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ORIGINAL RESEARCH

Lessons from a study in a rural community from southern Mexico: risk factors associated to transmission and reinfection of gastrointestinal parasites after albendazole treatment

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Purpose: To determine the prevalence of gastrointestinal parasites and evaluate the effect of a single dose of treatment with albendazole in a sentinel group from a rural community in southern Mexico.

Methods: Stool samples were collected from 1456 individuals aged ≥1 year during consecutive days, and examined for helminth infection using the modified Stoll dilution method. Additionally, 104 individuals were treated with a single dose of albendazole and evaluated over 21 weeks to assess reinfection. Questionnaires were administered to obtain individual and household-level data pertaining to behavior, demography, and socioeconomic status. Risk factors for reinfection after albendazole administration were determined using multiple logistic regression analyses.

Results: The prevalence of *Ascaris lumbricoides* was 73.9% (95% confidence interval [CI] = 71.56%–76.14%). Albendazole was 100% effective, but eggs began to be detected by 9–12 weeks posttreatment, increasing to 100% after 21 weeks. Logistic regression analysis revealed that all individuals from this study had a probability of reinfection of 1.65× each week after treatment. The prevalence of *Trichuris trichiura* was 57.2% (95% CI = 54.62%–59.77%) and chemotherapy was 34.7% effective. The prevalence for other minor gastrointestinal parasites ranged from 0.2% to 29.7%.

Conclusion: This was a comprehensive study on gastrointestinal parasites in a rural community from southern Mexico and, to the best of the authors' knowledge, is the first time that the effect of albendazole has been evaluated for a period of over 21 weeks following its administration. Risk factors associated with parasite transmission were linked to poverty and lack of hygiene, such as, defecating in open places, living customs (drinking piped water and/or from a well), and absence of knowledge about transmission life cycle of the parasites. Studies of this kind should be linked to health education and improvement of access to clean water and adequate sanitation to consolidate morbidity control and enhance sustainability.

Keywords: *Ascaris lumbricoides, Trichuris trichiura*, polyparasitism, risk factors, albendazole

Introduction

The gastrointestinal parasites *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), and *Necator americanus*/*Ancylostoma duodenale* (hookworms) are among the most widespread parasites worldwide. An estimated of 4.5 billion people are at risk of gastrointestinal parasitic infections, and more than 1 billion individuals are thought to be infected, of which 450 million (mainly children) suffer morbidity from their infection. An additional 44 million infected pregnant women suffer significant morbidity and mortality due to hookworm-associated anemia. Approximately 135,000 deaths

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occur per year, mainly due to infections with hookworms of *A. lumbricoides*.[1,2]

Broad-spectrum anthelminthics administered in a single dose have been shown to improve control of intestinal helminthiases in economically poor, endemic areas.[3] In particular, albendazole and/or mebendazole in a single dose significantly reduce infection and worm burden in school-age children harboring heavy intestinal infections.[2,4,5]

Since 1972, the Mexican National Health System (Sistema Nacional de Salud [SNS]) has provided mass chemotherapy with albendazole twice per year to school-age children in urban, suburban, and rural areas. This scheme has been of great health benefit to the country because prior to the implementation of the antihelminthic program, one of the main morbidities in children was related to gastrointestinal parasitoses (especially *A. lumbricoides* due to heavy burdens and malnourishment). It was also the main reason for surgery in children due to intestinal occlusion. After its implementation, child mortality diminished dramatically and the morbidity of the disease also decreased.[6] However, it is not known if this procedure is sufficient to reduce worm burden at community level because little is actually known about the real prevalence of gastrointestinal parasites in affected areas. Moreover, the fact that this mass chemotherapy is not administered to all inhabitants of the community raises suspicions that parasite transmission could rapidly increase after administration because the majority of dwellings lack sanitary facilities and, furthermore, because efficacy of treatment is not routinely assessed by microscopy.

With the potential shortcomings of the SNS strategy in mind, the objective of the current study was to determine the prevalence of gastrointestinal infections in a rural community in southern Mexico and to subsequently evaluate the effectiveness of the official albendazole dosage regime with a trial study of gastrointestinal parasite reinfection in a group of treated individuals.

Materials and methods

A cross-sectional study was undertaken from May to October 2008 in the village of Texán de Palomeque (20°90′N, 89°92′W), located 35 km northwest of Merida (20°59′N, 89°39′W), the capital of the state of Yucatan, Mexico.

