

Exposure of Seasonal Migrant Workers to *Onchocerca volvulus* on Coffee Plantations in Guatemala

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Abstract. Onchocerciasis (river blindness), which is close to being eliminated from Guatemala through semiannual administration of ivermectin, is still transmitted in one area of the country that coincidentally receives an annual influx of migrant workers to harvest coffee. Migrant workers generally are not included in semiannual ivermectin treatments, but if infected could serve as a reservoir. We report on two studies undertaken to measure the exposure to onchocerciasis (presence of IgG4 antibodies to a recombinant *Onchocerca volvulus* antigen, OV-16) among migrant workers. During two coffee harvest seasons, 170 migrant workers with a history of working in the disease-endemic area were tested and 1 (0.6%, 95% confidence interval = 0–3.2%) was seropositive. This low rate of exposure in migrant workers indicates that they are unlikely to play a significant role in transmission of onchocerciasis and may indicate that transmission in the last remaining disease-endemic area of Guatemala is decreasing significantly.

INTRODUCTION

Onchocerciasis (river blindness) is a parasitic disease caused by the filarial nematode *Onchocerca volvulus*. Onchocerciasis in the Americas was first discovered in Guatemala in an area now known as the onchocerciasis central endemic zone (OCEZ) in 1915 by Rodolfo Robles and was thereafter often known as Robles' disease. The condition is targeted for elimination in the six countries of the Americas where it is endemic (Brazil, Colombia, Ecuador, Guatemala, Mexico and Venezuela).¹ The Onchocerciasis Elimination Program of the Americas (OEPA) works with the national ministries of health to provide ivermectin (Mectizan®; donated by Merck & Co., Rahway, NJ) twice annually to the eligible population at risk. Although ivermectin does not directly kill adult worms, it is a microfilaricide that reduces human morbidity and transmission to the black fly (*Simulium* spp.) vectors.

Guatemala has 39% of the population at risk of onchocerciasis in the Americas, although recent evaluations have demonstrated that three of the four foci in Guatemala have interrupted transmission.^{2–4} Only the OCEZ, comprising the departments of Suchitupéquez, Sololá, and Chimaltenango (Figure 1), has recently documented transmission of onchocerciasis. Disease-endemic communities in this area are associated mainly with coffee plantations located at elevations of 500–1,500 meters along the slopes of several of the many volcanoes in the country.⁵ Treatment with ivermectin has been underway in Guatemala since 1996, with semiannual coverage rates > 85% (i.e., the target coverage rate) of the eligible population reported from all four disease-endemic foci of the country since 2002.⁶

Guatemala is a major producer of coffee, consistently ranking fourth or fifth globally in terms of production quantity. Despite decreases in the international coffee market, this

export is responsible for 12% of the gross domestic product of Guatemala.⁷ Most of the 33,000 coffee farms in this country are small family-operated holdings. However, production areas in the OCEZ tend to be large commercial plantations and these require considerable labor, especially from September through February for the harvest. Estimates from the Instituto Nacional de Estadísticas (National Statistics Institute) in 1989 placed the number of seasonal workers participating in the coffee harvest at 80,000 annually,⁸ although this is a difficult number to derive and other estimates have put the number higher. Approximately 60% of this labor comes from neighboring communities and the other 40% is migrant labor traditionally traveling from the indigenous highlands (elevation > 2,000 meters) of Guatemala, where onchocerciasis is not transmitted.⁸

The OCEZ in Guatemala contains approximately 105,877 persons at risk for transmission living in 321 communities (OEPA, 1006, unpublished data). A total of 211 of these communities are small farms or plantations, and although they comprise only 18% of the total population at risk in the OCEZ, they contain a disproportionate percentage (56%) of the population living in hyperendemic communities (these are communities that when first surveyed had a prevalence of microfilaria in skin biopsies > 60%). Although not all of these farms produce coffee, it is no coincidence that the communities with the highest transmission rates are also coffee plantations: the principal onchocerciasis vector in Guatemala (*S. ochraceum*) prefers to breed in the small, clean streams running down the sides of the volcanoes that are found at the elevation at which coffee grows best in this region.

