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John David Cowden

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EPIDEMIOLOGY OF *ASCARIS LUMBRICOIDES* IN TWO MEXICAN TOWNS: EFFECTS OF SCHOOL-BASED ANTHELMINTHIC TREATMENT.

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To investigate the effects of targeted anthelminthic treatment of children, the prevalence and intensity patterns of *Ascaris lumbricoides* infections were determined for two rural towns in Veracruz, Mexico during June and July 1999. The patterns in Tequila, where children aged 2-15 years had been treated tri-annually for six years with albendazole, were compared to those in Tlilapan, where no targeted anthelminthic treatment had occurred. A cross-sectional survey of one district from each town was conducted. Fecal specimens were collected from 96 residents of Tequila (72% of district) and 122 residents of Tlilapan (71% of district) and parasite egg counts were completed using the Kato-Katz method. In Tlilapan (untreated town), prevalence and intensity were highest in children and decreased with age. In contrast, the prevalence in Tequila (treated town) was lowest in children and increased with age, reaching 82% in those over 40 years old. Intensity decreased with age in Tequila, but older individuals had significantly higher intensities than those in Tlilapan (*p*=0.014). The association between targeted albendazole treatment and lower *A. lumbricoides* prevalence is evidence that the anthelminthic program has had positive effect in Tequila, but the classification of the town as highly-infected by WHO criteria suggests the effectiveness of the program could be improved. The high community intensity of infection in Tlilapan indicates that its citizens could benefit from inclusion in Mexico's anthelminthic program.

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Introduction

An estimated one billion people – one-fifth of the world's population – are infected with *Ascaris lumbricoides*, an intestinal parasite commonly known as the roundworm.[1] *A. lumbricoides* is a soil-transmitted nematode, or geohelminth, that primarily affects developing countries, where a combination of geography, climate, and living conditions allows for the spread of organisms transmitted by the fecal-oral route. Children are at special risk of significant infection and suffer adverse effects ranging from acute intestinal or biliary obstruction to chronic malnutrition and delays in physical and cognitive development. Heightened awareness of the extent of infection and of the subtle, insidious effects of geohelminths on children prompted the World Health Organization (WHO) in the early 1990s to begin promoting population-based de-worming programs as a cost-effective method to improve public health in developing areas of the world.[2]

Because children carry higher loads of intestinal parasites and suffer greater morbidity than adults, an effective and practical strategy for treating infected populations has been to target school-aged children.[3] Advantages of this approach include the ease of anthelminthic administration through the school system and the lower risk of selecting for drug resistance by treating only a limited segment of the population.[4] Evaluations of such targeted treatment programs have shown reductions in prevalence of infection and worm burden among school-aged children, with cure rates reported at 90% or more.[4-7] Such reductions have been associated with improvements in nutritional status and growth and possible improvements in cognitive function and school performance.[8-12]

In 1993, Mexico began including targeted anthelminthic treatment of children in tri-annual National Health Weeks designed to address a variety of public health issues. In a 1993 baseline survey of all Mexican states, the prevalence of *A. lumbricoides* among 9,337 children was 20.9%, ranging from 0%-84.9% depending on the community.[1] The heterogeneity of infection

levels was found to be associated with climate, altitude, and latitude of community, allowing high-risk areas across the country to be identified and selected for on-going treatment. Since then, selected communities have participated in a primarily school-based effort to treat all children aged 2 to 15 years with the anthelminthic albendazole. During National Health Weeks, nurses and doctors from community health centers have treated most children during school visits, but also have provided treatment for younger children and those not attending school by visiting homes in the community.

Evaluation of the Mexican program has been limited, but early evidence from pre- and post-treatment fecal examinations in selected areas suggested that albendazole had been highly effective in reducing prevalence and intensity of geohelminth infections among children.[1] Prevalence was reduced by 70%-90% and intensity by 99.9% during five National Health Weeks from 1993 to 1996. Clinical improvement of infected individuals and improved community support of treatments were also reported. This evidence established the early success of the Mexican treatment program, but the effectiveness of the program after six years of implementation has not been evaluated. This study employs epidemiologic data from a cross-sectional survey to describe the state of *A. lumbricoides* infection in two towns: one that had been included in National Health Week de-worming and one that had not. The comparison of towns is then used to address the question: how effective is the Mexican anthelminthic effort?

