

LARGE-SCALE ENTOMOLOGIC ASSESSMENT OF *ONCHOCERCA VOLVULUS* TRANSMISSION BY POOLSCREEN PCR IN MEXICO

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Abstract. To study the impact of mass Mectizan treatment on *Onchocerca volvulus* transmission in Mexico, entomological surveys were carried out in the endemic foci of Oaxaca, Southern Chiapas, and Northern Chiapas. Collected flies were screened by polymerase chain reaction (PCR) for *O. volvulus* parasites. The prevalence of infected and infective flies was estimated using the PoolScreen algorithm and with a novel probability-based method. *O. volvulus* infective larvae were not detected in flies from 6/13 communities. In 7/13 communities, infective flies were detected, with prevalences ranging from 1.6/10,000 to 29.0/10,000 and seasonal transmission potentials ranging from 0.4 to 3.3. Infected and infective flies were found in a community in Northern Chiapas, suggesting that, according to World Health Organization criteria, autochthonous transmission exists in this focus. These data suggest that *O. volvulus* transmission in Mexico has been suppressed or brought to a level that may be insufficient to sustain the parasite population.

INTRODUCTION

Onchocerciasis, which is caused by infection with the filarial parasite *Onchocerca volvulus*, remains a significant health problem in Africa and Latin America. The elimination of onchocerciasis as a serious health problem is the overall goal of two major international programs: the African Program for Onchocerciasis Control (APOC) and the Onchocerciasis Elimination Program of the Americas (OEPA). The strategy used by both programs relies on mass distribution of Mectizan (Merck & Co., Decatur, GA), with APOC using an annual distribution strategy and OEPA a semi-annual distribution approach.¹ In addition to eliminating ocular and skin disease resulting from *O. volvulus* infection, the goal of OEPA is to reduce transmission below the level that is necessary to maintain the parasite population (i.e., to reduce transmission below that required to maintain the reproductive rate at 1.0 or above). Unfortunately, the level of transmission necessary to reduce the minimum reproductive rate below 1.0 has not been accurately determined. In the absence of this information, the World Health Organization (WHO) has developed a series of criteria to certify that an area is free of onchocerciasis.^{2,3} Those criteria focused on entomology are based on showing the “absence or near absence” of L3 infection rates (transmission suppression) in *Simulium* black flies for a period of 12 years.^{2,3}

Two different types of measures of transmission suppression are defined in the WHO criteria: in areas where data exist on the level of transmission before the beginning of Mectizan distribution, transmission suppression is defined as a 99% reduction of transmission potential in relation to pre-control levels,³ and in areas where no pre-control data exist, the WHO criteria have specified an “absence or near absence” of infection in the vector population. In practical terms, this criterion has been interpreted to mean a prevalence of infective flies of less than 1/10,000. Infective black flies are defined as vectors carrying infective larvae stage larvae (L3).

Mexico has approximately 172,000 people at risk for *O. volvulus* infection and roughly 25,000 infected individuals

with documented infections.^{4,5} *O. volvulus* is endemic to three foci in Mexico: Southern Chiapas, Northern Chiapas, and Oaxaca. The most extensive endemic area is located in Southern Chiapas, contiguous to Huehuetenango, Guatemala. In this region, onchocerciasis is associated with coffee cultivation, and the period of maximum transmission coincides with the early dry season, which is in turn is associated with the presence of older age-structured populations of the main vector, *Simulium ochraceum* s.l.⁶ It has been suggested that the onchocerciasis in the Southern focus of Chiapas was introduced as a result of the migration of plantation workers from Guatemala after the expansion of coffee cultivation and that the Northern Chiapas focus similarly resulted from the annual seasonal in-migration coffee workers from Southern Chiapas.^{7,8} It has been further hypothesized that onchocerciasis infections in Northern Chiapas are solely a result of this seasonal in-migration and that no independent transmission of the parasite occurs in this focus.^{7,8} However, there have been no reports of large-scale entomological studies to test if independent transmission of *O. volvulus* is occurring in the Northern Chiapas focus.