Although resident populations in the OCEZ receive ivermectin twice per year, and regularly reach coverage rates above the target of 85% of the eligible population, ivermectin treatment of seasonal migrant workers appears to be infrequent and sporadic. The timing of the ivermectin rounds (normally in September and June) is largely responsible for the lack of inclusion of migrant workers because treatment rounds occur before and after the coffee harvest season. In a study carried out in the late 1980s, approximately 15% of migrant workers sampled on coffee farms in the OCEZ were found to have microfilaria in skin biopsies (Luján R,

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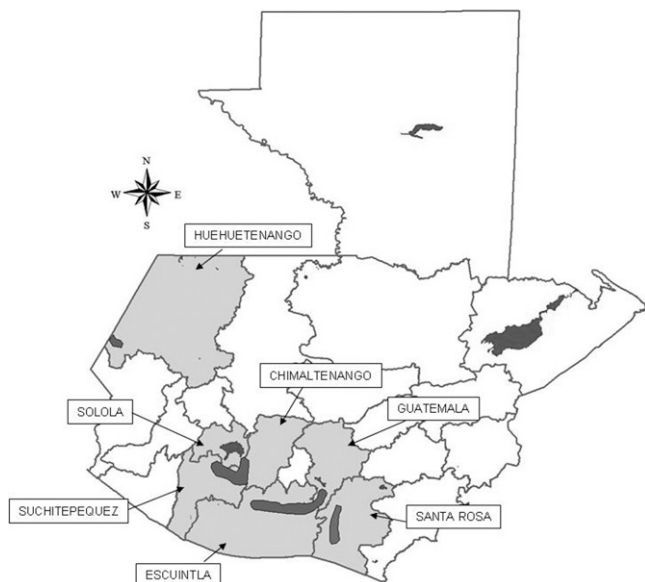


FIGURE 1. Four onchocerciasis foci in Guatemala (dark gray) in the disease-endemic departments (light gray). The onchocerciasis central endemic zone consists of the disease-endemic area in the departments of Suchitpeque, Sololá, and Chimaltenango.

Richards F, unpublished data). A study of migrant workers from 1997 through 1999 in the onchocerciasis focus in southern Chiapas, Mexico, which regularly received migrant workers from Guatemala, found that between 3% and 23% of the migrant workers were microfilaria positive.⁹ More recently, subcutaneous nodules suspicious for onchocerciasis have been detected in migrant workers on plantations in the disease-endemic piedmont regions (Mendoza C, unpublished data), and isolated cases of such nodules have been reported in health centers in highland communities that are not endemic for onchocerciasis that provide migrant labor. None of these nodules has been confirmed histologically as being caused by *O. volvulus*.

Migrant workers are present in largest numbers in the OCEZ during the coffee harvest months of December to February, which coincides with the period of peak onchocerciasis transmission (i.e., January–March¹⁰). In addition, the most intense exposure to blackfly bites occurs among the coffee trees.¹¹ Therefore, migrants are likely to be exposed and infected with *O. volvulus*. If there is an appreciable rate of infection among migrant workers who are not included in semiannual ivermectin treatment rounds, eventual elimination of onchocerciasis transmission in the OCEZ could be threatened by a reservoir of infection that returns annually to the disease-endemic area.

To assess the level of transmission of onchocerciasis to seasonal migrant workers, we conducted two evaluations on disease-endemic plantations in the onchocerciasis OCEZ. The first pilot study, which compared the seroprevalence of onchocerciasis between plantation residents and seasonal migrant workers, was limited in terms of study period because it covered only one month during the coffee harvest season in 2005, but enabled us to pilot the sampling method and establish recruitment procedures. The second survey, which included only migrant workers, was designed to rectify the limitations of the first study to increase the calendar period covered and

the sample size of migrant workers with previous exposure to the disease-endemic area.