Village meetings were held and village authorities and villagers were given detailed explanations about the aims, procedures, potential risks, and benefits of the study. It was clearly explained that participation was voluntary and that all individuals could withdraw from the study at any time. Written and oral informed consent was obtained from all adult subjects and from parents or guardians of minors. The study was approved by the Committee of Bioethics at the Scientific Research Council of the University of Yucatan, Merida.

After informed consent was obtained, a questionnaire was conducted by two members of the research team (one interviewer and one laboratory technician) to each participating household to record personal data, demographic data, and household general knowledge of parasites as well as to learn the status of their sanitary facilities. Details of this widely used approach have been presented elsewhere from other field studies in Guatemala, Mexico, and Indonesia.[7–9]

Finally, disposable plastic containers were prepared for all members of each study household. Participants' names and unique identifiers were marked on the plastic containers and distributed to the heads of households with detailed instructions on how to collect a fresh morning stool sample. All study participants were asked to provide a sufficiently large stool sample (at least 5 g). Containers were then collected after they were filled. Stool samples were collected daily during the field study and transported on ice to the Laboratory of Immunology and Molecular Biology at Centro de Investigación y de Estudios Avanzados del IPN Unidad Mérida. Stool samples were processed in the laboratory within a maximum of 3 hours after collection by experienced laboratory technicians. Stool specimens were examined by direct microscopy at 10× and 40× using the modified Stoll dilution method. To estimate the worm burden using this dilution method, approximately 1 g of the sample was dissolved in 14 mL of 0.1 N sodium hydroxide. Then 0.15 mL of the suspension was placed on a slide, the eggs under the entire cover slip were counted, and the number of eggs was multiplied by 100 to give an estimation of the number of eggs/mL and, subsequently, eggs/g.[10]

Treatment with albendazole (Zentel®; SmithKline Beecham SA de CV, Mexico City, Mexico) was provided to all willing individuals in the form of a single dose of albendazole (according to SNS recommendations, with 200 mg given to children aged 2–8 years and 400 mg to individuals aged ≥9 years). Dosages were administered by two trained nurses under the direct supervision of a local clinician attached to the project. Subject age groups were categorized using the SNS statistical categories of children (1–10 years old), teenagers (11–19 years old), and adults (≥20 years old).[6]

Additionally, a 21-week follow-up trial study was done to assess the impact of treatment. For the initial sampling after albendazole treatment, the aim was to enroll at least 320 individuals (standard error >0.015; P = 90%). This was based on the criterion that they were negative for *A. lumbricoides* after albendazole treatment before starting the follow-up study. The sample size was selected based on the premise that all inhabitants (n = 1456) had the same chance of reinfection. However, given that the majority of the inhabitants were reluctant to continue the follow-up study over 21 weeks, only 104 (33.43%) individuals finished the trial. The remaining 216 individuals only provided a stool sample during the first 2 weeks. They were negative by microscopy, but, given their reluctance to participate, they were excluded from the study. The 104 individuals who agreed to participate during the entire follow-up study represented 7% from the treated total population of 1456 individuals.

Samples were collected weekly in the morning after treatment, transported on ice, and examined as before. Subjects were considered positive when *A. lumbricoides* eggs were detected in their fecal sample. This was evaluated qualitatively based on the presence of *A. lumbricoides* eggs in the slides and quantitatively by the Stoll method.

Statistical analysis

All data were recorded using the Microsoft Excel 7.1 (Microsoft Corp, Redmond, WA) and Epi-Info 6.0 (Centers for Disease Control and Prevention, Atlanta, GA) computer software programs. Differences in proportions were evaluated using a chi-square test with 95% confidence intervals (CI). Odds ratios (OR) for relative risk, with corresponding CI and P-values, were calculated using Epi-Info, to identify the risk factors associated with *A. lumbricoides* transmission. In the same way, a logistic regression analysis using procedure proc logistic in SAS[11] (v9.0; SAS Institute Inc, Cary, NC), was applied to the *A. lumbricoides* reinfection data, using the variable reinfection (1 = yes; 0 = no) as the dependent variable (Y) and the other 13 as the covariates (X). These covariates were: (1) age, (2) age group, (3) gender, (4) defecation outdoors, (5) week posttreatment, (6) drinking of piped water, (7) drinking of boiled water, (8) drinking of water from a well, (9) treatment of well water with $AgCl_2$ before drinking, (10) eating of raw vegetables, (11) receiving treatment for worms once per year, (12) receiving treatment for worms twice per year, and (13) sight of parasites in feces. This regression analysis was performed to select the significant variable(s) affecting the likelihood of reinfection after treatment with albendazole. The linear logistic regression model was transformed to the exponential form by calculating the anti-Log (ex) of the linear parameter, and the likelihood of reinfection with *A. lumbricoides* was estimated for each posttreatment week with the following formula:

$$P(X) = \frac{e^{(-3.0506+0.5005X)}}{1+e^{(-3.0506+0.5005X)}} \quad (1)$$

The P-values were plotted on the Y-axis against the total posttreatment week range in the X-axis. Finally, concordance between the observed and expected values for reinfection with *Ascaris* for each volunteer was calculated and compared using the Hosmer–Lemeshow test.[12] Prevalence was reported as the percentage of positive individuals from the studied population.[13] The associated 95% exact CIs of the proportion of individuals harboring intestinal parasites were determined using the method of Miettinen as described in Armitage and Berry.[14]

Results

Environmental and demographic characteristics

The local economy of Texán de Palomeque is based mainly on agriculture and "backyard" farming of cattle, pigs, and chickens. Census carried out at the time of sampling recorded 1607 inhabitants (708 men and 899 women) living in 341 houses distributed in 65 land blocks ("manzanas" in Spanish). Each house was home to 1–25 (mean = 13) inhabitants. Ninety-five percent of the population was literate and 5% illiterate. There is one main paved road at the entrance of the village and unpaved paths connect the majority of the houses. The village has no drainage and 99% of the houses had electricity and piped water. Medical care was available from a social security hospital about 10 km west of the village. There, a doctor and a nurse visited the community twice a week to assist 20 social workers from the community. One house was reported as having a latrine and the remaining houses had no sanitation facilities. Villagers normally defecated outdoors in their backyards in areas designated for this purpose.

In the questionnaire, the members of 280 (82%) households said they had not seen any kind of parasites in their feces and 61 (18%) said they had seen some parasites; the parasite descriptions (ie, reddish round worm, measuring 13–17 cm long) matched that of *A. lumbricoides.* Likewise, 14 (4%) households said that they had regularly received anthelminthic treatment once per year and eight (2%) said that they had received treatment twice per year. None of the villagers had received anthelminthic treatment three times per year.

From the total population, 1456 (90.6%) persons (559 children, 337 teenagers, and 560 adults) from 331 families provided fecal samples and, from these, 104 persons (39 children, 25 teenagers, and 40 adults; 60 female and 44 male) from 11 families finished the 21-week follow-up study.

Prevalence of intestinal parasites

In the initial cross-sectional study, *A. lumbricoides* was found in 73.9% (1076/1456; 95% CI = 71.56%–76.14%) samples and *T. trichiura* in 57.2% (833/1456; 95% CI = 54.62%–59.77%). Age-stratified prevalence for both intestinal parasites is summarized in Table 1. For *A. lumbricoides*, a 42.5% (457/1076) prevalence was found in the childhood group, 19.5% (210/1076) in the teenager group, and 38% (409/1076) in the adult group. When comparing these groups, significant differences were found (χ^2 test for trend = 19.23; $P = 0.00001$). For *T. trichiura*, a 46.8% (390/833) prevalence was found in the childhood group, 19.6% (163/833) in the teenager group, and 33.6% (280/833) in the adult group. Significant differences were found among these groups (χ^2 test for trend = 69.58; $P = 0.001$). No difference ($P = 0.895$ and 0.554, respectively) was found by gender: prevalence of *A. lumbricoides* was 74.0% (596/805; 95% CI = 70.86%–77.03%) in females and 73.7% (480/651; 95% CI = 70.17%–77.07%) in males. Prevalence of *T. trichiura* was 56.5% (455/805; 95% CI = 53.01%–59.97%) in females and 58.0% (378/651; 95% CI = 54.16%–61.88%) in males.

The main risk factor associated with transmission of *A. lumbricoides* was linked to the habit of defecating in open places ($\chi^2 = 122.50$; OR = 6.61; CI = 4.50–9.71; $P = 0.1 \times 10^{-6}$). Another risk factor is related to community members' lack of knowledge; the prevalence of infection was 57% (95% CI = 54.18%–59.59%) in the group of people who were familiar with the parasite versus 73.35% (95% CI = 67.86%–78.36%) in those not familiar with the parasite ($\chi^2 = 395.31$) (OR = 0.07; CI = 0.05–0.10; $P = 1 \times 10^{-6}$).

No statistical difference was found between people who received regular treatment once per year and people who received treatment twice per year ($\chi^2 = 0.12$; $P = 0.60$), as 100% prevalence was found in both groups at the time of sampling.