Statement of purpose and hypothesis

The purpose of this study is to describe the prevalence and intensity patterns of *A. lumbricoides* infections in two rural towns and to use these data to investigate the effects of targeted anthelminthic treatment of children in Mexico. We hypothesize that individuals in the town previously treated with albendazole will have lower prevalence and intensity of infection than those in the untreated town, suggesting that treatments have been effective.

Methods

Tequila (18°74′ N, 97°04′ W) and Tlilapan (18°48′ N, 97°06′ W) are rural towns located in the mountains south of Orizaba, Veracruz, Mexico. Though separated by 10 km and approximately 500 m of elevation, the towns share a temperate humid climate with a rainy season extending through the summer and early fall months. Annual rainfall is approximately 100-200 cm and annual temperatures average 19-21°C. Tequila, at an elevation of 1660 m, is situated among mountain slopes while Tlilapan, at 1160 m, sits in a nearby river valley. The 2000 Mexican Census reported the populations of Tequila and Tlilapan to be 3179 and 2707, respectively. The towns are composed of a mixture of concrete houses and cardboard shacks with few latrines. Defecation commonly occurs in wild vegetation or in fields. Coffee, fruit and sugarcane are grown on the slopes surrounding the towns, and farming is the primary occupation of the inhabitants. Residents also work in factories in nearby cities.

Fieldwork was completed in June and July 1999, approximately one month after the most recent anthelminthic treatment cycle. In each town, a district with a population of approximately 125-150 was chosen for sampling (Cotlajapa, Sector 1 in Tequila and Sector 6, Block 1 in Tlilapan). A team of health workers from the regional health jurisdiction (Jurisdicción Sanitaria No. 7, Orizaba) and town-based health centers visited every house in each district, describing the study and distributing a small plastic fecal sample container to each family member. Verbal consent was obtained according to Protocol 11009 approved by the Yale University Human Investigations Committee, and a similar protocol approved by the Hospital Infantil de México. Each participating family member was assigned a code specifying town, family and individual. Fecal samples were collected for three consecutive mornings in each town and examined using the Kato-Katz method of fecal egg quantification during the afternoon in a private laboratory in Orizaba.[13] Two slides were prepared for each fecal specimen and the average of the two egg

counts calculated. One author (JC) directed all fieldwork and prepared and examined all slides. Results were reported to study participants and anthelminthic treatment was offered to infected individuals.

SPSS 10.1.0 software (SPSS Inc., Chicago, Illinois) was used to analyze all data. Ages of sample populations were compared using the Student's *t*-test. Associations of prevalence and intensity with towns and age groups were analyzed using the chi-square test and Fisher's exact test. Egg counts were converted to eggs per gram (EPG) of feces and then classified according to WHO intensity categories as light (1-4,999 EPG), moderate (5,000-49,999 EPG), or heavy (≥50,000 EPG) infections.[14] Age groups were categorized by decade of life and by treatment group (2-15 year-olds and ≥16 year-olds). In comparisons of intensity between towns and age groups, the moderate and heavy intensity categories were combined, as new WHO recommendations combine these groups for classification of a community's risk of morbidity from geohelminth infection.[3] Intensity was also analyzed as a continuous variable, allowing mean EPG to be compared between groups of participants using the Mann-Whitney *U* test. A two-tailed p value less than 0.05 was considered statistically significant for all analyses.