MATERIALS AND METHODS

Study area. The OCEZ in Guatemala is comprised of 321 communities or plantations in 11 *municipios* (similar to counties) in 3 departments (Suchitpeque, Sololá, and Chimaltenango). This area is located southeast of Lake Atitlán (Figure 1) and is characterized by moderate altitude (500–1800 meters above sea level) and a mountainous terrain. Studies of OCEZ plantations in the 1970s and 1980s demonstrated community microfilaria prevalence rates as high as 90%.¹² In a recent (2007) evaluation by the Ministry of Public Health and Social Welfare (MSPAS in the Spanish acronym) of established sentinel communities, the maximum community prevalence of microfilaria in the skin was 15% (Catú E, unpublished data).

In the pilot study, which was conducted in January 2005, six plantations were purposively selected among those still reporting migrant workers on-site and asked to participate in the study. In the second survey, which was conducted from October 2006 through January 2007, we used expert opinions (OEPA and MSPAS staff) to develop a list of historically hyperendemic plantations that cultivated coffee. We contacted the plantation owners in September 2006 to estimate the number of migrant workers they were likely to contract for the harvesting season. Only plantations that were likely to contract ≥ 100 workers during that period were included in the second survey.

Study population. In the pilot study, we compared year-round residents of the plantation to seasonal migrant workers, whereas the second survey included only migrant workers. Migrant workers traditionally arrive in organized groups, called *cuadrillas*, from the indigenous highland areas of Guatemala (mostly the departments of Quiché, San Marcos, and Huehuetenango) and receive short-term contracts of 30–60 days: Guatemalan labor laws do not encourage plantations to establish longer contracts. Migrant workers are paid according to the amount of coffee harvested, which can vary from a high of \$8 per day at the peak of the harvest period to a low of \$1 per day, during the early and late phases. Families accompany the migrant workers and wives and older children may be contracted officially by the plantation, but younger children, although they may also work with their parents in the harvest, are not officially contracted.

Sample size and selection. The sample size for the pilot study was purposively selected to be able to complete surveys and blood samples for all participants in a selected plantation in one day. For the second survey, we set the criteria for deciding whether migrant populations pose a threat to the elimination of onchocerciasis in Guatemala equal to a prevalence of antibodies to a recombinant *O. volvulus* antigen (OV-16) $> 1\%$ in repeat migrant workers (i.e., those persons who have worked on a plantation in a disease-endemic area at least once during the period 1–5 years before the survey). Using a one-sided test, finding 0 positive persons in 300 repeat migrant workers gives a 95% probability that the true prevalence rate is $< 1\%$. Based on the pilot study, we estimated 30% would refuse to participate. Therefore, the final sample size was 429.

In both surveys, all plantations were able to provide current payroll lists of resident and migrant workers. In the pilot

study, 15 migrant workers and 15 resident workers more than 18 years of age were randomly selected from the payroll list in each plantation. In the second survey, the number of migrant workers estimated to be contracted for the coffee harvest was collected from each plantation; the sampling fraction was calculated as the target sample size divided by the estimated total number of migrant workers in the selected plantations. This sampling fraction was applied to the number of migrant workers on payroll at each plantation each month to generate a random sample.

Procedures. In the pilot study, all randomly selected individuals ≥ 18 years of age listed on the plantation payroll as either a migrant worker or a regular resident who provided written informed consent were included in the study. All participating workers were administered a brief questionnaire requesting a three-year history of plantation work, knowledge of onchocerciasis, and information on their participation in the mass drug administration (MDA) program. Additionally, all workers were requested to provide a fingerprick blood sample.

In the second survey, a more precise definition of migrant worker was used: persons with contracts for ≤ 60 days and their family members who had not and did not intend to stay on the plantation for more than four months. Those persons who qualified were asked for verbal consent to complete a brief questionnaire requesting a five-year history of contract labor, knowledge of onchocerciasis, and participation in the MDA program. Migrant workers and their family members who had at least one previous contract in the past 1–5 years on a plantation in an onchocerciasis-endemic area were asked for written informed consent to take a fingerprick blood sample. Children less than 18 years of age who satisfied the inclusion criteria were asked for written informed assent.