Recently, we reported the results of a study that used a polymerase chain reaction (PCR)-based method to monitor transmission of *O. volvulus* in seven sentinel communities located in the three foci in Mexico.⁹ In that study, we found that transmission had been reduced significantly. In the Oaxaca and Northern Chiapas foci, transmission was very low or undetectable in the five communities examined. However, in the previously published study, 30,900 vector flies collected from the seven sentinel communities were examined, and flies collected from six additional communities were not examined at all. In this study, we report the results of an expanded study using the PCR-based method to evaluate transmission in a total of 87,500 flies from all 10 sentinel communities and 3 axillary communities located in the three endemic foci in Mexico. We also present a new method for more efficiently screening flies in areas subject to control and provide a new analytical approach for estimating the prevalence of infected flies in a community, without having to test all of the flies collected from the community in question.

MATERIALS AND METHODS

Selection of collection sites and fly collection. Ten of the communities selected for the study were those designated as

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the sentinel communities by the National Onchocerciasis Program of Mexico. These included four communities in the Oaxaca focus and six in the Southern Chiapas focus. The mean prevalence of skin microfilaridermia before the start of control in the Oaxaca communities was 7%, whereas in the communities in Chiapas, it was 16%. Apart from these designated sentinel communities, an additional community (Las Golondrinas) in Southern Chiapas was selected for inclusion into the study. Las Golondrinas was selected because it represents a formerly hyperendemic community from which pre-control epidemiologic data were available. Pre-selected sentinel communities were not in place in the Northern Chiapas focus, because autochthonous transmission was thought to not occur in this focus. Thus, two communities (Altagracia and El Ambar) were selected for inclusion, based on the advice of the coordinator and brigade commander of the local onchocerciasis control program. The population affected in Oaxaca and Northern focus of Chiapas is indigenous consisting of a mixture of diverse ethnic groups, whereas the population of Southern Chiapas is of mixed heritage and includes indigenous descendants of Mames. The most important economic activity in these communities is coffee cultivation.

The onchocerciasis program in Mexico began treatment with Mectizan in 1989, initially providing treatment only to patients registered by the program. From 1991 to 1994, treatment with Mectizan was offered, mainly to hyperendemic communities, under a scheme of annual distribution. From 1995 to the present, the strategy has been to provide mass semi-annual treatments of Mectizan to every qualified resident in the at-risk communities. In 2001, when the black flies for this study were collected, 17 rounds of Mectizan treatment had been provided to the study communities.¹⁰ The average Mectizan coverage in 2001 in the study communities (in the eligible population) was approximately 88%.¹⁰

Black flies were collected following standardized procedures,^{9,11,12} during the peak *O. volvulus* transmission season lasting from February to May 2001. Collections were carried out during the first 50 minutes of each hour, beginning at 11:00 AM and ending at 4:50 PM. Collectors received Mectizan 1 week before beginning the collection process. This procedure was 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. This landing rate was taken as an estimate of the biting rate. It is possible that the landing rate over-estimated the biting rate, because some proportion of the landing flies might not actually successfully bite.¹³ Thus, the transmission potential calculations provided below may be over-estimated 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. There, the *S. ochraceum* s.l. were separated by morphologic examination, and the few flies that were found to have taken a fresh blood meal discarded. *S. ochraceum* s.l. females were divided into aliquots of 50 specimens each for further processing for DNA, as described below.

DNA purification and amplification by PCR. The heads and bodies of the individual pools were separated by agitation and sieving as previously described.^{9,14} The head and body pools were processed individually. DNA was prepared from the individual head and body pools by homogenization, pro-

tease treatment, organic extraction, and adsorption to a silica matrix as previously described.¹⁵ Purified samples were eluted into microtiter dishes into a final volume of 50 μ L in a buffer containing 10 mmol/L Tris-HCl, pH 8.0, and 1 mmol/L EDTA and stored at -80°C .

All PCR reactions were carried out in sets of 84 samples, in the bottom seven rows of a PCR microtiter plate. A total of 2.5 μ L of the purified genomic DNA from each pool was used as a template for the PCR amplifications carried out in a total volume of 50 μ L containing 0.5 μ mol/L of O-150 5' primer (5'-GATTYTTCCGRCGAANARCGC-3') and 0.5 μ mol/L of 5' biotin-labeled O-150 3' primer (5'-B-GCNRTRTAAATNTGNAATTC-3', where B = biotin; N = A, G, C, or T; Y = C or T; R = A or G), 60 mmol/L Tris HCl (pH 9.0), 15 mmol/L $(\text{NH}_4)_2\text{SO}_4$, 2 mmol/L MgCl_2 , 0.2 mmol/L each dATP, dCTP, dGTP, and TTP, and 2.5 units of Taq polymerase. Cycling conditions consisted of five cycles of 1 minute at 94°C , 2 minutes at 37°C , and 30 seconds at 72°C , followed by 35 cycles of 30 seconds at 94°C , 30 seconds at 37°C , and 30 seconds at 72°C . The reaction was completed by incubation at 72°C for 6 minutes. The top row of each microtiter plate was reserved for 10 negative controls and 2 positive controls. One positive control contained the minimal amount of positive control DNA found to be 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 reaction sets 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.