Results

Study Population

All 305 residents of the two chosen sectors were invited to participate. A total of 218 (71%) accepted, including 96 of 134 (72%) in Tequila, and 122 of 171 (71%) in Tlilapan. The ages of participants ranged from 1 week to 74 years in Tequila and 2 months to 93 years in Tlilapan. Participants in Tequila were significantly younger than those in Tlilapan (mean age in years 19.2 ± 13.6 vs. 24.2 ± 16.5, $p = 0.048$; median age 15 vs. 21), but this difference was accounted for by the ≥16 year-olds (32.2 ± 13.6 vs. 38.5 ± 16.5, $p = 0.037$; median 30 vs. 35). The 2-15 year-old groups were of similar age (mean 8.2 ± 4.3 vs. 6.6 ± 3.8, $p = 0.067$; median 8 vs. 6). Mean age of non-

participants was also similar (24.7 ± 15.3 vs. 26.7 ± 20.0, $p = 0.714$). Women predominated among participants in both towns (Tequila 59%, Tlilapan 61%). In Tequila, there was a significantly lower percentage of women in the 2-15 year-old group than in the ≥16 year-old group (48% vs. 69%, $p = 0.041$). In Tlilapan, the percentages were similar (62% vs. 64%, $p = 0.867$). Sample characteristics are summarized in Table 1.

	Town		
Category	**Tequila**	**Tlilapan**	***p* value**
N	96	122	
Age range	1 week – 74 years	2 months – 93 years	
Mean age in years ± SD	19.2 ± 13.6	24.2 ± 16.5	0.048†
2-15 year-olds	8.2 ± 4.3	6.6 ± 3.8	0.067
≥16 year-olds	32.2 ± 13.6	38.5 ± 16.5	0.037†
Median age in years	15	21	
2-15 year-olds	8	6	
≥16 year-olds	30	35	
% Female	59%	61%	0.861
2-15 year-olds	48%	62%	0.166
≥16 year-olds	69%	64%	0.573

Table 1. Sample characteristics for the towns of Tequila and Tlilapan, Veracruz, Mexico.
† Significance level set at p value < 0.05

Prevalence

Though the community prevalence of *A. lumbricoides* infection in Tequila was similar to that in Tlilapan (39% vs. 37%, $p = 1.000$), patterns of prevalence by age group differed between the towns. Analyzing prevalence by decade of life (Figure 1) showed two prevalence peaks in Tequila, one in the 20-29 year-old group, and another in the 40-49 and 50-59 year-old groups. In contrast, prevalence in Tlilapan was highest in the 0-9 and 10-19 year-old groups and tapered off

with age. Differences between towns were statistically significant in the 0-9 ($p = 0.049$), 40-49 ($p = 0.032$), and 50-59 ($p = 0.030$) year-old groups.

Analysis of prevalence in treatment-based age groups (Figure 2) showed that in Tequila, prevalence was significantly lower in the albendazole-treated group (2-15 years old) than in the untreated group (≥16 years old) (27% vs. 53%, $p = 0.015$). In Tlilapan, where no one received treatment, the opposite was observed – prevalence was significantly higher in the younger group (51% vs. 32%, $p = 0.042$). Comparing age groups between towns revealed that the prevalence of *A. lumbricoides* infection among younger individuals (2-15 years old) was significantly lower in Tequila than in Tlilapan (27% vs. 51%, $p = 0.017$). Prevalence in the older age group (≥16 years old) was significantly higher in Tequila (53% vs. 32%, $p = 0.023$).

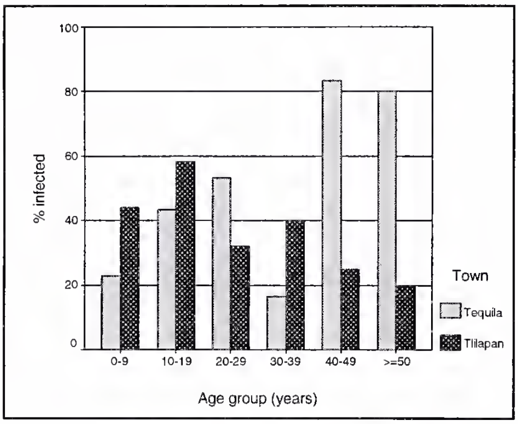

Figure 1. *Ascaris lumbricoides* prevalence by decade of life in Tequila and Tlilapan.