In both studies, fingerprick blood samples were taken using sterile procedures and 4–5 drops were placed on a labeled square of Whatman (Maidstone, United Kingdom) No. 2 filter paper. Filter paper blood samples were dried and stored in plastic bags in coolers at 4°C until they could be transferred to a refrigerator. Blood samples were analyzed for IgG4 antibodies to a recombinant *O. volvulus* antigen (OV-16) using standard procedures.⁴ Data were analyzed with SAS version 9

(SAS Institute Inc., Cary, NC). Dichotomous variables were compared between migrant and resident workers in the pilot study using the chi-square statistic. One-sided 95% confidence intervals [CIs] for prevalence of antibodies to Ov16 in migrant workers in the pilot study were calculated using the SAS FREQ procedure with the EXACT statement and the BINOMIAL option, and an alpha level of 0.10.

Human subjects. The protocols for the pilot study and the second survey received appropriate review by the institutional review board of the Centers for Disease Control and Prevention (Atlanta, GA) and the ethics committee of the Universidad del Valle de Guatemala (Guatemala City, Guatemala). The MSPAS (Guatemala City, Guatemala) reviewed and approved both protocols. All persons who provided blood samples signed written informed consent forms. Children less than 18 years of age were read and signed an assent form and a parent or guardian signed the consent form.

RESULTS

In the pilot study, we recruited 85 migrant and 93 resident workers at the 6 participating plantations (Table 1). There were slightly more female migrant workers than female residents on the payroll, but the difference was not statistically significant. However, there were significant differences in age (there were proportionally more migrant workers between the ages of 18 and 19 years than resident workers) and home area (migrant workers were more likely to come from the highlands). Migrant workers were considerably less likely to have ever heard of the disease onchocerciasis, or *filaria*, as it is often called in Guatemala, and 10 times less likely to have ever been offered ivermectin during a treatment round. Although the acceptance rate for those who were offered ivermectin was statistically lower in migrant workers, the number of migrant workers who had ever been offered ivermectin was too low to draw conclusions about their participation rate.

During the 2006–2007 coffee harvest season, the weather was cooler and wetter than in previous years and as a consequence, the harvest was delayed and required less labor than anticipated. As a response to the low numbers of migrant workers

TABLE 1

Characteristics of migrant and resident workers participating in the pilot study of onchocerciasis, January 2005, and migrant workers participating in the second survey, October 2006–February 2007, Guatemala*

| Characteristic | Pilot study | | Second survey Migrant workers (n = 226) No. (%) |
|--|-------------------------------------|--------------------------------------|---|
| | Migrant workers (n = 85) No. (%) | Resident workers (n = 93) No. (%) | |
| Female sex | 22 (25.9) | 17 (18.3) | 79 (35.0) |
| Age, years† | | | |
| 0–17 | 0 (0) | 0 (0) | 89 (39.4) |
| 18–19 | 14 (16.7) | 4 (4.4) | 16 (7.1) |
| 20–29 | 19 (22.6) | 28 (30.4) | 50 (22.1) |
| 30–39 | 20 (23.8) | 25 (27.2) | 42 (18.6) |
| ≥ 40 | 31 (36.9) | 35 (38.0) | 29 (12.8) |
| Home area† | | | |
| Potential onchocerciasis-endemic areas | 2 (2.4) | 59 (64.1) | 0 (0) |
| Adjacent to onchocerciasis-endemic areas | 9 (10.6) | 10 (10.9) | 0 (0) |
| Highlands | 70 (82.4) | 19 (20.7) | 226 (100.0) |
| Other | 4 (4.7) | 4 (4.4) | 0 (0) |
| Heard of onchocerciasis† | 19 (10.7) | 89 (50.0) | 7 (3.1) |
| Ever offered ivermectin† | 7 (8.2) | 84 (90.3) | 2 (0.9) |
| Accepted ivermectin when offered† | 5 (83.3) | 83 (98.8) | 2 (100.0) |
| Seropositive for OV-16† | 0 (0) | 27 (30.3) | 1 (0.8) |

* Percentages are the proportion of available data.