PCR amplification products were detected by PCR enzyme-linked immunosorbent assay (ELISA) essentially as previously described.⁹ Briefly, 10% of each PCR amplification reaction (5 μ L) was bound to a streptavidin (1 μ g/mL) coated ELISA plate, and the DNA strands denatured by alkali treatment. The bound PCR fragments were hybridized to a fluorescein-labeled *O. volvulus* specific oligonucleotide probe (OVS2: 5'-AATCTCAAAAAACGGGTACATA-FL-3'), and the bound probe detected with an alkaline phosphatase-labeled anti-fluorescein-labeled antibody (Roche Diagnostics, Indianapolis, IN). Bound antibody was detected using the ELISA amplification reagent kit from Invitrogen/BRL (Carlsbad, CA) following the manufacturer instructions. Color development was stopped by the addition of H_2SO_4 to a final concentration of 0.1 mol/L, and the plates were read in an ELISA plate reader set at 450 nm. The cut-off for classifying a sample as putatively positive was set at the mean of the 10 internal negative controls plus 3 SD or 0.1, whichever was greater. A second independent PCR and ELISA were carried out on putatively positive samples. Samples scoring positive in both independent reactions were scored as confirmed positive.

Experimental plan and data analysis. In previous studies using the O-150 PCR to measure the prevalence of infection in vector black flies, all of the head pools and body pools were screened.^{9,15} The prevalence of infection in the body pools and head pools and associated 95% confidence intervals (CIs) were determined using the algorithms available in the computer program PoolScreen v2.0.¹⁶ In these studies, it was noted that, as expected based on the biology of the parasite,

the prevalence of infection in the bodies of the vectors (which contain non-infectious L1 and L2 stages) was uniformly several-fold higher than the prevalence of infection in the head pools (which contain only the infectious third-stage larvae).^{9,15} For this reason, a modified strategy for PCR analysis of the head and body pools was implemented. Here, 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 a given community was taken as evidence for potential ongoing transmission, and screening of the body pools from that location was discontinued. All of the head pools from that community were screened, and PoolScreen was used to estimate the prevalence of infectious flies in the community and the associated 95% CI. If all of the body pools from a given community were screened and none were found to be positive, it was concluded that there was no evidence for ongoing transmission in the community. The prevalence of infected (and infectious) flies was therefore taken to be zero, and the upper bound of the 95% CI for the prevalence of infected (and infectious) flies was calculated using the PoolScreen algorithm. Head pools collected from villages where no evidence for infection in the vector population was found were not analyzed further.

PoolScreen could be used to calculate the prevalence of infectious flies in those villages for which evidence of infection was found in the body pools, because all head pools from such villages were screened. Similarly, PoolScreen could be used to calculate the upper bound for the prevalence of infected (and infectious) flies in those communities where all the body pools were screened and all were found to be negative. However, the model on which PoolScreen is based does not support an experimental plan where screening of pools is discontinued once evidence for infection is found. In the case where the screening of body pools was halted when a confirmed positive body pool was found, a probability-based model was used to obtain an estimate of the infection prevalence. In this model, pools are collected and tested until a certain pre-selected number of positive pools are observed (in this case 1). Using this approach, the statistic of interest is the number of negative pools observed before seeing the pre-selected number of positive pools. If the probability that an individual insect is infected is denoted by p and the number of insects in a pool is N , it follows from probability theory that the probability that a pool is negative is $(1 - p)^N$, whereas the probability it is positive is $1 -$ this quantity. If we sample until we observe m positive pools, the statistic Y , defined as the number of failures before observing a total of m successes, has a negative binomial distribution.

Using this model and observed data, several approaches can be taken to finding a point estimate of p , the prevalence of infection, and/or a CI for the parameter p . One is the classic methods of maximum likelihood and the Clopper-Pearson (C-P) approach to CIs of discrete distributions; the second is by the Bayesian approach to inference. Although individual statisticians often prefer one approach over the other, it is often instructive to use both methods and to compare the results in making a statistical judgment. We have taken that approach here.