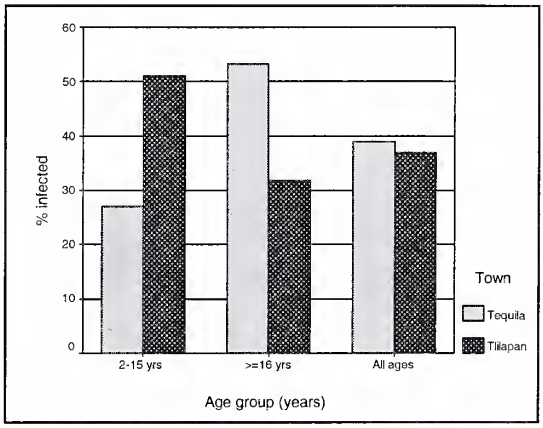

Figure 2. *Ascaris lumbricoides* prevalence by age group in Tequila and Tlilapan.

Intensity

In Tequila, infections of moderate intensity predominated overall and in both treatment-based age groups. Though the mean EPG was significantly lower among 2-15 year-olds than among ≥16 year-olds (164 ± 380 vs. 292 ± 613, $p = 0.031$), analysis of intensity categories revealed no significant association between intensity and age ($p = 0.260$) (Table 2, Figures 3-5). In Tlilapan,

light infections predominated, and an association of lighter intensity with increasing age was shown in both mean EPG and categorical analyses. The mean EPG among 2-15 year-olds was significantly higher than among ≥16 year-olds (272 ± 569 vs. 186 ± 689, $p = 0.019$), and the younger group had more moderate and heavy infections ($p = 0.010$).

Comparing intensity categories between towns showed a significantly higher number of moderate and heavy infections in Tequila ($p = 0.046$) (Figure 3), though mean EPG was similar in the two towns (219 ± 501 vs. 215 ± 629, $p = 0.599$). Analyzing age groups between towns showed similar intensity patterns in the 2-15 year-old groups (categorical $p = 0.137$, mean EPG 164 ± 380 vs. 272 ± 569, $p = 0.063$) (Figure 4), but infections of higher intensity in the ≥16 year-old group from Tequila (categorical $p = 0.034$, mean EPG 292 ± 613 vs. 186 ± 689, $p = 0.011$) (Figure 5). In the categorical analysis, dividing this group into those between 16 and 39 years old ($p = 0.020$) and those 40 years or older ($p = 0.667$) showed that the difference in intensity pattern between towns was accounted for by the 16-39 year-olds.

Community/ age in years (N)	Total infected N (%)	Light[A] N (%)[B]	Moderate N (%)	Heavy N (%)	EPG mean (+/- SD)[C]	EPG median[C]
Tequila						
All ages (96)	37 (39)	12 (32.4)	23 (62.2)	2 (5.4)	219 (501)	0
0-1 (3)	0 (0)	----	----	----	----	0
2-15 (48)	13 (27)	2 (15.4)	10 (76.9)	1 (7.7)	164 (380)	0
≥16 (45)	24 (53)	10 (41.7)	13 (54.7)	1 (4.2)	292 (613)	19
Tlilapan						
All ages (122)	46 (37)	25 (54.3)	17 (36.9)	4 (8.8)	215 (629)	0
0-1 (8)	1 (13)	----	1 (100)	----	----	0
2-15 (45)	23 (51)	9 (39.1)	13 (56.5)	1 (4.3)	272 (569)	15
≥16 (69)	22 (32)	16 (72.7)	3 (13.6)	3 (13.6)	186 (689)	0

Table 2. Prevalence and intensity of *Ascaris lumbricoides* infection by age group in Tequila and Tlilapan.

[A] Intensity categories defined by WHO criteria – Light = 1-4,999 EPG, Moderate = 5,000-49,999 EPG, Heavy = ≥50,000 EPG.[14]
[B]The denominator for intensity percentages is total infected individuals in a given age group.
[C]The arithmetic mean and median of EPG among all participants in a population.

