

Evidence for Suppression of *Onchocerca volvulus* Transmission in the Oaxaca Focus in Mexico

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Abstract. Entomologic and serologic surveys were performed in four sentinel communities in the Oaxaca focus in southern Mexico to assess the level of transmission and exposure incidence to *Onchocerca volvulus*. All communities have been receiving ivermectin mass treatment twice per year since 1997. In one community, parasite DNA was detected by polymerase chain reaction–enzyme-linked immunosorbent assay in 2004 in one pool of 50 vector heads of 170 such pools (8,500 flies) examined, which indicated an estimated transmission potential of 6.7 third-stage larvae/person/year. No evidence for transmission was found in the three other communities in 13,650 flies examined. All persons in a cohort consisting of 117 children in the four communities remained serologically negative for antibodies recognizing a cocktail of recombinant antigens over a four-year period from 2001 to 2004, which indicated an exposure incidence of 0%. Taken together, these data suggest that transmission has been suppressed in the four communities.

INTRODUCTION

The overall goal of the Onchocerciasis Elimination Program in the Americas (OEPA) is to eliminate onchocerciasis as a public health problem, culminating in the elimination of the infection in the six disease-endemic countries of Latin America. To assist in this process, the World Health Organization (WHO) has developed a series of guidelines to certify that an area is free of onchocerciasis. Those guidelines focused on entomology are based on demonstrating transmission suppression in *Simulium* spp. Two different measures of transmission suppression were recommended in the WHO guidelines. In areas where pre-treatment data were available, suppression of infectivity was defined as a 99% reduction in the annual transmission potentials (ATPs) from pre-treatment rates. In the foci where pre-treatment data were not available, transmission suppression was defined as an “absence or near absence” of third-stage infection in the vector population. The WHO did not specify quantitative metrics to the term “near absence”. In practical terms, scientists advising OEPA recently recommended that a prevalence of less than 1 infective fly per 2,000 flies would meet the criterion of “absence or near absence”. This criterion was derived from the one developed by the African Program for Onchocerciasis control (APOC), which set a limit of a prevalence of infective flies at maximum of less than 1 infective fly in 1,000 parous flies.¹ In Latin America, parity rates are approximately 50%, which means that a prevalence of 1 infective fly in 2,000 is functionally equivalent to the APOC standard of 1 infective fly in 1,000 parous flies.

Onchocerca volvulus is endemic in three foci in Mexico: Southern Chiapas, Northern Chiapas, and Oaxaca. The Oaxaca disease-endemic focus does not have any obvious epidemiologic link with the other foci in Chiapas and Guatemala. Its origin may be due in part to human movements from Oaxaca to and from the disease-endemic areas of Chiapas or

Guatemala (i.e., Esquipulas) to perform religious pilgrimages.² In the Oaxaca focus, the sentinel communities are relatively isolated (between 115 and 140 km apart, with the exception of Lalopa and La Chichina, which are 20 km apart). Unlike the situation in the other major focus of Mexico (Southern Chiapas), migrant coffee workers are not present in Oaxaca and thus play no role in the epidemiology of the disease in this focus.

Mass ivermectin distribution in the Oaxaca focus was initiated in 1994. From 1997 to the present time, the strategy has been to provide mass treatments twice a year to every eligible resident residing in the at-risk communities. Ivermectin coverage of the eligible population has remained greater than 85% every year from 2001 through 2006, with a mean coverage of 91.6% (range = 86.5–94%).

In the first large scale entomologic study conducted in Oaxaca in 2001, the data suggested that transmission was still occurring.^{3,4} The data in this report are from an entomologic and serologic follow-up study conducted in 2004, as part of a series of periodic prospective in-depth surveys evaluating the impact of mass distribution of ivermectin. As judged against current OEPA standard for an “absence or near absence” of transmission, the data suggest that *O. volvulus* transmission in the Oaxaca was suppressed by 2004, and may have reached a level that is likely to be insufficient to maintain the parasite population.