In the classic approach to statistical inference, the preferred point estimator is generally the maximum likelihood estimator (MLE) because of its excellent statistical properties. The

Bayesian approach does not use the concept of a point estimate in the same way as the classic approach, because what is viewed in the classic approach as a fixed, but unknown parameter is viewed in the Bayesian approach as a random variable. The Bayesian approach, however, often uses some statistic associated with the distribution of the random variable after the data is observed to serve as a "point estimate like" quantity. Such statistics might be the mean of the random variable, the median, or the mode. Similarly, in the classic approach, CIs are calculated by some algorithm, and the reported probability (e.g. 95%) is the percent of the time given a very large number of sets of data that the algorithm yields an interval containing to true value of the parameter. On the other hand, the Bayesian credibility interval is a probability statement about the quantity of interest based on prior knowledge and the currently observed data. Further details concerning the mathematics involved in the development of the probability-based method may be found in a technical report available at www.soph.uab.edu/bsthome.asp?ID=15.

The prevalence estimates were used to calculate estimates of seasonal transmission potential. Because *S. ochraceum* s.l. females were not collected throughout the year, it was not possible to precisely calculate the annual transmission potential (ATP). However, in Mexico, the level of transmission estimated during the peak of greatest transmission was very low (because of the effect of multiple rounds of treatment with Mectizan). Therefore, the value of transmission potential outside of the peak transmission period is probably zero or near zero. Therefore, the seasonal transmission potential (transmission occurring during the peak transmission season of February through May) probably represents a fairly accurate estimator of ATP. The seasonal transmission potential was calculated as the product of the seasonal biting rate, the proportion of flies carrying L3 larvae in the study season (from February through May), and the average number of L3 larvae in each infective fly. Following established methods,^{2,3} the seasonal biting rate was calculated as follows. First, the monthly biting rate was calculated as the product of the geometric mean number of flies gathered per person during each collection hour (50-minute sampling unit adjusted by hour), the number of hours sampled in a given day, and the number of days sampled in a given month. Once monthly biting rates were calculated, these values were summed to obtain the estimate of the overall seasonal biting rate. The overall proportion of flies carrying L3 larvae was calculated using PoolScreen v2.0, as described above.

In areas of Central America in which *S. ochraceum* s.l. is the vector, the average number of L3 larvae present in each infective fly before Mectizan distribution was estimated to be in the range of 1.5–2.0.¹⁷ Other studies have suggested that number of L3s in each infected fly decreases as the skin microfilarial load is reduced by Mectizan treatment.^{12,18} For these reasons, we estimated that after 17 rounds of ivermectin treatment, the number of infective larvae present in each infective fly would be close to 1.

RESULTS

Individual *S. ochraceum* s.l. were collected during the peak transmission period of February through May 2001 from 13 communities in the three foci in Mexico endemic for *O. volvulus*. The flies were grouped into pools of 50 individuals by

month and community. A total of 1,750 such pools, representing 87,500 individual flies, were prepared. The heads and bodies in each pool were separated and screened by PCR for the presence of *O. volvulus* larvae, using the screening strategy described in Materials and Methods. Using this strategy, 1,094 body pools and 1,288 head pools were examined.

Of the 13 communities, positive body pools were not detected in 5. The estimated prevalence of infected flies in these five communities was therefore zero. The upper bound for the 95% CI for the prevalence of infected flies in these five communities ranged from 3.0/10,000 flies to 10.4/10,000 flies, a number that depended on the number of pools screened from each community (Table 1).

PCR-positive body pools were detected in the remaining eight communities enrolled in the study. In these, two different methods of calculating the prevalence of infected flies were used. In the three communities where all body pools were screened, PoolScreen v2.0 was used to calculate the prevalence of infected flies. In these communities, the prevalence of infected flies ranged from a low of 4.9/10,000 in Altigracia to a high of 86.2/10,000 in Ampliación Malvinas (Table 1).

In the remaining five communities, positive body pools were found before all of the body pools were tested, and testing of body pools was therefore discontinued. As discussed above, PoolScreen could not be applied to calculate the prevalence of infected flies when screening was discontinued after the first confirmed positive pool was found. To calculate the prevalence of infected flies from these communities, an alternative method of calculating the prevalence of infected flies was used, as described in detail in the Materials and Methods. This method is based on a probability model that considers the number of negative pools screened before a positive pool is obtained. Two methods of obtaining an estimate of the prevalence of infected flies in Las Golondrinas (the classic maximum likelihood with the C-P approach and the Bayesian approach) were used. The classic and Bayesian point estimates were found to be quite close to one another (Table 2). However, the CIs derived from the two methods differed somewhat, with the intervals calculated by the Bayesian method smaller than those calculated using the conventional method (Table 2).