† Chi-square test comparing migrant and resident workers in the pilot study, $P < 0.05$.

encountered on the selected plantations, we attempted to recruit all migrant workers present rather than take a random sample. We interviewed 226 migrant workers and accompanying family members from 6 plantations. Because the second survey included accompanying family members less than 18 years of age, the migrant workers in the second survey were younger than those in the pilot study (Table 1). They were also less likely (3.1%) to have ever heard of onchocerciasis or *filaria*, and even less likely (0.9%) to have had been offered ivermectin during a treatment round than the migrant workers or the residents from the pilot study. These results did not change significantly when only adults were analyzed.

In the pilot study, 51 (60.0%) of the migrant workers had previously worked on a plantation in the 1–3 years prior to the study. In the second survey, the proportion was higher (82.7%), possibly because the second survey began earlier in the coffee harvest season. However, the proportions of persons who had worked previously on a plantation in a disease-endemic area were similar between the two surveys (51.8% in the pilot survey and 55.8% in the second survey), although the work history for the second survey covered a longer period. In the pilot study, migrant workers reported 174 contracts over the past 3 years, an average of 0.7 contracts per person per year. During the 2006–2007 coffee harvest season, 432 contracts were reported for the previous 5 years, an average of 0.4 contracts per person per year. Most of these contracts (94.2% in 2005 and 73.0% in 2006–2007) were in onchocerciasis-endemic areas. More than 95% of the contracts in onchocerciasis-endemic areas in both surveys began during October–January. The average contract length in disease-endemic areas was 36–40 days. In the pilot study, 92.4% of the contracts reported in disease-endemic areas had concluded by the end of January. Because of a longer survey period, this percentage was lower (74.9%) for the contracts in disease-endemic areas reported in the second survey, with almost 25% of the contracts concluding in February.

In the pilot study, there were no migrant workers of the 85 sampled (one-sided 95% CI = 0–3.8%) found to have IgG4 antibodies to the recombinant antigen OV-16 in comparison to 30.3% (27 of 89, 95% CI = 21.0–41.0%) of the resident workers ($P < 0.0001$). However, only 44 of the migrant workers had previously worked on a plantation and, therefore, had enough time to potentially generate antibodies to an infection with *O. volvulus*. In the second survey, 1 of the 126 migrant workers who had had enough time to generate antibodies was positive for antibody to OV-16 (0.8%, 95% CI = 0–4.4%), which indicated active infection or previous exposure to *O. volvulus*. That worker was a man 45 years of age who reported previously working in OCEZ disease-endemic zone plantations every year for at least the past five harvest seasons. No further evaluation of that positive worker took place.

In summary, in the two surveys, we tested 170 migrant workers who had the potential for exposure to onchocerciasis infection (i.e., had previously worked on a plantation in a disease-endemic area) and had sufficient time to mount an immune response. One person was positive, resulting in a combined prevalence estimate of 0.6% (95% CI = 0–3.2%).

DISCUSSION

Although we confirmed that migrant workers are present in OCEZ disease-endemic coffee plantations during the known period of peak blackfly biting (i.e., January–March¹⁰),

we did not find strong evidence that these workers are being frequently exposed to onchocerciasis. Most of the migrant workers included in these two surveys were residents of the indigenous highlands of Guatemala where onchocerciasis is not transmitted. Therefore, their only exposure could come from temporary periods working in disease-endemic areas, such as participating in the coffee harvest. However, the short duration of most contracts for migrant workers and the decreasing force of infection of *O. volvulus* in the Guatemalan OCEZ caused by semiannual treatment of the resident population with ivermectin have probably contributed to a significant reduction in the level of infection in migrant workers compared with that in earlier studies (Lujan R, Richards FO, unpublished data), in which skin snip infection rates among the migrant workers reached 15%.