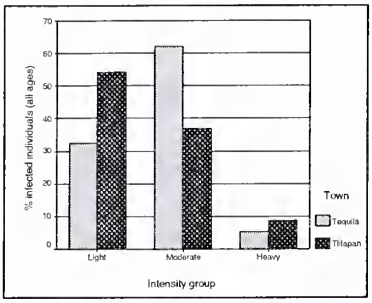

Figure 3. Intensity patterns for all ages in Tequila and Tlilapan using WHO criteria.[14]

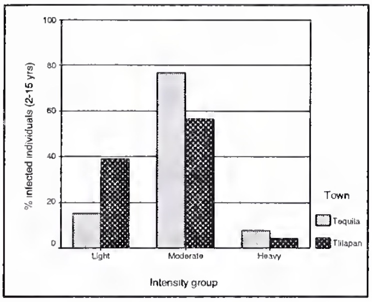

Figure 4. Intensity patterns in 2-15 year-olds from Tequila and Tlilapan using WHO criteria.[14]

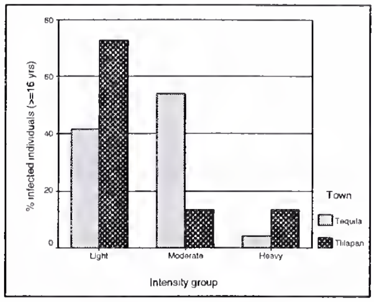

Figure 5. Intensity patterns in ≥16 year-olds from Tequila and Tlilapan using WHO criteria.[14]

The distribution of *A. lumbricoides* among the participants in the two towns was over-dispersed, with most individuals having no infection or infections of low intensity, and a few harboring disproportionately high worm burdens (Figures 6 and 7).

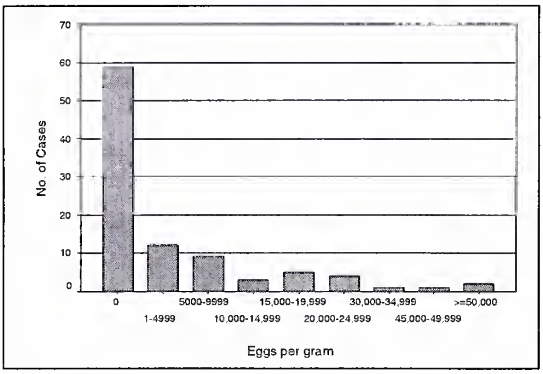

Figure 6. The distribution of *Ascaris lumbricoides* infection intensity among the residents of Tequila.

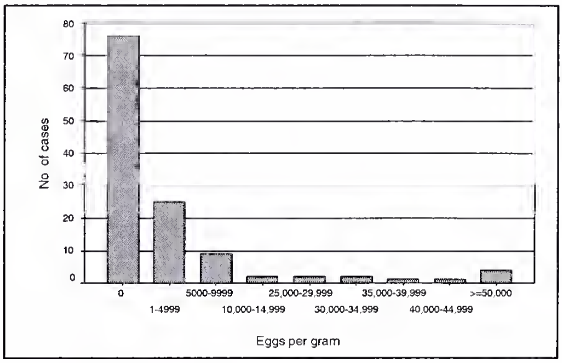

Figure 7. The distribution of *Ascaris lumbricoides* infection intensity among residents of Tlilapan.

Discussion

Prevalence and intensity data from a small cross-sectional survey of similar towns are not sufficient for a comprehensive evaluation of an anthelminthic treatment program. Ideally, a larger, longitudinal study would focus on the pre- and post-treatment epidemiology of infection over several treatment cycles in a town such as Tequila. Nevertheless, the prevalence and

intensity patterns described in this study raise important questions about the administration and effectiveness of the targeted anthelminthic campaign in Mexico.