Table 1 The prevalence by age group of major intestinal parasites (*Ascaris lumbricoides* and *Trichuris trichiura*) in Texán de Palomeque, Yucatan, Mexico

Age group (y)	*A. lumbricoides*[a]	*T. trichiura*[b]
Children (1–10)	42.5 (39.49–45.48)	46.8 (43.38–50.27)
Teenagers (11–19)	19.5 (17.18–22.01)	19.6 (16.92–22.42)
Adults (≥20)	38.0 (35.10–40.98)	33.6 (30.40–36.93)

Notes: [a]χ^2 test for trend; *P*-value = 19.23; 0.00001. [b]χ^2 test for trend; *P*-value = 69.58; 0.001. Values represent point prevalence expressed as percentage and values in parentheses represent 95% upper-limit confidence interval surrounding point estimate.

Prevalence of other minor intestinal parasites

From the 1456 fecal samples analyzed, 161 (11.0%) revealed no gastrointestinal parasites, one species was detected in 322 (22.1%) samples, two species in 523 (35.9%) samples, three species in 282 (19.6%) samples, four species in 156 (10.7%) samples, and five species in twelve (0.8%) samples.

The prevalence for minor intestinal parasites is summarized in Table 2. With regard to age-stratified prevalence, significant differences were found. Prevalence was higher in the childhood group than in the teenager and adult groups for *Hymenolepis nana* (χ^2 test for trend = 7.26; $P = 0.007$), and *Giardia lamblia* (χ^2 test for trend = 27.80; $P = 0.0001$). While no significant statistical difference was found between the childhood and other age groups for *Taenia* spp. (χ^2 test for trend = 1.30; $P = 0.253$), *Entamoeba histolytica* (χ^2 test for trend = 2.06; $P = 0.155$), and *Entamoeba dyspar* (χ^2 test for trend = 0.85; $P = 0.355$).

Effectiveness of treatment

The follow-up study of albendazole chemotherapy included 104 individuals (7% of total population) who finished the trial study. Before treatment, 100% prevalence (104/104) was found (X = 936 epg [eggs per gram]), which dropped to 0% (0/104) after albendazole treatment, but later returned to 100% (104/104) (X = 567 epg) by the end of the 21-week follow-up period. *Ascaris* eggs were detected at week 9 posttreatment in the teenager group (35 epg), at week 10 in

Table 2 The prevalence by gender of minor intestinal parasites in Texán de Palomeque, Yucatan, Mexico

Parasite species	Females (n = 805)	Males (n = 651)	Total	*P*-value[a]
Entamoeba dyspar	31.5 (28.35–34.88)	27.5 (24.09–31.09)	29.7 (27.39–32.16)	0.092
Entamoeba histolytica	30.0 (26.91–33.36)	27.5 (24.09–31.09)	28.9 (26.59–31.31)	0.282
Giardia lamblia	21.0 (18.58–24.36)	20.6 (17.54–23.89)	21.0 (19.01–23.27)	0.715
Hymenolepis nana	09.31 (07.39–11.53)	10.1 (07.92–12.71)	09.6 (08.21–11.31)	0.598
Taenia species	0.2 (0.030–0.89)	0.1 (0.003–0.85)	00.2 (00.04–00.60)	0.579

Note: [a]χ^2 test for trend to test prevalence of females and males. Values represent point prevalence expressed as percentage and values in parentheses represent 95% upper-limit confidence interval surrounding point estimate.

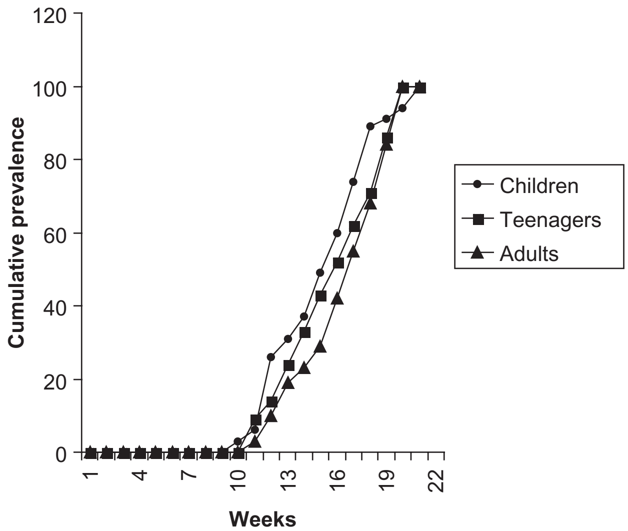

Figure 1 Cumulative prevalence of *Ascaris lumbricoides* cases in three age groups (children, teenagers, and adults) over a 21-week period after albendazole treatment.