TABLE 1

Results of screening pools of bodies for *O. volvulus* infection in communities where all body pools were examined

Community	Total no. body pools screened	No. positive body pools	Prevalence of infected flies*
Northern Chiapas			
Altigracia	125	3	4.9 (1.0–14.1)
El Ámbar	45	0	0 (0.0–8.5)
Southern Chiapas			
Ampliación Malvinas	37	13	86.2 (43.0–151.8)
Nueva Reforma Agraria			
Agraria	78	2	5.2 (0.6–18.7)
Estrella Roja	37	0	0 (0.0–10.4)
Nueva Costa Rica	65	0	0 (0.0–5.9)
Primer de Mayo	117	0	0 (0.0–3.3)
Oaxaca			
Santa Maria La Chichina	129	0	0 (0.0–3.0)

* Number of infected flies/10,000. The number in bold represents the estimated prevalence and the range in normal type the 95% CI surrounding the point estimate.

TABLE 2

Classical and Bayesian estimates for the prevalence of infected flies in villages where all bodies were not screened, using the probability-based method

Village	Total body pools available	Percent body pools screened	Classical*	Bayesian†
Southern Chiapas				
Morelos	128	58%	80.7 1.7–362	79.4 and 55.2 2.03–298
Las Golondrinas	297	26%	7.0 0.17–26.3	7.0 and 4.9 0.2–25.8
Oaxaca				
Santiago Teolaxco	150	32%	137 2.5–711	136 and 92 3.4–480
Santiago Lalopa	182	27%	7.5 0.18–28/3	7.5 and 5.2 0.2–27.8
La Esperanza	292	73%	2.61 0.06–9.7	2.61 and 1.81 0.06–9.6

* The top number in bold represents the point estimate for the prevalence of infected flies and the lower range the 95% CI surrounding the point estimate. Point estimates calculated by the maximum likelihood estimator and CIs using the Clopper-Pearson method. Values are expressed as the number of infected flies/10,000.

† The first number on the top row represents the mean point estimate and the second number the median point estimate using the Bayesian approach. The bottom row presents the 95% credibility range for the Bayesian point estimates. Values are expressed as the number of infected flies/10,000.

The results from the PCR screening of the head pools (representing flies containing infective larvae) in the eight communities in which infected flies were found are summarized in Table 3. In one of the communities (Nueva Reforma Agraria), no PCR-positive head pools were found, leading to a calculated prevalence of infective flies of zero, with an upper bound of 4.8/10,000 flies. In the remaining seven communities, infective flies were detected, leading to prevalence estimates ranging from 1.6 to 29.0 infective flies per 10,000.

Based on the fly collection data, it was possible to calculate the seasonal transmission potential for each community. Because the collection period coincided with the peak transmission season, and after 17 rounds of Mectizan transmission,

TABLE 3

Results of screening pools of heads for *O. volvulus* infection

Community*	Total no. of pools screened	No. of positive pools	Prevalence of infective flies†	Seasonal transmission potential
Northern Chiapas				
Altigracia	125	1	1.6 (0.05–8.3)	0.4
Southern Chiapas				
Ampliación Malvinas	37	5	29.0 (9.0–67.6)	3.3
Morelos	128	3	4.7 (0.9–13.8)	1.3
Las Golondrinas	295	3	2.0 (0.4–5.9)	1.5
Nueva Reforma Agraria	79	0	0 (0–4.8)	0
Oaxaca				
Santiago Teotlaxco	150	6	8.2 (3.0–17.7)	2.7
Santiago Lalopa	182	5	5.6 (1.8–13.0)	2.3
La Esperanza	292	3	2.1 (0.4–6.0)	0.8

* Head pools were only screened from communities in which positive body pools were detected, as described in the text.

† Number of infective flies/10,000. The top number in bold represents the estimated prevalence and the range below the 95% CI surrounding the point estimate.

was generally very low even in the peak season, the seasonal transmission potential probably provided a fairly accurate estimate of the annual transmission potential. In the six communities in which no infective flies were detected, the seasonal transmission potential was estimated to be zero. In the seven communities where infective flies were detected, the seasonal transmission potential ranged from 0.4 to 3.3 (Table 3).