Guidelines for the identification and treatment of communities at risk of morbidity from geohelminths have been published by the WHO, most recently in the guide *Helminth Control In School-age Children*.[3] Recommended control strategies for a given community depend on a combination of community prevalence and community intensity data. Community prevalence is defined as the percentage of infected individuals in a population (Table 3), and is categorized as low (<50%), moderate (50%-70%), or high (>70%). Community intensity is defined as the proportion of heavily infected individuals in a population, categorized as low (<10% of individuals with moderate or heavy infections) or high (≥10% moderate/heavy infections). Communities can be classified into any of three groups: 1) high prevalence and/or high intensity, 2) moderate prevalence and low intensity, or 3) low prevalence and low intensity (Table 3). Each group merits a different level of intervention. Targeted treatment of school-aged children, the basis of the Mexican campaign, is recommended for the first two groups, along with improvement in sanitation and health education. Only the latter improvements are recommended for the third, less-infected group.

Community Category	Recommended treatment strategies
I. High prevalence (>70%) and/or high intensity (≥10% of individuals with moderate/heavy infections)	Targeted treatment of school-aged children 2-3 times/yr, health education, improvements in sanitation, water supply, and waste management
II. Moderate prevalence (50%-70%) and low intensity (<10% moderate/heavy infections)	Targeted treatment of school-aged children at least 1 time/yr, health education, improvements in sanitation, water supply, waste management
III. Low prevalence (<50%) and low intensity (<10% moderate/heavy infections)	Health education, improvements in sanitation, water supply, waste management

Table 3. WHO treatment recommendations based on classification of communities with endemic *Ascaris lumbricoides* infection.[3]

At the time of the study, Tequila and Tlilapan qualified as low prevalence communities (37% and 39%) but had high community intensities (26% and 17% moderate/heavy infections), earning them both classification in the first, most-infected group. Tlilapan had not been included in the Mexican anthelminthic campaign based on the program's identification of high risk areas by climate, altitude, and latitude. Its classification by WHO criteria suggests that the town is at risk of morbidity and could benefit from targeted treatments. In Tequila, the exact effects of six years of anthelminthic treatments are impossible to determine without pre-treatment data, but the finding that one-quarter of all participants in this study had a moderate or heavy infection brings the effectiveness of the anthelminthic campaign into question.

Prevalence

In Tlilapan, the prevalence pattern by age was somewhat atypical for a treatment-naïve community – prevalence was highest in children and gradually tapered off with age. Anderson and May have examined numerous *A. lumbricoides* prevalence patterns, finding that when sample sizes are adequate from communities where the worm is endemic, prevalence typically rises quickly after infancy and is maintained at a high level throughout adulthood.[15] This common pattern allows the classification of communities based on overall prevalence, such as the WHO guidelines above, to represent the likely classification among the communities' children, too. In this case, though, Tlilapan's overall prevalence of 39% does not represent the prevalence among 2-15 year-olds. The younger sub-group, which would be the target of anthelminthic treatment if Tlilapan were included in the Mexican campaign, had a prevalence of 51%, making it a moderate prevalence population.

The prevalence pattern in Tequila showed evidence of six years of targeted treatment. Prevalence in treated children was significantly lower than in untreated adults and also lower than in the untreated children of Tlilapan. It is reasonable to attribute these differences to

anthelminthic treatment, as reduced prevalence in treated groups is an oft-reported result of de-worming programs.[4] By WHO definition, prevalence of 27% in Tequila's 2-15 year-olds is low, but its relation to pre-treatment prevalence is unknown. Interpretation of the figure is complicated by the timing of the fecal sample collection in relation to treatment, discussed below in the section on limitations.

The high prevalence of infection in Tequila's older age group is not unexpected, assuming that the pre-treatment pattern in this community was typical for *A. lumbricoides*. If prevalence were previously as high as 70%-80% from childhood on, repeated treatment of the younger groups could result in the patterns illustrated in Figures 1 and 2. The dip in prevalence among 30-39 year-olds is curious, but may be a result of small sample size for each decade group. The analysis by treatment-based group gives a less-detailed, but more statistically reliable picture of the high adult prevalence in Tequila.