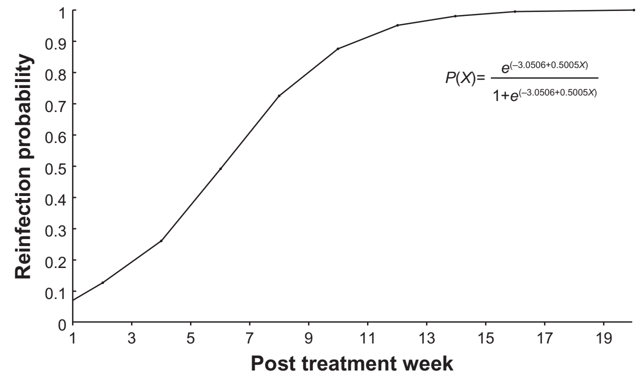

Figure 2 *Ascaris lumbricoides* reinfection probability, estimated by a logistic regression in function of posttreatment weeks, in 104 volunteers from Texán de Palomeque, Yucatan, Mexico, where X = probability of reinfection in week X.

the childhood group (X = 120 epg), and at week 12 in the adult group (X = 12 epg). By week 20, 100% prevalence was observed in the teenager and adult groups and at week 21 in the childhood group (Figure 1).

In contrast, albendazole treatment was only 34.7% effective against *T. trichiura*. Pretreatment prevalence was 45.2% (47/104); immediately posttreatment, prevalence was 30.8% (32/104), which increased to 42.3% at the end of the study. Twelve new cases of *T. trichiura* (X = 45 epg) were detected (seven children, three teenagers, and two adults; comprising seven males and five females).

From the 13 variables analyzed in the logistic regression analysis, only the variable posttreatment week affected significantly the probability of reinfection with *A. lumbricoides* to all people involved in the study of any gender and any age group ($\chi^2 = 108.037$; degree of freedom = 13; $P = 0.0001$). The Hosmer–Lemeshow test was highly significant ($\chi^2 = 3.9079$; degree of freedom = 8, $P = 0.8653$).[12] The concordance between the theoretical probabilities and the observed results was 99%. According to the test, all volunteers involved in the follow-up study had a probability of reinfection of 1.65× each week with *A. lumbricoides* each week after treatment (Figure 2).

Discussion

A rural community from southern Mexico was chosen to evaluate the prevalence of gastrointestinal parasites and to evaluate risk factors associated with transmission of main parasitic infections like *A. lumbricoides*. This community, among many others from Mexico, shared some similarities. The majority of the people defecated in open places and the cultural behavior of the community, in conjunction with the lack of sanitary services and absence of health education, promoted transmission of parasites in endemic areas.[8,15,16] Prevalence of *A. lumbricoides* was 73.9% and 57.2% for *T. trichiura*. The highest prevalence was found in children, followed by teenagers, and adults. Exposure to soil-transmitted helminth infections has been correlated to age-related behavioral as well as environmental factors where younger children carry heavier infections than adults.[2,17,18] Polyparasitism was observed in 66.8% of the inhabitants. Though worm burden was not quantified in the community-wide study, overdispersion was observed (data not shown), with many people carrying infections of light intensity and only a few substantially heavier parasite infections.[13,18]

The OR for relative risk was identified as defecating in open places and the behavior of people as potential risk factors that could contribute to the high prevalence of *A. lumbricoides* infection in the community. Similar observations have been addressed in other studies.[18,19] Regarding treatment, some households received regular treatment once or twice per year. Apparently, this strategy did not aim to reduce parasitic infection because 100% prevalence was found in both interviewed groups at the time of sampling. The findings might suggest that even though albendazole treatment is highly effective, the impact of chemotherapy alone is short lived and is not strong enough to prevent further reinfections.[5,20,21] To support this observation, a follow-up study of 21 weeks was performed on a group of 104 volunteers. No serious side effects were reported after treatment.[6] The albendazole dosage had an immediate effect on *A. lumbricoides* but did not prevent further reinfections as prevalence increased to 100% within 4–5 months posttreatment. Moreover, it seems that people probably became reinfected soon after treatment as the *A. lumbricoides* prepatent period lasts 60–70 days.[22]

In the same way, regression analysis predicted that the possibility of reinfection with *A. lumbricoides* in all volunteers (from any gender and any age group) can increase up to 1.65× each week (Figure 2). Effectiveness of treatment for *T. trichiura* infection was only 34.7% in the same group of volunteers. These results are in accordance with those from a meta-analysis study where a single dose of albendazole resulted in cure rate of 88% for *A. lumbricoides*.[5] In contrast, the treatment of *T. trichiura* was unsatisfactory. This is also in accordance with a study by Keiser and Utzinger,[5] which found that the efficacy of single-dose albendazole for *T. trichiura* was 28%.