In Morelos and Las Golondrinas in Southern Chiapas, entomological data were available that had been collected before Mectizan treatment was initiated. In Morelos, 6,819 *S. ochraceum* s.l. were collected and dissected in 1980 and 1981, to provide an estimate of the annual transmission potential.¹⁹ Similarly, in Las Golondrinas in 1991, 11,900 *S. ochraceum* s.l. were collected and dissected to obtain an estimate of the transmission potential before initiating Mectizan treatment.¹² When these data were compared with the post-Mectizan seasonal transmission potential estimated as described above, a greater than 90% reduction in pre-treatment values was found. In Morelos, the transmission potential was reduced from 19.8 to 1.3 (Figure 1A), whereas in Las Golondrinas, the transmission potential was reduced from 19.0 to 1.5 (Figure 1B).

Similar findings were obtained from Nueva Reforma Agraria in Southern Chiapas. In this community, no PCR-positive head pools were detected, leading to an estimated

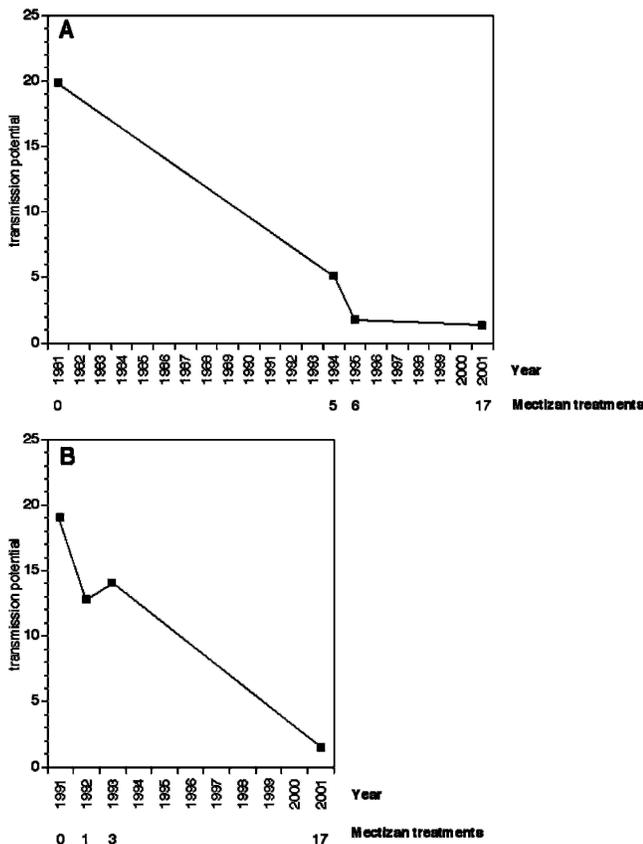


FIGURE 1. Effect of repeated Mectizan treatments on the seasonal transmission potential in two communities in Southern Chiapas. The year that each entomological evaluation was undertaken and the number of rounds of Mectizan treatment that occurred before the evaluation are shown on the abscissa. **A**, Morelos community. **B**, Las Golondrinas community.

prevalence of infective flies of zero, with an upper bound of 4.8/10,000 (Table 3). In this community, no pre-treatment entomological data were available. However, in an entomological study done in 1995 (after six rounds of Mectizan distribution), 9,440 *S. ochraceum* s.l. were collected and dissected (unpublished data). From these data, the prevalence of infective flies was estimated to be 7.4/10,000 (95% CI = 2.9–15.2).

In previous studies, the prevalence of infected flies was always greater than that of infective flies, a finding on which the screening protocol used in this work was based. To confirm that this was the case here, the ratio of the prevalence of infected flies to the prevalence of infective flies was calculated for those communities where infective flies were detected. The ratios of the upper bound of the 95% CIs surrounding the point estimates were also determined. As expected, the prevalence of infected flies was greater than that of infective flies for all communities examined, leading to infected/infective ratios greater than 1 in all cases (Table 4). The infected/infective ratio for the point estimates ranged from 1.2 to 17.2, with a mean of 6.6, whereas the ratios of the upper bounds of the 95% CIs ranged from 1.6 to 27.0, with a mean of 8.6 (Table 4).