The significant difference in prevalence between the older groups in Tequila and Tlilapan was most likely caused by a factor other than the treatment program. Several known risk factors for geohelminth infection were thought to be similar in the two towns, but were not formally surveyed in this study. These include percentage of workforce in agriculture, use of night soil in farming, water supply, sanitation habits, type of floors in homes, and soil characteristics in the towns and surrounding fields.[16, 17] One or more of these may have contributed to the higher prevalence of infection in Tequila's adults. A more rigorous study of these variables would be required to understand this discrepancy.

Intensity

Analyzing intensity data for the two towns adds to the concern that anthelminthic treatments are needed in Tlilapan and might be improved in Tequila. In Tlilapan, though infections of light intensity predominated overall, 31% of all 2-15 year-olds had moderate or

heavy infections. This number is analogous to the community prevalence for this sub-group, clearly high at well above 10%. Among adults in Tlilapan, most infections were of light intensity, consistent with well-described *A. lumbricoides* intensity/age associations – intensity is usually highest among children and tapers off in adults.[18] Even so, treating children in Tlilapan could result in a side-benefit for the town's adults. Chan et al. published a model predicting that a reduction in children's worm burden could eliminate a significant contributor to the transmission of infective eggs to the rest of the population, resulting in lower intensities among untreated adults.[19] Three field studies supported this model, reporting reduced intensities of *A. lumbricoides* infection among untreated adults after targeted anthelminthic treatment of school-aged children.[20-22]

In Tequila, moderate infections predominated in both age groups. This finding suggests that even though the level of infection in Tequila may have been higher prior to the initiation of treatments, the potential benefits of the anthelminthic campaign may not have been fully realized. Six years of effective treatment can be expected to reduce intensities dramatically in the treated group, and moderately in the untreated group.[20-22] Without pre-treatment intensity data, a confident conclusion about the effectiveness of Tequila's treatments cannot be made. But regardless of where the infection levels began, by the time of this study, they had not been reduced enough to move Tequila out of the most-infected WHO classification.

Because of the typically over-dispersed distribution of geohelminths in a community, mean EPG is not as reliable as categorical grouping for the assessment of community intensity of geohelminth infections, and it is not a common basis for group comparison in the literature. Mean EPG of different populations can be useful if compared using non-parametric analyses such as the Mann-Whitney *U* test.[17] In this study, such data play a supporting role to the standardized WHO categories. The observed differences in mean EPG between the age groups in

both towns support the conclusions made above: in Tequila, children have lower intensity than adults, and in Tlilapan vice versa. The discrepancy between the categorical and mean EPG analyses of intensity between towns highlights one of the pitfalls of using mean EPG to evaluate a community. The mean EPG in Tequila and Tlilapan was nearly identical, but there were significantly more moderate infections in the former town. Thus, the categorical analysis shows that there are more individuals at risk of morbidity in Tequila, despite the similarity in average burden per individual.

Conclusions

This evaluation of the Mexican anthelminthic campaign yielded two primary conclusions: 1) the anthelminthic campaign had not included all towns that might benefit from treatment, and 2) repeated treatments of children in Tequila may not have reduced the community worm burden as much as predicted by epidemiological models and previous studies.

The efficacy of tri-annual treatments with albendazole for the reduction of individual and community infection by *A. lumbricoides* in Mexico has been well-established[1]. But the effectiveness of the Mexican National Health Week anthelminthic campaign depends on the accurate selection of high risk areas and the coordinated administration of treatments in numerous communities across the country. The Mexican program is a large-scale effort, and success in these activities naturally will vary over space and time. The data presented here suggest that the residents of Tlilapan might benefit from future inclusion in the Mexican anthelminthic program. In Tequila, the effectiveness of anthelminthic treatments could probably be improved and may have been compromised by a number of factors, including poor coverage due to school absenteeism, poor compliance of parents and/or children, and incomplete delivery of treatments by limited health personnel. The potential for further reduction of *A. lumbricoides*

infection in Tequila and Tlilapan will depend on more-detailed evaluations of how and where the Mexican anthelminthic program is administered.