MATERIALS AND METHODS

Study area. Historic parasitologic baseline data were used to classify the communities in Oaxaca with regard to their pre-treatment disease-endemic status. Eleven communities (11%) were classified as historically mesoendemic (moderate transmission and an *O. volvulus* microfilarial prevalence as detected by skin biopsy of greater than 20% but less than 60% in a sample of 30 adults who had resided in the community for at least five years). A total of 87 (89%) communities were classified as historically hypoendemic for onchocerciasis (scanty transmission and a proportion of microfilaria-positive skin biopsy specimens \leq 20%).⁵ No historically hyperendemic communities (with high transmission and *O. volvulus*

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prevalence in skin biopsy specimens > 60%) were found in the Oaxaca focus.

The present entomologic study was conducted during the 2004 transmission season, and the serologic study was carried out on samples collected in both 2001 and 2004. The four mesoendemic sentinel communities included in this study were La Esperanza (17°37'40"N, 96°22'10"W, elevation = 1,600 meters), Santiago Lalopa (17°25'4"N, 96°14'54" W, elevation = 1,200 meters), Santiago Teotlaxco (17°26'45' N, 96°19'14' W, elevation = 1,225 meters), and Santa María La Chichina (17°26'23"N, 96°17'8"W, elevation = 1,360 meters). The population in the Oaxaca focus is predominantly indigenous, consisting mainly of Zapoteca and Chinanteco ethnic groups. The most important economic activity in these communities is coffee cultivation.

Black fly collection and polymerase chain reaction (PCR). Black flies (6,868 *Simulium metallicum*, 6,812 *S. calidum*, and 21,250 *S. ochraceum* females) were collected by using standardized procedures^{3,6,7} during the peak *O. volvulus* transmission season lasting from January to April 2004. In 2004, mass ivermectin distribution was conducted just before the peak transmission began. Black fly collections were conducted simultaneously in two sites in each community (one inside the community and another within a nearby coffee plantation). The collections were carried out during the first 50 minutes of each hour, beginning at 11:00 AM and ending at 4:50 PM.⁸ Collectors received ivermectin one week before beginning the collection process. This procedure was reviewed and approved by the Ethics and Biosecurity Committee of the National Institute of Public Health of the Health Secretariat of Mexico (Cuernavaca, Mexico).

Black flies were collected before they began feeding. The landing rate measured from the collections was taken as an estimate of the biting rate. It is likely that the landing rate overestimated the biting rate because a proportion of the landing flies in a natural setting do not successfully obtain a blood meal. The proportion of flies successfully obtaining a blood meal cannot be easily estimated from the landing rate because this is not a constant and appears to be a factor of vector density (with the probability of success in obtaining a meal declining at high vector densities).⁹ Thus, the transmission potential calculations provided below are likely to be overestimated by a factor proportional to the number of flies that land but do not bite.

Field-collected black flies were preserved in isopropanol at room temperature and returned to the laboratory. *Simulium ochraceum* s.l. flies were separated by morphologic examination, and the few flies that were found to have taken a fresh blood meal were discarded. *Simulium ochraceum* s.l. females were divided into aliquots of 50 specimens each for further processing. The flies were placed in liquid nitrogen and subjected to vigorous agitation to separate the heads and bodies (i.e., thoraces and abdomens). The heads were purified from the bodies by passage through a 25-mesh sieve and each fraction (heads and bodies) was processed separately.

The separated head and body pools were tested for *O. volvulus* parasites by using a PCR assay specific for *O. volvulus*. Details of protocols for genomic DNA purification, primer sequences, PCR conditions, and detection of PCR products by ELISA have been previously presented.^{3,4} DNA extractions were carried out in sets of 20 samples each, with each set containing 18 fly pools and 2 sham extractions that

served as contamination controls for the DNA extraction process. All O-150 PCRs were carried out in sets of 84 samples, in rows B–H of a PCR microtiter plate. Row A was reserved for 10 PCR-negative controls and 2 positive controls. One positive control contained the minimal amount of positive control DNA consistently detected by the PCR amplification conditions, as determined by an initial titration study. This control was carried out to ensure that all of the reactions were operating at peak efficiency. The second positive control contained the same minimal amount of positive control DNA mixed with 2.5 μ L of a DNA preparation from a pool that tested negative in a prior set of reactions. This control ensured that no inhibitors were present in the fly DNA preparations.