DISCUSSION

This study represents the most comprehensive entomological evaluation of the effectiveness of semi-annual Mectizan treatment on *O. volvulus* transmission in the three onchocerciasis endemic foci of Mexico reported to date. The data document that semi-annual Mectizan distribution has succeeded in dramatically reducing transmission. Of the 13 communities examined, no evidence for ongoing transmission was detectable in 5, and no infective flies were detectable in 6. Furthermore, transmission has been dramatically reduced even in those communities where infective flies were detected. In the two communities where pre-control entomological data were available, transmission after 17 rounds of Mectizan distribution was reduced by 93%.

As mentioned above, WHO criteria for suppression of transmission are defined as a 99% reduction in the prevalence of infective flies from pre-control levels where pre-control data are available, or an “absence or near absence” of infection in the vector population where pre-control data are not available.³ This latter criterion has practically been interpreted to mean a prevalence of infective flies of less than 1/10,000. Although not a part of the WHO criteria, we used

TABLE 4
Ratio of prevalence of infected flies to infective flies

Community*	Point estimate infected/infective ratio	Upper bound infected/infective ratio
Northern Chiapas		
Altagracia	3.1	1.7
Southern Chiapas		
Ampliación Malvinas	3.0	2.2
Morelos	17.2	21.5
Las Golondrinas	3.5	4.4
Oaxaca		
Santiago Teotlaxco	16.7	27.0
Santiago Lalopa	1.3	2.1
La Esperanza	1.2	1.6

* Only communities in which positive head pools were found are included.

biting rates and the prevalence of infective flies to estimate transmission potentials. These values also suggest that transmission is currently at very low levels in all three foci. Seasonal transmission potentials ranged from 0.4 to 3.3 in communities where infective flies were found. Assuming that the seasonal transmission potentials are representative of the annual transmission potential (which is likely given the low level of transmission seen during the peak transmission season from which the seasonal transmission potential was calculated), these values are even more suggestive than the WHO criteria that progress toward elimination of onchocerciasis in Mexico has been excellent. Modeling studies have suggested that in the savanna zone of West Africa, reducing the annual transmission potential to less than 100 was sufficient to eliminate the pathologies associated with *O. volvulus* infection as a public health problem.²⁰ While the annual transmission potential necessary to maintain reproduction at a level sufficient to sustain the population (the minimum reproductive rate or R_0) is not known, previous modeling studies using data derived from West Africa have suggested that the ATP necessary to maintain a reproductive rate of 1.0 is approximately 29.²¹ If this is correct, this suggests that control efforts in all three foci in Mexico have been more than sufficient to bring transmission below the level necessary to maintain the minimum reproductive rate.

Consistent with previous studies,^{9,15} the prevalence of infected flies (i.e., those with parasites in the body) were higher than the prevalence of infective flies (i.e., those with parasites in the head). This confirms the hypothesis that the prevalence of infected flies is the most sensitive indicator of parasite-vector contact, and thus body pool PCR positivity can be used effectively to monitor for the parasite-vector contact in communities subject to control. However, because the life cycle stages in the body of the flies are not infectious, the prevalence of infected flies is not an accurate measure of transmission, which is the indicator of most significance to the control programs. Here, we have adopted a strategy that combines the advantages of screening both heads and bodies, screening head pools from only those communities where the presence of positive body pools provides evidence for parasite-vector contact, and discontinuing screening of bodies as soon as a confirmed body positive pool is found. By using the probability-based approach described in Materials and Methods, it was also possible to obtain an estimate of the prevalence of infected flies from the data collected by the approach of screening body pools until a positive pool is found. Using this strategic approach results in a significant savings in both time and money, with little or no loss in the information obtained.

The prevalence of infected flies is not an important indicator of the level of ongoing transmission and therefore is not a significant metric for control activities. However, this parameter can be useful in other respects. Because infected flies are more common than infective flies, the prevalence of infected flies provides a more sensitive measure of the degree of parasite-vector contact than does the prevalence of infective flies. Thus, this parameter may become a useful measure of vector-parasite contact when the prevalence of infective flies (and thus transmission) reaches undetectable levels.

Two methods of deriving point estimates and associated 95% CIs for the prevalence of infected flies in the communities in which only a portion of body pools were examined were used in this study. These were the classic method and the

Bayesian method. It is interesting to note that, whereas the classic and Bayesian point estimates for the prevalence of infected flies were quite close to each other, the CIs calculated by the Bayesian method were consistently tighter than those calculated by the classic method. This is most likely because the C-P intervals are known to be very conservative (generally too wide, especially when the actual prevalence is small), and as a result, are generally not recommended for this reason. Other types of CIs can be considered in the classic setting, but these rely on large sample arguments and are known not to be exact. In any case, a sample size of one is hardly large, and so such intervals are totally inappropriate in the example used here.