One person was found to be positive for antibody to OV-16 in the study, which could indicate either active infection or past exposure to *Onchocerca* antigens. That person was not examined further to determine if he had microfilaria in his skin and was therefore capable of infecting blackflies. We believe that the migrant population in the OCEZ as a whole has a low prevalence of infection, and those infected probably have low density microfilarial counts in skin and thus are likely to be minimally infective to the relatively inefficient *S. ochraceum* blackflies that are the known vector in the area. As long as transmission in the OCEZ continues to decrease, it is unlikely that migrant workers will pose a threat to the eventual success of the elimination effort, even when excluded from the ivermectin treatment program. Migrant workers likely serve as sentinel canaries for the OCEZ and show the success of the treatment program in halting transmission.

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REFERENCES

1. Onchocerciasis (river blindness), 2005. Report from the Fourteenth InterAmerican Conference on Onchocerciasis, Atlanta, Georgia, United States. *Wkly Epidemiol Rec* 80: 257–260.
2. Cruz-Ortiz N, Rizzo N, Gonzalez R, Sauerbrey M, Zea-Flores G, Dominguez A, Oliva O, Catu E, Castro J, Lindblade KA, 2008. *Evaluación Entomológica, Serológica y Oftalmológica para Demostrar la Eliminación de la Transmisión de Onchocerca volvulus en el Foco de Huehuetenango*. Guatemala City, Guatemala: CDC-CAP.
3. Gonzalez RJ, Cruz-Ortiz N, Rizzo N, Richards J, Zea-Flores G, Dominguez A, Sauerbrey M, Catu E, Oliva O, Richards FO, Lindblade KA, 2009. Successful interruption of transmission of *Onchocerca volvulus* in the Escuintla-Guatemala focus, Guatemala. *PLoS Negl Trop Dis* 3: e404.
4. Lindblade KA, Arana B, Zea-Flores G, Rizzo N, Porter CH, Dominguez A, Cruz-Ortiz N, Unnasch TR, Punkosdy GA, Richards J, Sauerbrey M, Castro J, Catu E, Oliva O, Richards FO Jr, 2007. Elimination of *Onchocerca volvulus* transmission in the Santa Rosa focus of Guatemala. *Am J Trop Med Hyg* 77: 334–341.
5. Yamagata Y, Suzuki T, Garcia Manzo GA, 1986. Geographical distribution of the prevalence of nodules of *Onchocerca volvulus* in Guatemala over the last four decades. *Trop Med Parasitol* 37: 28–34.
6. Onchocerciasis (river blindness), 2006. Report from the Fifteenth InterAmerican Conference on Onchocerciasis, Caracas, Venezuela. *Wkly Epidemiol Rec* 81: 293–296.
7. Plant R, 1996. *Hacia la Reconstrucción de la Sociedad Civil: Las Organizaciones de Trabajadores Rurales en Guatemala*. CUESTIONES DE DESARROLLO. Geneva: International Labour Organization.
8. Baumeister E, 1993. Guatemala: *Los Trabajadores Temporales de la Agricultura*. Gómez S, Klein E, eds. Los Pobres del Campo: el Trabajador Eventual. Santiago: FLACSO/PREALC.
9. Rodriguez-Perez MA, Segura-Cabrera A, Lizarazo-Ortega C, Basanez MG, Davies JB, 2007. Contribution of migrant coffee labourers infected with *Onchocerca volvulus* to the maintenance of the microfilarial reservoir in an ivermectin-treated area of Mexico. *Filaria J* 6: 16.
10. Porter CH, Collins RC, 1988. Seasonality of adult black flies and *Onchocerca volvulus* transmission in Guatemala. *Am J Trop Med Hyg* 38: 153–167.
11. Porter CH, Collins RC, 1988. Biting activity of black flies in Guatemala: parity rates and differences between localities and habitats. *Am J Trop Med Hyg* 38: 142–152.
12. Brandling-Bennett AD, Anderson J, Fuglsang H, Collins R, 1981. Onchocerciasis in Guatemala. Epidemiology in fincas with various intensities of infection. *Am J Trop Med Hyg* 30: 970–981.