The infected proportion in the vector population was calculated from the proportion of body (thorax plus abdomen) pools positive in the PCR assay, and the infective proportion in the vector population was estimated from the proportion of head pools positive in the PCR assay. These proportions were expressed per 2,000 flies examined. Because the prevalence of infection in bodies (which contain the non-infectious L1 and L2 stages) is consistently higher than the prevalence of parasite in the head pools (which contain only the infective third-stage larvae),^{3–10} the body pools from the flies collected in the individual communities were screened until a confirmed body positive pool was obtained. A confirmed body pool from the flies collected from any site in a given community (thus taking into account the spatial heterogeneities associated with the prevalence of infected flies and parity⁸) was taken as evidence for potential ongoing transmission, and screening of the body pools from that location was discontinued. All head pools collected from that community from all sites were then screened, and PoolScreen version 2.0¹¹ was used to estimate the prevalence of infective flies in the community and the associated 95% confidence intervals (CIs). If all body pools from a given community were screened and none were positive, it was concluded that there was no evidence for ongoing transmission in the community. The prevalence of infected (and infective) flies was therefore taken to be zero, and the upper bound of the 95% CI for the prevalence of infected (and infective) flies was calculated using the PoolScreen algorithm. Head pools were generally not analyzed from villages in which no evidence for infection in the vector bodies was found,^{3,4} with the exception of La Chichina, where a randomly selected sample of head pools was also tested.

Serologic assays. A total of 1,133 individuals of both sexes and all ages participated in the 2001 survey, a population that included 286 children \leq 10 years of age. A cohort of 117 of these children (including 61 untreated children less than 5 years of age in 2001 and 56 treated children who were 5–6 years of age in 2001) were retested in 2004 using the same methods. The 61 untreated children in the 2001 cohort had received between one and five treatments by 2004. The 56 children in the 2001 cohort who had been treated prior the start of the study had received between two and six treatments by 2001, and had received 7–11 treatments by 2004. Human blood sample collection, use of specific recombinant antigens (OvMBP16, OvMBP11, and OvMBP10),^{12–14} enzyme-linked immunosorbent assay (ELISA), and data interpretation were carried out as previously described.¹⁵ Fusion of parasite antigens to MBP greatly facilitated isolation of recombinant peptides. However, antibodies in serum samples

directed to the MBP might have confounded the assay. To control for this, anti-MBP assays were carried out in parallel and subtracted from the recombinant antigen values. The mean of eight optical density values of positive control sera from a pool of high-responding Mexican onchocerciasis sera was used to correct all ELISA values for each plate. We previously described the utility of this ELISA for detecting exposure to infection in a sentinel cohort from Las Golondrinas of the Southern Chiapas focus under treatment with ivermectin.¹⁵ The ELISA is a sensitive tool for detection of children pre-patently infected or who have been exposed to infective larvae because the Ov7 and Ov11 antigens are present in third-stage larvae.^{16,17}

Antibody prevalence was defined as the proportion of antibody-positive results among persons who had optical values equal to or greater than the cut off value. The cut-off for classifying a sample as positive was set at the mean of the 30 negative controls plus 7 standard deviations.¹⁸ Test specificity at this cut-off value was 100% and sensitivity was 96% when compared with the skin snip test,¹⁹ and 97% when compared with a seropositive reference collection.²⁰

Data analysis. Because *S. ochraceum* s.l. females were not collected throughout the year, it was not possible to precisely calculate the ATP. However, in Mexico, the level of transmission during the peak of transmission season was very low (because of the effect of 11 years [22 rounds] of treatment with ivermectin). The value of transmission potential outside of the peak transmission period (when the fly population is low) is therefore probably zero or near zero. Therefore, the seasonal transmission potential (transmission occurring during the peak transmission season of February through April) probably represents a fairly accurate estimate of the ATP. For the purposes of seasonal transmission calculation, we assumed that after multiple rounds of ivermectin treatment, the number of third-stage larvae present in each infective fly would be close to 1.²¹