A primary objection that classic statisticians have with the Bayesian approach is the need to provide a synthesis of prior knowledge in the form of a "prior" distribution, that is, before having gathered the current data. At some point, however, one has no prior experience and so a "reference or noninformative" prior must be used. Yang and Berger²² provided a catalog of noninformative priors. For the negative binomial, the Jeffrey's prior is one of the two possibilities. This prior has the advantage that it is robust with respect to re-parameterization of the probability model and so was chosen over the simple uniform model for our work. The posterior distribution of p (the prevalence of infection) for our data is a rather complex distribution that can be found in closed form. It is a β distribution with parameters $(Y + \frac{1}{2}, 1)$ and the quantity $(1 - p)^N$.²³ Because of the known conservativeness of the C-P interval, we believe that the Bayesian interval is the more accurate interval in this case.

Although the probability-based method reported here was used to calculate the prevalence of infected flies, there is no reason that this method might not be applied to calculating the prevalence of infective flies as well. One might envision a screening strategy in which head pools from a given community are screened until X positive pools are obtained. Once X positive pools are found, screening is stopped and the prevalence of infective flies calculated from the number of pools that were screened before X positive pools were found, using the probability-based method described here. Using such an approach, one might be able to obtain an accurate estimate of the prevalence of infective flies without screening all of the pools obtained from a given community. This approach would have obvious advantages in terms of time and cost savings compared with a strategy of screening all head pools. However, for this approach to be practical, it will be necessary to determine the number of positive pools that will have to be detected to yield an acceptably accurate estimate of the true prevalence of infective flies. Studies addressing this question are currently underway.

In both states, it is clear that transmission was not evenly distributed, because the prevalence of infected and infective flies varied considerably in different communities from the same focus. For example, in Southern Chiapas, no evidence for any transmission was detected in two of the seven communities, but a high prevalence of infective flies was seen in one community (Ampliación Malvinas). The persistence of transmission in Ampliación Malvinas was in concordance with studies suggesting exposure of the human population to the parasite as judged by the presence of *O. volvulus*-reactive antibodies in sera collected from children ≤ 5 years old (unpublished data). Ongoing transmission at Ampliación Malvi-

nas cannot be ascribed to poor coverage with Mectizan, because coverage data showed that this community met its ultimate treatment goal in every one of the 5 years before 2001, when the flies used in this study were collected (data not shown). This suggests that, in some cases, transmission may persist even in the face of ostensibly good coverage with Mectizan. There are several potential reasons that this might occur, including the presence of infected seasonal workers who may be difficult to identify and target during Mectizan distribution or the existence of heavily infected individuals who may be refusing treatment and serving as parasite reservoirs. In such cases, entomological studies such as those presented here may be useful in pinpointing areas where transmission is continuing in the face of good coverage data, thus requiring additional attention.

In the initial study of a portion of the black flies collected in 2001 at three foci in Mexico, a single PCR-positive pool of heads from the community of Altagracia from Northern Chiapas was found.⁹ At that time, it was suggested that this might represent evidence for autochthonous transmission in this community, but that it was also possible that this result represented a laboratory artifact, and that an analysis of additional flies would resolve this issue. In this study, three PCR-positive body pools from Altagracia were found, providing additional evidence for autochthonous transmission in this community. In contrast, no evidence for transmission was found in El Ambar, the second community surveyed in Northern Chiapas. There are at least two possible explanations for this. First, the number of pools examined from El Ambar was small, and may have been insufficient to detect a low level of transmission. Second, the seasonal biting rate of *S. ochraceum* s.l. in El Ambar was low relative to that in Altagracia (740 versus 2793 bites/person/season, unpublished data). It is possible that this level of vector-host contact is not sufficient to maintain transmission in El Ambar in the face of continuing Mectizan pressure.

In summary, the data presented here show that substantial progress toward interruption of transmission of *O. volvulus* has been made in all three endemic foci in Mexico. No evidence for transmission was detected in 6 of 13 communities, and the seasonal transmission potential was extremely low even in those communities where vector infectivity was detected. In all communities, the level of transmission may have now been brought below the level necessary to maintain the minimum reproductive rate of the parasite population. Additional entomological and modeling studies will need to be carried out before it can be confirmed that transmission has been suppressed in Mexico. Such studies are currently underway.

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