of individual community idiosyncrasies or that results were affected by specific seasonal conditions.

Limitations include small sample size and the transient nature of the population. The small sample size and low prevalence of infection limited the study's power to fully explore associations between parasitic infections and growth. The transient nature of the population made sample collection and follow-up difficult. During each visit, we were able to obtain 2 stool samples from only a fraction of the participants, and only a fraction of those sampled during the first visit were resampled during the second visit. Because of the inherent difficulty of sample collection in the informal settlements, we employed extensive conservative adjustment in our analysis to make every effort not to underestimate population prevalence of clinically significant soil-transmitted helminths targeted by MDA. While most of the children in the study likely lived in the community, it is possible that some of the children were visiting, or that the family lived in 2 different places, a common practice in our target communities. Sometimes families are unwilling to share information on multiple domiciles for fear of the legal ramifications. Despite this limitation, the study children were still exposed to the risks of the new informal settlements' environment.

This study confirms that in our target communities, the prevalence of albendazole/mebendazole responsive helminthes is well below the WHO threshold for large-scale empiric treatment. While formal deworming programs may not be policy in Lima, it is our experience that there is widespread empiric treatment by organizations and physicians (domestic and foreign) active in these settlements. Even though these communities have many of the risk factors associated with STH, we consistently found low prevalence across multiple sites in different seasons.

An accurate estimation of the prevalence of STH intestinal infection in these communities will inform risk assessment by organizations active in the communities. Furthermore, in the current debate regarding deworming programs,[27,28] we seek to contribute an example of how resources are being misdirected in a group of marginalized communities, inappropriately treating and misleading patients regarding their health and disease processes.

Conclusion

The need for large-scale empiric treatment for soil-transmitted helminthes in the newly founded informal settlements in Lima, Peru, is not supported by our data. Continuing this practice leads to unnecessary medication exposure and poor resource allocation. More insidiously, it likely reinforces community misconceptions of disease burden and distracts from addressing more critical problems in these marginalized communities. This study serves as an example of how misapplied recommendations and/or policy can waste time and resources in a setting where both are sorely lacking.

Author Contributions

MTC: Contributed to conception and design; contributed to acquisition, analysis, and interpretation; drafted manuscript; critically revised manuscript; gave final approval; agrees to be accountable for all aspects of work ensuring integrity and accuracy.

RAS: Contributed to conception and design; contributed to acquisition; drafted manuscript; critically revised manuscript; gave final approval; agrees to be accountable for all aspects of work ensuring integrity and accuracy.

DMT: Contributed to design; contributed to analysis and interpretation; critically revised manuscript; gave final approval; agrees to be accountable for all aspects of work ensuring integrity and accuracy.

DB: Contributed to analysis and interpretation; critically revised manuscript; gave final approval; agrees to be accountable for all aspects of work ensuring integrity and accuracy.

HC: Contributed to analysis and interpretation; critically revised manuscript; gave final approval; agrees to be accountable for all aspects of work ensuring integrity and accuracy.

CG: Contributed to conception and design; critically revised manuscript; gave final approval; agrees to be accountable for all aspects of work ensuring integrity and accuracy.

CZ: Contributed to conception and design; contributed to acquisition; critically revised manuscript; gave final approval; agrees to be accountable for all aspects of work ensuring integrity and accuracy.

KW: Contributed to acquisition; critically revised manuscript; gave final approval; agrees to be accountable for all aspects of work ensuring integrity and accuracy.

MN: Contributed to design; critically revised manuscript; gave final approval; agrees to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Conflicting Interests

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