The prevalence of infection in the body pools and head pools and associated 95% CIs were determined using the algorithms available in the computer program PoolScreen version 2.0. The proportion of infective flies (prevalence of infection in head pools) was used to calculate estimates of seasonal transmission potential. The seasonal transmission potential was calculated as the product of the seasonal biting rate, the proportion of flies carrying third-stage larvae in the transmission season (from January through April during 2004), and the average number of third-stage larvae in each infective fly, which was assumed to be 1.0 as discussed above. The seasonal biting rate was calculated as the product of the geometric mean of Williams²² of the number of flies collected per person/day, and the total number of days in the transmis-

sion season (121 days for Santiago Teotlaxco, La Esperanza, and Santa María La Chichina and 91 days for Santiago Lalopa).

It is known that when the number of zeros is large (i.e., many 50-minute sampling units resulted in no flies collected), the addition of 1 before taking the log of the count may bias the geometric mean. Given that the number of zeros in the sampling units was small (i.e., only a few 50-minute sampling units without flies) it was not necessary to use a constant such as the *k* parameter of the negative binomial distribution of the number of flies per hour to adjust the sample.

Each raw 50-minute fly count was first adjusted to a 60-minute time period by dividing each fly count by 0.83 (i.e., 5/6). A constant value of 1 was added to each adjusted value, and the geometric mean number of flies per person per hour was calculated. This mean hourly value was multiplied by 10 (the mean number of daylight hours during the transmission season at this latitude)^{23,24} to obtain an estimate of the daily biting rate. Daily biting rates were then multiplied by the length of the transmission season (in days) to obtain a seasonal biting rate.

We verified that the log-transformation of the fly counts had normalized the data (using Smirnov Kolmogorov tests in SPSS software; SPSS Inc., Chicago, IL). Thus, the CIs for the hourly counts were calculated on the log-transformed data. The antilog of these intervals were calculated and the constant was subtracted from each the upper and the lower limit (95% CIs became asymmetric according to Kirkwood and Sterne).²⁵ The geometric mean and CIs (they are in the same units as the means)¹⁰ were then multiplied by 10 to obtain a CI for the daily biting rate. The confidence intervals for the ATP values were the product of the biting rates and the confidence intervals of the proportion of infective flies.^{26,27} The proportion of individuals with antibodies was calculated as the number of seropositive individuals divided by the total number examined and expressed as a percentage.

RESULTS

The results of the PCR screens were used to calculate a prevalence of infected and infective flies in the vector populations, together with the associated 95% CIs. The prevalence of infective flies was then combined with estimates of the biting rate (calculated from the fly collection data as described in the Materials and Methods) to calculate an estimated seasonal transmission potential. The results of these calculations are summarized in Table 1. In Santiago Teotlaxco (*n* = 49 pools or 2,450 flies), and Santa María La Chichina (*n* = 59 pools or 2,950 flies), all body pools were

TABLE 1

Prevalence of infected and infective flies (expressed as rate per 2,000 flies examined) and seasonal transmission potential (third-stage larvae per person per year) estimated during 2004 in four sentinel communities in the focus of Oaxaca, Mexico*

Community	Seasonal biting rate	Prevalence of infected flies	Prevalence of infective flies	Seasonal transmission potential
Santiago Lalopa	51,114 (42,658–61,204)	0.46 (UL = 1.0)	0 (UL = 0.46)	0 (UL = 14.1)
Santiago Teotlaxco	23,929 (20,047–31,417)	0 (UL = 1.6)	0	0
La Esperanza	37,324 (30,861–45,079)	0.35 (UL = 0.92)	0.35 (0.0004–0.92)	6.7 (0–17.2)
Santa María La Chichina	13,824 (12,377–18,326)	0 (UL = 1.3)	0	0

* Seasonal biting rate = geometric mean of the number of bites per person/day multiplied by the total number of days in the months of January through April (*n* = 121 days). UL = 95% upper limit. Values represent point estimates and values in parentheses represent 95% confidence intervals surrounding point estimates.

negative for *O. volvulus*, which suggested a low or non-existent rate of parasite-vector contact, and a corresponding lack of transmission. For this reason, most of the head pools from these communities were not examined further. However, a randomly selected sample of 28 head pools of La Chichina was screened to confirm this supposition. As expected, all of these pools were also negative in the PCR assay.