Intestinal ascariasis is associated with significant nutritional impairment, which is more or less proportional to the worm burden. The long-term effect of the malnutrition caused by ascariasis, among other effects, is growth impairment.[2,4,15,20,23,24] In this study, nutritional status and nutritional improvement linked to parasitism were not evaluated. However, it is probable that these act within the community as factors associated with low nutritional status; especially if food intake is poor in quality and quantity.

In this sense, the therapeutic efficacy of 400 mg of albendazole was evaluated in seven countries (Brazil, Cameroon, Cambodia, Ethiopia, India, Tanzania, and Vietnam) and the cure rates were very high for *A. lumbricoides* (99.5%) and hookworms (94.8%) but significantly lower for *T. trichiura* (50.8%).[2] The cure rates were also affected to different extents among the three species by the preintervention fecal egg counts and trial country but not by sex or age.

Based on these evaluations, results from this study are certainly encouraging. The use of 200 mg for children and 400 mg for adults as proposed by the SNS is not strong enough to prevent further reinfections. Even if this dosage is delivered twice per year, parasitic prevalence could increase up to 100% within 6 months. In hyperendemic communities, daily exposure is usual, so that new broods of larvae are migrating through the lungs and reaching the intestine to replace the previous mature ones as soon as they are lost. The interval between patent infections in such circumstances is 2–3 months.[13] Therefore, special considerations should be taken when planning a strategy for drug delivery. For instance, Bundy et al[25] provided a single dose of 400 mg of albendazole in two and four cycles of treatment per year. The reduction achieved by four cycles of treatment was greater than that achieved by two, which may indicate a cumulative reduction in the rate of reinfection. Also, long-term community-wide programs to improve living conditions, sanitation, water supplies, and health education will certainly help to prevent parasite transmission.[20,23] Hygiene behavior change is a long-term process. In rural Uzbekistan, for example, the minimum time needed for a hygiene promotion program to have a positive effect was 1 year because the results of hygiene promotion did not point to any significant improvement after 6 months.[19] This strategy, therefore, had similar effects to chemotherapy alone. In the same way, Cairncross[26] has demonstrated that most endemic transmission of enteric infections among poor communities in developing countries is not primarily via water, but instead through other routes such as contamination of hands, food, clothing, and other routes, which can be controlled by improving hygiene behaviors. The same strategy can be feasible in Mexico, as it has been shown that health education combined with chemotherapy reduced *Taenia solium* transmission in some rural endemic communities when compared with use of chemotherapy alone.[27,28] Likewise, stool examinations, which add obvious costs to any program, is necessary to estimate community prevalence and to assess effectiveness of treatment.[15] In this sense, the validation of the McMaster technique to assess worm burden over the Kato-Katz technique opens the gate to other tests for stool analyses.[29] Similarly, the use of generic forms of albendazole needs to be considered, as it has been shown that different forms have a different degree of efficacy.[21,30]

Additionally, there is no conclusive evidence that any anthelmintic currently used against intestinal ascariasis is effective against the larval worms migrating through the liver and lungs.[31] Further, even though albendazole is specific for nematode infections, polyparasitism in the community and its contribution to anemia, gastrointestinal illness, and malnourishment should not be ignored, as a 9.6% prevalence of *H. nana*, 28.9% of *E. histolytica*, 29.7% of *E. dyspar*, 21% of *G. lamblia*, and 0.2% of *Taenia* sp. were found. Similar observations have been reported in Laos.[18] Future studies, therefore, must not only include health education but also improve mechanisms to motivate the participation of community members in follow-up studies.

In conclusion, to the best of the authors' knowledge, this is the first comprehensive study on *A. lumbricoides* transmission in a rural community from southern Mexico. The follow-up trial showed evidence of the short-lived efficacy of albendazole on *A. lumbricoides* and low efficacy on *T. trichiura* due to its failure to prevent further reinfections. Policy decision makers should bear this in mind when planning or evaluating parasitic control programs.

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Disclosure

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