Limitations

Because the original study that produced these epidemiologic data was not designed to rigorously evaluate the effects of the Mexican anthelminthic campaign, the findings and conclusions from this study must be qualified. A cross-sectional study gives a picture of one moment in time. Any resulting conclusions about changes across time must include some amount of conjecture. Nevertheless, the above suggestions about the effectiveness of the anthelminthic treatments in Tequila and the need for treatment in Tlilapan are based on reasonable assumptions and well-studied principles of geohelminth infection and community treatment programs.

Though the comparison of infection patterns in the two towns resembles an experimental-control model, the data were not collected in a manner that allows for isolation of a variable such as town's treatment history. The differences in infection burden between Tlilapan and Tequila undoubtedly represent a combination of many variables unmeasured in this study. Even if both towns were treatment-naïve, such differences in prevalence and intensity in similar, nearby towns would not be unusual. Annan et al. and Crompton and Tulley have described impressive heterogeneity in the epidemiology of *A. lumbricoides* between neighboring communities with seemingly equal risks of infection.[23, 24] The value of the comparison in this study lies primarily in the illustration of this heterogeneity and the resulting need for careful identification of potential high-risk areas that could benefit from targeted treatment.

Specific problems encountered in this evaluation include the timing of data collection in relation to treatment cycles, small sample size in an important sub-group of treated children,

significantly younger population sampled in Tequila, a general under-sampling of men, and a lack of demographic data.

Evaluation of anthelminthic drug efficacy requires that fecal specimens be analyzed shortly after treatment to minimize the possibility that the effect of medication will be masked by re-infection. To evaluate the effectiveness of a geohelminth control program, however, the WHO recommends that specimens be collected just before a treatment cycle, when the extent of re-infection across the period between treatments can be estimated.[25] This also allows program planners to determine the proportion of heavily infected children just before treatment and to adjust program administration accordingly. In this study, because prevalence and intensity data were collected one month after the most recent de-worming, re-infection must be considered a contributor to prevalence and intensity figures in Tequila. But the collection occurred at least two months before the next planned treatment, suggesting that the recommended collection would have shown an even higher level of infection in the treated town.

Small sample sizes prevented the analysis of data from an important sub-group of the 2-15 year-olds. The cumulative effects of the anthelminthic campaign might have been seen best among 8-15 year-olds, the only individuals eligible for all six years' treatments. Though prevalence in this age group was 50% lower in Tequila than in Tlilapan (27% vs. 54%), the sample sizes were 26 and 13 individuals, and the difference was not statistically significant ($p = 0.157$). Assuming a beta error of 20%, a sample size of 54 individuals in each town would have been necessary to confidently assess this difference. Therefore, sample sizes were too small to confidently rule out a false negative result in this analysis.

The age difference between samples from Tequila and Tlilapan was accounted for by the ≥16 year-olds, and could have reflected a real difference in average age between the communities due to social factors such as employment opportunity or emigration. It also may have resulted

from the selection of sectors that did not accurately represent the larger communities' average age. The lack of general demographic data, addressed briefly below, prevented exploration of this issue. The towns' children were similar in age, and the primary conclusions about the effects of this school-based treatment program should not be significantly affected by a difference in age between the older groups.

Men were rarely present when the study was described to families, and their reluctance to provide fecal samples was a challenge that was not overcome during fieldwork. Any difference in infection patterns between men and women, possibly due to men's agricultural work, women's exposure to their children's feces, or an inherent gender difference in susceptibility to infection, would have affected the validity of this study's results.

Finally, the general lack of demographic data pertaining to factors such as age, sex ratio, employment, income, school attendance, night soil use, sanitation, and water supply in the two towns precluded a more rigorous comparison of their epidemiology. The collection of this information by questionnaire while enrolling subjects is a common practice in geohelminth risk factor studies, but was not included in this intentionally basic epidemiologic survey.

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