In Santiago Lalopa, one pool of bodies (of the first 152 pools or 7,600 flies tested) was PCR positive, which indicated parasite-vector contact in this community. However, no positive head pools were found (165 pools screened or 8,250 flies), which resulted in a prevalence of infective flies of 0 (95% upper limit [UL] = 0.46/2,000 flies).

In La Esperanza, as in Santiago Lalopa, a body pool was positive in the initial screening, which suggested parasite-vector contact. Subsequent screening of 169 head pools (8,450 flies) resulted in a single confirmed positive pool, which resulted in a calculated prevalence of infective flies of 0.35/2,000 flies (95% UL = 0.92/2,000 flies), and a calculated transmission potential of 6.7 third-stage larvae per person per year.

The number of flies collected in each community ranged from 2,450 to 8,500, which was less than 10,000. Thus, when separated by community, the number of vectors collected was not sufficient to comply with the WHO guideline of having at least 10,000 flies tested from each community. However, in all cases, the sample was sufficient to exclude 1/2000 in the UL of the 95% CI. Taken together, the 367 head pools (18,350 flies) were screened from the four sentinel communities of the Oaxaca focus, which resulted in an overall prevalence of infective flies of 0.27/2,000 (95% CI = 0.01–0.6/2,000) flies, which met the OEPA current criterion for “absence or near absence” of transmission. None of the 117 children < 10 years of age seroconverted in the four sentinel communities of the Oaxaca focus (Table 2), which resulted in an estimated exposure incidence of 0%.

DISCUSSION

The data in our study suggest that transmission of *O. volvulus* has been suppressed in the Oaxaca focus of Mexico. During the four-year period encompassed by this study (2001–2004), none of the initially seronegative children seroconverted as assayed by ELISA had seroconverted. The absence of contact with the parasite in this cohort of children ≤10 years of age (i.e., subjects born after the implementation of the ivermectin distribution program) indicates that none had been exposed to *O. volvulus*, which suggested that the level of exposure to the parasite is now quite low in this area. This finding is consistent with those of a previous parasitologic study, which demonstrated that consecutive treatment with ivermectin has resulted in a dramatic decrease of the prevalence of skin microfilariae and nodules in Oaxaca, be-

ginning as early as 1998.²¹ Despite these promising findings in the human population in 1998, the first large-scale entomologic study of transmission in this area, which was conducted in 2001 after six years of mass administration of ivermectin, demonstrated that three of four sentinel communities still had evidence for ongoing transmission.⁴ In 2001, the prevalence of infective flies was 1.6/2,000 (UL = 3.5) in Santiago Teotlaxco, 1.1/2,000 (UL = 2.6) in Santiago Lalopa, and 0.4/2,000 (UL = 1.2) in La Esperanza. The corresponding seasonal transmission potentials were 2.7, 2.3, and 0.8 third-stage larvae per person, respectively. No evidence for transmission was found in Santa María La Chichina in 2001.⁴ Judging from the data reported above, transmission had apparently decreased or ceased in all of these communities by 2004 because infective flies were only detected in La Esperanza in this year.

When a parasite population is at endemic equilibrium (i.e., before introduction of vector- or ivermectin-based control), the effective reproductive ratio is equal to 1 (regardless of the value of the basic reproduction ratio, which would have been greater than 1 for introduction and persistence of the infection). Once control starts, the parasite population is moved away from this endemic equilibrium and density-dependent constraints are relaxed. This relaxation may make the effective reproductive ratio increase to greater than one initially, but the ratio will decrease in the face of an effective control regimen, eventually becoming less than one. If maintained at this level, the parasite population will eventually become extinct in the area under control. Therefore, what an elimination program such as OEPA wants to achieve is to reduce and maintain the effective reproduction ratio below 1. The reproduction ratio will be determined by the force of infection, which may be measured by the ATP. Unfortunately, the exact relationship between the ATP and the effective reproduction ratio is not known, and the threshold ATP necessary to maintain the reproductive ratio below one is controversial. However, previous deterministic modeling studies using data derived from west Africa and Latin America have suggested that this threshold probably lies somewhere between 5 and 20 third-stage larvae per person per year. All seasonal ATPs in the sentinel villages were within this range in 2004, which suggested that if conditions remain unchanged, the parasite population is likely to be on the path to elimination. The transmission potential in La Esperanza was 6.7 third-stage larvae per year (under endemic unstable equilibrium). In the other communities, the estimated transmission potential was zero. Taking the product of the upper bounds for the 95% CIs for the prevalence of infective flies and the biting rate, the maximal possible transmission potential for La Esperanza is estimated to be 17.2 larvae per person per year, within the 5–20 estimates of previous studies.

It must be emphasized that even when transmission has been suppressed, treatment cannot be discontinued immediately. Transmission may be suppressed by treatment, but it may rebound if the pressure on the population is removed. Thus, it is necessary to maintain control activities until the level of transmission is so low that any rebound in transmission that occurs when control activities end will not reach a level that will cause the reproduction ratio to increase above the breakpoint. Unfortunately, it is difficult to predict to what extent transmission will increase once control activities are ended. This is because the degree of the increase will depend in part upon the competence of the vector, which may in turn

TABLE 2

Number of persons becoming serologically positive (% of incidence of exposure to onchocerciasis) from 2001 (children ≤ 7 years of age) through 2004 (children ≤ 10 years of age) in four sentinel communities in the focus of Oaxaca, Mexico

Santiago Lalopa	Santiago Teotlaxco	La Esperanza	Santa María La Chichina	Total
0/47 (0%)	0/28 (0%)	0/20 (0%)	0/22 (0%)	0/117 (0%)

depend upon microfilarial skin densities, with vectors that lack a cibarial armature, such as *S. ochraceum* being quite competent at low densities.^{10,28,29} Unstable equilibria will also exist because the parasite has separate sexes (e.g., mating probabilities), which again make it difficult to predict with certainty when treatment may be safely stopped. These issues can be explored with relevant stochastic models, which will have to be individually tailored to the ecology of each focus in the Americas.

The serologic data presented above also suggest that transmission may have been brought to undetectable levels throughout much of Oaxaca. However, serologic data do not provide precise estimations of infection rates because some persons exposed to the parasite may develop specific antibodies but never get infected. Thus, detection of circulating antibodies to *O. volvulus* in an exposed population cannot be used to define the presence and level of infection, but these data do have potential utility as an epidemiologic tool to provide an estimate of exposure. In this regard, sampling sentinel cohorts as done here, instead of carrying out mass sampling, could save considerable time, cost, and effort.³⁰

The plan for certification of the elimination of onchocerciasis developed by OEPA is made up of four phases.²³ Phase I includes ivermectin treatment for 2–4 years, which results in suppression of transmission. In phase II, suppression is maintained through treatment of the mean reproductive lifespan of the adult female (approximately 13–14 years). After this, (in phase III), it is expected that the adult parasite population would die by senescence and maintaining the suppression of transmission will no longer be dependent on ivermectin distribution. Thus, in phase III, ivermectin distribution will cease and intensive surveillance will be conducted to document that transmission will not re-develop. Finally, in phase IV, the elimination of the *O. volvulus* infection will be certified. The entomologic data presented here show that no evidence for transmission was detected in three sentinel communities of the Oaxaca focus, while that of La Esperanza was apparently below the level currently accepted as the benchmark for transmission suppression by OEPA. Transmission suppression was supported by the serologic data, which showed no evidence for new infections in children in the sentinel communities. More studies are needed in extra-sentinel communities in this focus (i.e., in San Miguel Tiltepec where the first cases of onchocerciasis were discovered in 1924)³¹ before we may conclude that transmission of onchocerciasis in Oaxaca has been suppressed throughout the state. Studies of these extra-sentinel communities are currently underway.

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